Modulation of Spatial Processing By Somatosensory Inputs In The Rat

Thomas Gener

PhD report

2011

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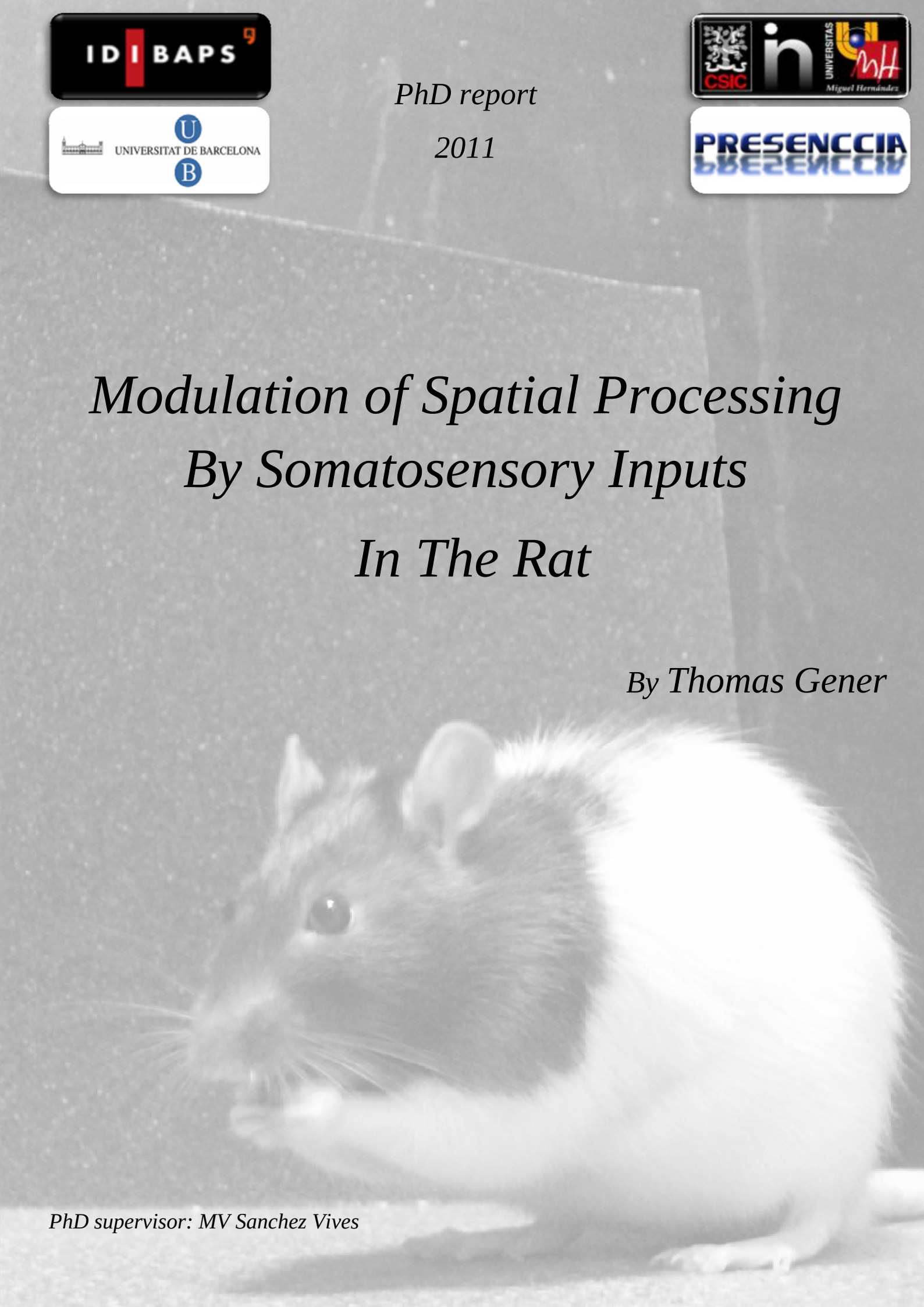

PhD supervisor: MV Sanchez Vives

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I would like to thank my parents which let me choose the hard and difficult way of science. To all my family and friends to support my never ending studies.

Petka, you know already everything.... ***Thank you***.

ABREVIATION

BD: Basal ganglia

BPN: Brainstem premotor nuclei

CA: Cornus Ammonis

CA1: Cornus Ammonis 1 of the hippocampus

CA2: Cornus Ammonis 2 of the hippocampus

CA3: Cornus Ammonis 3 of the hippocampus

CEnt: Caudomedial entothinal cortex,

Cer: Cerebellum

BCx: Barrel cortex

DG: Dentate Gyrus

DIEnt: Dorsal intermediate entorhinal cortex,

DLEnt: Dorsolateral entorhinal cortex,

EC: Entorhinal Cortex

Ect: Ectorhinal cortex

EEG: Electroencephalogram

fMRI: Functional Magnetic Resonance Imaging

FN: Facial nucleus

GD: Grid cell

GrDG: Granular layer of the Dentate Gyrus

Hp: Hippocampus

IO: Inferior olive

LFP: Local field potential

LMol: Lacunosum moleculare layer of the Hippocampus

LTD: Long term depression

LTP: Long term potentiation

M1: Somatomotor 1

MCx: Primary motor cortex

MEnt: Medial entorhinal cortex

MoDG: Molecular layer of the Dentate Gyrus

MoS: Molecular layer of the Subiculum

Or: Oriens layer of the Hippocampus

PaS: Parasubiculum

PC: Place cell

PET: Positron emission tomography

PF: Place field

Pn: Pontine nuclei

PoDG: Polymorph layer of the Dentate Gyrus

POm: Medial sector of the posterior nucleus of the thalamus

PRh: Perirhinal cortex

PrS: Presubiculum

Py: Pyramidal cell layer of the hippocampus

Rad: Radiatum layer of the hippocampus

RN: Red nucleus

S1: Somatosensory cortex 1

S2: Secondary somatosensory cortex

SC: Superior colliculus

SLu: Stratum lucidum of the hp

TeA: Temporal association cortex

TG: Trigeminal ganglion

TN: Trigeminal nuclei of the brainstem.

VIEnt: Ventral intermediate entorhinal cortex

VL: Ventrolateral thalamic nucleus

VPM: Ventroposteromedial nucleus of the thalamus

VPMdm: Dorsomedial VPM,

VPMvl: Ventrolateral VPM

VS: ventral subiculum

ZI: Zona incerta

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SUMMARY

The generation of cognitive maps is influenced by different senses such as vision, audition or smell. However, the tactile information system -a highly developed system in the rat- and its influence on spatial processing, has hardly been studied. The availability of precise tactile information in the hippocampus (Pereira *et al.*, 2007) is highly suggestive of a possible influence of tactile information on spatial processing. In this study we aimed to test if somatosensory information contributes to the cognitive map creation and spatial representation. The deprivation of the tactile sense without the possibility of using other senses (total darkness, homogeneous odour and uniform white noise), should then affect the coding of spatial information and could be detected as an alteration in place cell properties such as firing rate, location and/or extension of the firing fields. These types of changes would demonstrate that somatosensory inputs are involved in the cognitive map creation.

To carry out this study we developed three kinds of experiments. First, we developed a paradigm (Gener *et al.*, 2009) to temporarily deprive the tactile input using locally applied local anaesthesia (lidocaine). In a second part, we demonstrate that this deprivation was effective in the awake animal, altering the behaviour during tactile discrimination protocols and reducing successful trials from 88% to chance (48%). Finally, we applied the deprivation technique to characterise the cognitive map creation. With that purpose, we first demonstrated that place cells recorded in a controlled environment were sensitive to tactile cues, such that the rotation of the cues induce the rotation of the firing fields. Next, when tactile information was deprived, the place cells' fields showed changes in their compactness and size.

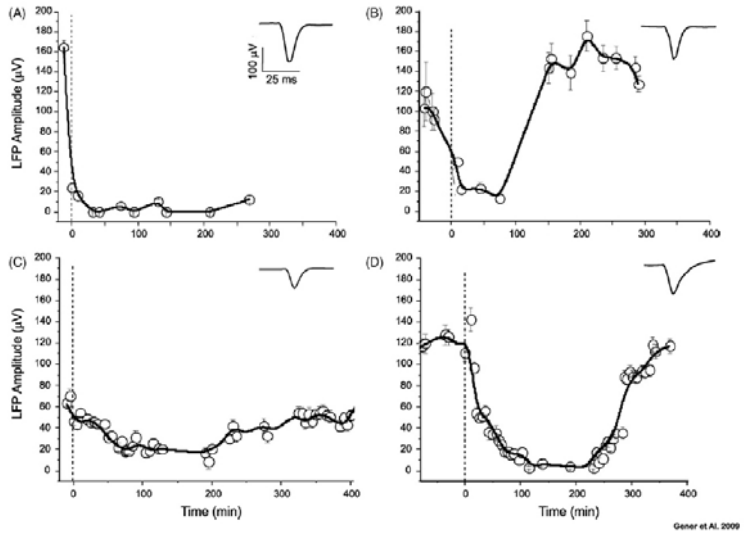
The results of this study suggest that somatosensory input information transduced by the whiskers contributes to the cognitive map creation. Those findings respond to some of the questions about hippocampus integration's of sensory information.

RESUMEN

La generación de los mapas cognitivos espaciales (O'Keefe and Nadel, 1978) está influenciada por sentidos como la visión, la audición o el olor. Teniendo en cuenta, anteriores estudios y, en particular, uno que describe la conectividad entre la información táctil y el hipocampo (Pereira *et al.*, 2007), nuestro objetivo aquí ha sido descubrir si que la información somatosensorial está implicada en la creación del mapa cognitivo y en la representación espacial del sujeto en el entorno. Para realizar nuestro objetivo estudiamos las bases neuronales de la representación espacial, las células de lugar. Son neuronas que disparan solo cuando el animal esta en un sitio determinado de sus entorno. En el resto del espacio, estas neuronas son relativamente silenciosas. Si la hipótesis de que hay modulación táctil de los campos de la células de lugar es cierta, la pérdida del tacto, sin la posibilidad de utilizar los otros sentidos (oscuridad total, olor homogéneo y ruido blanco uniforme), debería afectar la codificación de la información espacial y como resultado se podría observar en las células de lugar un cambio de los campos receptores y cambios de propiedades como la frecuencia de disparo, la localización. Estos cambios demostrarían que las entradas somatosensoriales se ven efectivamente involucradas en la creación del mapa cognitivo.

Para llevar a cabo este estudio, hemos desarrollado tres tipos diferentes de experimentos para tener así una visión completa del sistema. Hemos desarrollado un nuevo paradigma (Gener *et al.*, 2009) para privar temporalmente las entradas táctiles utilizando un anestésico local (la lidocaína). Se registro la corteza de barriles mientras se estimulaba los bigotes del animal con y sin aplicación de lidocaína. Después, hemos demostrado que la privación del sentido del tacto con la técnica desarrollada afectaba las habilidades de un animal despierto. El animal entrenado a discriminar entre dos texturas dejaba temporalmente de poder lleva a cabo esta tarea con la aplicación de lidocaína en los bigotes. Finalmente, determinamos las implicaciones del tacto en la creación del mapa cognitivo registrando células de lugar y aplicando la técnica de deprivación del tacto en uno de los dos protocolos.

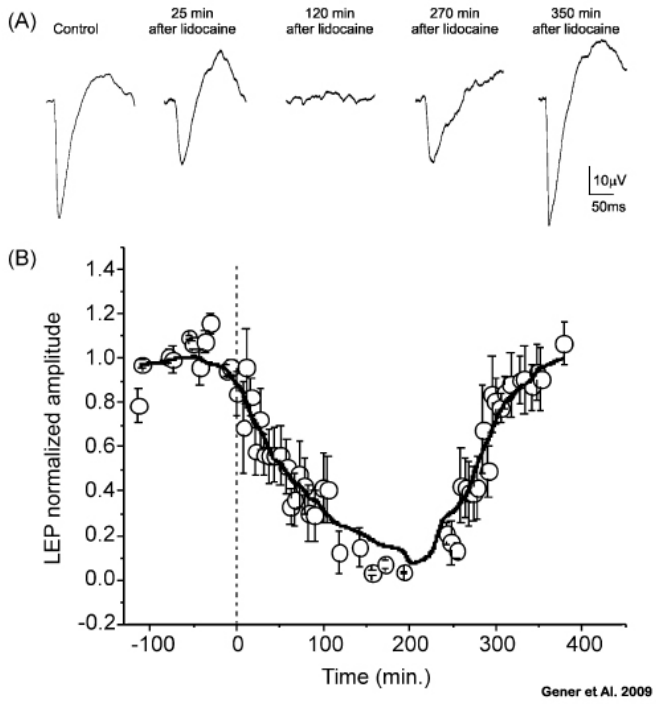
La técnica de deprivación fue realizada registrando la corteza de barril con y sin presencia del anestésico local, la lidocaína, mientras estimulábamos los bigotes con un soplo de aire.



Curso temporal de la acción de los cuatros tipos de aplicaciones de la lidocaína. El cero en abscisas (línea punteada) representa el tiempo de aplicación de la lidocaína. Cada circulo representa el promedio de la amplitud de 60 respuestas sensoriales (0.2 Hz). (A) Inyección subcutánea de lidocaína (10%, 0.2 ml). (B) Aplicación de la lidocaína en crema, 1 g (25 mg/g). (C) Aplicación de la lidocaine en espray (10%, 2 espray, 0.2 ml). (D) aplicación gotas Liquidas de lidocaine (10%; 0.4 ml).

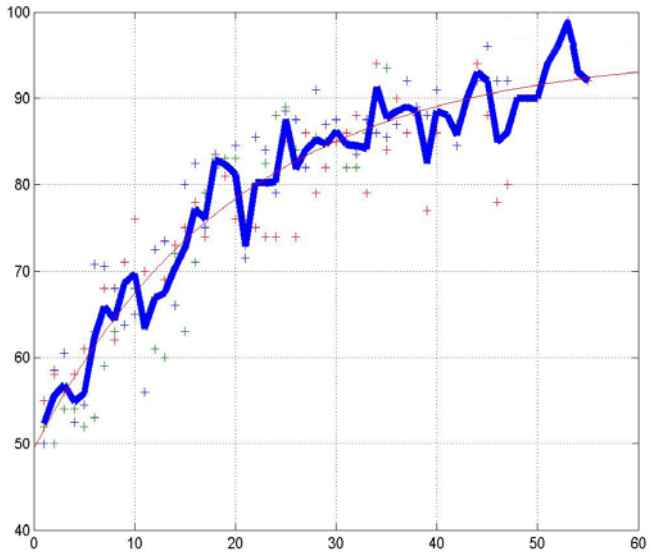
El soplo de aire fue ajustado para tener una respuesta extracelular significativa entre 50 y 100µV y una respuesta intracelular entre 5 y 10mV en la condición de control. Se probaron diferentes técnicas de aplicación de la lidocaína: en crema, inyecciones líquidas, espray líquido o gotas líquidas en la base de los bigotes. La técnica funcionó en todos los casos, aunque cada una de las formas de aplicación tiene sus ventajas

La técnica elegida como la más adecuada es la aplicación de lidocaína líquida en la base de los bigotes de la rata. Esta técnica, es la más estable a lo largo de los diferentes experimentos y la más segura en cuanto a su uso con animales despiertos.



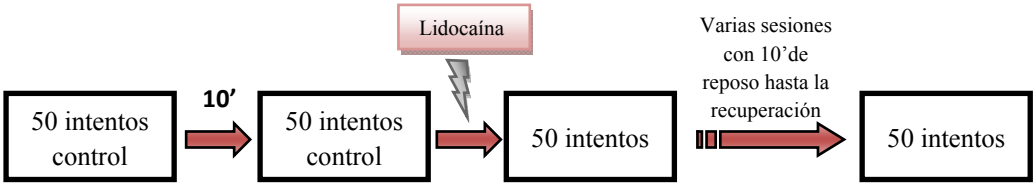
Efectos de la aplicación de gotas de lidocaína 10% (0.4 ml) en la base de los bigotes. (A) Forma del promedio de la respuesta extracelular inducida antes y después de la aplicación de lidocaína. (B) Promedio global del curso temporal de la aplicación de lidocaína (n = 6). Cada punto representan el promedio de 60 amplitudes de respuestas evocadas. La respuesta evocada es normalizada con respecto a la respuesta control.

Para estar seguros que la técnica de deprivación del tacto descrita previamente fue efectiva en animales despiertos, se realizó un estudio de discriminación táctil con y sin aplicación de lidocaína. La pérdida de la información proveniente de los bigotes debería disminuir la capacidad de discriminar la textura y permitirnos ver el curso temporal de la acción de la lidocaína en animales despiertos. Para el protocolo de discriminación táctil los animales se entrenan para discriminar entre dos texturas. La rata aprende a reconocer una textura a su sitio asociado para así recibir una recompensa (una gota de agua) y si se equivoca en vez de la recompensa recibe un castigo (sonido repulsivo). Para realizar la tarea se realizó tanto el "set-up" como el software para controlarlo y se diseñó un protocolo de aprendizaje de la tarea. Una vez que el animal consigue discriminar entre las dos texturas con un porcentaje de éxito superior al 80% se inicia el protocolo de privación.



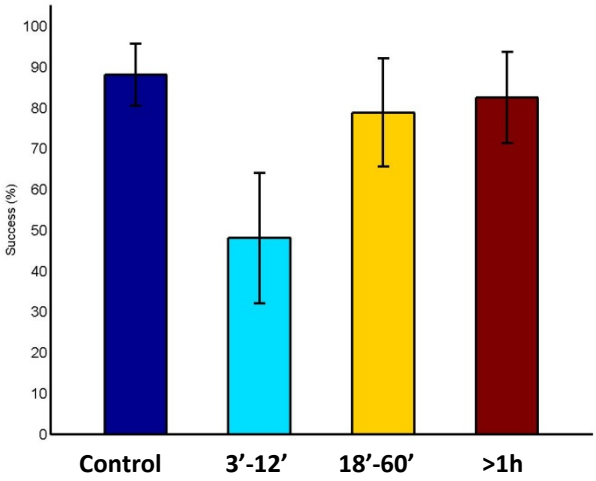
Curva de aprendizaje de la tarea de discriminación táctil. Para 3 animales: cada punto representa 50 intentos de una misma sesión (eje X representa el número de sesión, eje Y representa el % de éxito).

Primero se realiza dos sesiones de control para tener un nivel básico de respuesta y luego se aplican unas gotas de lidocaína en la base de los bigotes del animal. El animal realiza la discriminación táctil en esas condiciones hasta recuperar el nivel control de porcentaje de éxito de realización de la tarea previo a la privación. Cada sesión consta de 50 exposiciones a la textura.



Esquema del protocolo de la aplicación de la lidocaína.

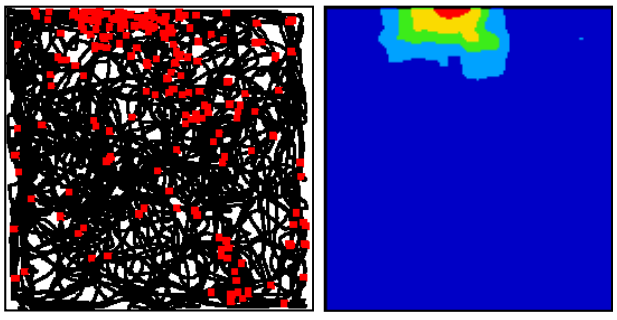
Los resultados confirman nuestra hipótesis reduciendo de 88% a 48% el porcentaje de respuestas correctas.



Porcentaje de éxito durante la privación somatosensorial. 1^{era} columna (en azul oscuro), promedio de las condiciones de control. 2^{nda} columna (en azul claro), después de la aplicación de lidocaína (3-12 min). 3^{era} columna (en amarillo), el primer período de recuperación (18-60 minutos) y la 4ta columna (en rojo), la recuperación completa (más de 60 min.).

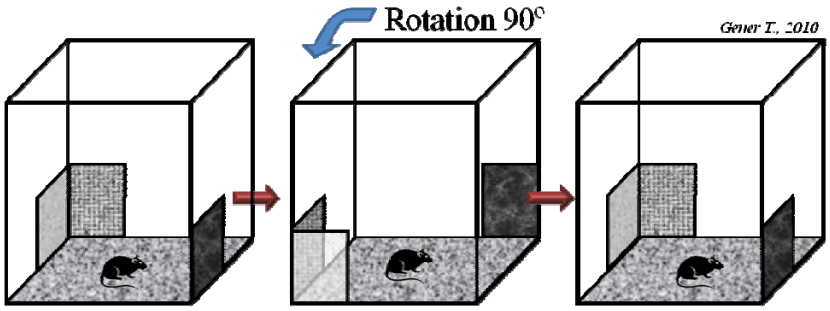
Se observa que una vez la acción de la lidocaína y en consecuencia el fin de la deprivación del tacto, la discriminación de las texturas vuelve a niveles semejantes a los de la condición de control. Además de observar el porcentaje de éxito, se calculó también el tiempo de reacción del animal desde la exposición hasta la recompensa así como el número de time-outs realizado (número de veces que el animal empieza un intento pero no lo acaba). El factor de Fano (un índice de dispersión de los datos) se calcula también para tener una idea de la variabilidad de cada una de las variables estudiadas. Todos los resultados confirman la hipótesis de que la lidocaína impide al animal realizar correctamente la tarea temporalmente.

Una vez desarrollada y validada la técnica de privación somatosensorial en animales despiertos realizando una tarea de discriminación táctil se aplicó en uno de los experimentos de electrofisiología conductuales. El objetivo de esos experimentos es demostrar que la información táctil es una parte integrante del proceso de creación del mapa cognitivo y también el de caracterizar la actividad de las células de lugar con y sin esta información. Para llevar a cabo este objetivo, un estudio de las células de lugar de la región CA1 del hipocampo fue realizado en dos diferentes protocolos.



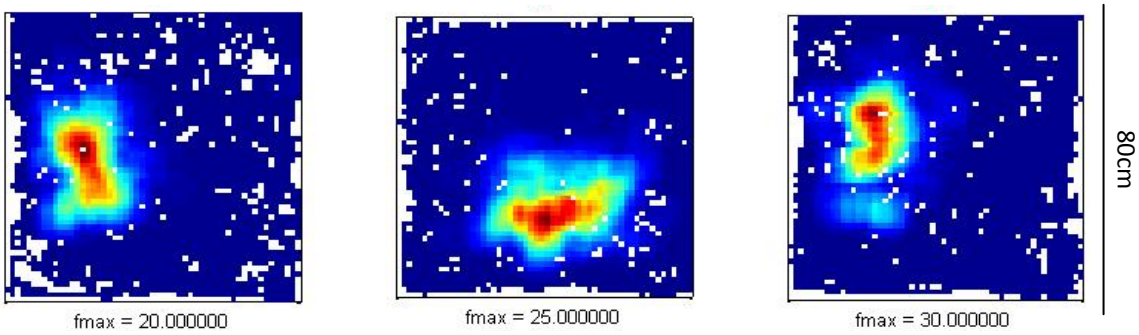
Representación de las células de lugar. A la izquierda: localización de los disparos de una neurona con la ruta recorrida por el animal. A la derecha: representación de la zona de disparo en el entorno. El código de color muestra la frecuencia de disparo corregida por el tiempo de permanencia, rojo para el máximo y azul oscuro para el mínimo (de mis datos).

El primer protocolo consistió en el registro de células de lugar en un entorno cuadrado enriquecido con pista táctiles (tres papeles de lija de diferente rugosidad) en 3 condiciones diferentes. Una condición control, después una condición de rotación, el entorno con las pistas táctiles es girado 90º, y para acabar una última condición control de nuevo. Los registros se realizan en las condiciones más controladas posibles. Es decir, como estudiamos el tacto, solo este sentido puede estar activo mientras que los demás deben estar, en cuanto posible, controlados. Con ese fin se limpia el entorno con alcohol perfumado entre cada registro además de que durante el registro se emite un sonido blanco uniforme y finalmente durante todo el registro se mantiene una oscuridad total. Este protocolo está diseñado para determinar si las células de lugar utilizan efectivamente el sentido del tacto para generar su zona de disparo.



Esquema del protocolo de rotación.
Las pistas táctiles (papel de lija) están rotadas de un ángulo de 90º.

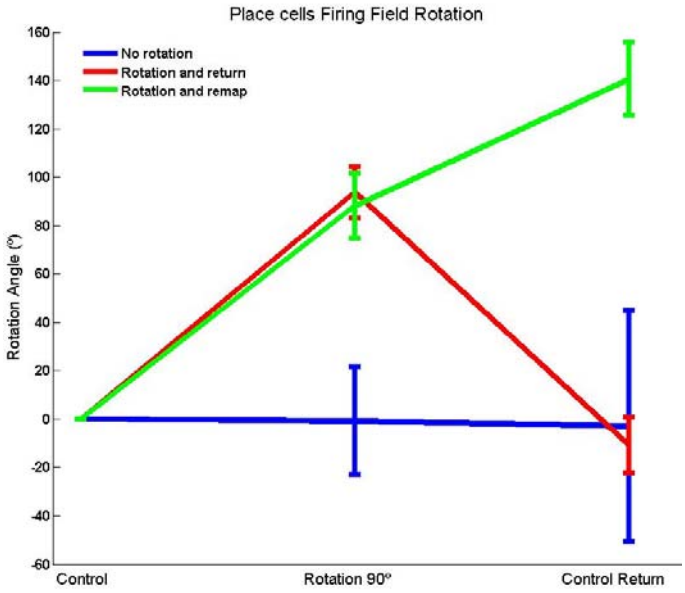
Los resultados demuestran que la mayoría de las células de lugar giran 90º su zona de disparo de acuerdo con las pistas táctiles. Sin embargo, tres grupos diferentes de células de lugar pueden ser formados debido a su comportamiento durante el protocolo. El primero está formado por las células que giran de acuerdo con las pistas táctiles (rotación y retorno). Este grupo representa 71.4% del total de las células de lugar registradas.



Ejemplo de "rotación y retorno" de una célula de lugar.
La zona de disparo ha rotado de 90º de acuerdo con las pistas táctiles (rata RF registros: RF2101T4C1).

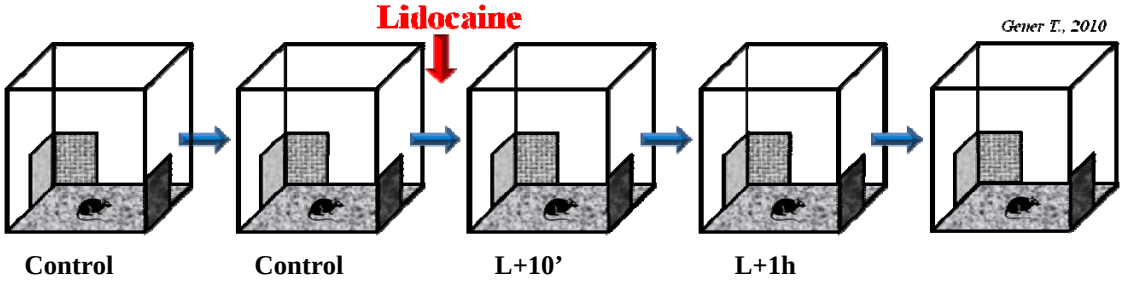
El segundo grupo incluye las células cuyas zonas de disparo que giran con las pistas táctiles la primera vez pero no vuelven. Son el grupo de células rotación y reasignación. Este grupo representa 19.04% del total de las células de lugar registradas. El último grupo de células está formado por células de lugar cuyos campos nunca rotan con las pistas táctiles (no rotación). Este grupo representa 9.52% del total de las células de lugar registradas.

Estos resultados confirman la hipótesis de que al menos algunas células de lugar utilizan la información recibida de los aferentes táctiles (pistas táctiles) para generar su zona de disparo.



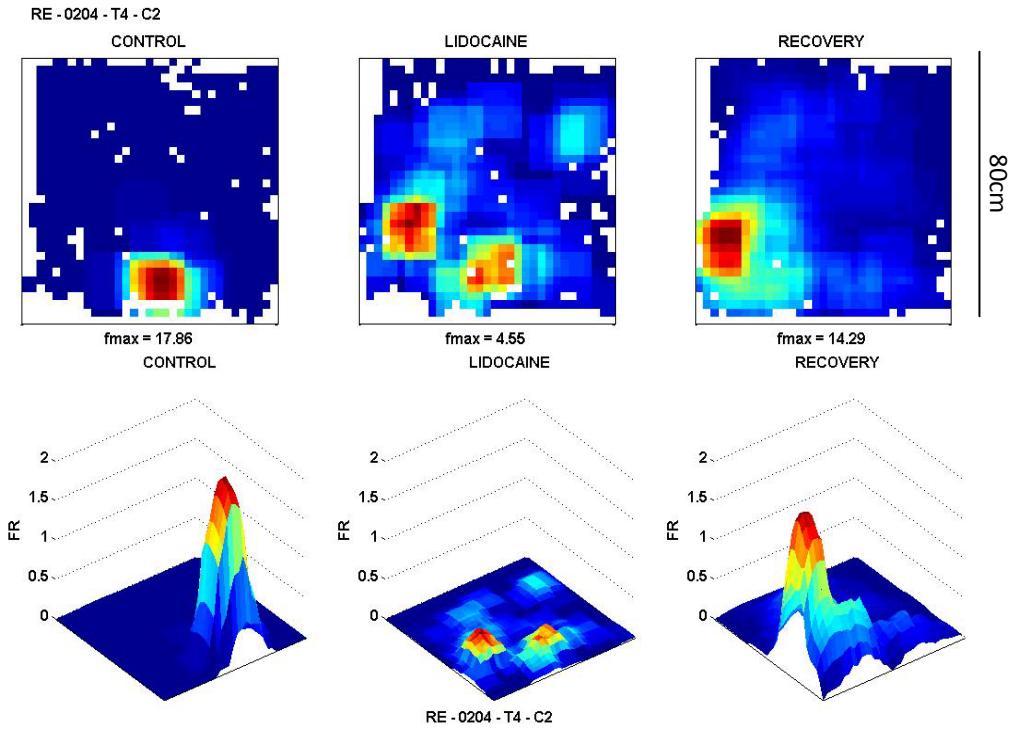
Ángulo de rotación. Superposición de los grupos de "no rotación" en azul, "Rotación y retorno" en rojo y "rotación y reasignación" en verde. En el eje x las condiciones del Protocolo; en el eje y los ángulos de rotación normalizados respecto a la condición de control.

El segundo protocolo consiste en registrar células de lugar en el mismo entorno que el descrito anteriormente, es decir con las mismas condiciones controladas y enriquecidas táctilmente. El protocolo se compone de dos partes, la primera son registros controles, después, se aplica la lidocaína en la base de los bigotes del animal tal fue desarrollada la técnica descrita anteriormente. Se registra las células de lugar en condición de privación sensorial hasta más de 3 horas después.



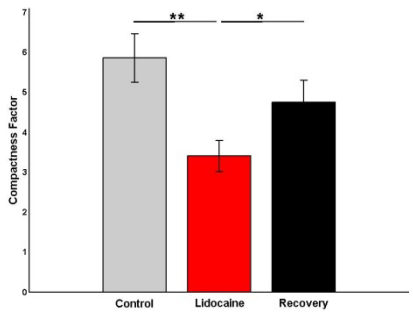
Esquemas del protocolo de privación sensori...
3 papeles de lijas (P360, P180, P40) son colocados de manera asimétrica en el laberinto.

Todas las células de lugar registradas fueron analizadas y varios factores de espacialidad y de no espacialidad fueron calculados: la frecuencia máxima (en Hz), la simetría, el área 20 (% del área total que supera en más del 20% la frecuencia media de disparo), área 60 (idem pero por encima del 60%), la frecuencia media (en Hz), el índice Skaggs (contenido de información espacial), el vólumen del FF (Firing field o zona de disparo), la compacidad, la amplitud del disparo y la anchura del disparo.



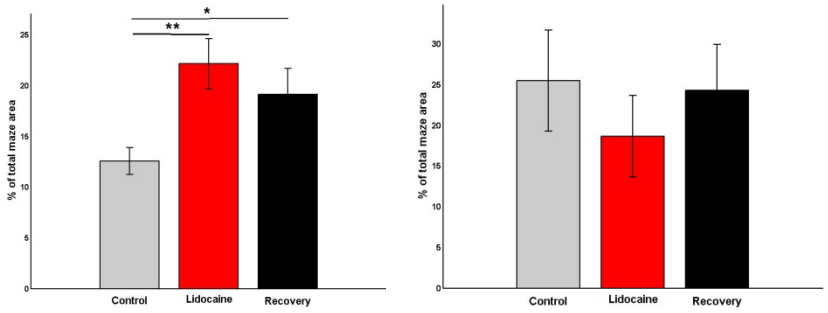
Ejemplo de células de lugar (rata E). En la parte superior, representación de la células de lugar en condición control, con lidocaína, y después de la recuperación. En la parte inferior, la misma célula en una representación 3D (eje Z, la frecuencia de disparo).

Al calcular estos factores con todas las células seleccionadas, se encontraron diferencias significativas para algunos de ellos (compacidad, simetría, area20).

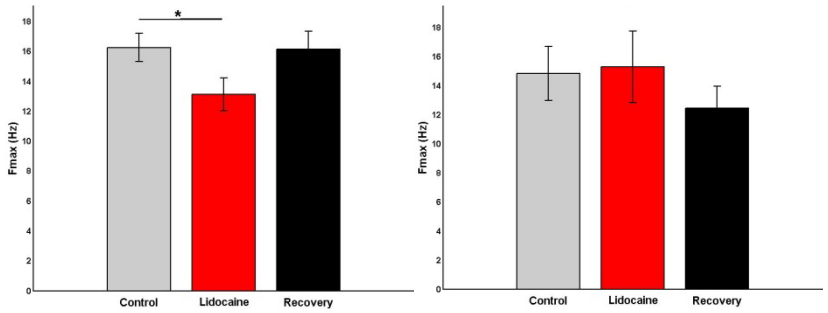


Compacidad para todas las células de lugar. Comparación entre condiciones de control, lidocaína y recuperación (p= 8.2026e10-4, * p= 0.0445).**

Al promediar toda la población de células se pueden ocultar cambios opuestos en diferentes grupos. Por ese motivo se crearon dos grupos usando el factor de compacidad. Si una célula de lugar tiene una compacidad superior en la condición de control que en la de lidocaína, forma parte del grupo uno. El grupo dos, inversamente, está compuesto de las células de lugar que tienen una compacidad superior en condiciones de lidocaína que en condición de control. Una vez establecidos los grupos se vuelve a calcular todos los parámetros para los dos grupos de células de lugar. El grupo uno está compuesto por 51 células que representan a su vez el 78,5% de las células de lugar mientras que el grupo dos está compuesto por 14 células que representan un total de 21,5% del grupo de celdas de lugar.



Area20 para los grupos durante las condiciones. A la izquierda, grupo 1 (p=9.58e10-4); a la derecha, grupo 2.**



Fmax para los grupos durante las condiciones. A la izquierda, grupo 1 (*p=0.032); a la derecha, grupo 2.

Una vez los grupos están separados, las diferencias son más importantes y más numerosas. Así, la compacidad, el area20 y area60, la frecuencia máxima y la simetría son significativamente diferentes. Todos los factores tienen la tendencia de volver una vez el efecto de la lidocaína ha pasado.

Los resultados muestran que las células de lugar registradas en un entorno enriquecido táctilmente son sensibles a las pistas táctiles (reasignación por rotación). A su vez, cuando la lidocaína es aplicada, las células de lugar muestran cambios en la manera de disparar como por ejemplo en la compacidad o el tamaño del campo de disparos.

El estudio realizado en esta tesis sugiere que la información táctil proveniente de los bigotes es una parte del sistema de creación del mapa cognitivo. Estos descubrimientos, gracias a las técnicas conductuales utilizadas hoy en día, responden a ciertas preguntas sobre las propiedades de integración de la información sensorial por parte del hipocampo.

INTRODUCTION

To fully understand the study realised during this PhD, several scientific notions and concepts are needed. In this introduction these information will be defined briefly.

1. CONCEPT OF SPATIAL NAVIGATION

Different of species such as mammals (human or rodents for example), birds or even insects need to have spatial "sense" to survive. This means looking for food, escaping from predators, protecting themselves from weather (finding a refuge) or getting some social interaction (reproduction or social relationship). This sense is defined as the capacity to orientate oneself in a known or unknown environment, to be able to recognize it and to navigate in it. Most of the animals can do that by instinct (Tolman, 1948). To demonstrate this faculty, scientists invented protocols to simulate natural environments and natural tasks as the radial maze (maze of 8 arms for example) (Olton, 1979) or the water maze (swimming pool where a platform is hided) (Morris *et al.*, 1982) for the most known and commonly used. Those tools allow scientists to demonstrate that different strategies are used by animals to identify the environment. The development of electrophysiological techniques in the last quarter of 20th century mainly with the multi-single unit recordings techniques (McNaughton *et al.*, 1983; O'Keefe and Recce, 1993; Wilson and McNaughton, 1993) increases the possibilities of spatial navigation research. To explain the concept of spatiality, many theories have been published, but the start point for all of them is called "the cognitive map". This theory is based on the fact that spatial behaviour is not the result of stimulus – response associations but of a spatial representation of the environment. The revolution, even if Tolman in 1948 (Tolman, 1948) expressed this concept, came with the publication in 1978 by O'Keefe and Nadel of "The Hippocampus as a cognitive map" (O'Keefe and Nadel, 1978).

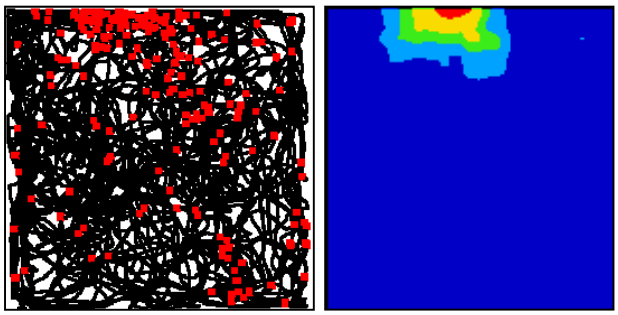


Figure 1: Place cell representation.
On the left: spike localisation of a neuron with animal's path. On the right: place field (PF) representation, spike frequency with respect to position (from my own data).

This book, more than hypothesising the concept, by reviewing all the previous knowledge of the field, tried to demonstrate the physiological basis of the navigation, spatial representation and also localised in the brain this function. This was made possible by the previous discovery of place cells (PCs) (O'Keefe and Dostrovsky, 1971) in 1971 by O'Keefe and Dostrovsky.

Place cells are pyramid cells of the hippocampus (Hp) which fire only when the animal is in a specific place of the environment (Figure 1). This fundamental discovery shows that in the hippocampus exist cells which are active only when the animal is in a specific place of the environment. Recently, in 2005, Hafting demonstrated that other cells in the hippocampal complex, more precisely in the entorhinal cortex (EC), have another type of spatiality than the place cells that he called grid cells (GDs) (Figure 2).

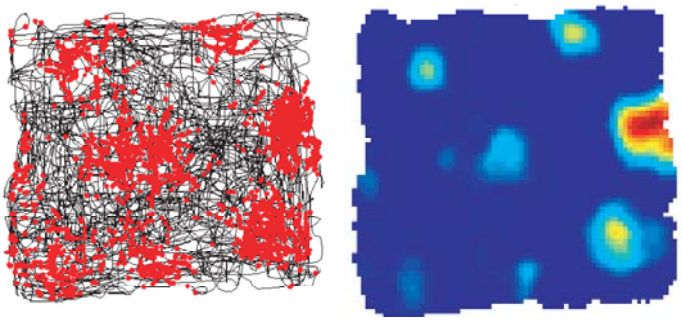


Figure 2: Grid cell representation.
On the left, spike localisation and path, on the right multi place field representation. T*aken From Hafting et al. (Hafting et al., 2005).*

"Grid cells are neurons that fire when a freely moving animal traverses a set of small regions (firing fields) which are roughly equal in size and arranged in a periodic triangular array that covers the entire available environment" (Hafting *et al.*, 2005). The hippocampal complex, with mainly PCs and GCs but also with other cells as head direction cells (HDCs) (Ranck, 1984; Taube, 2007) (cells that are active when animal is orientated in one precise direction), should be the centre or a part of the spatial representation.

2. THEORICAL BASES OF SPATIAL NAVIGATION

Spatial orientation is the ability to use the environment in order to know at each time one's own position and how to move optimally into this environment. Mammals, and more precisely rodents, on which the studies were mainly performed, can build some internal spatial representation of the environment. Those representations are used

to solve several orientation problems such as finding some food in a labyrinth or realising a spatial task (Gallistel, 1990; Gallistel, 1990).

To explain how navigation in an environment is possible, two main strategies were proposed. The allocentric and egocentric navigation are two distinct but not opposite modes.

The allocentric navigation is based on the principle that animals use external cues to generate a topographical representation of the environment. External cues are usually relevant landmarks. Natural external cues could be geographic artefacts as trees, plants, rocks, mountains, water sources or stars. Experimental external cues could be colour marks, geometric forms, or objects placed in a maze for example. It has been demonstrated in behavioural studies that animals use landmarks to orientate and find a platform (Morris *et al.*, 1982) or to find food previously hidden (Biegler and Morris, 1993; Biegler and Morris, 1996). More than that, neurons in Hippocampus complex, as Place cells, are sensitive to landmarks, a simple change of the colour of the landmark in the environment can change the electrophysiological properties (Bostock *et al.*, 1991).

Allocentric navigation and spatial representation are one of the ways animals use to orientate themselves but it will be nonsense to pretend that animals navigate using only external cues. If those cues, for any reason, are not available, animals could be able to orientate themselves in another way. The other way, alternative or complementary to the allocentric navigation, use a sequential strategy. Animals know the position of its own body regarding all the elements of the environment from a starting point. The number of steps done in a direction with respect to some fix point could be remembered, estimated and finally known. If an object is on the right or left, behind or in front of him, gives some of the information an animal can get to orientate to start it trajectory and more generally it spatial representation. The movement itself of the animal is then a vector. This vector is continuously moving with the animal movement. Each time the animal moves, the vector position is actualised allowing the determination of the distance covered. This is the egocentric navigation, a sequential strategy (McNaughton *et al.*, 1996). This strategy integrates internal cues as vestibular, motor and orientation information. During spatial foraging, the animal records the information of its own path to create trajectories. Egocentric navigation is also called path integration navigation (McNaughton *et al.*, 1996; Etienne and Jeffery, 2004;

Jeffery, 2007; Jeffery, 2007). In this kind of orientation, more than just the hippocampus seems to be involved, for example, the parietal cortex. This strategy, more efficient and rapid in some cases, did not allow as much flexibility as the allocentric one and could generate some error or imprecision in the trajectory.

Trying to localise the biological origin of this concept, several behavioural experiments damaging parts of the brain have been carried out. The partial or complete damage of the hippocampus complex, (Morris *et al.*, 1982; Schenk and Morris, 1985; Morris *et al.*, 1990) shows that this anatomical brain structure is involved in the spatial process. Studies using the technique of functional Magnetic Resonance Imaging (fMRI) show that the hippocampus complex is involved in human navigation suggesting that "place cells like" could be present in the human hippocampus (Aguirre *et al.*, 1996) which were confirmed several years after (Ekstrom *et al.*, 2003; Ekstrom *et al.*, 2005).

The biological basis of spatial navigation even if not completely known involve more than just place cells. Others specific cells have been discovered, GCs (Hafting *et al.*, 2005) and HDCs (Ranck, 1984) seem to be involved more specifically in the path integration navigation. Some evidence has been also discovered in human virtual navigation (Wolbers *et al.*, 2004).

3. HIPPOCAMPUS COMPLEX (ANATOMY, ORGANISATION AND FUNCTIONS)

Because everything point to the hippocampus as the main structure involved in spatial representation, we will describe shortly, in this part, anatomic and functional role of the hippocampus.

3.1. ANATOMY

The hippocampus comes from the archicortex. After its development, is situated down the posterior and temporal cortex. It is part of the telencephalon. It is a symmetrical bilateral structure. The Hp is part of the limbic system. This one includes the hippocampus, cingulate cortex, olfactory cortex, and amygdala. The limbic structures are part of the neural basis of emotion, behaviour, long term memory and olfaction (MacLean, 1952).

The fact that Hp is present in most of the species is currently accepted. Hippocampus' size increases with animal size, twice bigger for humans than for

monkeys, but the relative size is inverted proportionally to the evolution level of the species. Humans have a small hippocampus regarding his brain's size and in rodents as rats, it is the opposite (Figure 3). This is probably due to the increase of the cortex's size during species' evolution. Even with those differences of size, Hp organisation is not different from one to another species (O'Keefe and Nadel, 1978). An interesting fact is that the organisation of the Hp is similar in all the species suggesting the importance of its role in the nervous system.

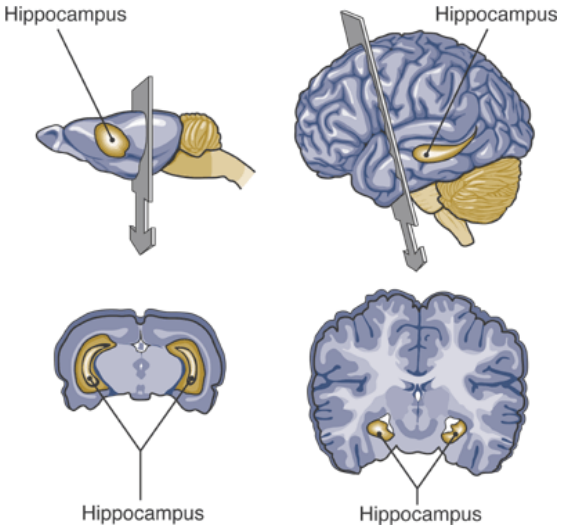


Figure 3: Brain Model.
On the left, rat brain. On the right, human brain. *Taken from Hiller-Sturmhöfel et al.* (Hiller-Sturmhöfel and Scott Swartzwelder, 2004).

ORGANISATION

In mammals, the hippocampus has the shape of a curved tube in form of big C, which has been analogized variously to a seahorse, which it takes its name, to the ram's horns of Amun in Egyptian mythology (*Cornu Ammonis*, CA), which give the name of the subdivisions (CA1 to CA4). It is formed by two layers which are one inside of the other. The granular cell layer of the dentate gyrus is the first of the cell layers. The second one, composed by the subiculum and the pyramidal cells of the *Cornu Ammonis*, is considered as the real Hippocampus.

DENTATE GYRUS

The dentate gyrus (DG) is composed by three layers of neurons (Figure 4): polymorphic, granular and molecular (reviewed in Lopes da Silva *et al.*, 1990; Andersen *et al.*, 2007). The middle layer is the most prominent and contains granule

cells that project to the CA3 subfield of the hippocampus. These granule cells project mostly to interneurons, but also to pyramidal cells and are the principal excitatory neurons of the DG. The major input to the dentate gyrus (perforant pathway) is from layer 2 of the entorhinal cortex and the dentate gyrus receives no direct inputs from other cortical structures as much as the literature describes. The perforant pathway is divided into the medial perforant path and the lateral perforant path generated, respectively, at the medial and lateral portions of the entorhinal cortex. The medial perforant path creates synapses onto the proximal dendritic area of the granule cells, whereas the lateral perforant path does so onto the distal dendrites of these same cells.

The dentate gyrus layers are:

- *The polymorphic layer* is the superficial layer of the DG. This layer contains mainly interneurons and the axons of the dentate granule cells pass through this stratum on the way to CA3.
- *Stratum granulosum* is composed by the cell bodies of the dentate granule cells.
- *Stratum moleculare*, is divided in two parts. The first one is where commissural fibres from the contralateral dentate gyrus run and form synapses as well as where inputs from the medial septum terminate on the proximal dendrites of the granule cells. The second part is the deepest of the strata, sitting just superficial to the hippocampal fissure. The perforant path fibres make excitatory synapses with the distal apical dendrites of granule cells.

HIPPOCAMPUS

The hippocampus (Hp) is composed by multiple subfields (Figure 4). In rodents, the hippocampus is positioned so that, roughly, one end is near the top of the head (the dorsal or septal end) and other end near the bottom of the head (the ventral or temporal end). The *Cornu Ammonis* regions are also structured in clearly defined strata (or layers) (reviewed in Lopes da Silva *et al.*, 1990; Andersen *et al.*, 2007):

- The *alveus* is the most superficial layer. It contains the axons from pyramidal neurons. It is one of the major outputs of the hippocampus.
- *Stratum oriens* is just under the *alveus*. The cell bodies of inhibitory basket cells and horizontal trilaminar cells are located in this stratum. The basal dendrites of pyramidal neurons are also found here, where they receive inputs from other

pyramidal cells (recurrent connections, especially in CA3), septal fibres and commissural fibres from the contralateral hippocampus. In rodents, the two hippocampi hemispheres are highly connected (Notice that in primates for example, this commissural connection is weaker).

- *Stratum pyramidale* contains the cell bodies of the pyramidal neurons. Those neurons are the principal excitatory neurons of the hippocampus. In region CA3, this stratum contains synapses from the mossy fibres which course through stratum lucidum (see below). This stratum also contains the cell bodies of many interneurons, including axo-axonic cells, bistratified cells, and radial trilaminar cells.
- *Stratum lucidum* is one of the thinnest layers in the hippocampus. Mossy fibres from the dentate gyrus granule cells pass through this stratum in CA3.
- *Stratum radiatum,* as well as *Stratum oriens*, contains septal and commissural fibres. It also contains Schaffer collateral fibres. Those fibres project forward from CA3 to CA1. Some interneurons which can be found in more superficial layers can also be found here, including basket cells, bistratified cells and radial trilaminar cells.
- *Stratum lacunosum* is a thin stratum. It contains Schaffer collateral fibres, but it also contains perforant path fibres from the superficial layers of entorhinal cortex. Due to its small size, it is often grouped together with stratum moleculare into a single stratum called stratum lacunosum-moleculare.
- *Stratum moleculare* is the deepest stratum in the hippocampus. Here the perforant path fibres form synapses with the distal and apical dendrites of pyramidal cells.
- *The hippocampal sulcus* or fissure is a cell-free region that separates the CA1 field from the dentate gyrus. The phase of recorded theta rhythm varies systematically through the layer. The fissure is often used as a fixed reference point for recording Electroencephalogram (EEG) because it is easily identifiable.

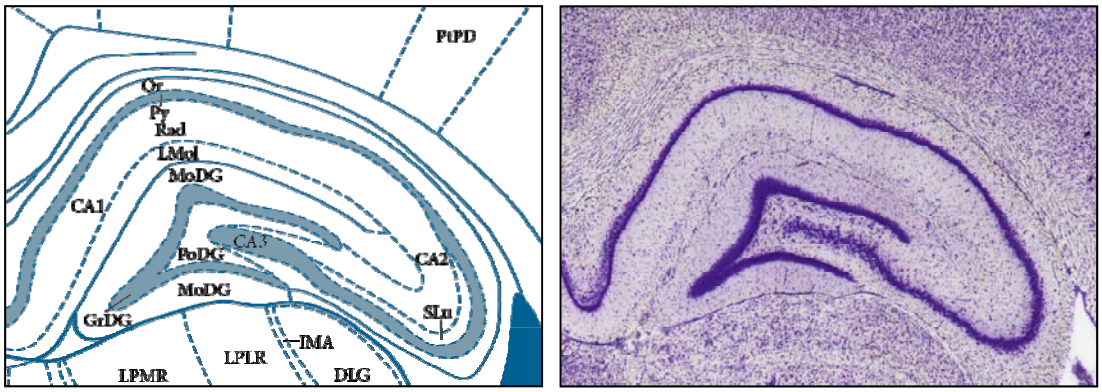


Figure 4: Rat hippocampus zone at the coordinate -3.96 mm from bregma.
On the left scheme of the hippocampus (Hp). Or: oriens layer of the hp, Py: pyramidal cell layer of the Hp, Rad: radiatum layer of the Hp, LMol: lacunosum moleculare layer of the Hp, MoDG: molecular layer of the DG, PoDG: polymorph layer of the DG, MoDG: molecular layer of the DG, GrDG: granular layer of the DG, SLu: stratum lucidum of the Hp, CA1: field CA1 of the Hp, CA2: field CA2 of the Hp, CA3: field CA3 of the Hp. On the right, photo of hippocampus stain with NISSL technique. *Taken from Paxinos (Paxinos and Watson, 2005).*

In the hippocampus anatomy field of investigation it has to be noticed that the first to investigate were Ramon y Cajal (1893) and later Lorente de Nõ (1933), using what is now "classical Golgi" studies. They proposed the first classification still used nowadays. The differences are done on the criteria of the presence or not of body cells, of axons and of dendrites. Four regions, CA1, CA2, CA3, and CA4 were originally described. CA1 is the upper region formed by small pyramidal cells. CA2 and CA3 are inferior regions, but nowadays most of the authors do not distinguish those two regions and refer to it as CA3 with the particularity to be formed by big pyramidal cells.

All the layers of the hippocampus are highly inter-connected (Amaral and Witter, 1989). All the connections are well known and many studies focus on this preparation *in vitro*. The granular cells project their axons on the dendritic's tree of the CA3 pyramidal cells via the mossy fibres.

ENTORHINAL CORTEX

The entorhinal cortex (EC) is situated at the end of the temporal lobe in the rodents (Figure 5). It is usually divided into medial and lateral regions with three bands with distinct properties and connectivity running perpendicular across the whole area. The entorhinal cortex has a major importance because it serves as an interface between the hippocampus and the neocortex. The entorhinal cortex also maintains connections with neocortical association areas (as well as with the amygdala, hippocampus, septal nuclei, olfactory bulb, etc.) but not directly with primary sensory areas (Leichnetz and

Astruc, 1976). A distinguishing characteristic of the EC is the lack of cell bodies where layer IV should be; this layer is called the *lamina dissecans*. The superficial layers - layers II and III - of EC project to the dentate gyrus and hippocampus: Layer II projects principally to dentate gyrus and hippocampal region CA3; layer III projects primarily to hippocampal region CA1 and the subiculum. These layers receive inputs from other cortical areas, especially associational, perirhinal, and parahippocampal cortices, as well as prefrontal cortex.

The entorhinal cortex processes inputs from every sensory modality, as much as inputs relating with cognitive processes. The deep layers, especially layer V, receive outputs of the hippocampus. Rodent's entorhinal cortex shows a modular organization. In 2005, it was discovered that entorhinal cortex contains a neural map of the spatial environment in rats with the grid cells (Hafting *et al.*, 2005). Neurons in the lateral entorhinal cortex exhibit little spatial selectivity (Hargreaves *et al.*, 2005), whereas neurons of the medial entorhinal cortex (MEnt), exhibit multiple "place fields" (PF) that are arranged in an hexagonal pattern, and are therefore called "grid cells." These fields and spacing between fields increase from the dorso-lateral MEnt to the ventro-medial MEnt (Fyhn *et al.*, 2004). Recently, some evidence shows the same kind of organisation in humans with cells active only for one path :"path cell" (Jacobs *et al.*, 2010).

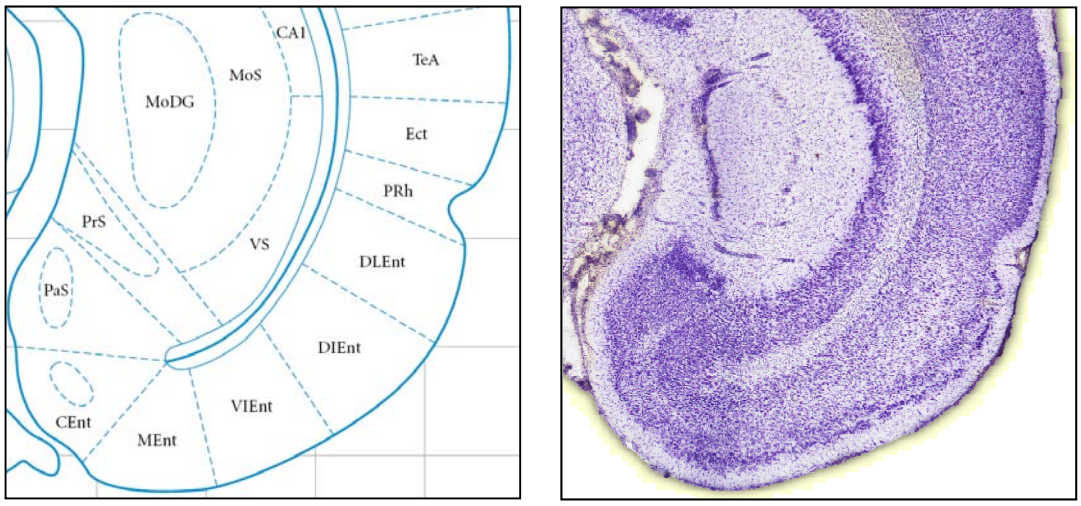


Figure 5: Entorhinal Cortex zone at the coordinate -6.84 mm from bregma.
On the left, scheme of the entorhinal cortex, CA1: field CA1 of the Hp, MoS: molecular layer of the subiculum, MoDG: molecular layer of the DG, PrS: presubiculum, VS: ventral subiculum, PaS: parasubiculum, CEnt: caudomedial entothinal cortex, MEnt: medial entorhinal cortex, VIEnt: ventral intermediate entorhinal cortex, DIEnt: dorsal intermediate entorhinal cortex, DLEnt: dorsolateral entorhinal cortex, PRh: perirhinal cortex, Ect: ectorhinal cortex, TeA: temporal associatin cortex. On the right, photo of entorhinal cortex stain with NISSL technique. *Taken from Paxinos (Paxinos and Watson, 2005).*

HIPPOCAMPAL CIRCUIT

We will start the description of the hippocampal circuit with the dentate gyrus and following the functional path (Figure 6). The dentate gyrus (DG) is a tight layer of small granule cells wrapped around the end of the proper hippocampus, forming a semicircle. Next comes a series of Cornu Ammonis areas: first CA4 (which underlies the dentate gyrus), then CA3, then a very small zone called CA2, then CA1. The CA areas are all filled with densely packed pyramidal cells. After CA1 comes an area called the subiculum. Then come two areas called the presubiculum and parasubiculum. Finally, the circuit finishes with cortex, mainly the entorhinal cortex (EC).

The hippocampal formation is a loop receiving input from the EC (layer 2/3) via the axons called perforant path and sending outputs to EC (layer 4/5). The perforant path originates in layer 2 of the EC, and terminates in the dentate gyrus and CA3. The other inputs from EC layer 3 are connected directly to CA1. Granule cells of the DG send their axons (called "mossy fibres") to CA3. Pyramidal cells of CA3 send their axons to CA1. Pyramidal cells of CA1 send their axons to the subiculum and deep layers of the EC. Subicular neurons send their axons mainly to the EC. The perforant path-to-dentate gyrus-to-CA3-to-CA1 is called the “trisynaptic circuit” and justifies the lamellar hypothesis of the hippocampus which proposed that the hippocampus can be thought of as a series of parallel strips, operating in a functionally independent way (Andersen *et al.*, 1969; Andersen *et al.*, 2007).

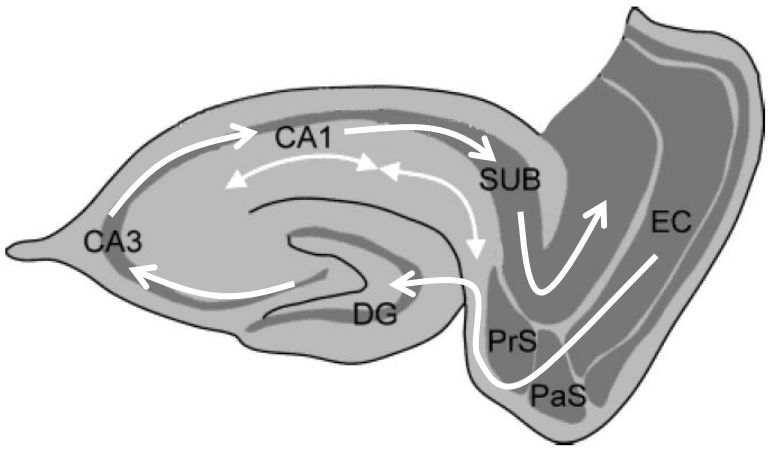


Figure 6: Scheme of the hippocampus circuit.
Adapted from Bannerman et al. (Bannerman and Sprengel, 2007).

Perforant path inputs from EC layer II go into the dentate gyrus and is relayed to region CA3 (and to mossy cells, located in the hilus of the dentate gyrus, which then send information to distant portions of the dentate gyrus where the cycle is repeated). CA3's region combines this input with signals from EC layer II and sends extensive connections within the region and also sends connections to region CA1 through a set of fibres called the Schaffer collaterals (reviewed in Ishizuka *et al.*, 1990).

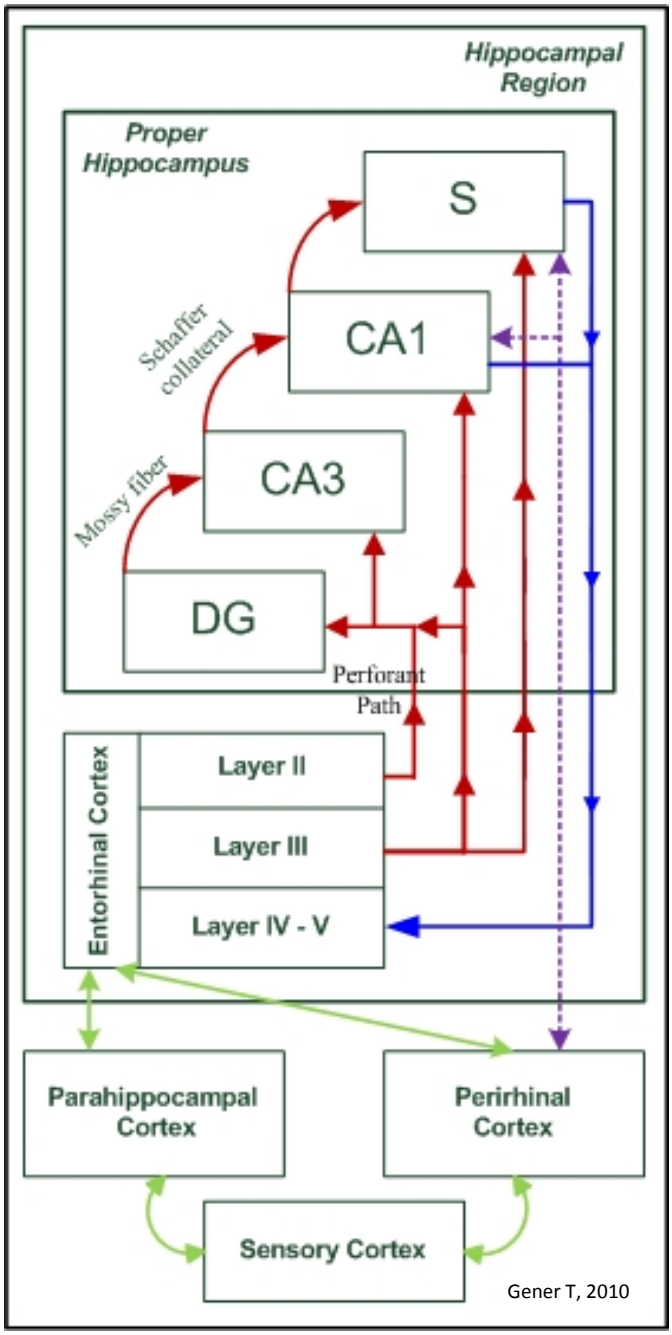


Figure 7: Scheme of the hippocampal region.
Scheme inspired from Amaral et al. and O'Mara (O'Mara, 2005; Amaral and lavenex, 2007).

Region CA1 receives input from the CA3 subfield, EC layer III and the nucleus reuniens of the thalamus (which project only to the terminal apical dendritic tufts in the *stratum lacunosum-moleculare*). In turn, CA1 projects to the subiculum as well as sending information along the aforementioned output paths of the hippocampus. The subiculum is the final stage in the pathway, combining information from the CA1 projection and EC layer III to also send information along the output pathways of the hippocampus (Figure 7). The hippocampus also receives a number of subcortical inputs.

HIPPOCAMPAL INPUTS

The main input of the hippocampus as we already mentioned, comes from the entorhinal cortex. It is an important structure of the limbic system. It receives a lot of information from several neocortical areas. It links to the prefrontal, cingulate, temporal, piriform and visual cortex. There is also a direct projection from the olfactory bulb to the entorhinal cortex (Kerr and Dennis, 1972). The layer II of the entorhinal cortex projects perforant fibres to the granular cell of the dental gyrus and CA2/CA3 (Steward and Scoville, 1976; Tamamaki and Nojyo, 1993). Layer II of the entorhinal cortex projects perforant fibres to CA1 (Steward and Scoville, 1976) and the subiculum (Witter and Amaral, 1991). The hippocampus receives also inputs from the brain stem (locus coeruleus, median raphe nucleus), and from septum via the fornix (Risold and Swanson, 1997).

The hippocampus has a complex structure organization. This organisation was explained thanks to two different theories, the "laminar organisation" theory and the "tri-synaptic circuit". The lamellar concept is still sometimes considered to be a useful organizing principle, but more recent data, showing extensive longitudinal connections within the hippocampal system, have required it to be substantially modified (Andersen *et al.*, 2000). Hippocampal' structure more than bidimensional is definitely tridimensional.

HIPPOCAMPAL OUTPUT

Hippocampus projects outputs to many structures. Pyramidal cells from CA1 send projection to the subiculum (Amaral *et al.*, 1991) which is the main output of the hippocampus. This one sends projections mainly on the entorhinal cortex. EC receives also some output to it layer V from CA1 (Calderazzo *et al.*, 1996). Via the fornix, the subiculum is connected to the thalamus through mammillary bodies and the amygdale

complex (which receives direct projection from CA1). Subiculum projects also to the septum (which receives direct projection from CA1 and CA3) and accumbens nucleus (Groenewegen *et al.*, 1987).

We remind that the main input, output and the organisation of the hippocampus, which was originally supposed simple and laminar, is more complex than that. The existence of a loop in CA3 with a lot of interconnection contrasts with a CA1 low interconnected. Furthermore, many of the interneurons of a huge axonal's tree extend transversally and longitudinally. Hippocampus is highly connected with other parts of the brain, notably with the cortex as it is interconnected with the several parts of the hippocampus complex.

Finally, the right and left hemispheres of the hippocampus are also exchanging information through the commissural fibres. Pyramidal cells of CA1 and CA3 project on the opposite side of hippocampus.

Until recently, hippocampus was considered as a functionally uniform structure. This fact in the latest years changed radically. Several studies in the hippocampus demonstrated a strong spatial organisation of some functions as the ability to evoke sustained long term potentiation (LTP) (Segal *et al.*, 2010) or the fact that rhythm theta is not uniformly distributed in the hippocampus (Lubenov and Siapas, 2009).

3.2. HIPPOCAMPUS FUNCTION

In this part, we will resume the most relevant functions of hippocampal structure. Even if our interest is focused in spatial learning and spatial memory, it will be very reducing to say that hippocampus is only dedicated to those functions. Hippocampal functions are diverse and multiples. Nowadays, it is accepted that the functions of hippocampus are learning and memory in general and spatial learning in particular (Schmajuk, 1984). We will do a short non exhaustive description of the several functional aspects in which the hippocampus is involved.

Memory process is conceptually constructed in three steps: Encoding (receiving information); Storage (save the encoded information); Retrieval (remember the stored information). Those several steps in memory are characterized in term of memory by three temporal memory types: sensory memory (the very beginning of the perception), short term memory (few times after the information perception, working memory is one

aspect of this), long term memory (long time memory storage). In humans, since the first proofs that hippocampus complex were involved in memory, many theories have been developed. In 1957, Scoville and Milner published a medical case about a patient that had an important hippocampal lesion (patient HM). This patient had a severe anterograde amnesia and some spatial and non spatial memory deficits (Scoville and Milner, 1957). More recently thanks to new methods as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), it has been confirmed that the human hippocampus is responsible of some spatial processes (Aguirre *et al.*, 1996) and by stimulating those structures, spatial learning is possible in a virtual town (Maguire *et al.*, 1998; Maguire *et al.*, 1999). From all those theories emerges a concept that memory is divided in several subfields (Figure 8). Memory is organized in two general concepts which appear in the beginning of the second part of the 20th century with Ryle (Ryle, 1949); One responding to "knowing that", the declarative memory (also name explicit memory); The other one in contrast, the non-declarative memory (also call procedural memory, or implicit memory), responding to "knowing how". Furthermore, the declarative memory is separated in the semantic memory (memory of meanings, understandings, and other concept-based knowledge unrelated to specific experiences) and in the episodic memory (memory of autobiographical events and referring to specific events).

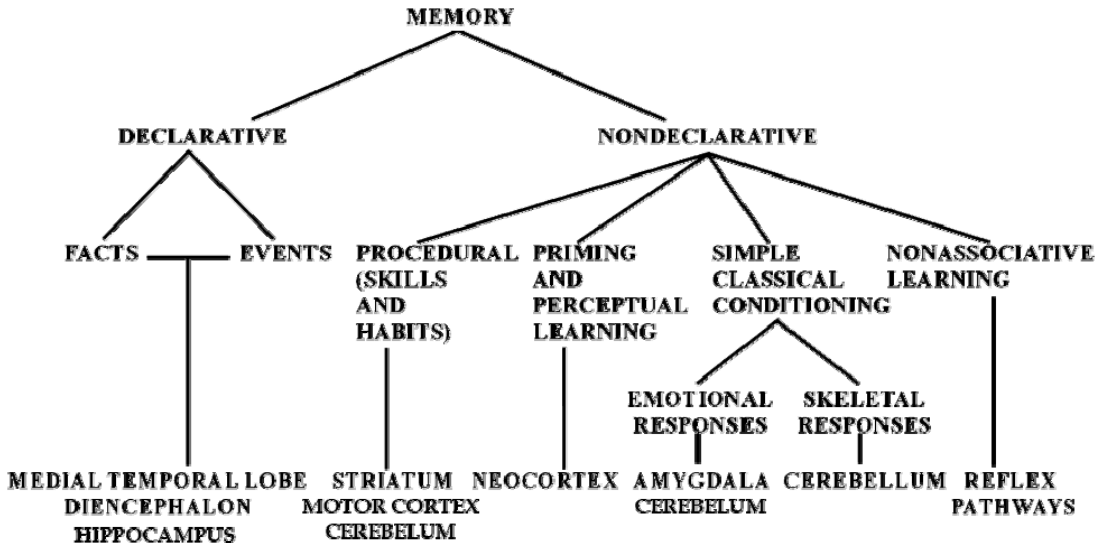


Figure 8: Taxonomy of mammalian memory systems.
Adapted from Whishawet al. [72].

Even if the hippocampus is not only dedicated, to the spatial memory, it is an essential function of this structure. Spatial memory is the part of memory responsible for recording information about environment and spatial orientation. One of the most effective ways to involve a structure in some functions is to inactivate it or to damage this structure. Hippocampus after lesion reveal to be involved, for example, in the consolidation of the memory (Winocur, 1990) or in some task that uses working memory (Olton, 1979). It has been also demonstrated in the last decade that damage in the hippocampus complex or similar depending on the species, leads to difficulties in food localisation tasks (in birds, Hampton and Shettleworth, 1996; Fremouw *et al.*, 1997). In rodents, depending on the damaged zone, deficits are distinct and levels of seriousness are different but mainly involved in the spatial memory.

In the radial maze, damaged animals were incapable to remember which arms they already visit (Olton *et al.*, 1982), in the water maze damaged animals could hardly find the platform (Morris *et al.*, 1986; Good and Honey, 1997). In some different experiences, damaged animals had some difficulties to learn where the platform was if the start point was changed but not if the platform was visible (Eichenbaum *et al.*, 1990; McDonald and White, 1994) or if the start point was stable, this showing some evidences for the path integration theory. On the same principle, if some inputs of the hippocampus region are damaged, as Fimbria – Fornix, the subject is incapable to come back to his start point (Whishaw and Tomie, 1997; Whishaw and Maaswinkel, 1998) or has some impossibility to realize task in radial maze (Olton *et al.*, 1982) or water maze (Sakamoto and Okaichi, 1998). In other species, as the monkey, hippocampal's lesions also lead to some deficits in realisation of spatial tasks (Baylis and Moore, 1996).

As it is obvious that the hippocampus is not only dedicated to spatial memory, it is also demonstrated that spatial memory is not only localised in the hippocampus. Other brain structures are also involved in spatial control. Lesions of those structures lead to similar or identical effects of hippocampal's lesions. For example, parietal cortex (Kolb and Walkey, 1987; Save and Moghaddam, 1996), median frontal cortex (Kolb and Tees, 1990) or septum (Kelsey and Landry, 1988) lesions lead to deficits in spatial tasks in water maze. Apart from the mnemonic function, hippocampus seems to be involved in further other functions. For example, a rat with hippocampal lesions leads to a decrease of olfactory capacities (Eichenbaum *et al.*, 1986) and to some alterations of the temporal evaluation of events (Olton *et al.*, 1987). Hippocampus seems to be also involved in the

behaviour inhibition which could be named anxiety (Kimble, 1968; Gray, 1982; Davidson and Jarrard, 2004). Behavioural inhibition refers to a pattern of behaviour involving withdrawal, avoidance, fear of the unfamiliar and over-arousal of the sympathetic nervous system. Sensory motor functions are also present in the hippocampus (Vanderwolf and Cain, 1994). Even if cerebral lesions do not eliminate the basic motor patterns involved in instinctive behaviour, the overall organization and control of such motor patterns is disrupted and animals cannot perform properly any motor tasks. Hippocampus should be also involved in the novelty environment detector (Gray, 2000). The hippocampus seems to be involved in the stress process. Pharmacological lesions demonstrated this, showing that glucocorticoids are capable to damage or destroy hippocampal neurons (Sapolsky, 1985). Recent evidences on glucocorticoids suggest that stress related cognitive impairments involve declarative memory. They are probably related to the changes that they cause in the hippocampus, whereas the stress-induced catecholamine effects on emotionally laden memories are postulated to involve structures such as the amgydala (McEwen and Sapolsky, 1995; de Kloet *et al.*, 1999).

The main molecular biological studies in the later years were focused on the discovery of long term potentiation (LTP) (Bliss and Lomo, 1973) and long term depression (LTD) (Martin *et al.*, 2000; Bliss *et al.*, 2004), which are proposed to be the biological base of the memory in general. Those findings were recorded for the first time in the hippocampus.

The principle theories of hippocampal functions in memory processing such as declarative (Squire, 1992), spatial (O'Keefe and Nadel, 1978), recognition (Gaffan, 1974) memory, stimulus configurations (Rudy and Sutherland, 1989; Sutherland *et al.*, 1989) or relational processing (Cohen and Eichenbaum, 1993) and the development of the episodic-like memory theory of 'automatic recording and retrieval of attended experiences' (Morris *et al.*, 2003; Morris, 2006) are now well accepted and demonstrated. It is nowadays admitted that the hippocampus is mainly involved in the formation and the acquisition of the spatial memory but not in the storage of this one. The resolution of a spatial task previously learned to a lesion is possible (McNaughton *et al.*, 1986; Poucet *et al.*, 1991). In any case the hippocampus seems to not be the structure which store the spatial long term memory (reviewed in Poucet, 1993).

3.3.HIPPOCAMPUS AND RHYTHMS

In this part, we will provide a short description of brain rhythms. How we record them, why they are important and specially the theta rhythm will be discussed.

Brain rhythms are present in all the brain. They seem to have some important functions of synchronisation. An easy way to record them is the electroencephalography (EEG). Historically, the origins of the electroencephalography are in 1875, when an English physician named Richard Caton published his discovery about an electrical phenomena (Caton, 1875). The first human EEG was performed by a German physiologist and psychiatrist called Hans Berger in 1920. Nowadays, the EEG is common and daily used in medical diagnostic applications like diagnostic case of epilepsy, coma, encephalopathy, or brain death.

The generation of those rhythms is generally due to the synchronic oscillations of the brain neurons, of the global network. Cells discharge at the same moment creating a powerful signal recordable from the skull. As mentioned previously, several rhythms are present in the brain, such as Alpha (α) from 8Hz to 12Hz in human, Beta (β) between 12Hz and 30 Hz in human, Gamma (γ) between 30Hz and 100 Hz in human, Delta (δ) between 0Hz and 4Hz in human and Theta (θ) between 4Hz and 7 Hz in human. Another and more recent discovered rhythm present in the hippocampus is the sharp wave – ripples complex with a high frequency about 140 to 200Hz. The frequencies of those rhythms are different regarding the species, for example in human theta frequency is to 4 to 7 Hz and in the rat is to 6 to 12 Hz.

In the rest of this part, we will focus on the hippocampal brain rhythms and particularly in theta rhythm which is the most studied of the hippocampus, due to its predominance and its correlation with behavioural activity.

In the hippocampus, six main groups of oscillatory patterns are described: four rhythmical as theta (6-12Hz), beta (12-30Hz), gamma (30-100Hz), ripples (100-200Hz) and two non rhythmical as LIA (Large Irregular Amplitude activity) and SIA (Small Irregular Amplitude activity) (Vanderwolf, 1969). Focusing on the most studied rhythm, hippocampal theta, with a frequency range of 6–12 Hz that appears when a rat is engaged in an active motor behaviour such as walking or foraging, and also during REM sleep. Theta waves, with a lower frequency range (6–7 Hz), are observed when a rat is motionless but aware of the environment. Theta rhythm could also be classified in

two subtypes on the basis of the pharmacology. A-Theta, or theta type 2, is present when the animal is in state of arousal and attentive, this rhythm is atropine sensitive (anticholinergic). T-theta or theta type 1 is present when the animal has a motor activity as locomotion or other movement, and during the REM sleep period. This rhythm could be serotoninergic or glutamatergic and unaffected by atropine (Kramis *et al.*, 1975).

Vanderwolf and his colleagues described a strong relationship between theta and motor behaviour and argued that theta rhythm was related to a sensorymotor processing (Vanderwolf, 1969). Another theory, led by John O'Keefe, suggested that theta is part of a mechanism that animals use to generate cognitive map of the environment. The most popular theories, however, link the theta rhythm to mechanisms of learning and memory and spatial cognition (O'Keefe and Dostrovsky, 1971).

The relation between theta rhythm and the hippocampal activity is another interesting property that should be detailed. This is the temporal coding of the place cells. The theta rhythm is correlated with the activity of place cells. When animal enters in the place field, cell's activity seems to synchronise its burst activity to a frequency of 6-8 Hz corresponding to theta rhythm. This phenomenon is called "phase precession" (Figure 9) and have been brought to light by O'Keefe and Recce (O'Keefe and Recce, 1993).

When the animal enters in a place field, the activity of place cells starts to be in a precise phase of the theta rhythm. Then if the animal follows the place field, the burst of activity shift gradually in time following theta rhythm until the animal leaves the place field. In other terms, complex spike cells does not fire in a continuous pattern when the animal runs thought a place field but fires as burst with a frequency close to the EEG theta rhythm. Because the firing rate is associated with the speed of the animal, some studies use a treadmill (Fox and Ranck, 1975; Buzsaki *et al.*, 1983) or a circular track to record place cells (O'Keefe and Recce, 1993; Yamaguchi *et al.*, 2002; Huxter *et al.*, 2003). From those works, it has been demonstrated changes of theta phase, in which place cell's spike fired changed in a systematic way. In other words, when the rat entered the cell's place field, the cell began firing at a particular phase of theta. However, as the animal progressed though the field, the burst of unit firing occurred on an earlier phase of each successive theta cycle. This phase precession phenomenon is partly explained by cells firing rhythmically at a frequency higher than the one of theta

(O'Keefe, 2007). This phenomenon has been well studied but it is still not completely understood. Some evidences show a relation between the field size and the oscillation (Maurer *et al.*, 2005), others demonstrate that the stimulation reset the phase precession (Zugaro *et al.*, 2005).

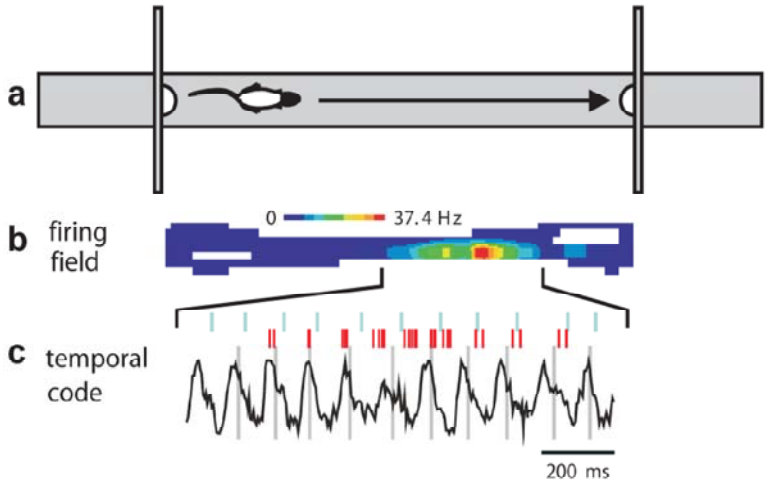


Figure 9: Phase precession scheme.
A: scheme of the maze, B: Place cell, C: timing between spike and theta of this neurone. *Taken from Huxter et al. (Huxter et al., 2003).*

4. PLACE CELLS PROPERTIES

Place cells are neurons as mentioned previously which fire when the animal enters in a specific zone of the environment. This specificity in spatial firing is essential for animals (rodent or human) to localise themselves in the environment but one place cell does not give the full cognitive map. A single place cell could not explain how work the mechanisms of this ability. Only a complex network of cell as the hippocampus could give this perception.

In the rest of the thesis, I will refer to pyramidal cells for both pyramidal cells and complex cells and to interneurons for theta cells and interneurons (Ranck, 1973; Klausberger *et al.*, 2003).

In the Hp as we saw beforehand, there are several anatomic layers. One of them is constituted by pyramidal cells, recognizable by their anatomical shape and their activity. Those excitable cells are neurons known as PCs. Interneurons have a homogenous high mean discharge rate. The pyramidal cells have a mean discharge rate low with (if they are PCs) a peak rate at least two times superior to mean rate.

As mentioned previously, most of the place cells are pyramid cells, but are all pyramid cells place cells? It has been demonstrated that 30% to 70% of the pyramid cells have a place field in one environment or another (Wilson and McNaughton, 1993; Gothard *et al.*, 1996). Considering this fact, silent cells (pyramid cells firing with a very low rate) could be potential place cells whose level of inhibition is high in the "silent" environment perhaps due to enhanced excitatory inputs to its inhibitory interneurons from anatomically close place cells with maintained fields in this environment (Thompson and Best, 1989).

The first observation about place cells was performed by O'Keefe (O'Keefe, 1976). He was interested in observing any changes in the cells firing depending on the conditions. Those neurons were not sensitive directly to any sense as vision or odour (we will detail this point later). PCs fire in a specific place but in any directions of motion or attentive state. It seems that PCs did not respond to any motivation or task changes. Since then, new studies have demonstrated that those affirmations were not totally correct (see below).

One of the first questions about place cells was how the system is organised anatomically. In other terms, were place cells and their firing field organised regarding their position in the hippocampus or not? After several studies and contradictory theories (Eichenbaum *et al.*, 1989; Hampson *et al.*, 1999), Redish *et al.* demonstrated that there was no anatomical tendency to fire in one place or in other (Redish *et al.*, 2001) and that there was no tendency that cells anatomically close fired in close vicinity. In order to explain such phenomena, the authors speculated that maybe one of the functions of the inhibitory interneuronal network is to allow some pyramidal cells to capture a territory in an environment and to exclude their neighbours from firing in that region (Thompson and Best, 1989). The size of place field have been also studied and seemed to have a high variability (larger place field in the vicinity of the wall and more compact in the centre of a maze) (O'Keefe and Burgess, 1996). Place cells, if they seem to have not any anatomical organisation regarding the place field localisation, have an organisation regarding the size of place field. Place fields are small in the dorsal hippocampus and bigger in the ventral part (Jung *et al.*, 1994) (Figure 10). In the CA1 of the hippocampus, there is rarely more than one PF for one neuron in one experimental environment.

A cell is considered as a place cell if it has a place field. Then, the criteria fluctuate regarding the studies and the group of research. To be considered as a place field some criteria are commonly accepted for example: the minimum peak firing rate should be >1Hz and maximum peak should be at least twice the mean frequency.

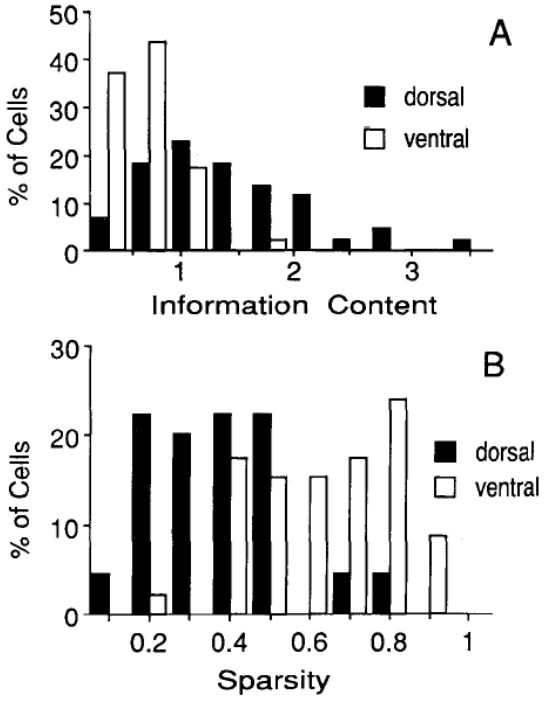


Figure 10: Differences between dorsal and ventral hippocampus.
Place cells differences in dorsal and ventral Hp. ***Taken from Jung et al. (Jung et al., 1994).***

Once defined what a place cell is, we need to define how those place cells generate place fields. To determine this, several studies were carried out. They concluded that the stabilisation of place fields in a new environment is about 2 to 15 minutes (Hill, 1978; Wilson and McNaughton, 1993; Frank *et al.*, 2004) which is a fast process. During the place field creation authors described that the firing rate can increase or decrease. They also described that in a new environment place cells can adopt a remapping behaviour. What is called remapping is the fact that a same place cell will fire in different places in function of the environment. What is generating the place field creation? Is it the external cue or the pathway integration? Many studies have tried to validate one or the other theory but until now no final proof has been brought out (the most probable is that both processes are involved depending on the cue available at the moment).

Many observations have been reported to explain cognitive map creation. One of them is that place field in an open maze (square or circular) are non-directional (Muller *et al.*, 1994), but in a linear track or in radial arm maze (high polarity of the maze) place

cells are highly directional (McNaughton *et al.*, 1983; Markus *et al.*, 1995; Gothard *et al.*, 1996). This means that in the open maze place cells are firing whichever the direction of the animal entering in the place field (Figure 11-A). In a linear track, place cells fired only if the animal enters in a specific direction in the place field (Figure 11-B).

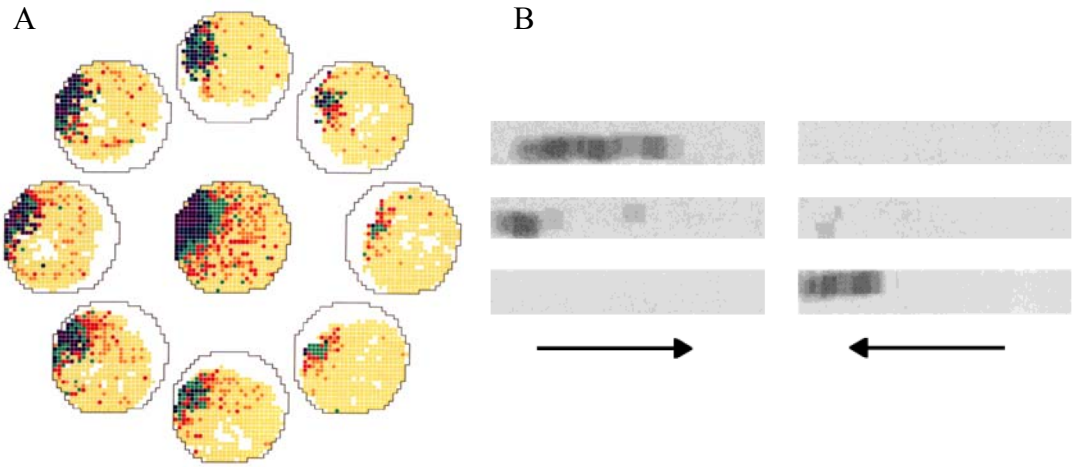


Figure 11: Place cell polarity.
(A) No polarity of place cell in circular maze. *Taken from Muller et al. (Muller et al., 1994)*; (B) Polar place cell in a linear track. *Taken from O'Keefe (O'Keefe, 1999).*

It has been mentioned that the cognitive map could be generated by two different theories, allocentric or egocentric navigation. Several studies demonstrating the allocentric theory have been carried out, as the rotation of the visual cue out of the maze (extramaze) (O'Keefe, 1976; Olton *et al.*, 1978; Jeffery, 1998; Jeffery and O'Keefe, 1999) or in the maze with a "cue-card" (intramaze) (Muller and Kubie, 1987). On the contrary, O'Keefe and Burgess describe some evidence that place fields are controlled by the distance to the wall of the maze (O'Keefe and Burgess, 1996). In the last decade, a study mixed allothetic and idiothetic cues to characterize them, and results showed that most of the time two kinds of cue are needed to create a place field (Rossier *et al.*, 2000). As John O'Keefe described, when place cells were discovered (O'Keefe, 1976), two types of processes are responsible for place cell, path integration and landmark from the environment. For the second type, perception is needed, and lots of experiments were carried out on senses. The main sense's influence studied was vision (O'Keefe, 1976; Quirk *et al.*, 1990; Markus *et al.*, 1994). Results of those studies showed that vision could influence place cells' remapping or not, a stable firing field. More studies based on senses were performed trying to eliminate one or more sense inputs. Etienne Save recorded place cells from blind animals since birth (Save *et al.*,

1998) demonstrated that vision is not essential for place cells. In the same way, place cells have been also recorded in animal without vision and audition (Hill and Best, 1981). In the opposite if just one white card is used as landmark it could be enough to create a valid cue. The white card could be enough to create an angular location but not to create distance cue depending where it is placed (Cressant *et al.*, 1997). As we saw, place cells are sensitive to their environment but also to the arrangement of it.

To confirm that place cells are also influenced by idiothetic cue as it is mentioned by integration path theory and by several experimental evidences. Protocols as passive rotation have been performed demonstrating that place fields rotate with this cues (Sharp *et al.*, 1995; Jeffery *et al.*, 1997; Jeffery and O'Keefe, 1999).

Others sources of information could influence place cells discharge. A positive or negative valence on the place field, as punishment (Moita *et al.*, 2004), reward, or a task (goal) have been tested. Some of those protocols showed some changes (Breese *et al.*, 1989; Kobayashi *et al.*, 1997; Hollup *et al.*, 2001) and others not (Speakman and O'Keefe, 1990; Zinyuk *et al.*, 2000), showing that the context seems to be more important than rewards.

Another interesting question to answer is what determines the shape and the size of the place cells. We saw that hippocampus do not have any place cells topological organisation, so what influences the size and the shape of a place field? Muller and Kubie described that if the size of the maze changes, then the size of the place field can also change (Muller and Kubie, 1987). As place cells could be influenced by visual cue or not, the size of the maze could influence or not those cells as demonstrated by Wilson (Wilson and McNaughton, 1993). More studies tried to determine what the causes of those phenomena were and two theories emerged. The first one was that the animal changes its reference from the closer one (box) to the bigger one (room) (Gothard *et al.*, 1996). The second one supposes that the connectivity between place cells were lost and acted as independent maps (O'Keefe and Burgess, 1996).

After having reviewed the main properties of hippocampal place cells, it has to be mentioned that spatial navigation is a complex system where hippocampal place cells play a role but are not the only source of information. Many cells of the hippocampal complex are involved as head direction cells (Ranck, 1984; Taube *et al.*, 1990), or more

recently discovered, as grid cells of the entorhinal cortex (Hafting *et al.*, 2005). Cells seem also to fire faster in relation with the animal's speed (speed cells) (McNaughton *et al.*, 1983; O'Keefe *et al.*, 1998). To sum up, information about animal's head direction and speed of movement through the environment is available to the hippocampal formation and this information is likely combined in the medial entorhinal cortex with grid cells (O'Keefe, 2007). The integration system suffers to some errors get accumulated from the position of the animal itself, and animal position should be reinitialised constantly. Etienne Save suggested that place fields can be maintained for only 1 to 2 min on the basis of path integration information alone (Save *et al.*, 2000), another system should then relay the information. In human, an experiment by Ekstrom (Ekstrom *et al.*, 2003) making navigate in virtual streets some patients showed that the temporal and frontal lobes are activated in the navigation. Recently, experiment in human navigating in a virtual environment in darkness demonstrated that integration path is used (Tcheang *et al.*, 2011). Regarding these findings, it is still not clear how goals or rather goal's location is stored or in the hippocampus or in others parts. It seems that the rewards are used by the system as a cue but in a different way that physical one. Several potential candidates are nowadays investigated as the lateral septum subiculum, *nucleus accubens*, or the prefrontal cortex (Hok *et al.*, 2005).

Between both theories, allocentric navigation and egocentric navigation, experimental evidences have shown that both are possible. The most probable is that both approches are used by rat's hippocampus regarding the context. In human, recent studies describe the existence of grid cell like (Doeller *et al.*, 2010) and the existence of entorhinal cortex path cells (Jacobs *et al.*, 2010) which will validate all the theories for humans.

5. SOMATOSENSORY SENSE IN THE RAT

In this part, we will explain how rat can "feel" its environment. The somatosensory path will be described. Rats are nocturnal food foragers, and have their navigation depending heavily on whiskers information. The vibrissae is known as the main source of sensory inputs for the rat and other rodents for as long as one century (Vincent, 1912). In the rodents, whiskers are compensating for a general poor vision. The physiological explanation is that the sensory cortex part dedicated to whiskers is the largest (Welker, 1976). This mechanism can be compared in human to the hands (Figure 12). They allow them to have information about their environment more or less 5-10 cm around. This precise system is able to discriminate between texture very similar (30 µm deep and with a space intervals of 90 µm) (Carvell and Simons, 1990). The rodent's whisker is a complex and very well designed system to observe the environment. In this part we will describe the organisation of this system and explain the basics of its mechanism.

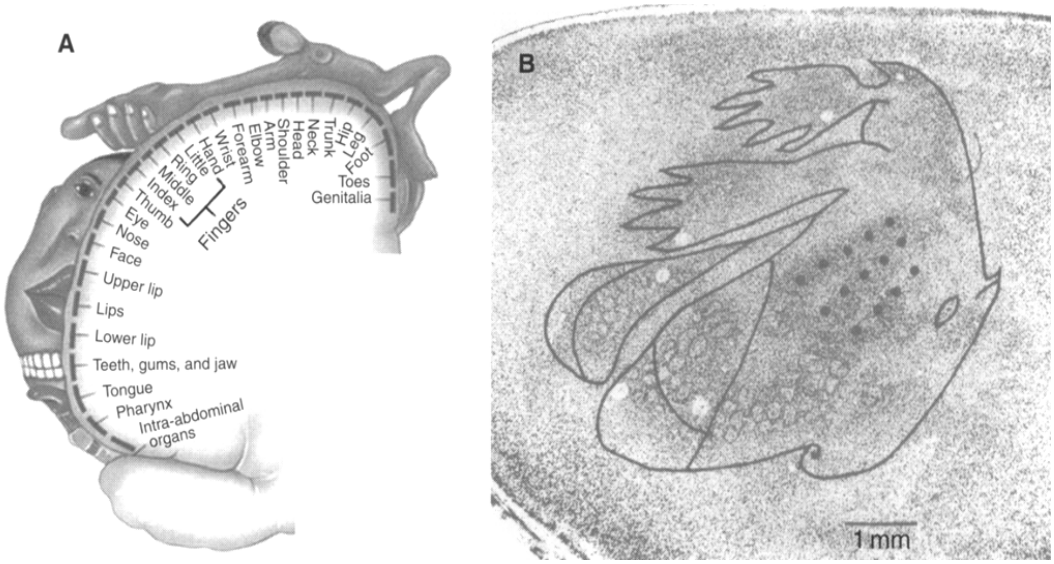


Figure 12: Representation of the sense on the cortex.
A in Human, B in Rat. *Taken from Welker (Welker, 1976).*

5.1. WHISKERS, THE SOMATOSENSORY INPUTS

Whiskers, or *vibrissae*, are the inputs of the somatosensory system. Whiskers are specialized hairs which are usually used for tactile sensation. These "hairs" grow around the nostrils of the animals. Whiskers are organised in two kind, macrovibrissea and

microvibrissea. The microvibrissea can be viewed as an object recognizing sense organ. In contrast, the mystacial macrovibrissea can be viewed as a distance detecting/object locating sense organ (Brecht *et al.*, 1997). In this study, no discrimination between macrovibrissea and microvibrissea will be done.

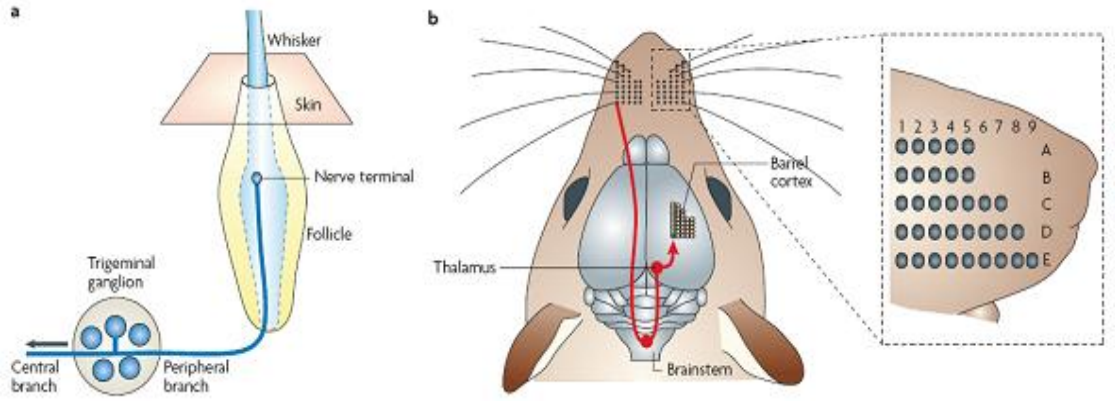


Figure 13: Scheme of the somatosensory pathway from the whisker to the barrel cortex. (a) Mechanoreceptor terminal and trigeminal ganglion. (b) On the Right 2D-Whiskers grid, On the Left, pathway of the information to the Barrel cortex. *Taken from Diamond et al. (Diamond et al., 2008).*

Whiskers are well organized on the snout of the rodents. Whiskers organization forms a grid of 5 rows and 9 columns (see Figure 12-b, Figure 13-b, Figure 18). Each whisker will be represented in the same way in the associated cortex (barrel cortex). The whisker is growing in a follicle which is highly specialised (Figure 14). This follicle act as a mechanoreceptor responding and sending information of direction, velocity, duration of movements, and forces (Rice *et al.*, 1993). It takes 8–11 days for a new vibrissae to appear (Ibrahim and Wright, 1975) and several days to grow to their normal length at 1mm/day.

The particularity of vibrissae is that it is an active system always in movement to detect form and distance. Whiskers are moved rhythmically between 5 to 15 Hz to explore objects in the environment, including textures (Carvell and Simons, 1990; Kleinfeld *et al.*, 2006). Two different theories try to explain how whiskers can provide information about shape and distance of an object place in the near environment. The first one is a model into which the texture is encoded temporary by a unique temporal pattern of movement. This is the kinetic signatures and it is based on the frequency and amplitude of the whiskers movement (Arabzadeh *et al.*, 2005). The second, the resonance model, derives from the observation that whiskers are resonant beams, with characteristic resonance frequency inversely related to whisker length (Hartmann *et al.*, 2003; Neimark *et al.*, 2003). Wolfe, in 2008, tried to determine finally which theory was

the true one and using an elegant protocol (rats were trained to whisk against sandpapers of different grain size while recording whisker motion with a linear array of optic sensors) (Wolfe *et al.*, 2008) demonstrated that his results agreed with the kinetic signatures. However, a study of Diamond in 2008 (Diamond *et al.*, 2008) argued those results. The problem stays not completely solved and is the centre of many scientific discussions.

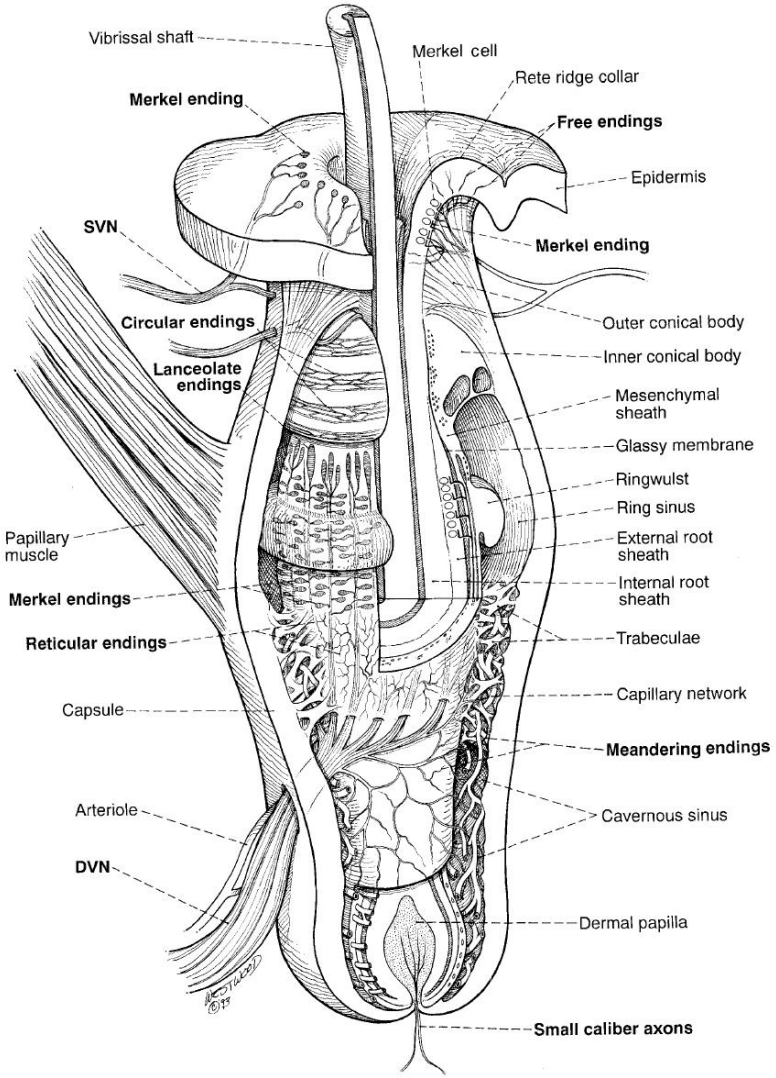


Figure 14: Scheme of a rat follicle.
Taken from Rice et al. (Rice et al., 1993).

5.2. SOMATOSENSORY PATHWAY

Once whiskers touch some object or surface from the environment, the movement is transformed by the follicle into information. This information is transmitted to the higher structure of the brain to be analysed (Figure 13). Once the signal is analysed by

the cortex, motor regions react in function of this signal (Figure 15), this imply a connection between somatosensory (S1) and somatomotor (M1) systems.

The first step of the pathway is the trigeminal ganglion (TG). The ganglion sends then a branch to the trigeminal nuclei (TN) of the brainstem. In this nucleus, synaptic transmissions are realized and send some projections to ventral posterior nucleus medial (VPMdm), to ventro-lateral (VPMvl) and to the medial sector of the posterior nucleus (POm) of the thalamus. Another synaptic transmission is done at this stage and sends projections into the primary and secondary somatosensory cortex (Figure 16, Figure 17).

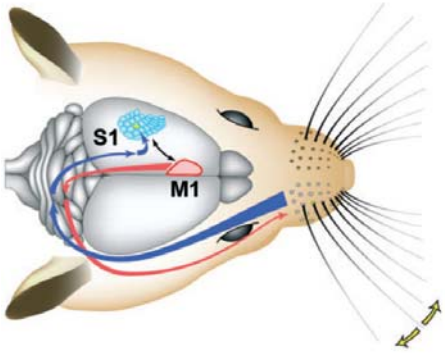


Figure 15: Signalling pathway from whisker to cortex.
S1: Somatosensory barrel cortex, M1: Primary motor cortex. ***Taken from Aronoff et al. (Aronoff et al., 2010).***

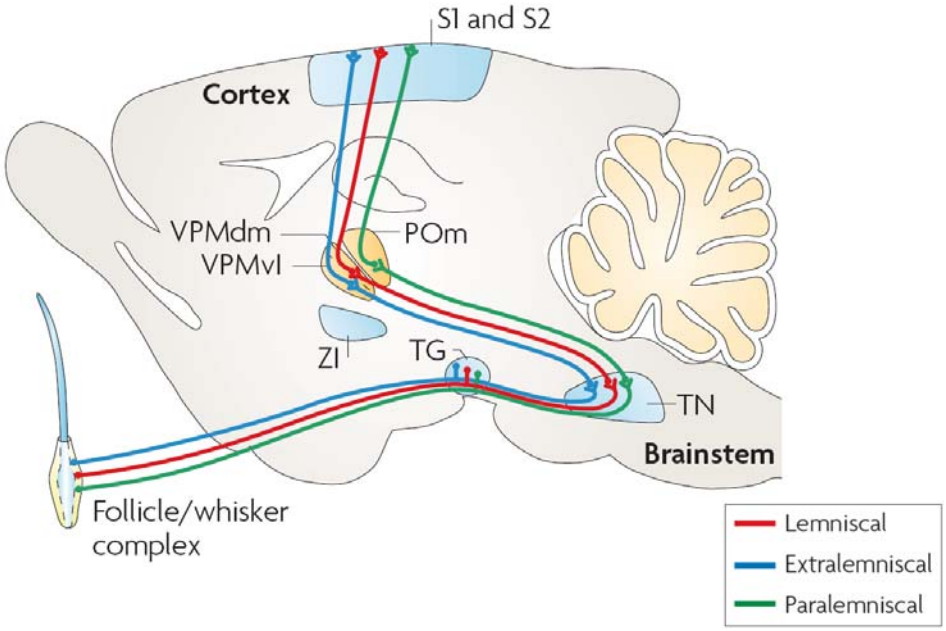


Figure 16: Somatosensory pathway.
Taken from Diamond et al. (Diamond et al., 2008).

The afferent inputs are divided in three different ways: lemniscal, extralemniscal and paralemniscal (for review (Diamond *et al.*, 2008)).

Lemniscal pathway (Figure 16, Figure 17): In the first stage of the path, neurons are organised in "barrelettes" in the TN. The axons of second order projected to the 'barreloids' of the dorsomedial section of the ventral posterior medial nucleus (VPMdm) of the thalamus. Barrelettes and barreloids respect the whiskers organisation. Axons of VPMdm neurons project to the layer IV of the primary somatosensory cortex (S1), where the termination are organised in 'barrels' (dense clusters of small neurons).

Extralemniscal pathway (Figure 16, Figure 17): This pathway concerns the neurons of the caudal part of TN. They are also organised in barrelettes respecting the whiskers pad organisation. They project their axons to the ventrolateral domain of the VPM (VPMvl) where, as in the lemniscal pathway, they are organised in barreloids. The septum between the barrels of S1 and the secondary somatosensory cortex (S2) receives the axons of VPMvl neurons.

Paralemniscal pathway (Figure 16, Figure 17): Neurons from the rostral part of the TN are not spatially organised as others pathways. They project to the medial sector of the posterior nucleus (POm) and to the zona incerta (ZI). The axons of POm neurons project directly to the barrels, in layer Va of S1, S2 and to the primary motor cortex (MCx) for the descent back loop. As mentioned, the paralemniscal pathway is not organised spatially as the two other pathways and seems to integrated multiple-whisker information.

The descent back loop to the whisker involves mainly the motor pathway and is composed principally by the, basal ganglia (BG), the brainstem premotor nuclei (BPN), the cerebellum (Cer), the facial nucleus (FN), the inferior olive (IO), the pontine nuclei (Pn), the red nucleus (RN), the superior colliculus (SC) and the ventrolateral thalamic nucleus (VL) as detailed in Figure 17.

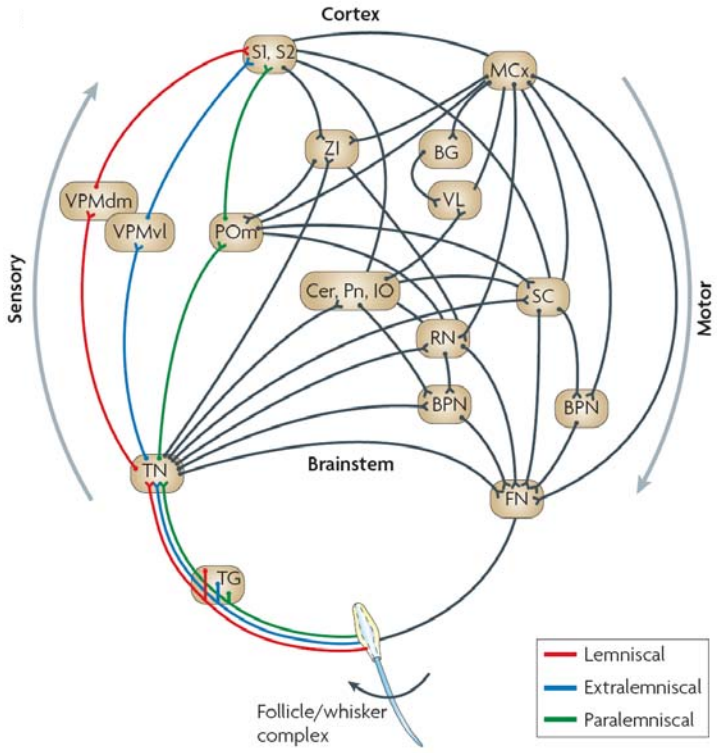


Figure 17: Detail somatosensory pathway.
Taken from Diamond et al. (Diamond et al., 2008).

5.3. BARREL CORTEX

The Barrel cortex (BCx) is part of the somatosensory cortex and receives information from whiskers of the contralateral side and processes it. The input to this system is layer IV. The organisation of this layer gives its name to the entire cortex (barrel cortex) because it is organise in barrel (Figure 18). As we mentioned previously the entire path arriving to the layer IV is composed by only three synapses. This highly organised structure has been put in evidence and described in the 1970's (Woolsey and Van der Loos, 1970). The area of the BCx in rat is between 4.7 and 6.4 mm^2. Barrel cortex is arranged as the snout pad (Figure 18) in 5 rows usually labelled from A to E.

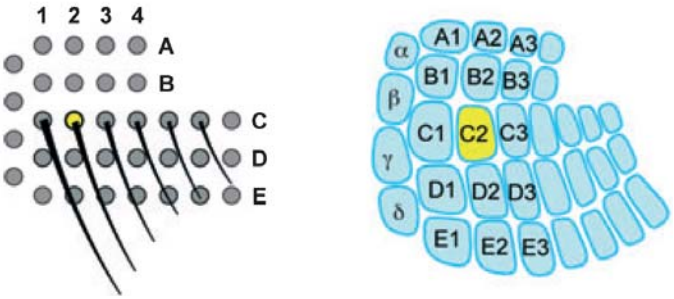


Figure 18: Similarity between whiskers and barrel cortex organisation.
On the left: whisker pad, on the right, barrel cortex. ***Taken from Aronoff (Aronoff et al., 2010).***

The end of somatosensory input pathway is the BCx. In this structure, each whisker is represented by a small and well-defined structure in layer IV. These barrels are somatotopically arranged in an almost identical fashion to the layout of the whiskers on the snout. This structural organization appears in the early phase of the development.

The connections of the BCx are, as in general in the cortex, multiple and complex, that is why we will not develop this part but it has to be at least mentioned. The barrel cortex makes a lot of interconnection with several layers. Barrel cortex also creates connections with other sensory cortices (sensory loops). Other connections are present for the sensory motor loops (Figure 19). Those loops concern the feedback performed during whisking.

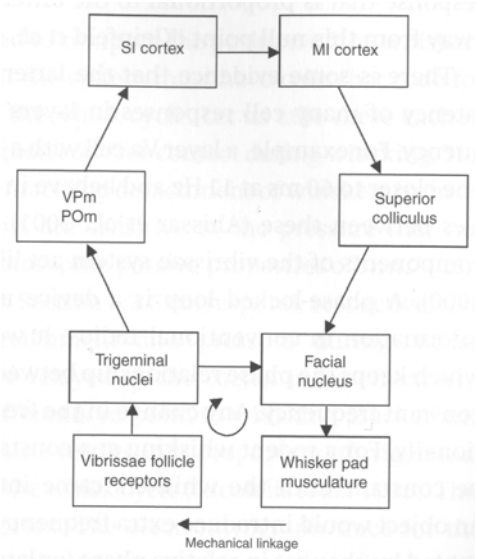


Figure 19: Sensory-motor loops.
Taken from Fox (Fox, 2008).

Our study focuses on the possible relation between the hippocampus and specially the place cells, responsible for the cognition maps creation and the somatosensory information, which are represented by the whiskers and all the pathways finishing in the barrel cortex. Even if both sides of this relation are well defined as we review aforementioned in the introduction, it is not well known how is treated the somatosensory information in the hippocampus. A recent study explored this relation between hippocampus and barrel cortex. Pereira *et al.* demonstrated that trigeminal inputs from the whiskers reach the CA1 region through thalamic and cortical relays associated with discriminative touch (Pereira *et al.*, 2007). Finally authors concluded that tactile information associated with fine whisker discrimination is readily available to the hippocampus for dynamic updating of spatial maps.

Based on those findings our goal was to investigate the possible influence that somatosensory inputs could have on the cognitive map creation. To do so, we recorded the biological base of the cognitive maps which are the place cells. It has to be demonstrated clearly that somatosensory inputs are used by the cognitive map system and if so we have to characterise how it influences the spatial maps creation. These cells should be recorded in an environment in which the animal uses principally the tact. Place cells should be then recorded with possibility to use this sense and without this possibility. We have to develop a technique to remove temporary the somatosensory inputs in order to monitor as well the recovery.

HYPOTHESIS

The main hypothesis of this PhD work was that the tactile information is relevant to the process of orientation and navigation in the rat.

Cognitive map generation is influenced by senses as vision, audition or smell as it has been recalled in the introduction. Based on those descriptions and on the study performed in the laboratory of Nicolelis (Pereira *et al.*, 2007), in which it is described the connection between the tactile information and the hippocampus, we hypothesised that somatosensory information is part of the cognitive map creation and spatial representation.

Supposing that this hypothesis is valid, the loss of the tactile sense should affect the coding of spatial information and could be observed in place cells' properties as firing rate, location and / or extension of the firing field. Alterations in these parameters will demonstrate that somatosensory inputs are involved in the creation of the cognitive map.

OBJECTIVES AND GOALS

GENERAL OBJECTIVE

Demonstrate if the somatosensory pathway is involved in the creation of cognitive maps and if it is, to characterize its properties.

SCIENTIFIC OBJECTIVES

In order to test our hypothesis, we carried out three kinds of experiments.

The scientific objectives were:

1. IDENTIFICATION OF A METHOD FOR TACTILE DEPRIVATION AND ITS CHARACTERIZATION

It was necessary to find the means to temporarily remove the somatosensory information in the anaesthetised animal.

2. BEHAVIOURAL CONSEQUENCES OF TACTILE DEPRIVATION ON TEXTURE DISCRIMINATION TASK

To validate that the method developed previously was effective in the awake animal and during behavioural tasks.

3. TACTILE INFLUENCE ON THE COGNITIVE MAP OF SPACE

To observe any influence that tactile deprivation has on the cognitive maps creation. To demonstrate if tactile information is part of the creation process of the cognitive map and to characterise place cells' properties with and without tactile information.

TECHNICAL OBJECTIVES

To complete the scientific objectives, several technical's challenges have been carried out. Along the development of this work, we found the need to achieve a number of technical objectives:

FOR ACUTE EXPERIMENTS

- Set up the technique of extracellular and intracellular recordings in the barrel cortex of an anaesthetised animal.
- Evoke a response in the barrel cortex by stimulating the whisker.

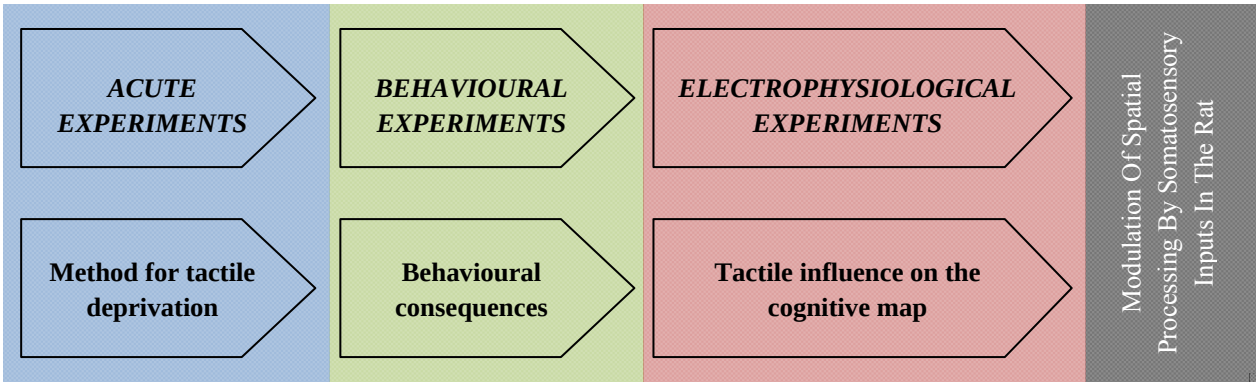
- Achieve temporary deprivation of somatosensory input by local anaesthetic (lidocaine).

FOR BEHAVIOUR EXPERIMENTS

- Develop an original behavioural protocol (tactile discrimination task)
- Design and Built a set up for tactile discrimination task.
- Create the software to control the tactile discrimination task set up (software→Labview™).
- Train animals → create a shaping protocol for animals to learn the tactile discrimination task.

FOR ELECTROPHYSIOLOGICAL BEHAVIOUR EXPERIMENTS

- Develop protocols to observe place cells in an enriched environment to exhort tactile information as main references to the creation of the cognitive map.
- Improve place cells recording technique.
- Improve the place cells data clustering.



MATERIAL AND METHODS

1. ACUTE EXPERIMENTS

1.1. SUBJECTS

The animals used for recordings in somatosensory cortex (S1) were eleven adult "Wistar" rats with a weight of 250-300 grs when they arrived in our animal facilities. They were housed in the animal care facility with food and water ad libitum. Rats were cared for and treated in accordance with the EU guidelines on protection of vertebrates used for experimentation (Strasbourg 3/18/1986).

1.2. SURGERY

Anaesthesia was induced by intraperitoneal injection of ketamine (100 mg/kg; Imalgene 1000, Merial Laboratorios SL) and xylacine (8-10 mg/kg; Rompun 2%, Bayer). Atropine 0.05mg/kg (Atropina Braun 1mg, B. Braun Medical SA) was injected to avoid secretions during the anaesthesia. The animals were not paralyzed. Maintenance dose of ketamine was 75 mg/kg/h by intraperitoneal injection. Anaesthesia levels were monitored by the recording of electroencephalogram (EEG) and the absence of reflexes. Body temperature was maintained at 37°C thanks to water blanket (T/pump, Gaymar) to avoid any electrical noise. Heart rate (ECG) was monitorised and maintained between 250-300 bpm (Capnograph SurgiVet, Wisconsin, USA). Once in the stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA), the skull was exposed and a craniotomy of 2x2 mm was made at coordinates AP –1 to -3 mm from bregma, and laterally, L 4.5-6.5 mm (Paxinos and Watson, 2005) above the barrel cortex. After opening the dura, exposition of the cortex, and electrodes insertion, recording could be performed.

1.3. WHISKER STIMULATION

A puff of air given through a 1 mm tube placed in front of the whiskers (10-15 mm) was used for stimulation (Figure 20, Figure 21). The air puff (5-10 ms) was controlled by a stimulator (Master 8, A.M.P.I., Israel) and delivered by a Picopump (WPI, Sarasota, FL). Its pressure was adjusted (5-10 psi) such that it would evoke a response that was of 50-100 μV in the extracellular recordings and between 5 and 10 mV in the intracellular recordings. For stimulus response curve, the pressures used were from 2 to 35 psi. The whisker displacement was not monitored and the time zero for the stimulus was taken as the initiation of the air puff.

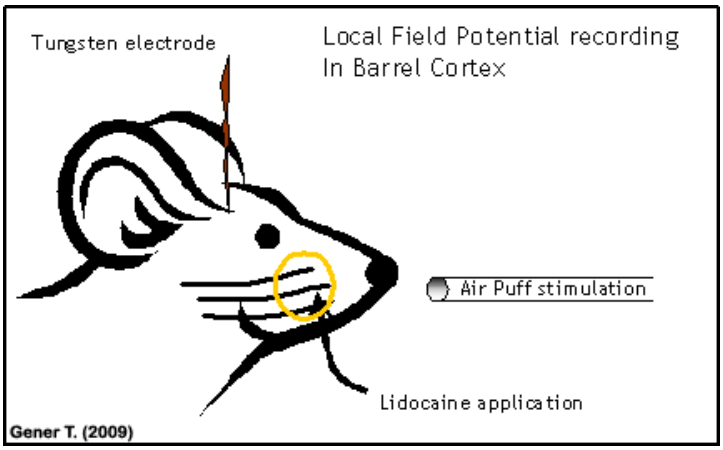


Figure 20: Scheme of whiskers stimulation during recordings.

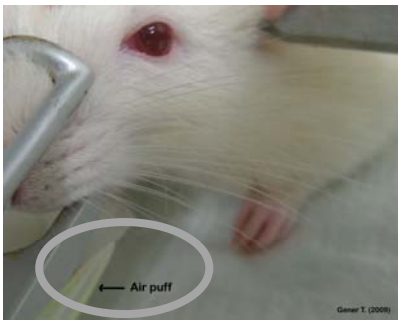


Figure 21: Photo of whiskers stimulation.

1.4. WHISKER PAD ANAESTHESIA

Local application of lidocaine was made at the base of the whiskers of anesthetized rats (Faggin *et al.*, 1997; Ahissar *et al.*, 2001). Several kinds of lidocaine applications have been tested to find the most efficient and convenient ways to temporary block somatosensory input.

- Cream: lidocaine cream (EMLA crema, 25 mg/grs) (Juhlin and Evers, 1990) was applied to the base of the whiskers (1 or 2 grs). To improve lidocaine skin absorption, a little massage of the snout was done.
- Injection: liquid lidocaine (Xilonibsa, 10%) was injected in the snout (subcutaneous injection, 4-5 injections, 50 µl each).
- Aerosol: liquid lidocaine (Xilonibsa, 10%, laboratorios Inibsa S.A.) was vaporized on the snout in the whiskers zone (2 sprays).

- Liquid drop: a drop of liquid lidocaine (Xilonibsa, 10%, laboratorios Inibsa S.A.) was applied to the base of the whiskers with a pipette (0.4ml).

1.5.RECORDINGS

Firstly, barrel cortex's extracellular recordings were obtained with a tungsten electrode (FHC, Bowdoinham, ME, USA, impedance to 300-500 KOhms). For stability and to avoid desiccation agar at 4% (AGAR, Scharlau Chemie SA, Spain) was used to cover the area. Extracellular recordings were also used to adjust whisker stimulation. Then, barrel cortex's intracellular electrodes were made of borosilicate glass capillaries 1 mm O.D. x 0.5 I.D. (Sutter Instruments puller, up to an impedance of 50-100 Mohms). Intracellular recordings were obtained within <1 mm from the extracellular recording electrode. Data have been recorded with a CED commercial acquisition board (Cambridge Electronic Design, UK), data acquisition has been processed with the commercial software Spike 2 (Cambridge Electronic Design, UK).

1.6.DATA ANALYSIS

Once acquired, data were analysed off line with the commercial software Spike 2 (Cambridge Electronic Design, UK). A custom-made script by Lionel Nowak (Slope 2) code in Spike 2 was used to detect evoked responses, and allowed us to determine the value of the parameter we were interested in: peak's amplitude of the LFP, the delay from stimulus, the width of the response. All plots and graphic were performed with Microcal Origin® 6.0.

2. BEHAVIOURAL EXPERIMENTS

2.1. SUBJECTS

In these experiments, six "Lister Hooded" rats with a weight of 350-380grs were used. Animals were housed in groups of two to three animals in standard size cages (480 x 265 x 210 mm) keeping in an enriched environment (paper and plastic toys) under a 12 hr light/dark cycle, with food and water ad libitum. When the behavioural training started, rats were housed separately in the same conditions but water deprived to motivate the learning process. After all sessions, animals had access to water ad libitum during 15 min, and got reward with strawberry gelatine. Rats were cared for and treated in accordance with the EU guidelines on protection of vertebrates used for experimentation (Strasbourg 3/18/1986). Animals were trained daily until the task was learned.

2.2. SET UP

For these experiments, we designed and created a homemade apparatus for the tactile discrimination task set up (Figure 22). Two motors for water dispenser (12V Pinch solenoid valves, Sirai, Italy), one motor step by step for texture control (12V standard step by step motor), and 3 infrareds for touch and drink detection were used to built the set up. The design of the electric circuit was homemade (Figure 23). Set-up's structure was built in wood and plexiglas.

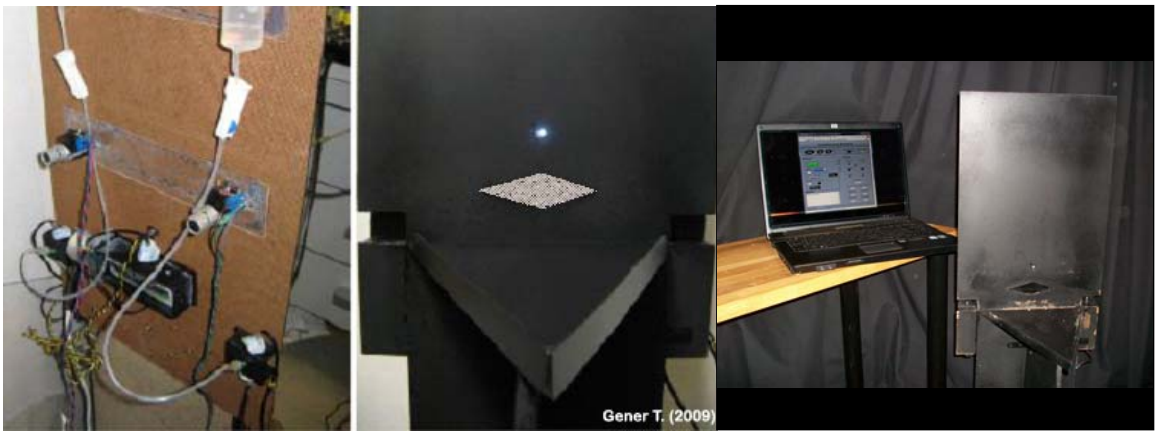


Figure 22: Photos of the behavioural setup.
Left, backside of the set up texture motor, water dispenser. Centre, front side of the set up: two water dispensers and in the middle the texture and LED. Right, set up and recording system.

New software has been developed in Labview™ (National Instruments™) to control motors (texture presentation and water dispensers), to save the time of reaction of the subject (timestamps), and do the analysis of each protocol (Figure 25). This application allowed us to set up all the parameters of the behavioural experiment, and the hardware acquisition card. Indicators show how the experiment is going in real time and what do the rat in each moment. One tool of the software gives us directly after the last trial the percentage of success and data are saved for later analysis.

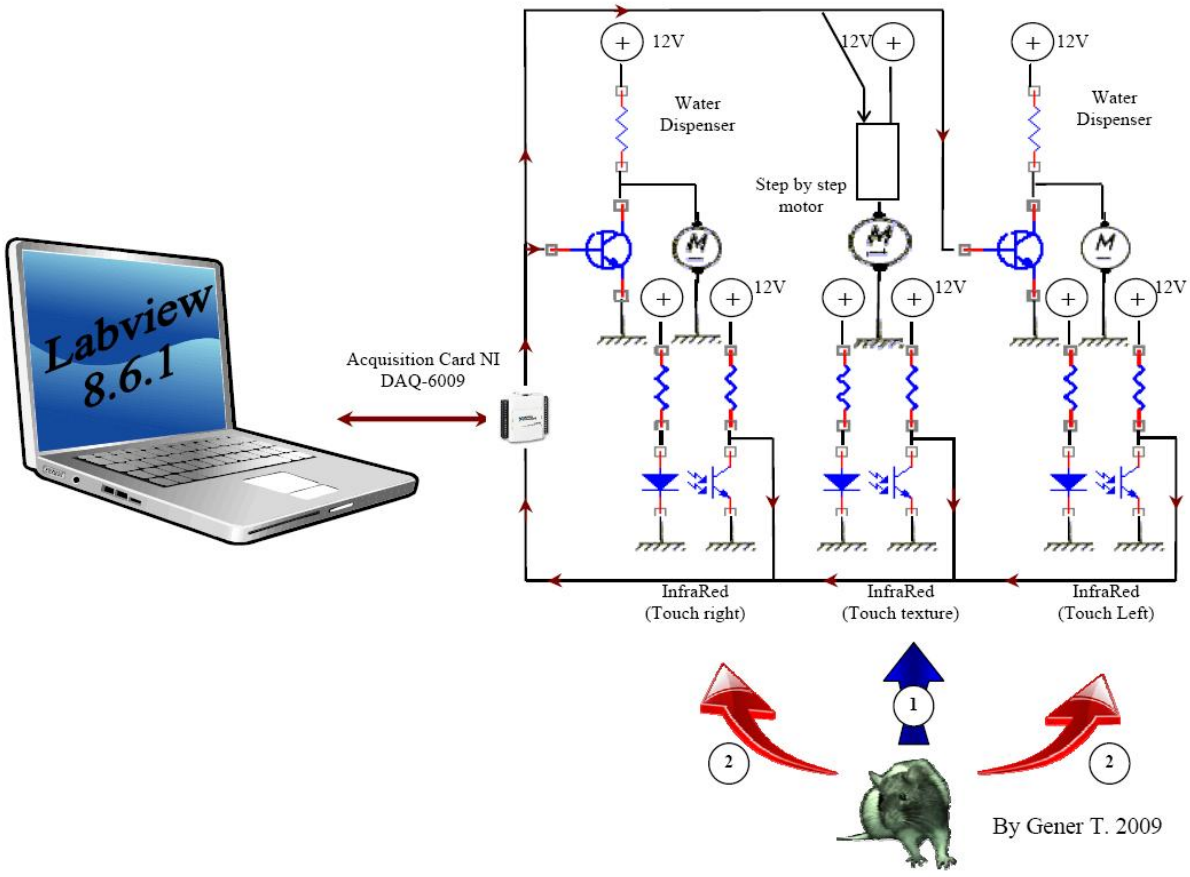


Figure 23: Scheme of the electrical circuit of the behavioural set up.

2.3. PROTOCOLS

We carried out a tactile discrimination task experiment (Diamond *et al.*, 2008). Animals should discriminate between two textures. According to the texture presented, the rat should decide to go to the left nose poke or to the right one. The animal should repeat the task touching first the texture then the nosepoke until the total number of trial was performed. Previously, the rat had been trained to associate a texture to a side to get a reward (Left side for smooth texture, right for a rough texture).

Once the subject learnt the association, the behavioural experiment was carried out. In our case the animal must touch the central panel, discriminate the texture and go to get the reward form the left or the right. If the wrong choice was made, a 90 db pure sound was produced as punishment for the subject. If the animal did not go to get his reward before a certain time (5-10 sec) the trial was over and the subject must touch again the central panel (Time out). A light (LED) indicates to the animal that the central panel is available and that a new trial could start.

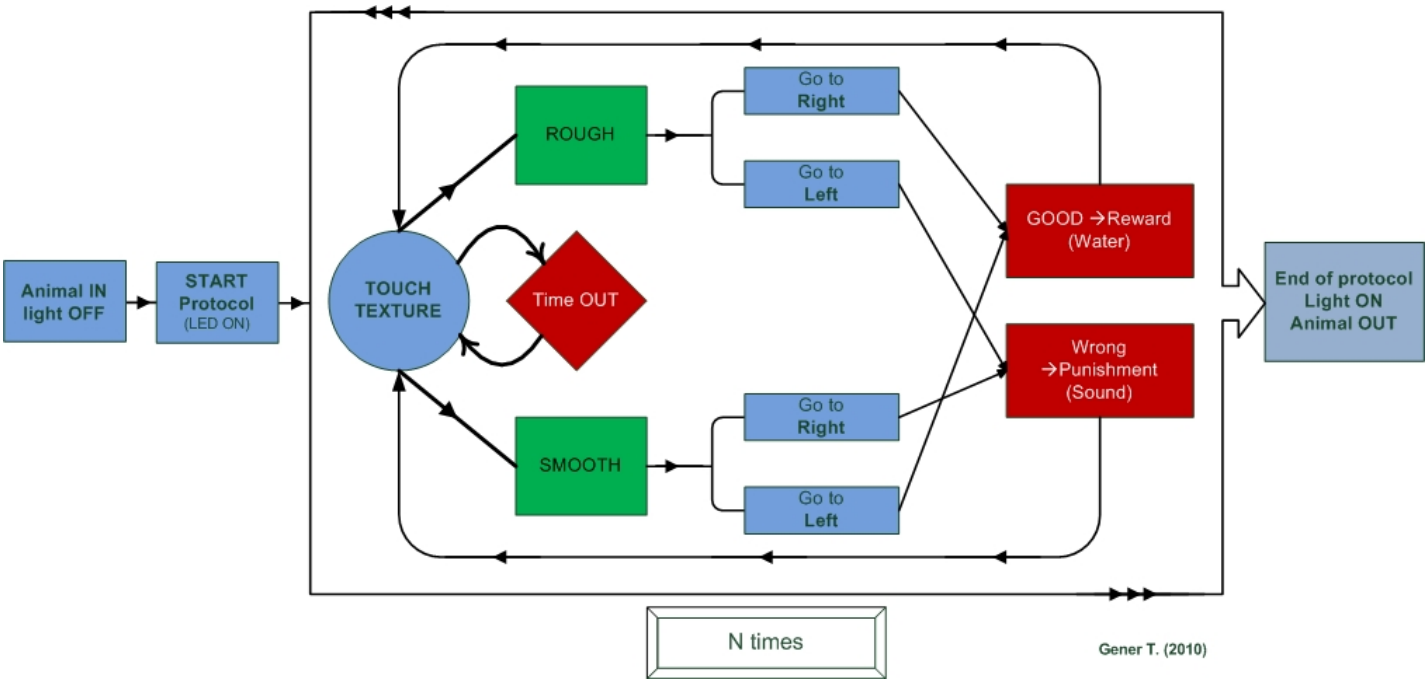


Figure 24: Scheme of the behavioural protocol session.

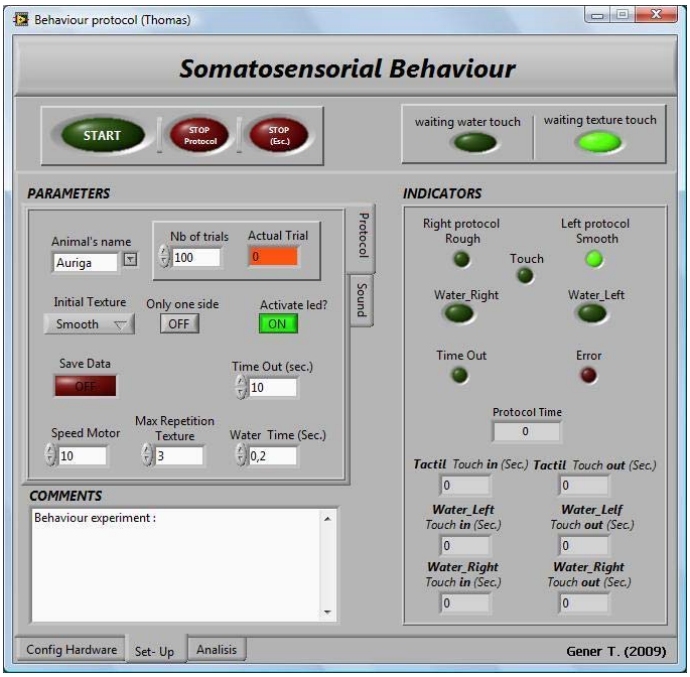


Figure 25: Screenshot of the somatosensory behaviour software. Software creates to control behavioural experiment. Right panel indicator, Left panel parameters of experiment (Nb of trial, sound level...).

One hundred to two hundred trials (Figure 24) were realized to let the animal learn the task and to have a good sample of data during the learning phase then during all the protocol time, one session representss 50 trials.

The tactile deprivation protocol could be carried out when the animal learnt the task and the level of success regarding missed responses was constantly (at least 3 consecutive sessions) above 80%. The discrimination task was then performed (50 trials) every ~10 min to characterise, in time as in performance, the loss of tactile sense (Figure 25). After a minimum of two control sessions, lidocaine, a local anaesthetic blocking sodium channels (Strichartz, 1973; Scurlock *et al.*, 1978; Starmer *et al.*, 1984), was applied to the base of the whiskers in order to deprive the somatosensory input (Gener *et al.*, 2009). Under the effect of lidocaine the task should be more difficult for the animal and it should be harder to get the reward.

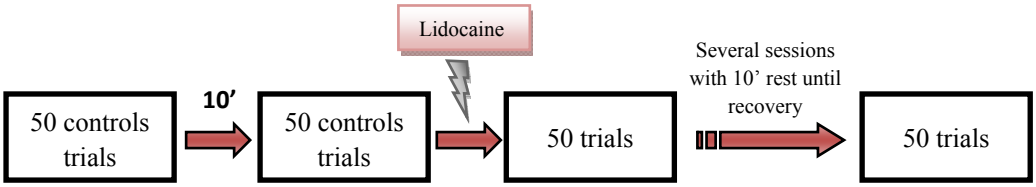


Figure 26: scheme of the lidocaine protocol.

2.4. ANIMALS TRAINING – SHAPING

To make rats learn the task properly, a strict protocol (shaping) was needed. Four phases were necessary to realise a good learning:

1. The first phase was made to create the association between the touch to the central nose poke and to get a reward from left or right. At this moment, only one nose poke was available. Animals got the reward just touching the central nose poke, indicated the beginning of the trial. The subjects were trained by block of trials (50 left sides, 50 right sides) until animals succeeded. This phase was completed in few sessions.

2. In the second phase, both nose pokes were opened. The animal still received the reward when the central nose poke (texture) was touched. In this phase, animals had the possibility to touch the right side or the left side but received a reward from only one side.

3. The third phase consisted in associating properly the texture and the side of the reward. The animal now got the reward only when entering in the correct nose poke regarding the texture presented in the central nose poke, and not anymore just touching the central one. The texture even if always the same was moving (random side and rotation) to get animal used to it.

4. Final phase, the animal was now subjected to a randomize choice of the texture. The randomisation was done to avoid any possibility of different association excepted with the texture itself (random presentation's number, random rotation's sense, and random rotation's number of the texture). The animal got an aversive stimulus (90 db pure sound) if a wrong choice was made and after a time of inactivity of the animal trial was stopped and started again from beginning (time out).

The learning curve started when the animal began phase four. During all the experimentation two textures presentation objects were created, one with sand paper stuck to a fine Plexiglas object: Smooth, P360 and Rough, P40; another one in Plexiglas, one side: smooth was sleek; other side: rough was a softly sanded (sand paper used P120).

2.5. LIDOCAINE APPLICATION

Lidocaine used was Xilonibsa, 10%, from the "laboratorios Inibsa S.A.". Regarding the datasheet of the drug, the effect starts 2 to 5 min from the topic application, and has an anaesthetic's effect duration from 10 to 20 min. Liquid lidocaine was applied on the snout of the animal with a syringe without needle to have a precise topic application. During this manipulation the animal was constraint in the hand of the experimentalist and the liquid lidocaine was applied gently from both side of the snout on all the whiskers' pad.

2.6. BEHAVIOURAL DATA ANALYSIS

We measured several parameters as the number of success, reaction time, number of time outs, the dispersion of data (Fano Factor) and the lidocaine effect. All the parameters were calculated by homemade script with interface (GUI) coded with Matlab®.

NUMBER OF SUCCESSES

This is the number of times when animal was doing correctly the task. The rat chooses the correct side and gets it reward. It is expressed in percentage:

$$\frac{(Number\ of\ Success\ \times\ 100)}{Number\ Total\ of\ Trials} = \%\ of\ Success$$

REACTION TIME

We measured the time needed by the animal to touch the central nose poke (texture), to decide to which side to go and to drink. The time to enter and to go out of those three events was recorded. Here, we take the time when the animal enters into the central nose poke and when it enters into one of the lateral nose poke.

$$Time\ Lateral\ Entry - Time\ Central\ Entry = Reaction\ Time$$

NUMBER OF TIME OUTS

We defined a time out as a started trial but not ended in the specific time. In other terms, if the animal touched the central poke but did not go to get his reward before a define time (5 sec.), it was a time out:

$$\frac{(Number\ of\ Time\ Out \times 100)}{Number\ Total\ of\ Trials} = \%\ of\ Time\ Out$$

DISPERSION OF DATA (FANO FACTOR)

It is a measure of the dispersion of a probability distribution. We determined it to characterise the distribution of the data's population: $\frac{\sigma 2W}{\mu W} = F$, where $\sigma 2W$ was the variance and μW was the mean of a random process in some time window W.

LIDOCAINE EFFECT

We determined several phases in time to measure the lidocaine effect over the time. These phases were: before the lidocaine application (CONT), the first part of the time course from 3' to 12' (LID1) then from 18' to 1h (LID2), and the last part considered as recovery from 1h and half (REV).

STATISTICS

For the several parameters calculated, we obtained the mean. We calculated the standard deviation (σ): $\sigma=\sqrt{\sum_{i=1}^{N}\frac{(X_i-\mu_x)^2}{N}}$ where N is the size of sample, X the value of the sample, and μ the mean of the sample. We also calculated the standard error of the mean (SEM): $SEM = \frac{\sigma}{\sqrt{N}}$ where σ is the standard deviation and N the size of the sample.

We wanted to determine if different groups of data were significantly different or not. For example, to determine if the percentage of success in control condition and after the application of lidocaine was significantly different. For that we used the two samples T-test. This statistic test is applied to compare whether the average difference between two groups is really significant or if it is due instead to random chance. This test was also chosen because t-test is highly robust to the presence of unequal variances (Markowski and Markowski, 1990). We use the function in Matlab® call ttest2. This function performs a t-test of the null hypothesis between two vectors of data (x and y). The null hypothesis is that the two groups are equal and there is no difference between them. Hypothesis equals to 1 suggested a rejection of the null hypothesis at α% significance level meaning that the two groups of data are significantly different. Hypothesis equals to 0 suggested a failure to reject the null hypothesis at α% significance level meaning that the two groups of data are not significantly different.

3. ELECTROPHYSIOLOGICAL BEHAVIOURAL EXPERIMENTS

3.1. SUBJECTS

The animals used for this study were Lister Hooded and Wistar (Harlan Iberica SL). Animal's weight when they arrived in animal facilities were 300-350 grs. Animals were housed in standard cages 60x40x30 cm (Rody Cavia, SAVIC) keeping an enriched environment under a 12 hr light/dark cycle, water and food *ad libitum*. During approximately a week, animals were diary handled to get used to be manipulated. Rats are usually caged in groups of 2 or 3 before starting experimentation. Once started, the animals were housed individually. After a week, and previously to the surgery, animals were placed in the maze several times and trained to food purchasing task (open maze). After recuperation from surgery animals were maintained to 80% to their original weight to motivate them to pursue sweet rice in the maze (see protocol).

3.2. TETRODES AND MICRODRIVES

Each tetrode (O'Keefe and Recce, 1993; Wilson and McNaughton, 1993) was made from four twisted strands of HM-L-coated 90% platinum-10% iridium wire of 17 and 25 µm diameters (California Fine Wire, Grover Beach, CA). Four tetrodes were held by a cannula that was attached to a microdrive supplied by Axona Ltd, St Albans, UK. The microdrive was reusable after a remounting process (Figure 28). This microdrive allowed a dorsal to ventral movement of the tetrodes in steps as accurate as 25 µm to search for new units. This very light device (1.5grs) allows animals to move freely. Microdrives were attached to the skull with dental cement and 7 stainless steel screws (Precision Technology Supplies LTD). One of them was used as a ground. In those experiments, two microdrives of 16 channels configurations were used: 2*8 electrodes and 1*16 electrodes connectors (Figure 27). Electrodes tips were gold plated to reduce impedances between 150 to 250kΩ at 1kHz.

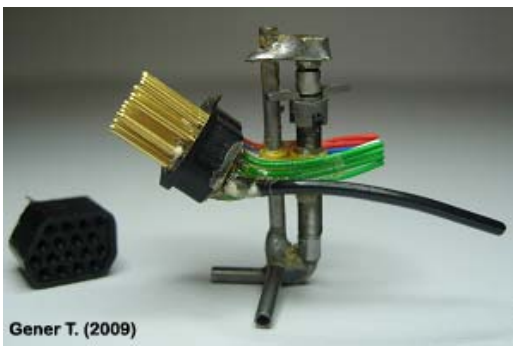


Figure 27: Photos of the microdrive. 16 channels unmount.

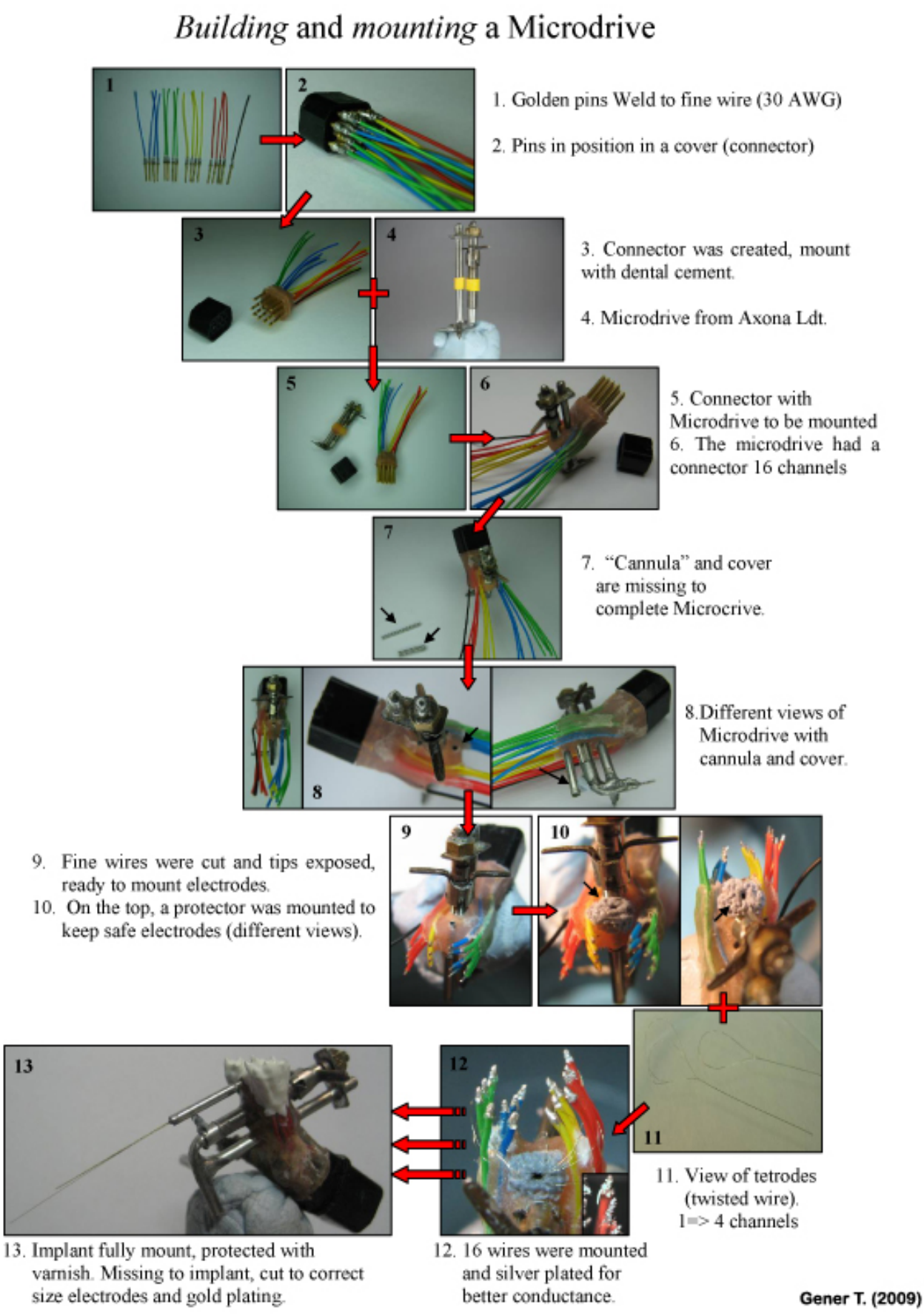


Figure 28: How to build and mount a microdrive.

3.3.SURGERY

We used two techniques to anesthetize animals, one in Alicante, and one in Barcelona. In the INA (instituto de neurociencias de Alicante), anaesthesia was induced using intraperitoneal injections of ketamine (100 mg/kg; Imalgene 1000, Merial Laboratorios SL) and xylacine (10mg/kg; Rompun 2%, Bayer). During the surgery the animal was deeply anesthetized using a mixture of isoflurane (0.5-1.2%) and oxygen (1.5 l/min). In IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona), anaesthesia was induced using intraperitoneal injections of ketamine (100 mg/kg; Imalgene 1000, Merial Laboratorios SL) and medetomidine (0.2 mg/kg, Domtor, Pfizer SA). During the surgery the animal was deeply anesthetized using a mixture of ketamine (100 mg/kg; Imalgene 1000, Merial Laboratorios SL) and medetomidina (0.5 mg/kg, Domtor, Pfizer SA). If needed, 20% of the induced dose was used as maintenance. The animal was then mounted in a stereotaxic apparatus and the skull was exposed. A trephine was used to make a 3 mm diameter craniotomy over parietal association cortex (PtA) at -4 A-P and 2.8 M-L. Seven holes were done in order to fix 7 stainless steel screws (Precision Technology Supplies LTD) on the head of the animal to hold the microdrive, one of them used as the ground. Body temperature was monitored through a rectal thermometer and maintained by means of an electric blanket.

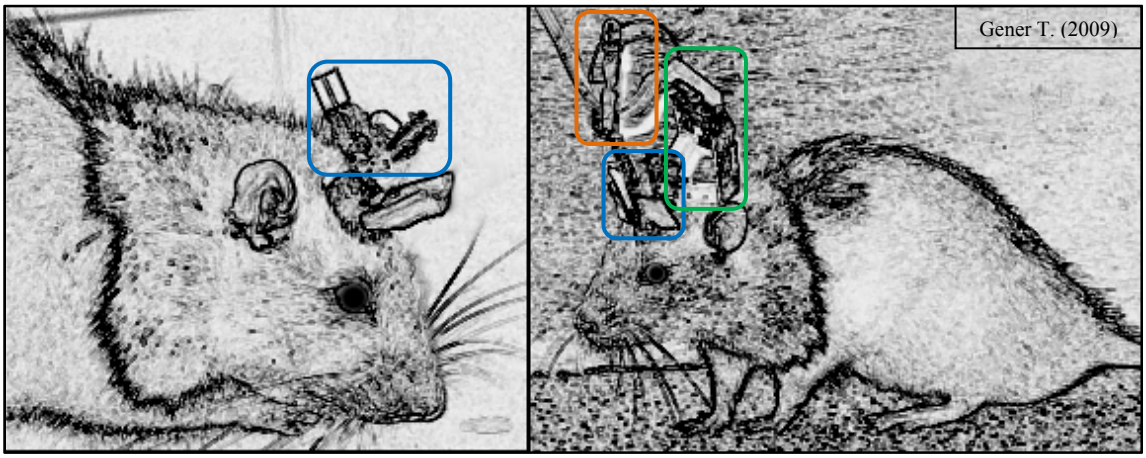


Figure 29: draw of implanted rats.
On the left draw, unconnected rat implanted with 2*8 electrodes (in blue the microdrive). On the right draw, the rat (implanted with 2*8 electrodes) is in the maze connected to the recording system by a cable (in blue the microdrive, in green the headstage, in orange the cable and infrared LED).

Heart rate (ECG) was also monitored and maintained between 250-300 bpm (Capnograph SurgiVet, Wisconsin, USA). Reflexes were regularly checked during surgery to assure deep anaesthesia. Other drugs were given during surgery and the recovery period (5 days) in order to prevent infection, inflammation and for analgesia:

antibiotics (enrofloxacin; 10mg/kg; s.c.) and topical application of neomycin and bacitracin in powder (Cicatrin®), analgesic (buprenorphine; 0.05mg/kg; s.c.), antiinflamatory (methylprednisolone; 10mg/kg; i.p.) and atropine (0.05mg/kg, s.c.; Atropina Braun 1mg, B. Braun Medical SA) to prevent secretions during surgery. Rats were cared for and treated in accordance with the Spanish regulatory laws (BOE 256; 25-10-1990) which comply with the EU guidelines on protection of vertebrates used for experimentation (Strasbourg 3/18/1986).

3.4. HISTOLOGY

Histology study has been performed after each animal's sacrifice, to verify that the implanted tetrode was in the right place, the CA1 of the hippocampus.

Animals, after the study, were perfused with paraformaldhyde 4%. The brain was extracted carefully and cut in a vibratome in slides of 100 µm. Then a classic Nissl staining was performed to be able to observe the pathway of the electrode. An electrical lesion was performed before extracting the brain of 5 µA during 10 seconds.

3.5. ELECTROPHYSIOLOGICAL RECORDINGS

The electrodes wires were AC-coupled to a headstage with a field-effect transistor (FET). Lightweight wires (2-3 m long of flexible low noise wire) connected these to a preamplifier (gain of 1000) and then to the filters and amplifiers of the recording system (Axona, St. Albans, UK). Signals were amplified (10,000-40,000), high pass filtered (360 Hz) and acquired using software from Axona Ltd (St Albans, UK) (Figure 30). The sample frequency was 48 KHz. When the signal raised a predefined user threshold, each spike waveform of 1ms (200 µm before the threshold and 800 µm after the threshold) were stored. One channel of the sixteen available was used to record EEG. The signal was continuously stored, with a sample rate at 250 Hz and 4800 Hz to see high frequency. Before every experimental session, tetrodes were "screened" for neuronal activity. Once spikes could be well isolated from background noise the experimental protocol started.

To resume, tetrodes of 17 µm were connected to the headstage by a golden connector. The headstage (FET) by a flexible low noise cable was connected to the preamplifier which is linked to the acquisition card of the Axona computer. In another

way, video camera is connected to the video acquisition card of the computer to record the track of animal. All those data are recorded by the Axona Ltd software (Figure 30).

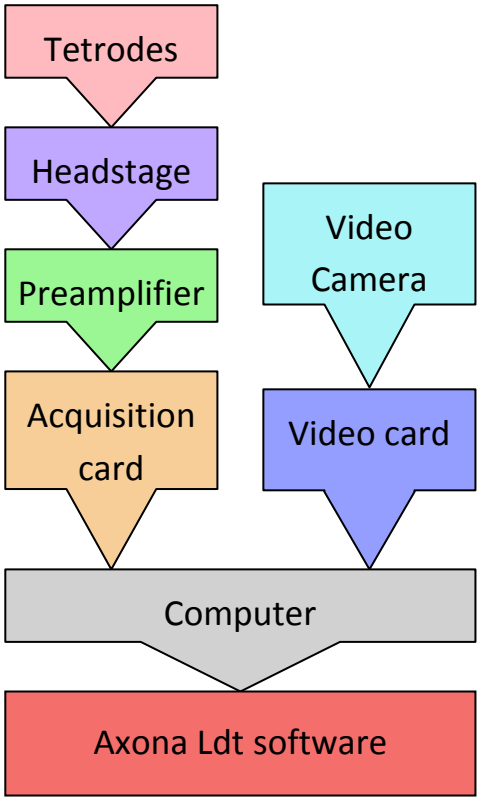


Figure 30: Scheme of recording system with Axona ldt hardware.

Electrophysiological recording were performed in a differential mode. The signals were recorded between two electrodes. The first channel was the one with the signal, the second channel with the local noise to eliminate (reference electrodes). The reference electrode was chosen manually for each recording (Figure 31) and for each electrodes.

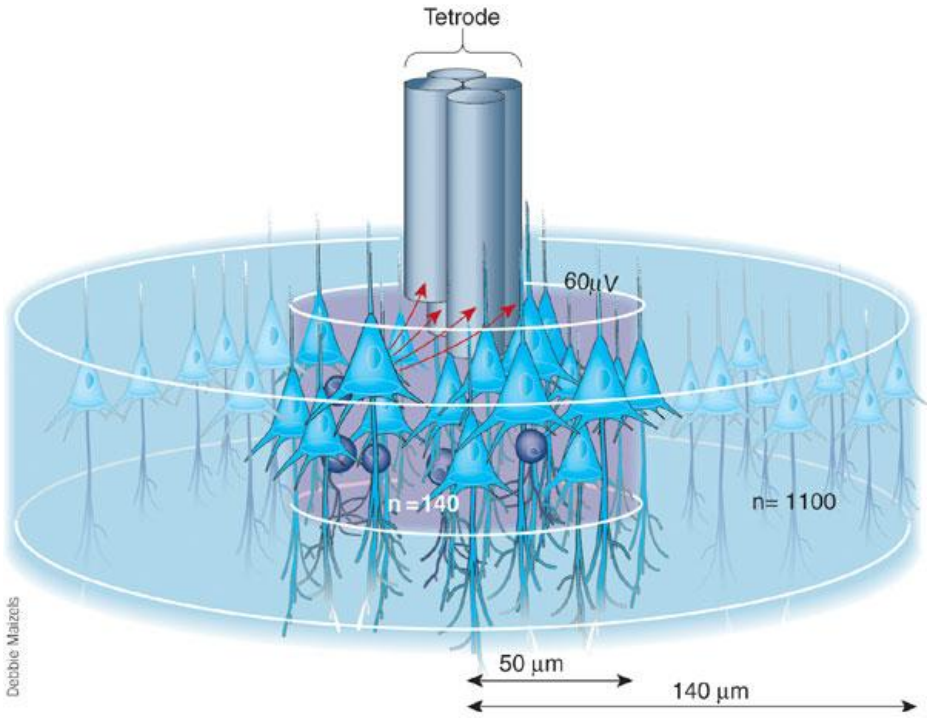


Figure 31: Scheme of a tetrode implants in the brain.
The four electrodes close to the pyramids cells. Electrodes can record the local field potential (LFP) or by differential recording single multi units. *Taken from Buzsaki (Buzsaki, 2004).*

The position of the animal was tracked using an infrared camera (Sony). On the head of the animal was placed an infrared LED (Light-Emitting Diode) to track the rat head in the total obscurity. Sampling frequency of the tracking system was 50Hz. The data of animal position will be then correlated with the brain activity (Spike), to determinate where and when animal had some activity or not.

3.6. PROTOCOLS

Several protocols and several maze sizes have been developed and tested, to try to find the best way to observe the influence of the somatosensory input on place cells.

The general design was to record PCs in control condition then induce a change (regarding the somatosensory input), record PCs under this condition and then record PCs after the effect. To carry out this design we developed a protocol and a maze. The protocol consisted on a minimum of three main conditions. Recordings were performed in total darkness, with homogeneous white noise (70 db) and clean floor (cleaned with alcohol 70% mixed with soap and water). Those precautions were taken to avoid that animals used any other senses to orientate apart from the tact. The control condition in which PCs baseline activity was determined, an experimental condition, in which recordings were performed under the effect of lidocaine and finally the last recording session, was carried out after the lidocaine effects disappeared. The number of recordings in the several phases was changing depending on PCs' stability. Recording time was between 15 min to 20 min to obtain a complete tracking recovering maze. Animals were motivated with sweet rice randomly through in the maze each 20 sec to keep the animal in movement. The resting time between two conditions was 10 min. After testing the basic protocol in some pilot experiments, we tried to enrich the environment in order to get more relevant information for whiskers to create the spatial mapping.

The first enriched maze had a barrier and an object in the middle. This posed problem being that the barrier and object could influence the place cell in a small environment. A second maze had a grid on one wall was also discarded to be not enough relevant for whiskers (the object was big enough to be recognized with the paw or body). The final protocol was chosen because it was enough enriched of different texture and not disturbing the animal in the foraging as the firing of cell. It was organised in three different sand papers (P360, P180, P40) in three different walls of the

maze. The maze dimension was 80 cm x 80 cm. Once the final organisation of the maze was obtained, we determined two protocols to study, the first one was the tactile cue rotation (to control that place cells were orientated by tactile cue) and the second one was the temporal somatosensory deprivation (to observe the influence on place of the tact).

PROTOCOL: TACTILE CUES ROTATION

This protocol was to determine if the animal used tactile cues to orientate in the maze. The concept of this protocol was to move the tactile cues and to observe if place fields will moved according to tactile cues. We rotated 90º the polarised tactile cues (Figure 32).

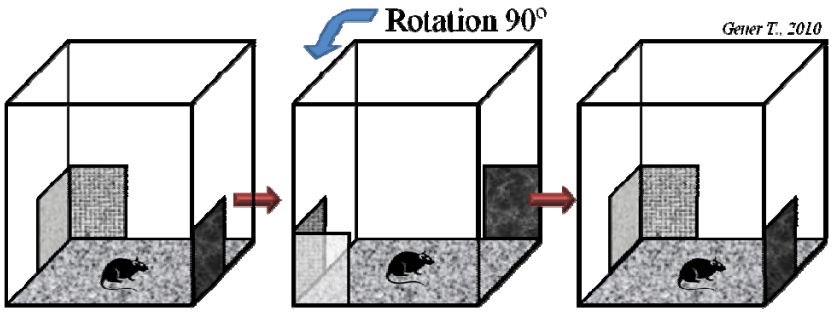


Figure 32: Scheme of the rotation protocol.
Tactile cues (sand paper) are rotated with an angle of 90º.

The protocol consisted in three recordings in an enriched maze as aforementioned. Animals were resting 10 min in their home cage between the several conditions. The first recording was a control recording to have a base line recording. Then a recording with the 90º rotated tactile cues was performed. The last recording was carried out with the tactile cues at the same place than the first control recording.

PROTOCOL: TEMPORAL SOMATOSENSORY DEPRIVATION

This protocol was to determine how the place fields are affected by the tactile deprivation. All the recordings were performed in an enriched maze. We first carried out two control conditions, to observe the stability of the PC. The lidocaine was applied to the snout of the animal to produce the tactile temporal deprivation. Then we carried out the first recording session 5 to 10 min after the lidocaine application. Several recordings were obtained after longer intervals to observe the effect of lidocaine on the place fields and the possible recovery (Figure 33).

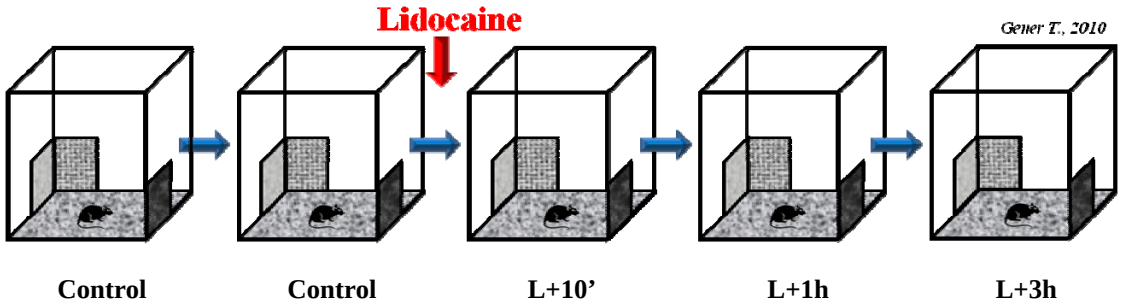


Figure 33: Scheme of the time protocol.
Three sand papers (P360, P180, P40) were place as tactile cue asymmetrically in a square maze.

During the analysis, we considered only three conditions: 1) Control, before the lidocaine application, 2) Lidocaine, after the lidocaine application (10'), 3) Recovery, after the lidocaine effect more than 2h after the application.

3.7.DATA ANALYSIS

The data were analysed in several steps. Each step required different software, some commercial and some homemade software and scripts were used.

UNIT ISOLATION

The first step was to verify if the signal recorded was a spike and more than that if it was place cell. For that, we must isolate the units recorded, and then the spike was correlated with the position of the animal. The entire first step was performed with commercial software (TINT) (Figure 34).

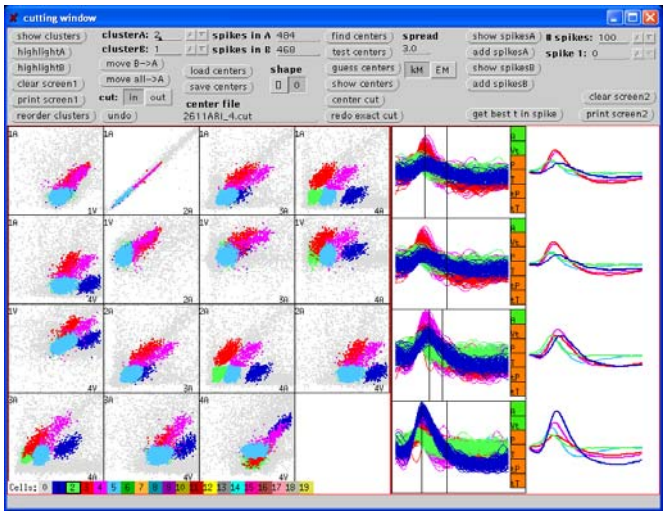


Figure 34: Example of the software TINT by Axona Ltd.
On the left, we can observe the cluster of different spike, on the right the shape of those spikes.

Data were analysed using a cluster cutting technique (Tint, Axona Ltd). This cutting was based on the spike amplitude, the time of the spike peak, etc. Units were classified by being assigned to different clusters. Interspike time histogram and autocorrelograms were plotted to control the refractory period (2 ms) and artefacts.

PYRAMIDAL CELLS – INTERNEURONS CLASSIFICATION

Classification between two general types of cells (pyramidal cell and interneurons) has been performed. Parameters such as auto-correlograms, spike width (spike waveforms), mean firing rate have been take in account (Csicsvari *et al.*, 1999) to distinguish putative place cells from interneurons.

- Spike width (peak to valley) should be superior to 300 ms to be a pyramidal cell.
- Spike mean rate should be low < 2 Hz for a pyramidal cell and higher for interneurons (Markus *et al.*, 1994; Csicsvari *et al.*, 1999).
- Maximum peak in the spike time autocorrelogram should be between 3 and 8 ms to be considered as a pyramid and should be more than 10 ms to be considered as an interneuron.

PLACE CELLS

A place cell is a neuron which has particular specifications. A place cell is in general a pyramid cell, which has one or multiple firing fields.

To be considered as a place cell, neurons should have some precise characteristics.

- The cluster isolated from a place cell should have more than 100 spikes.
- The spike's amplitude should be superior to 100 µV.
- The cluster should be clean from any noise and respect the refractory period of >2ms.
- Minimum peak firing rate should be superior to 1 Hz.
- To be considered as firing field, the firing rate should be superior to 20% maximal peak.
- Skaggs value (Skaggs, 1993; Markus *et al.*, 1994) >1.
- The ratio maximum frequency to mean frequency >2.

- The judgement of the experimentalist was also a factor taken in account.

FIRING MAPS GENERATION

To know if a neuron is a place cell, we have to correlate the cell firing with where in the space the neuron fired. Information about the animal localisation and the spike timing are needed. Animal tracking was performed using an overhead tracking camera which sampled the animal position (50Hz). Animal's Position is stored in X, Y coordinates and divided into bins to obtain a spatial map matrix. Frequency of firing at each position was then obtained by dividing the total number of spikes by dwelling time in each bin.

Once we recorded a pool of place cells and carefully analysed those, we used a complete custom system developed in Matlab® (Figure 35). Those scripts were created to be able to organise and analyse a big amount of place cells data. It was divided in three phases, the first one to extract data and to label all the protocol of each recording. The second phase was to classify all the cells recorded and to calculate all the parameters needed for further analysis. The final part was to analyse all the cells together by groups to have an overview of the effect of the protocol on the hippocampus cells properties.

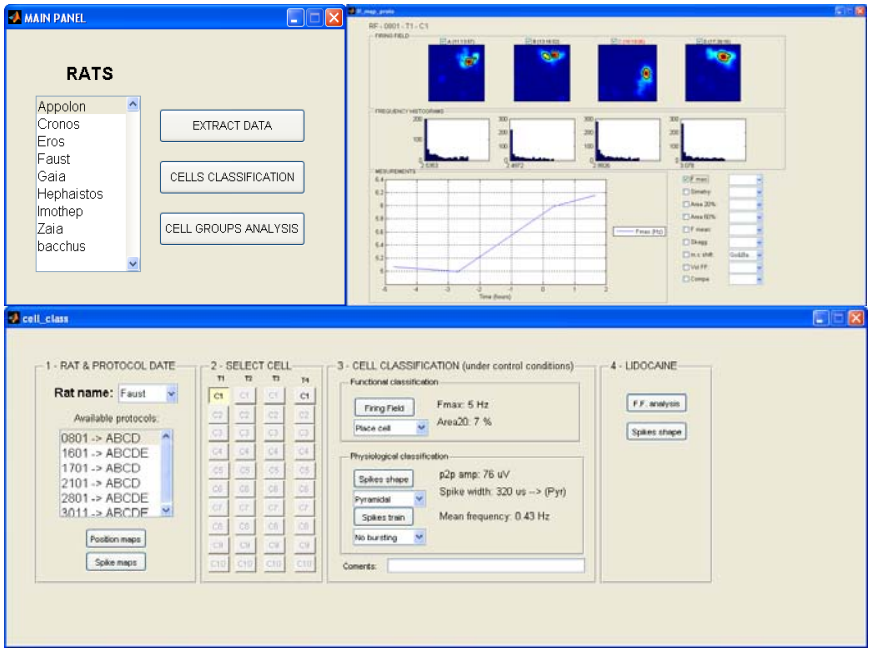


Figure 35: Example of the Matlab® script GUI develops for placeCells analysis.

During the phase two, we generated the firing map. For that, we calculated for each bin of 5x5 pixels (3.25pixels→1cm) the firing rate as the number of spikes by the

time spent in this place (bin). We carried out then a smoothing of the firing map with a 2D Gaussian filter of 7x7 bins with horizontal and vertical SD of 5 bins (Robbe and Buzsaki, 2009). It was excluded from the firing field any bin with an adjacent bin with a low firing rate ($0.9 < \frac{FR_{bin} - FR_{\text{adjacente mean bin}}}{FR_{bin}}$). The value of the bin exclude is set to the mean of all the adjacent bins. This was to avoid any artefact of recording.

FIRING FIELD

The place field is the location where neurons fired with a high rate. To be considered as a place field or firing field, it must have several characteristics as: the number of spike generating this firing field should be more than 50. The time spend by the animal in the place field zone should be superior to 500 ms. The surface of the place field should not exceeded more than 25% of the total maze (Robbe and Buzsaki, 2009).

The place field area represents the surface of the maze where the firing rate is higher than 20% of the maximum firing rate.

FIRING FIELD PARAMETERS

For all the place cell analysis we calculated all the followed parameters. They are common for the two protocols performed, the tactile cues rotation and the temporal somatosensory deprivation.

1. **Fmax** (in Hz): It is the maximal frequency recorded of the entire maze. It gives us the frequency of the peak of the place field.
2. **Symmetry**: It is the firing rate at the mass centre divided by the Fmax. $Sym = \frac{FR_{centroid}}{FR_{max}}$. This value gives an idea of the shape of the firing field. A value of 1 means that the mass centre and Fmax were in the same place. The firing field was symmetric.
3. **Area 20** (in % of total maze area): It is the percentage of the area which had a frequency superior of 20% of the Fmax. It gives an idea about how well defined the place field is.
4. **Area 60** (in % of total maze area): It is the percentage of the area which had a frequency superior of 60% of the Fmax. It gives a measure about how precise and sharp the place field is.

5. **Fmean** (in Hz): This is the mean frequency in each bin of the maze. This value gives us the general firing rate of the neuron. It is important to classify the cells and to understand how the global level of activity the cell is.
6. **Skaggs or spatial information content:** This factor reflected the entropy of the activity in the maze. It defines the quantity of information which provides each bin.

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

Where i is the number of bin, Pi the probability of occupancy of the bin i, Ri the firing rate of the bin i and R the Fmean (Skaggs, 1993; Markus *et al.*, 1994). The spatial information content is calculated on the smoothed firing maps.

7. **Vol FF**: This is the sum of the firing rate of the firing field. This reflects the quantity of the surface and the rate. It helps to observe the place of the cell firing and the level of firing of the cell at the same time.

$$Volume = \sum_{i=0} FR_i \quad \forall i \in FF$$

8. **Compactness**: This is a factor of firing field compactness.

$$Comp = \frac{1}{radius \cdot NFF}$$

Where radius is the distance between the mass centre and the furthest bin of the firing field, and NFF is the number of fields which are part of the firing field (as define previously).

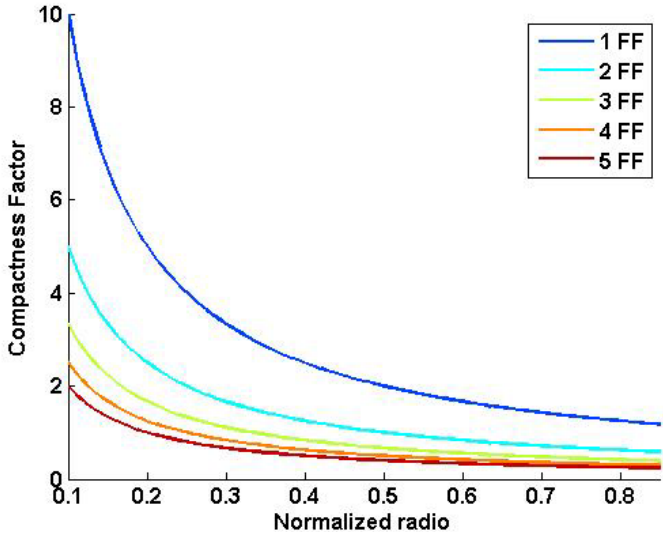


Figure 36: Theoretic Compactness. The radius is normalised to the maximum radius of the maze. In blue the one firing field, in red five firing fields.

This value is an important indicator of how the place field is compact or not. It takes into account the maximum size of the firing field and the number of place fields of the place cells. As aforementioned, a firing field has to respect some characteristics (more than 50 spikes, time spent in the FF > 500 ms, FF surface < 25% of total maze). Due to those restrictions, a value of compactness corresponds to only one place cell configuration. A high compactness factor means a small and unique place field.

SPIKE PARAMETERS

We calculated those parameters to be able to classify the cell type and also they give us an idea about the possible changes of the change in the cell properties changes.

9. **Spike amplitude** (μV): Mean of peak to peak spike amplitude. We calculated the mean of the minimum and the mean of the maximum then we do the subtraction.
10. **Spike width** (μs): This is spike width of the mean spike. It is the time between the spike peak and the spike valley (in this order).

A factor was just used for the tactile cues rotation. It was the shift of the max firing peak centre.

Centre shift or angle of rotation (in degrees): This is the shift of the bin where the maximum firing of the place field were respectively to the control condition (Fenton *et al.*, 2000). This is a measured as the angle made by the position of the maximum firing in rotation condition and in return condition versus the control condition. This gives us information about the remapping of the place field according to the rotation of the tactile cue.

This factor was used to create groups of the place field behaviour. In the rotation condition, if the angle was more than 45º, it was considered as rotation. If the angle was smaller, it was considered as stable. For the return condition, the angle should be inferior to 45º to be considered as return. Cells should complete both restrictions in the both conditions to be part of a group.

STATISTICS

For the several parameters calculated, the means were obtained. We calculated the standard deviation (σ): $\sigma=\sqrt{\sum_{i=1}^{N}\frac{(X_i-\mu_x)^2}{N}}$ where N is the size of sample, X the value of the sample, and μ the mean of the sample. We also calculated the standard error of the mean (SEM): $SEM=\frac{\sigma}{\sqrt{N}}$ where σ is the standard deviation and N the size of the sample.

Once the values of all the aforementioned factors calculated, we wanted to know if the groups in the several conditions presented some differences. More precisely, if the temporal deprivation changes significantly the place cells parameters. The way to realise this is to perform some statistical test. The test chosen in this study is the two sample t-test. This test was selected because of its simplicity and robustness (Markowski and Markowski, 1990). This test allows us to accept or reject the null hypothesis. The null hypothesis is that the two groups are equal and there is no difference between them. We wanted to compare the vector one versus another one separately and not all together that why we do not used any ANOVA test.

We used the function in Matlab® called ttest2. We use vectors X and Y (vectors of data value) and α (statistic error) were parameters of this function. The result returns for the hypothesis, 1 or 0 regarding if the test accept or reject the null hypothesis, P, the minimum level of probability where the null hypothesis is rejected and the CI, confidence interval return two values, the limits of this interval. We will not mention in the result the confidence interval. We will express the factor of significance.

RESULTS

1. ACUTE EXPERIMENTS

Acute experiments were performed in order to characterize a reversible and non-invasive method to block sensory transmission from the whiskers. They consisted on the recording of the local field potential (LFP) of the barrel cortex and in intracellular recording from the same barrel cortex. Those recording were performed in control conditions and with the application of lidocaine on the snout of the rat. Recordings from barrel cortex of 8 rats were included in this study. In 4 of the rats the complete study was performed in both hemispheres.

1.1. RECORDINGS

Intracellular recordings were obtained from a total of 13 neurons (Figure 37-A) recorded in close vicinity from the LFP recording. We obtained sensory evoked potentials by an air puff to the whisker (10 ms, 5-10 psi)(Reig and Sanchez-Vives, 2007)(Figure 37-B_Control).

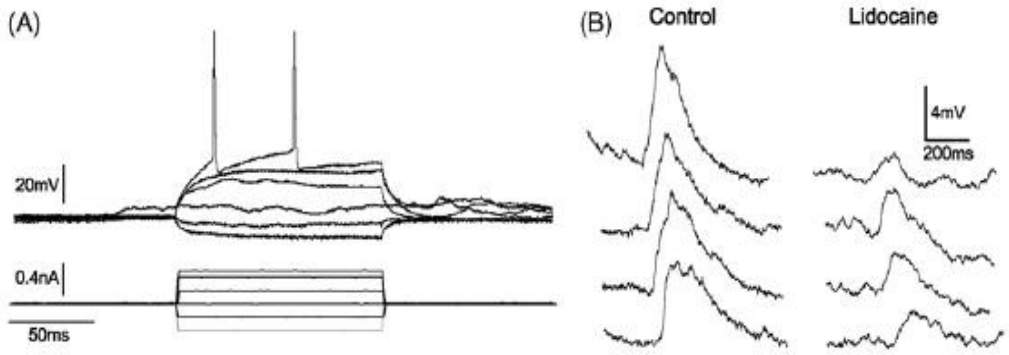


Figure 37: Blockage of intracellularly recorded whisker-induced synaptic responses by topical application of lidocaine.
(A) I–V protocol of the recorded neuron. Bottom, intracellularly injected current from −0.4 to 0.5 nA. Top, Vm responses to current injection. (B) Four raw traces of intracellular recordings in response to an air puff stimulation in control (left panel) and after application of topical lidocaine to the whiskers (right panel). Note the decrease of the amplitude to an 81%.

The average amplitude was 6.1±2.6 mV and their average latency 11.06±2.49 ms (range 8-15.6 ms). Figure 38 illustrates simultaneously recorded sensory evoked synaptic potentials in the LFP and in the intracellular recording. In this particular case the evoked synaptic potential was reduced from 6.4±2.0 mV to an average value of 1.2±1.5 mV (a decrease to 81.4±28% of the control amplitude) following lidocaine application. This decrease was also reflected in the LFP evoked synaptic potential from

58.5±31.37 μV to 7.8±21.0 μV (a decrease to 86.5±44% of the control amplitude). Once tested that the LFP accurately reflected the intracellularly recorded synaptic potentials, the following sensory responses were only evaluated through the LFP. Long (>300 min) and highly stable recordings were needed to monitor the action and recovery of lidocaine application.

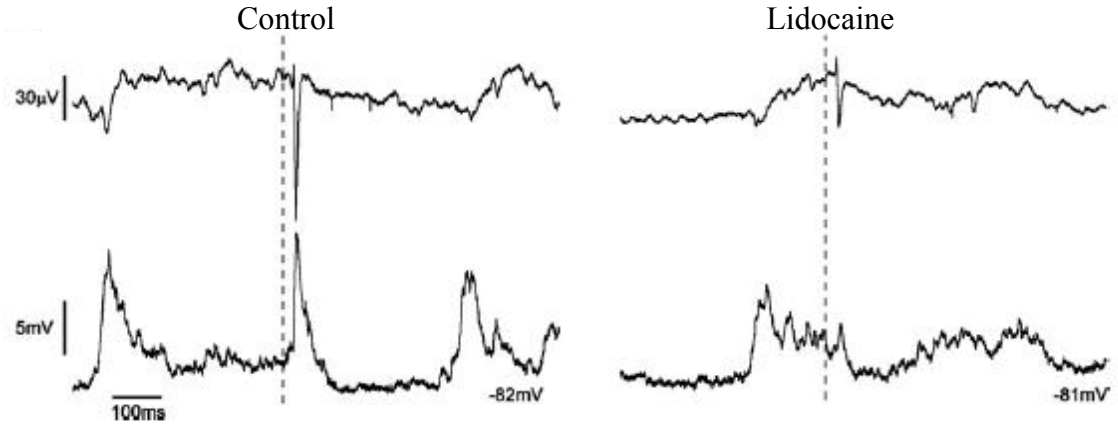


Figure 38: Blockage of intra and extracellularly recorded whisker-induced synaptic responses by topical application of lidocaine.
Traces of simultaneous LFP (top) and intracellular recording (bottom). The dash line represents the trigger of the air puff in both control (left panel) and in presence of lidocaine (right panel). The parameters of the stimulation were in both cases (control and lidocaine) 10 ms, 5 psi.

Whisker stimulation yielded a sensory-evoked response of an average peak of 80.9±5.2 μV (n=12) in the extracellular recordings. The whisker response evoked in this way was repeated at 0.2 Hz and each of the measured responses were the result of averaging 60 stimuli (300 s). This pattern of stimulation was continued for several hours (3-6 h) in order to monitor the time course of action of lidocaine and its recovery.

1.2. LIDOCAINE APPLICATION EFFECT

Lidocaine was locally delivered in different forms (local injection, cream, aerosol, drops) and concentrations (2-10%) to the base of the whiskers (see Methods). Each time, previously to the lidocaine application, a whisker induced response was obtained as control.

Local injection of lidocaine (4-5 injections, 50 μl, 10%) at the base of the whiskers induced the fastest (maximum effect in 30 min) and most complete (97%)

blockage (n=2; Figure 39-A). Five hours later the response was not yet recovered. Being an invasive technique it was found not to be ideal for awake behaving animals, even when it induced an effective blockage and thus other forms of application were explored.

Lidocaine cream was topically applied to the base of the whiskers (dose 1 grs; 25 mg/gr). The maximum blockage by cream was achieved 65 minutes after application (n=2), during which it decreased to 87% with a recovery time of 200 minutes. Cream application had the advantage of causing less stress than injection to an awake rat, but posed the problem of staying in layers of different thickness, therefore losing the control over the dosage and maybe affecting the movement of the vibrissae (Figure 39-B).

Aerosol application was carried out in two experiments (n=2). The maximum blockage by aerosol application was 55% with a maximal effect after 105 minutes and a recovery time of 320 minutes. Even when effective, the aerosol technique presented also a deficit in the control of the applied dose, not so much regarding the amount being sprayed, but regarding the distribution of the product over the desired area, the whisker pad (Figure 39-C).

A drop of lidocaine (0.4 ml, lidocaine 10%) was found to induce a reliable blockage of the sensory evoked synaptic potentials (Figure 39-D). Apart from being an easier method to apply to an awake rat (unpublished observations), it did not present the dosage problems reported of the other forms of application. Thus, for a thorough characterization of the lidocaine action, 3 experiments were performed while doing this form of lidocaine application, in all cases the recordings were done in both hemispheres while stimulating whiskers on both sides.

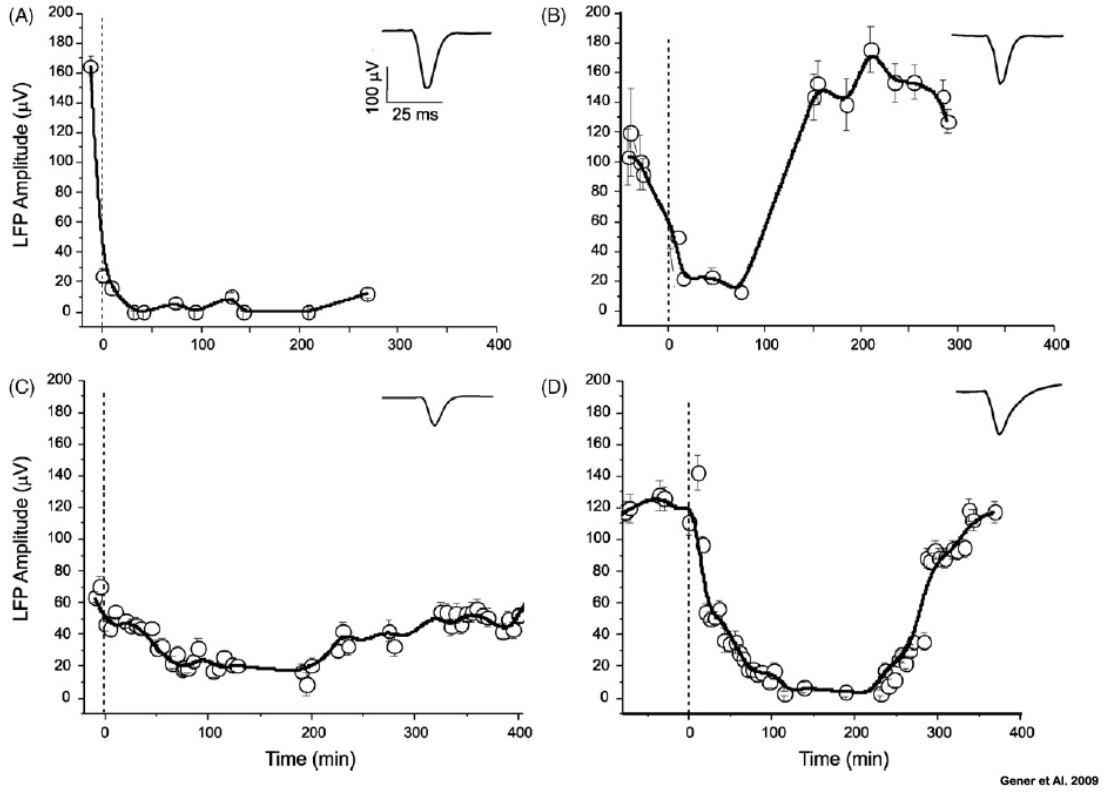


Figure 39: Time course of action of four different forms of lidocaine application.
Zero (dash line) represents the time of lidocaine application. Each circle represents the average amplitude of 60 sensory responses (0.2 Hz) as measured in the LFP in response to the air puff stimuli (5 psi, 5–10ms duration). (A) Subcutaneous injection of lidocaine (10%, 0.2 ml). (B) Lidocaine in cream, 1 g (25 mg/g). (C) Lidocaine in aerosol application (10%, 2 sprays, 0.2 ml). (D) Liquid drop (10%; 0.4 ml). Insets represent the averaged stimulus-evoked response before lidocaine was applied.

The grand average of the effect in these 6 cases is represented in Figure 40-B. The average decrease of the response amplitude was of 91.1% the control response in 150 minutes and with a recovery time of 300 minutes (Figure 40). We also focused on the slopes (descendant and ascendant) of the response. The descendant slope was changing during the time course; while the down ward slope in control conditions was 2.1±0.8 mV/second, during the action of lidocaine decreased to is 0.9±0.6 mV/second, and following the recovery was of 2.5±1.7 mV/second; and ascendant slope was constant during the time 0.20±0.15 mV/second.

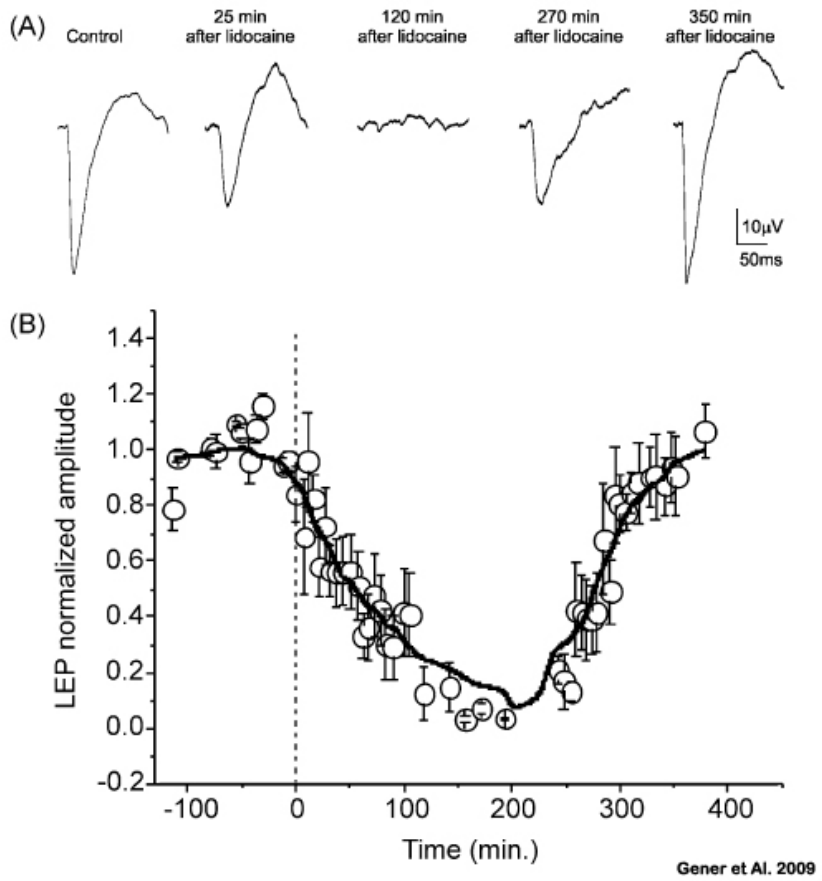


Figure 40: Effect of topical application of a drop of 10% lidocaine (0.4 ml) to the whisker pad. (A) Waveform average of extracellularly recorded evoked response in control condition, after lidocaine application and during recovery. The stimulus parameters were constant and consisted of 5ms duration and 5 psi pressure. Each trace represents the average of 60 stimuli occurring at 0.2 Hz. (B) Grand average of the time course of action of topic lidocaine (n = 6). Each point represents the average peak amplitude evoked by 60 stimuli per recording, therefore 360 responses in grand average. The sensory-evoked responses were normalized with respect to the average of the control response. The parameters of the air puff stimulation were the same in all cases (5ms).

The stimulus-response relationship was generated by random modifications of the puff pressure delivered to the whiskers and the measurement of the evoked response (Figure 41). Such stimulus-response curve was carried out before and after lidocaine application (n=6). The amplitude of the sensory response was represented against the intensity of the stimulus delivered to the whiskers. While the responses were noticeably reduced, the slope of the stimulus-response relationship is maintained in the presence of lidocaine. Following the end of lidocaine action (>300 min), the stimulus-response relationship returned to control levels.

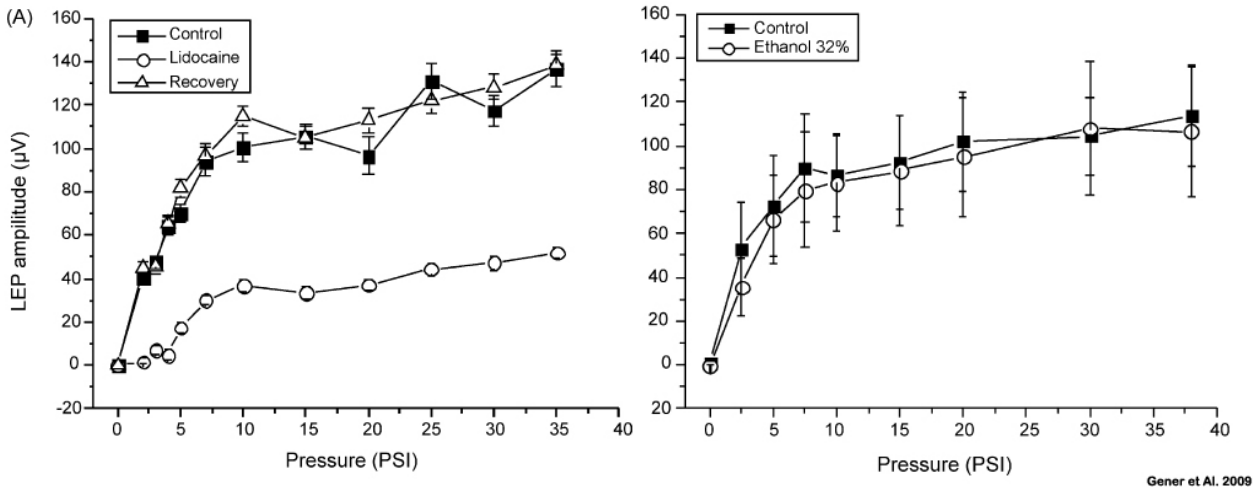


Figure 41: Stimulus–response relationship in control and lidocaine.
(A) Stimulus–response graph to an air puff of 5ms and randomly varied pressure (0–38 psi). Responses were measured in control condition and with lidocaine (0.4 ml 10% lidocaine). Each point is the average of 60 stimuli. (B) Stimulus–response relationship in control and with ethanol (32%). random varied pressure (0–40 psi) was tested in both condition. Each point is the average of 60 stimuli.

We performed several control experiments in order to demonstrate that the lidocaine was effective. We applied on the base of the whiskers ethanol 32% (in saline), the solvent of the liquid lidocaine 10% (Figure 42). The response in function of the pressure of the stimulus (air puff on the whiskers) was measured. The result showed that there were no difference between the control response (without any ethanol 32% application, N=4) and the application of ethanol 32% on the base of the whiskers (N=6). This result demonstrated that only the lidocaine had a reversible deprivation effect. This effect was due only to the lidocaine and not to any mechanical causes or other artefacts.

We also performed other set of experiences cutting all the whiskers excepted one. The stimulation was done as before on only one whisker and applying lidocaine as previously. The lidocaine effect was also present in this condition of experimentation (N=4).

To conclude, topic application of liquid lidocaine applied at the base of the whiskers could induce a blockage in the barrel cortex sensory response of 91% the amplitude, and the recovery time could be achieved in approximately 3 hours after the maximum effect. This characterization should be of use to induce a reversible blockage of whisker information in both anesthetized and awake preparations. Longer blockages would require repeated topical applications.

Our results demonstrated that whiskers anaesthesia with lidocaine were successful to block the somatosensory input in a reversible way. This gives us a method to use in awake behaving rats in order to explore changes derived from a short term somatosensory deprivation.

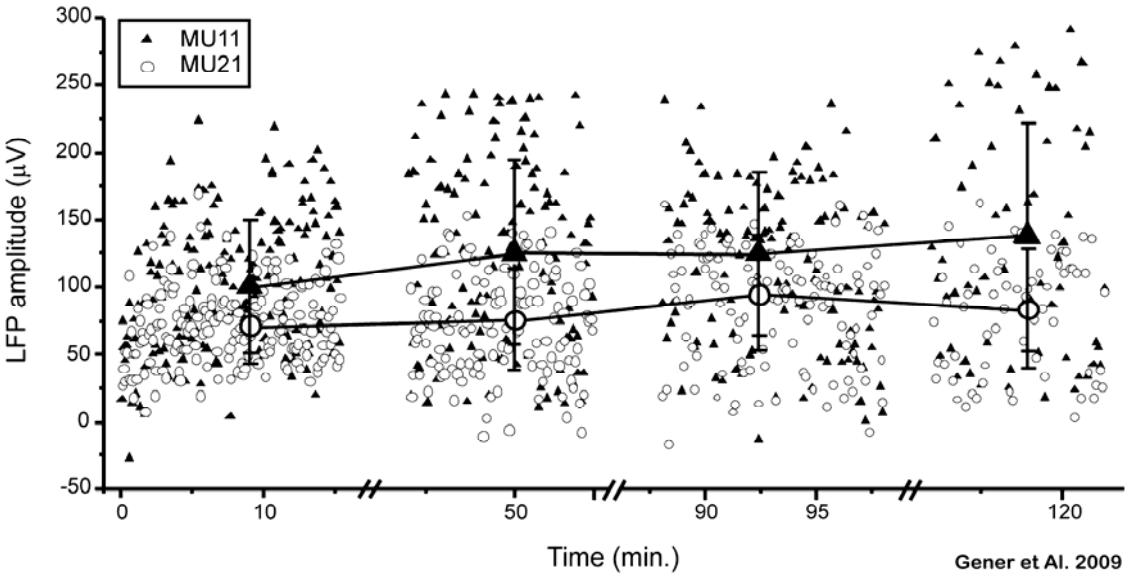


Figure 42: Recording over time of the LFP in response to a puff air stimulation in control condition. MU1 and MU2 are two different recordings (2 rats). Each point is a recording. The four full point (full square and circle) represented the average of the measurement regarding its time of recording. The repetitive stimulation was done over 120 min. stimuli duration was 5ms, pressure was 10 psi and the frequency of stimulation was 0.2 Hz.

These results were very suggestive that application of lidocaine on the base of an anaesthetised animal's whiskers would temporarily deprive the somatosensory inputs.

2. BEHAVIOURAL EXPERIMENTS

Behavioural experiments were carried out in order to define the action of lidocaine and to characterise this action on awake animals. We carried out a tactile discrimination task in the control condition and under the action of lidocaine. Details of the techniques and of the protocol were described in "Material and Methods". Here we will just remind the principle of the texture discrimination task.

Animals had to discriminate between two textures and got a reward if success, if not, they got a sound punishment (see scheme of the protocol in Materials and Methods). Animals were deprived of water and trained to the task until reaching 80% of success to the task. If not the animal was discarded.

Six animals were trained to the behavioural task. Three of them (rats A, D and F) were included for posterior data analysis because they reached the percentage of success required to be considered stable (80% during at least three sessions). The other three (rats B, C and E) were discarded for different reasons. Rat E did not success, after one month, to learn the phase one of the shaping (it did not associate to touch the texture and to get a reward in another place). Rat C learned very fast the task. It achieved all the phases in one week (from shaping to random texture exposure). The animal was getting a percentage of success of 90% minimum. During the protocol and lidocaine application, the animal's percentage of success was not affected by the anaesthetic. The subject was considered an outlier in every aspect. The last rat discarded was rat B. It was able to learn the task but after a training of two months, it did not reach the minimum level of success (superior of 80%).

It has to be noticed that these three discarded rats instead of the others were not naive rats. They were already part of some other learning protocols. This could explain why they did not complete with success the requirement of the new task.

2.1.LEARNING PROTOCOL

The protocol consisted, after the shaping phases (see Materials and Methods), on a random presentation of the texture. The animal must choose one of the reward places (right or left). If it was recognised the texture and went to the associated nose poke, a water reward was given, if wrong it got a sound punishment. We measured the percentage of correct responses (% of success), the number of time-outs responses and the reaction time response across the number of sessions performed until obtaining a high stable percentage of correct response (>80% for at least 3 sessions).

LEARNING CURVE

The learning curve is the representation of the animal's percentage of success since it started to realise successfully the task. Time is expressed in number of sessions.

We started the learning curve when the animal knew how to do the task but did not realise it properly. The presentation of the texture was random. The learning curve was performed with the data of each record (Figure 43). For each 50 trials, the percentage of success was calculated and gave us one point of the learning curve.

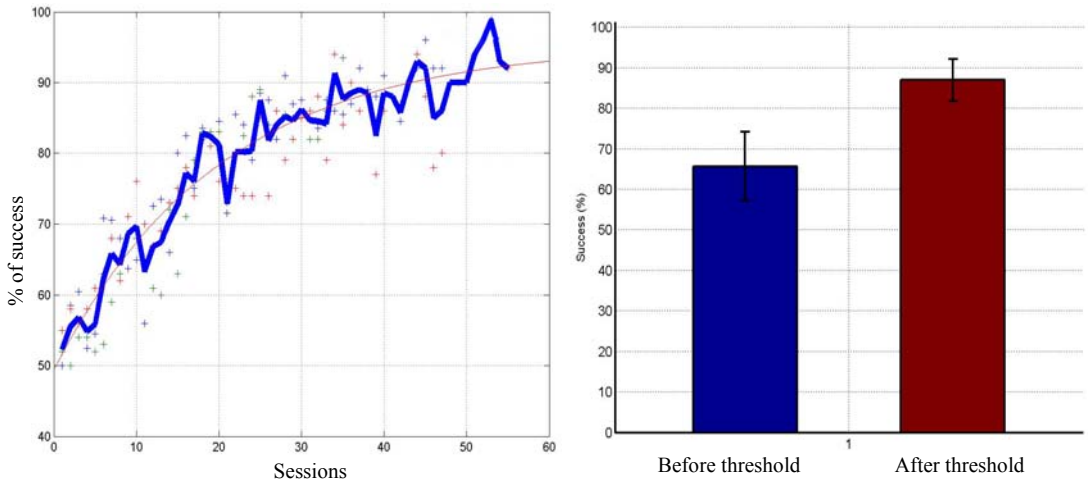


Figure 43: Learning curve of tactile discrimination task.
For the 3 animals: On the left: each point represents 50 trials of one recording over sessions (x-axis represents the number of session, y-axis represents % of success); on the right: Mean of the percentage of success before (in blue) and after (in red) the threshold of 80%.

The learning curve was obtained for the three animals included in the study. The animals have learnt the task when the task had been performed with a percentage of success superior of 80%. This level of 80% of success was reached, after 16 sessions for the rat A, 18 sessions for the rat D, and 18 sessions for the rat F. The average of the

three animals gave us a media of 17.3 sessions. The maximum mean percentage of success was 99%±0.0 and the lower was 52.3%±2.5. In Figure 43 (right), we could observe the mean percentage of success after the shaping phase but before the threshold of 80% and after. Before the level the percentage was 65.7% ±8.49 and after was 87.01%±5.13.

TIME-OUTS

During the learning process we also recorded the number of time-outs responses. A time out response was considered when the animal started a trial but did not finish it, in others words, animals did not try to get the reward (Figure 44).

The number of time-outs responses was higher when the animal did not reach the level of 80% of success with a mean before this threshold of 1.54±3.78 and 0.25±0.66 after this one. Time outs were reduced by 83.76% between animals which did not know the task and when they knew it.

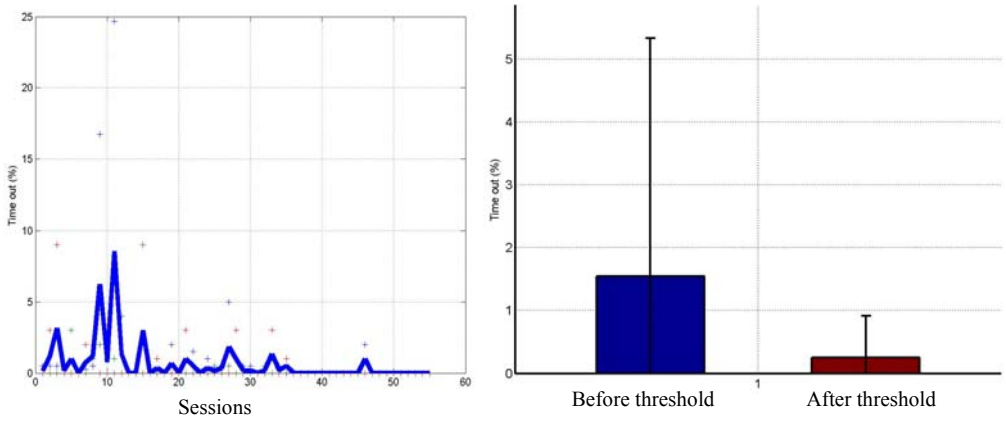


Figure 44: Number of time-outs during the learning process.
On the left: Each point represents 50 trials of one recording over sessions (x-axis represents the number of session, y-axis represents number of time out); On the right: Number of time-outs before (in blue) and after (in red) the level of 80% of success during the learning process

For rat A, the number of time-outs responses was 2.84±7.1 and 0.3±0.92 before and after the threshold, respectively. For rat D, the number of time-outs responses was before 1.16±2.28 and after 0.10±0.20. For rat F, the number of time-outs responses was before 0.94±2.2 and after 0.27±0.83. The data behaviour set was the same for the three animals.

REACTION TIME

During the learning process we also calculated the reaction time (Figure 45). The reaction time was the time that the animal took to enter in the central nose poke (start a trial) until it entered in one of the reward nose poke the animal (get it reward drinking water).

The reaction time was higher when the animal did not reach the level of 80% of success with a mean before this of 1.45±0.08 seconds and after the threshold of 1.07±0.25 seconds. Reaction time was reduced by 26.20% between when the animal was learning the task and when it knew it (Figure 45).

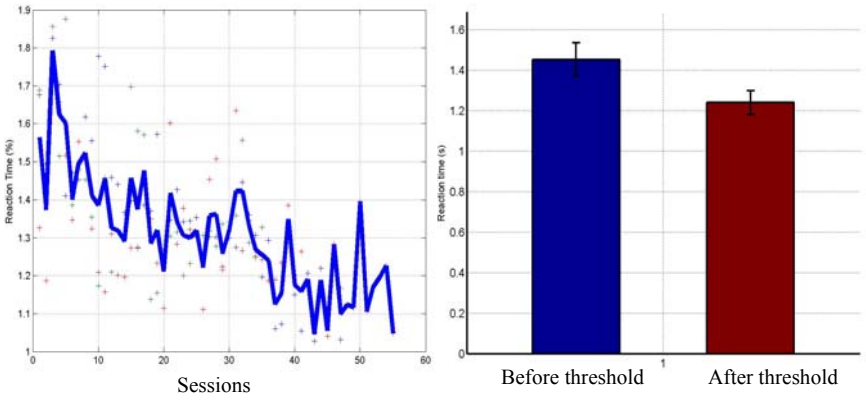


Figure 45: Reaction time during the learning process.
Each point represents 50 trials of one record (x-axis represents the number of session, y-axis represents the reaction time). Mean of the reaction time before (in blue) and after (in red) the threshold of 80% (3 animals).

For rat A, the reaction time was before the threshold, 1.52±0.16 seconds and after the threshold, 0.98±0.53 seconds. For rat D, the reaction time response was before 1.47±0.21 seconds and after 0.27±1.02 seconds. For rat F, the reaction time response was before 1.36±0.16 seconds and after 1.25±0.14 seconds. The data behaviour set was the same for the three animals.

2.2. LIDOCAINE PROTOCOL

The protocol was developed to measure the change in animal's behaviour when rats were deprived of the somatosensory inputs. We applied lidocaine (local anaesthetic) on the base of the whiskers to deprive animals. The protocol consisted on the tactile discrimination task as described previously, first in control condition and then with a lidocaine application on the snout of the rat to temporary inhibits the somatosensory input.

We measured as during the learning curve, the percentage of correct responses (% of success), the number of time-outs responses and the reaction time responses. The dispersion of those responses (fano factor) across time was also calculated to get a better representation of the data set. Time zero was the lidocaine application time. For the analysis we separated the time protocol in four different temporal parts. The data before time zero was considered the control. The data from 3 min to 12 min form the first period were considered as the peak of the lidocaine action (see Materials and Methods), then the period from 18 min to 60 min was the third one and more than one hour after the lidocaine application was the final period of the protocol.

TIME COURSE

Here, we focused on the evolution of the percentage of success across time. The application of lidocaine (Xilonibsa, 10%, "laboratorios Inibsa S.A.") reduced the percentage of success (Figure 46). The mean control for all three animals was 88.12%±7.57 of success. The maximum peak of reduction was between 3 and 12 min with a mean of percentage of success of 48.05%±145.95. This reduction was significantly different with a $p=2.87*10^{-39}$ to the control and represented a decrease of 45.44% of the total percentage of success. The first period after the peak from 18 to 60 minutes had a mean of 78.85±13.25%. The last period was 1 hour after the lidocaine application with a mean of 82.53±11.15%. We could observe that the temporal deprivation of somatosensory sense reduced the success of the rats doing the tactile discrimination task (Figure 46).

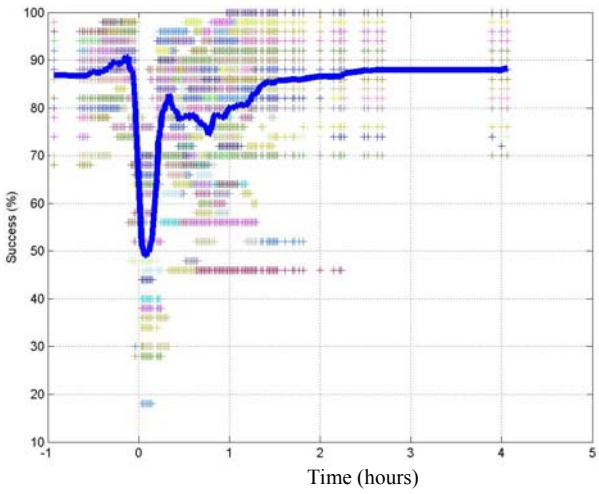


Figure 46: Time course of the percentage of success mean (N=3).
Lidocaine application time is T=0. Dots represented 50 trials, colour represented one protocol (x-axis represents the time is hour, y-axis represents the % of success).

In Figure 47, we can observe several time periods of the percentage of success. The maximum deprivation could be observed for the second period of time (3' to 12'). The third and fourth periods were significantly different from the lidocaine condition with, respectively, a p~0 and $p=1.06*10^{-24}$. This demonstrates the effect of lidocaine to deprive the somatosensory inputs of awake animal. We compared then the level of success of the discrimination task for the third and fourth periods that were hardly different from the control level. This revealed a tendency of those results to come back to the control value.

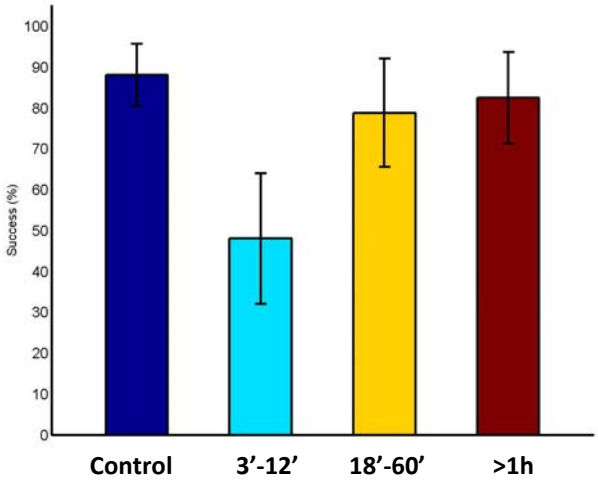


Figure 47: % of success during somatosensory deprivation.
Dark blue is in control condition, light blue is after lidocaine application (3-12min), yellow is first period of recuperation (18-60min) and the red is the complete recovery (more than 60 min).

TIME-OUTS

The mean of time outs before deprivation was 0.58±3.22. During the peak of the deprivation due to the lidocaine, the mean was 31.82±17.64 time-outs (Figure 48). This was significantly different with a $p=3.38*10^{-33}$. Then the third period the mean was 1.57±4.63 time-outs and for the last period, the mean of time-outs number was 1.84±6.37. These two last periods were not significantly different from one another (p=0.72), and the difference were also not significant between one this groups with the control (p=0.07 for group 1-3, p=0.09 for group 1-4). Time-outs for the lidocaine condition was significant for the two last periods ($p=4.46*10^{-40}$ for group 2-3 and $p=7.76*10^{-25}$ for group 2-4). Lidocaine application induced a temporal increase of the number of time outs. After the effect, the number of time outs came back to the control level.

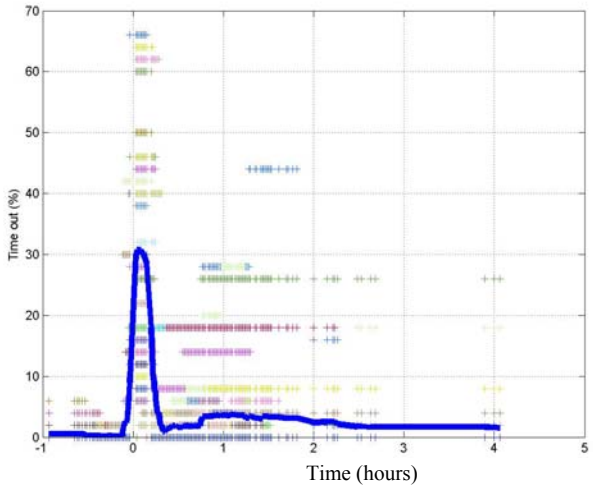


Figure 48: Time course of the number of time-outs during somatosensory deprivation. T=0 mean lidocaine application. X-axis represents the time in hours, y-axis represents the number of time out.

In Figure 49, we could observe the number of time-outs during the several time period of time represented. The maximum number of time outs could be observed for the second period of time (3' to 12').

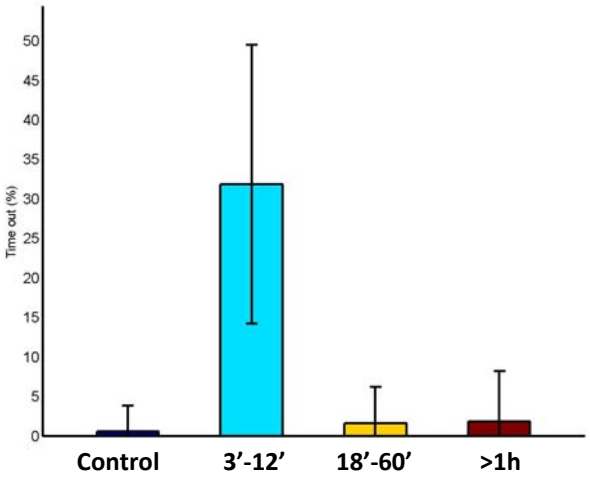


Figure 49: Number of time-outs during somatosensory deprivation by phase. Dark blue is in control condition, light blue is after lidocaine application (3-12min), yellow is first period of recuperation (18-60min) and the red is the complete recovery (more than 60 min).

REACTION TIME

Reaction time mean in control condition was 1.23±0.13 seconds, when the lidocaine was applied, the mean for the first period was 1.82±0.26 seconds. The third period had a mean of 1.50±0.26 seconds and the final one 1.42±0.24 seconds (Figure 51).

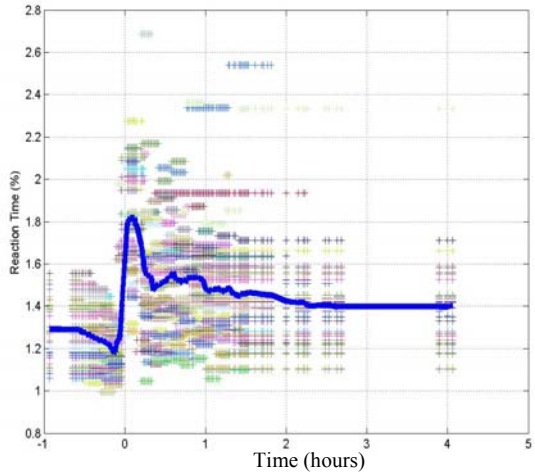


Figure 50: Time course of the reaction time during somatosensory deprivation. T=0 mean lidocaine application. X-axis represents the time in hours, y-axis represents the reaction time.

Here we observed that the reaction time is larger after lidocaine application but did not reach back the control level. In Figure 50, the time course of the reaction time could be observed. The difference between the control period and the first lidocaine one (3'-12') was significantly different with a $p=1.28*10^{-33}$. There were also some significant differences between the lidocaine period and the third and fourth one with respectively $p=1.44*10^{-09}$ and $p=1.80*10^{-12}$. The control condition, the third and fourth periods showed also significant differences even if the tendency showed a return to the control condition (Figure 51).

For the three main factors as the percentage of success, the number of time-outs and the reaction time, all the results were coherent. Lidocaine application on the whiskers pad interfered with the proper behaviour of the animals doing the tactile discrimination task. We focused now on how the data were distributed across the experiments by calculating the fano factor.

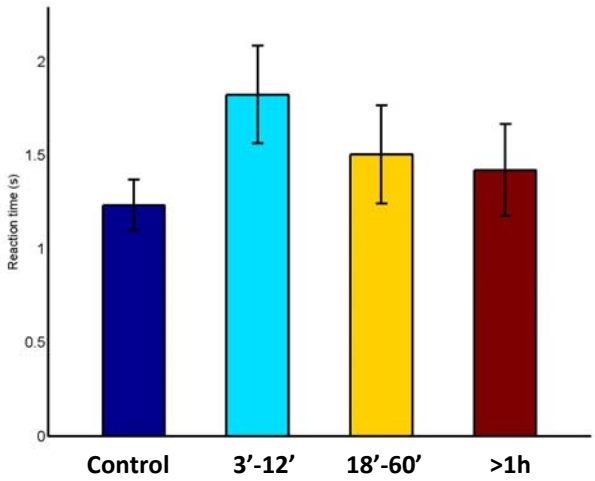


Figure 51: Reaction time during somatosensory deprivation.
Dark blue is in control condition, light blue is after lidocaine application (3-12min), yellow is first period of recuperation (18-60min) and the red is the complete recovery (more than 60 min).

FANO FACTOR

The fano factor represented the dispersion of the data. Due to the application of a drug on three different animals, even if the effect could be the same, the range of action could be different. The analysis of the fano factor will reveal us if the data dispersion were the same.

For the control, the fano factor for the percentage of success was 0.65 (Figure 53), for the number of time outs, it was 17.90 (Figure 52), and for the reaction time, it was 0.015 (Figure 54). During the period of maximum action of lidocaine, the percentage of success had a fano factor of 5.29, for the number of time-outs, a fano factor of 9.78, and for the reaction time, a fano factor of 0.037. For the third period, the fano factor was 2.22 for the percentage of success, 13.62 for the number of time-outs and 0.046 for the reaction time. The last period had a 1.50 for the percentage of success, 22.01 for the time-outs and 0.042 for the reaction time. The fano factor increased under lidocaine condition for the percentage of success and the reaction time but its only decrease after the effect for the % of success. For the number of time-outs, the fano factor stays relatively high. For the time-outs, the fano factor decreases under lidocaine condition and returns to control condition.

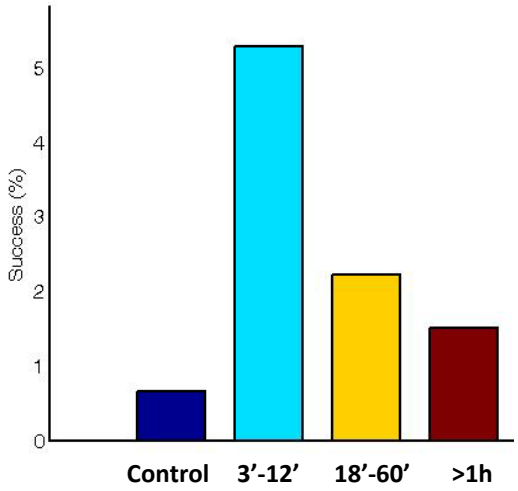


Figure 53: Fano factor of the % of success for the four phase of the somatosensory deprivation.
Dark blue is in control condition, light blue is after lidocaine application (3-12min), yellow is first period of recuperation (18-60min) and the red is the complete recovery (more than 60 min).

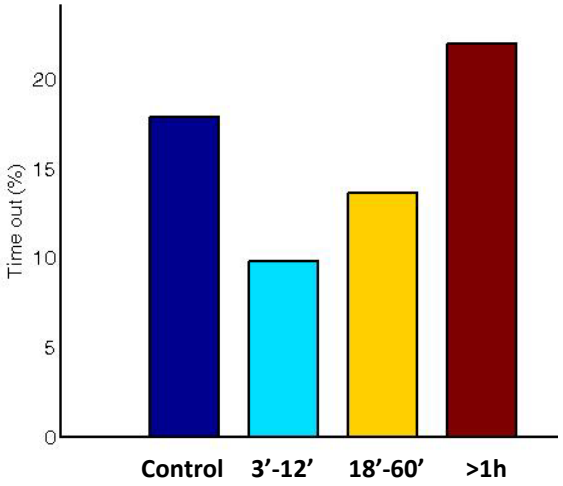


Figure 52: Fano factor of the time out for the four phase of the somatosensory deprivation.
Dark blue is in control condition, light blue is after lidocaine application (3-12min), yellow is first period of recuperation (18-60min) and the red is the complete recovery (more than 60 min).

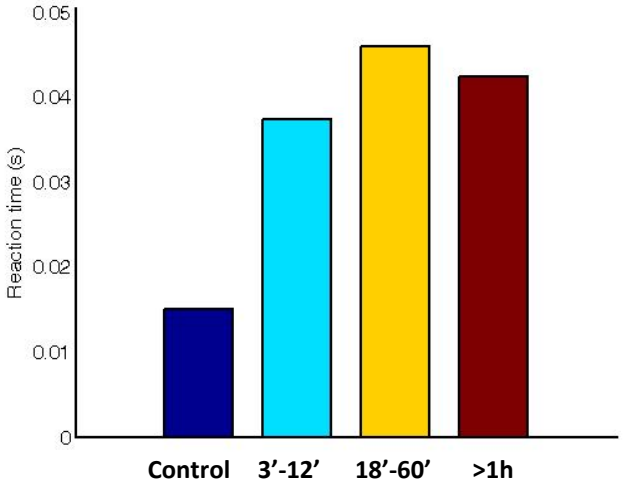


Figure 54: Fano factor of the reaction time for the four phase of the somatosensory deprivation.
Dark blue is in control condition, light blue is after lidocaine application (3-12min), yellow is first period of recuperation (18-60min) and the red is the complete recovery (more than 60 min).

These results were very suggestive that the application of lidocaine on the whisker temporary perturbed animals' behaviour which could not performed properly the tactile discrimination task.

3. ELECTROPHYSIOLOGICAL BEHAVIOURAL EXPERIMENTS

Once that we had characterised a deprivation method that was tested and validated in the awake animal, the main goal of those experiments was to test that the somatosensory pathway participates to the creation of the cognitive map using the deprivation method. To achieve this goal, we performed recordings of place cells in the CA1 hippocampus (see Materials and Methods). Two kinds of protocols were created and carried out.

- The first protocol consisted of doing a rotation of tactile cues (see Material and Methods) to determine that place cells integrated information from somatosensory inputs.
- The second protocol consisted on temporarily deprive whisker information (local application of lidocaine) using the technique developed and described previously. This protocol aimed to characterise the participation of the cognitive map creation of the somatosensory input.

3.1. ROTATION OF SOMATOSENSORY CUES

CELL PROPERTIES

We recorded from 38 cells in which the full protocol was performed, from 4 rats Lister Hooded and Wistar (rats F, G, I, U). From these cells, the analysis based on the spike's width, spike's amplitude and the spike train autocorrelogram (see Material and Methods), showed that 30 pyramidal cells were recorded which represent 79% of the total cells recorded and 8 unclassified cells was recorded representing 21% of the total cells recorded (Figure 55). It was not possible to safely classify interneurons with these three parameters. If there was a doubt on the possible type of the cell it was classified as "unclassified cells". From these 38 cells, 21 place cells were identified which represented 56% of the total cells recorded (see Material and Methods for criteria). The 17 remaining cells did not code for space, representing 44% of the total cells recorded.

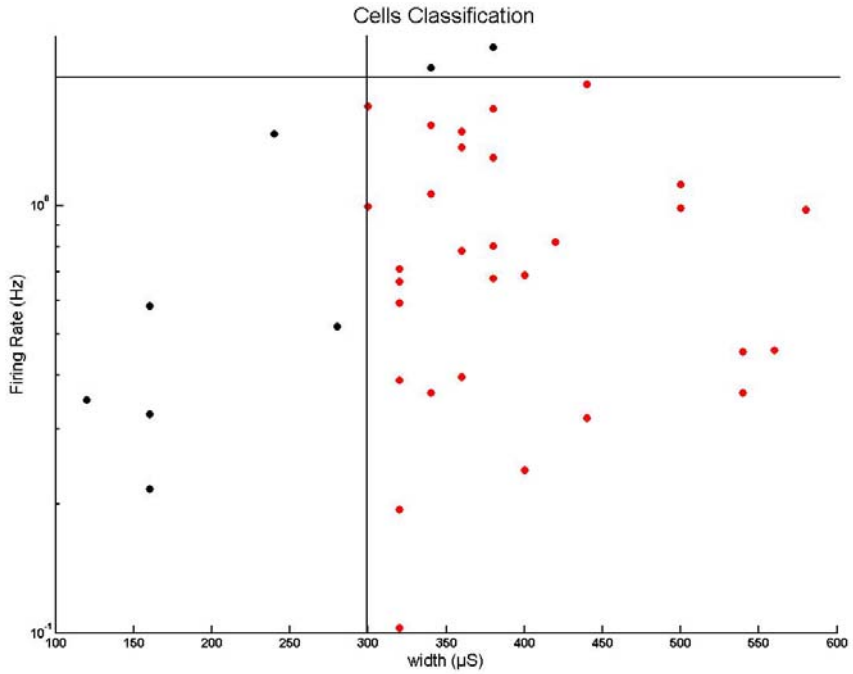


Figure 55: Cells classification of rotation protocol.
In red the pyramidal cells, in black the unclassified cells. X-axis represents the spike width in µS and Y-axis represents firing rate in Hz.

In Figure 56, we could observe some examples of autocorrelograms of the neurons classified as pyramidal cells and unclassified cells. The shape of the autocorrelogram (like a triangle) and the presence of a peak in between 3 to 8 ms are characteristic to the pyramidal cells (up of Figure 56). The interneuron autocorrelogram have also a characteristic shape (see Figure 67) but we did not observe clearly those characteristics (bottom of Figure 56). We decided then to not include the "unclassified" cells in the study.

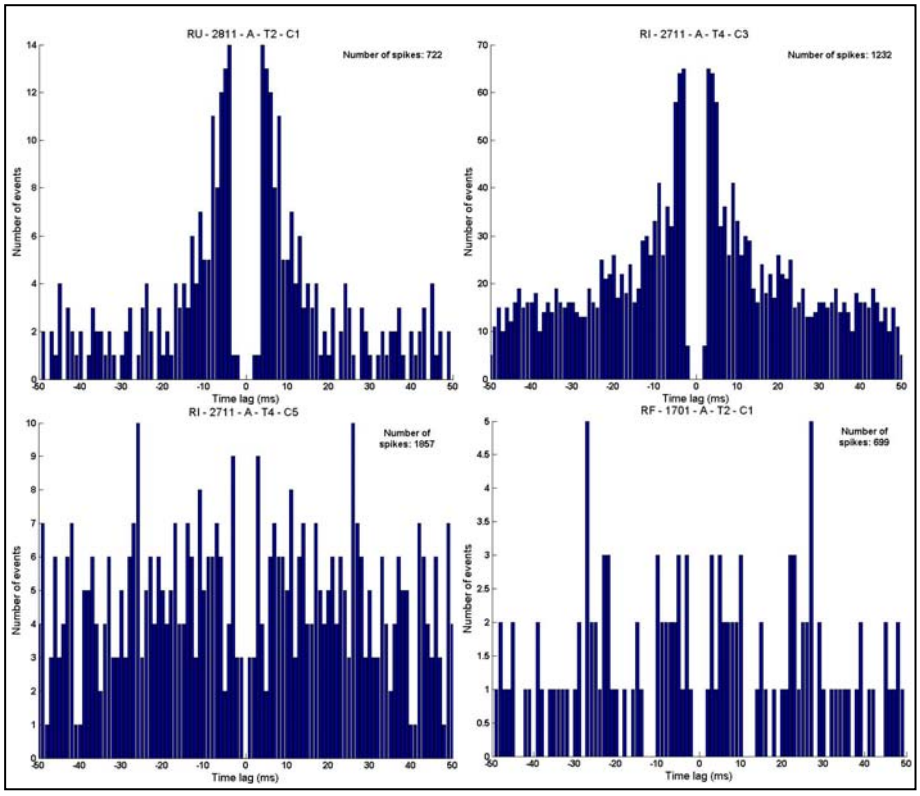


Figure 56: Example of autocorrelogram of cells for the rotation protocol. In the upper part autocorrelograms of pyramidal cells, in the lower part autocorrelograms of unclassified cells. X-axis represents the time between two spikes in ms and Y-axis represents the number of events. The 4 examples are from 4 different rats.

For these groups of cells, we calculated the mean of several parameters in control condition as maximum frequency of firing, symmetry of the firing field, the percentage of the area covered by the firing field for a threshold of 20% and 60% the maximum firing rate, the mean of frequency for all the maze, the spatial information content (Skaggs), the volume of the firing field and the compactness of the firing field (Table 1) (see Material and Methods). We calculated also parameters of the spike as the mean of the spike amplitude and the spike width in control condition (Table 2).

Notice that the key parameters of place cells as the spatial information content, the compactness and Fmax were higher in the groups of place cells, respectively 6.16±0.43, 3.37±0.82 and 16.78±1.75, than for "no place cells" groups ($p=0.0215$, $p=0.0021$, $p=0.0422$). The factors: percentage of area 20, percentage of area 60 and Volume of the FF were lower for the group of place cells that for the no place cells ($p=8.1863*10-7$, $p=0.0092$, $p=0.4216$) which correspond to a well defined place field. The parameters from the pyramidal cells were also the closer from the group of place cells which was coherent with the theory that pyramidal cells formed the place cells group.

Table 1: Mean of rotation control firing field parameters by groups.

	Total Cell N = 38	*Place Cells N = 21*	*No Place Cells N = 17*	*Pyramid Cells N = 30*	*Unclassified cells N = 8*
Fmax	14.72±1.14	16.78±1.75	12.17±1.10	15.53±1.24	11.70±2.60
Symmetry	0.12±0.01	0.14±0.02	0.08±0.01	0.11±0.01	0.13±0.03
% area 20	25.37±3.22	12.96±1.76	40.70±4.71	21.85±3.41	38.57±6.94
% area 60	3.37±0.42	2.40±0.35	4.57±0.76	3.41±0.51	3.24±0.63
Fmean	0.39±0.05	0.33±0.06	0.48±0.08	0.36±0.05	0.52±0.15
Skaggs	1.67±0.19	2.24±0.25	0.98±0.18	1.91±0.21	0.79±0.15
Vol FF	340.77±49.341	304.51±56.00	385.56±86.70	340.82±55.28	340.59±116.85
Compct	1.14±0.27	1.92±0.42	0.16±0.04	1.38±0.33	0.21±0.09

Table 2: Mean of spike parameters by groups.

	Total Cell N = 38	*Place Cells N = 21*	*No Place Cells N = 17*	*Pyramid Cells N = 30*	*Unclassified cells N = 8*
Amplitude (μV)	113.28±7.78	120.69±11.44	104.12±10.06	121.35±9.14	83.01±7.53
Width (ms)	364.73±17.49	410.47±21.45	308.23±22.56	402±14.04	225±32.90

The higher spike amplitude and the higher spike width were for the groups of place cells and pyramid cells.

In order to be able to compare several conditions of the protocol, we measured the aforementioned parameters in the three conditions: Control_1, Rotation 90º, Control_2. There were no significant differences between groups over the protocol. The fact that there were no differences demonstrates the stability of the cells recordings over time and that the rotation did not affect the spatial parameters of place cells and place cells properties.

For example, the Fmax was stable over the conditions with respectively 16.78±1.75, 16.60±1.81 and 16.45±1.84 for the Control_1, the Rotation 90º and the Control_2 conditions. The Skaggs was 2.24±0.25, 2.07±0.27 and 2.14±0.30 for the Control_1, the Rotation 90º and the Control_2 conditions (Table 3).

Table 3: Mean firing field parameters during rotation protocol.

Place cell N=21	***Control_1***	***Rotation 90º***	***Control_2***
Fmax	16.78±1.75	16.60±1.81	16.45±1.84
Symmetry	0.15±0.018	0.13±0.02	0.11±0.01
% area 20	12.96±1.77	14.32±2.40	13.94±1.42
% area 60	2.40±0.35	2.41± 0.34	2.46±0.23
Fmean	0.33±0.06	0.31±0.05	0.28±0.05
Skaggs	2.24±0.25	2.07±0.27	2.14±0.30
Vol FF	304.51±56.00	308.79± 72.21	293.32±53.94
Compct	1.92±0.42	1.44±0.38	1.69±0.36

Table 4: Mean of spike parameters during rotation protocol.

Place cell N=21	***Control_1***	***Rotation 90º***	***Control_2***
Amplitude (µV)	120.69±11.43	124.08±11.49	125.94±11.99
Width (ms)	410.47±21.45	405.71±24.39	391.42±21.90

The spike's amplitude and spike's width as place cells parameters were stable. There were no significant differences between those values over conditions (Table 4).

Furthermore, as we can observe in Figure 57, the autocorrelogram had a similar profile across the conditions. The characteristic peak of the pyramidal cells is quite stable over conditions showing a stable recording and unchanged cell properties.

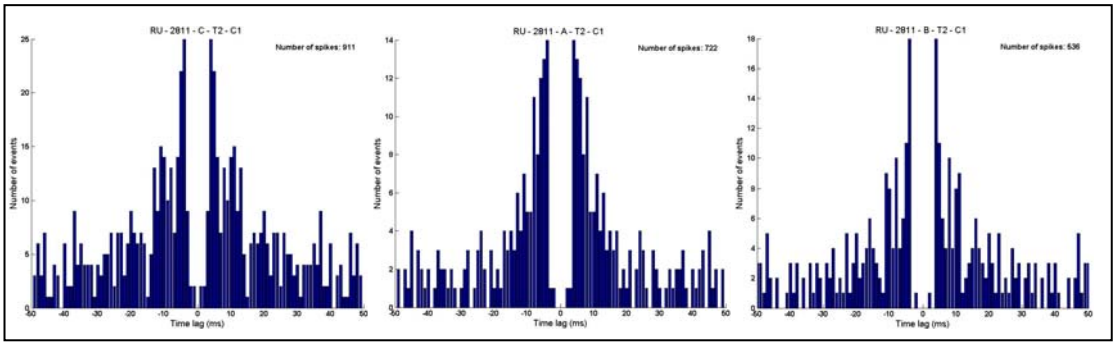


Figure 57: Example of autocorrelogram for one pyramidal cell over the rotation protocol. X-axis represented the time between two spikes in ms and Y-axis represented the number of events.

ROTATION EFFECT

We characterised the rotation by calculating the rotation angle of the FF's maximum peak (see Materials and Methods). The angle was normalised from the control condition one inducing that all the control condition had an angle of 0º. Three groups of place cells could be distinguished regarding the behaviour of their place fields: 1) "Rotation and return", 2) "Rotation and remap", 3) "No rotation".

1) "Rotation and return"

The first group had their place fields remapped according to the rotation of the tactile cues and rotated back with the tactile cues. From the 21 place cells, 15 cells were part of this group representing 71.4% of the total place cells. In the following examples, we can observe this behaviour of place cells (Figure 58, Figure 59). This group which "rotate and return" can be described by the fact that the place field rotated with the tactile cues and came back to the control place when the tactile cues came back to their original location (control condition). The rotation of tactile cues was 90º, and the calculated rotation angle was 93.7º±10.53 and in the return condition the rotation angle was 10.7º±11.47 (Figure 64). The angle between the rotation condition and the return one, was significantly different one to the other ($p = 4.04*10^{-7}$).

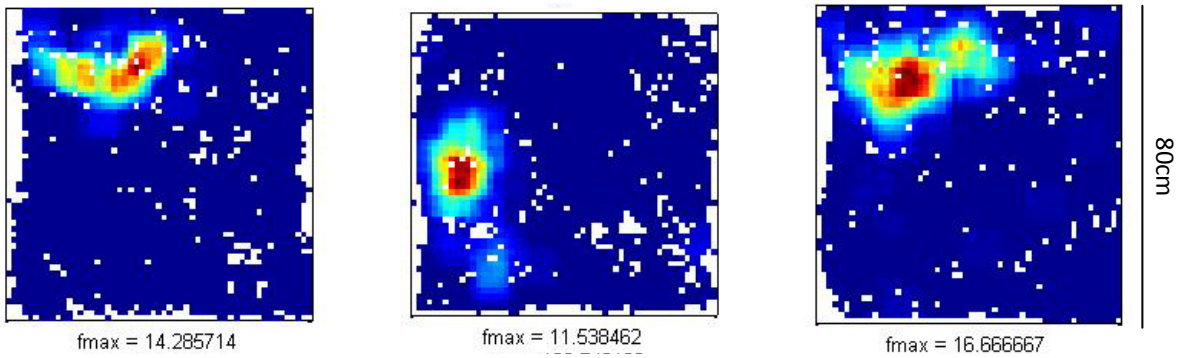


Figure 58: Example of place cell rotation.

Place field rotate 90 º according to the tactile cues. Data recorded in rat RF (RF0801T1C1). The middle condition is rotated 90º.

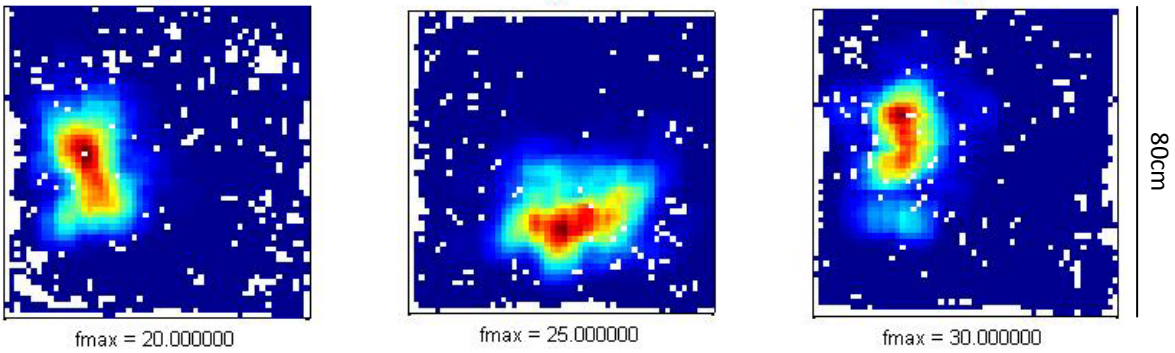


Figure 59: Example of place cell rotation.

Place field rotate 90 º according to the tactile cues. Data recorded in rat RF (RF2101T4C1). The middle condition is rotated 90º.

2) "Rotation and remap"

Another group could be described regarding the place field behaviour. In this group, place fields rotated with the tactile cues but did not rotate back with the tactile cues. From the 21 place cells, 4 cells were part to this group representing 19.04% of the total place cells. In the following examples, we can observe the behaviour of place cells (Figure 60, Figure 61). The angle of rotation was 88.07º±13.28 for the rotation condition and in the return one was 140.5º±15.18 (Figure 63). For this group it was not possible to obtain some robust statistical test due the low number of cells (N=4). Even like this, the difference of the angle due to the rotation of the tactile cue and the behaviour of the place field when the tactile cues came back to the initial place was quite different over conditions and in comparison to the other groups.

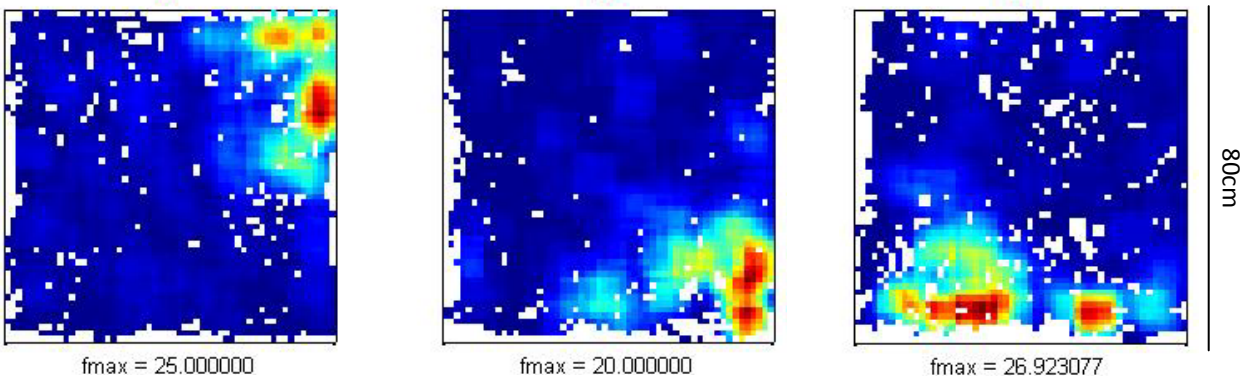


Figure 60: Example of place cell without return.
Place field rotate 90 º according to the tactile cues but did not come back to the control place when cue rotate back. Data recorded in rat RU (ru2811T1C1).

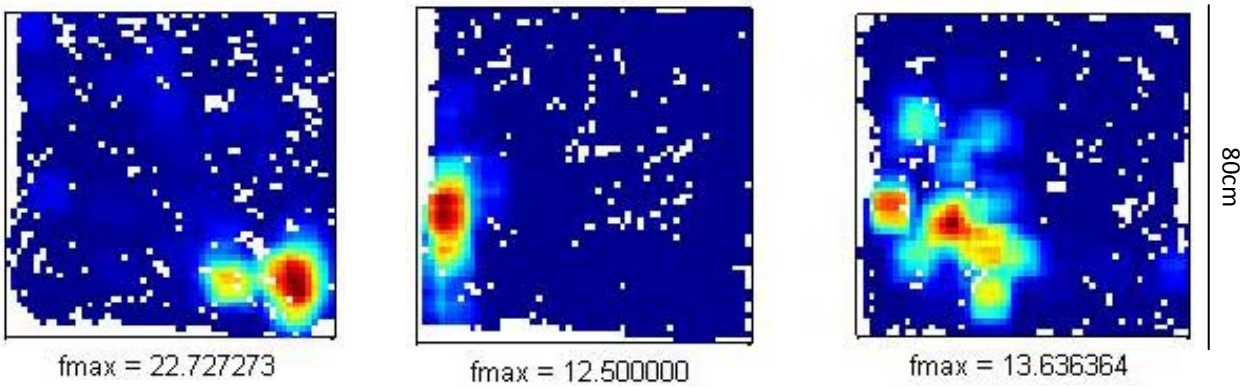


Figure 61: Example of place cell without return.
Place field rotate 90 º according to the tactile cue but did not come back to the control place when cue rotate back. Data recorded in rat RF (rf3011T4C1).

3) "No rotation"

The last group that could be described was formed by cells which their place fields did not rotate with the tactile cues at all. From the 21 place cells, 2 cells were part to this group representing 9.52% of the total place cells. In the following example, we can observe the behaviour of place cells (Figure 62). The angle of rotation was 0.85º±22.21 for the rotation condition and in the return one was 2.98º±47.82 (Figure 63). For this group it was also not possible to realise some statistic test due the low number of cells (N=2). In spite this, the difference of the angle due to the rotation of the tactile cues and the behaviour of the place fields when the tactile cues came back to the initial place was not different over conditions. The behaviour of place fields in comparison to the other groups was quiet different suggesting no remapping with or without the tactile cues rotation.

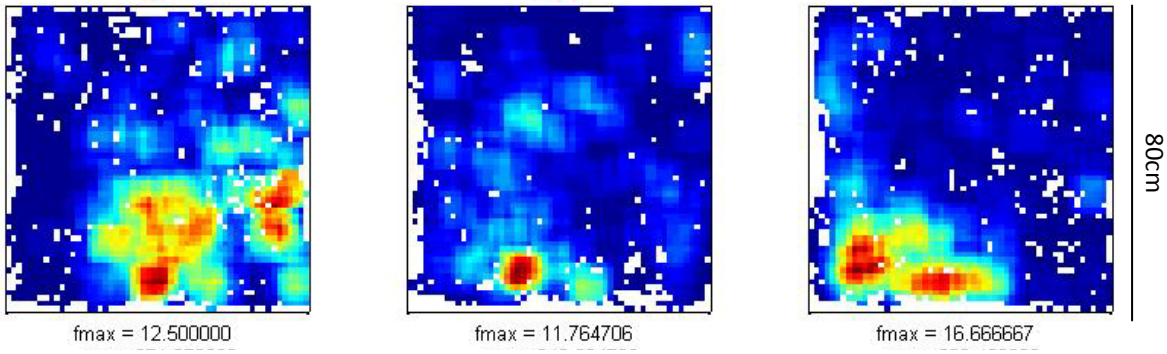


Figure 62: Example of place cell with no rotation.
Place field do not rotate 90 º according to the tactile cue. Data recorded in rat RU (ru2811T2C1). The middle condition is rotated 90º.

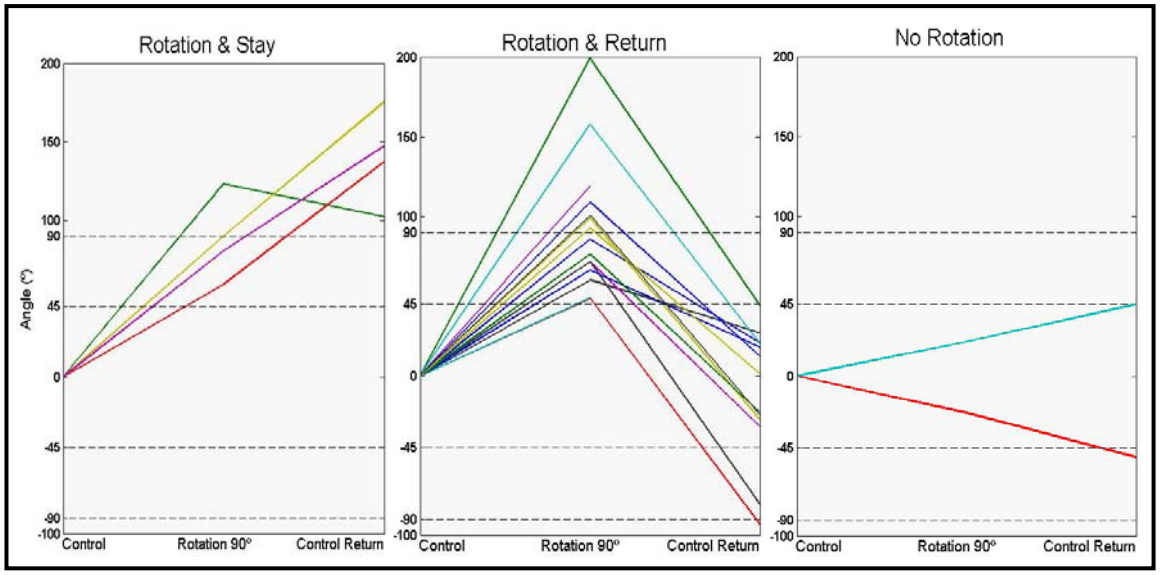


Figure 63: Groups of place cell rotation.
Each colour line represents a protocol. On the left place cells which rotate and stay (N=4), in the middle, place cells which rotate and return (N=15), on the right, place cells which did not rotate at all.

In Figure 64, we can observe the mean of the rotation angle for all the groups. We can clearly observe three different behaviours of the cells regarding the rotation angle. The red line represents the group which "rotate and return". The 90º tactile cues rotation induced each time the rotation of place field firing peak. This rotation was about the same angle as the tactile cues rotation. As we can observe in the results, when place cells had this behaviour, the error was minimum. In addition, the group, represented in green in Figure 64, rotated according to the tactile cues but did not when it came back. The firing field remains in the same place or remap randomly. This group had for the rotation the same rotation angle than the "rotate and return" group which rotate always according to the tactile cues. The last group which could be described was a group of

cells which not change at all over the conditions (blue line in Figure 64). Those cells did not follow the rotation of tactile cues.

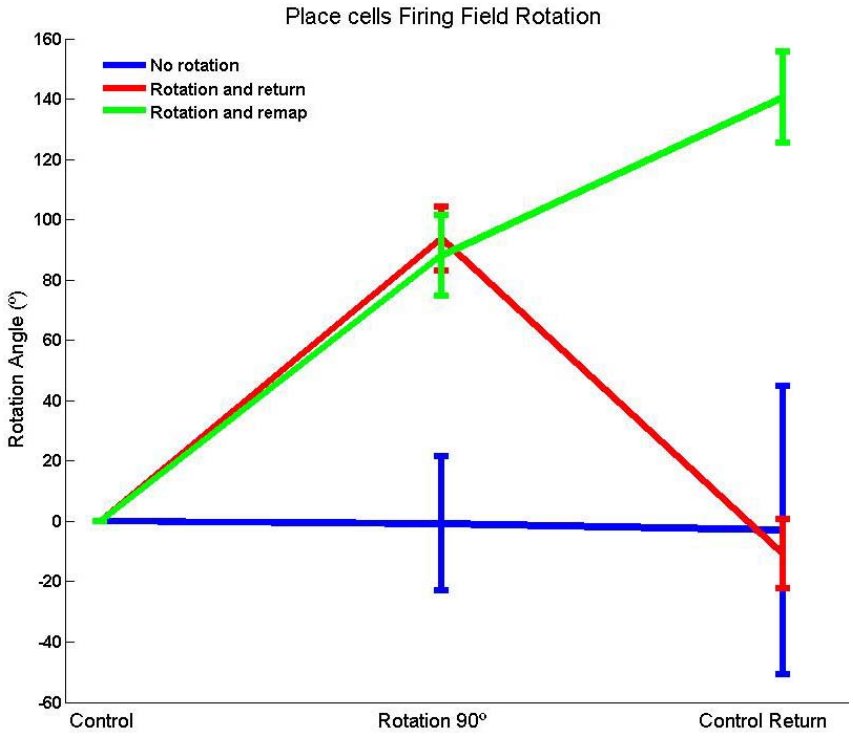


Figure 64: Rotation angle.

Superposition groups, "no rotation" in blue, ”Rotation and return” in red and "Rotation and remap” in green. X-axis was the protocol conditions; Y axis was the rotation angles normalised to the control condition.

These results were very suggestive that place cells used the somatosensory inputs (tactile cues) to generate their firing fields.

3.2. TEMPORAL SOMATOSENSORY DEPRIVATION EFFECT ON SPATIAL COGNITION

CELL PROPERTIES

We recorded from 202 cells, from 8 rats Lister Hooded and Wistar (rats A, B, C, E, F, G, H, I). These cells were classified regarding their class type (pyramidal cells or interneurons). The analysis based on the spike's width, the spike's amplitude and the spike train auto-correlograms (see Material and Methods), showed that 127 pyramidal cells were recorded which represent 63% of the total cells recorded and 33 interneurons were recorded which represent 16% of the total cells recorded (Figure 65). 42 recorded cells could not be classified in one of the possible classes. We placed them all in a group of unclassified cells representing 21% of the total cells recorded.

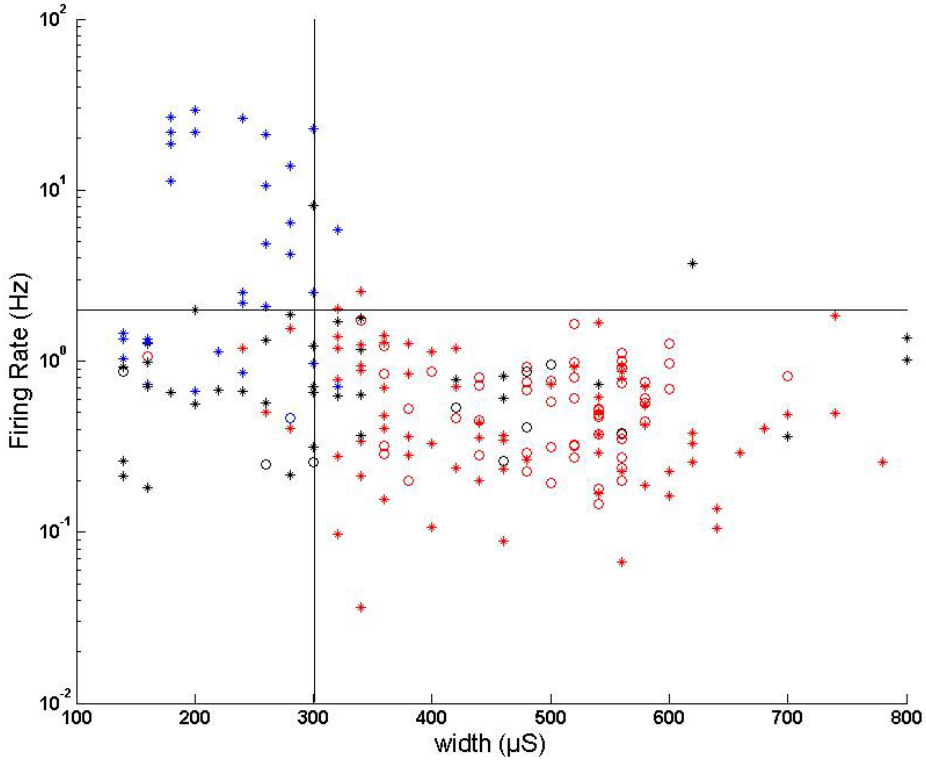


Figure 65: Cells classification of lidocaine protocol.

Circles are place cells, cross are no place cells; red dots are pyramidal cells, blue dots are interneurons, black dots are unclassified cells. X-axis represented the spike width in µS and Y-axis represented firing rate in Hz. Note that place cell which are not in the group of pyramidal cell in this graph were check with others factor to be consider as one.

From these 202 cells, 97 cells fulfilling the criteria of spatial coding (see Material and Methods for criteria) were identified which represented 48% of the total cells recorded out of which we selected and included in the analysis only 65 cells that fulfilled additional criteria of stability (see Material and Methods for criteria) which

represented 32% of the total cells recorded. We then considered 137 no place cell representing 68% of the total cells recorded.

We determined the electrophysiological type of the several cells recorded regarding three factors: 1) the firing rate, 2) the spike's width (Figure 65) and 3) the characteristics of the autocorrelogram: the shape and the peak (Figure 66, Figure 67). The shape of the autocorrelogram (like a triangle) and the presence of a peak between 3 to 8 ms are characteristics of the pyramidal cells (Figure 66). The interneurons autocorrelograms have also a characteristic shape (Figure 67) with a peak later than 10 milliseconds. Cells which were not characterised clearly were considered unclassified cell type.

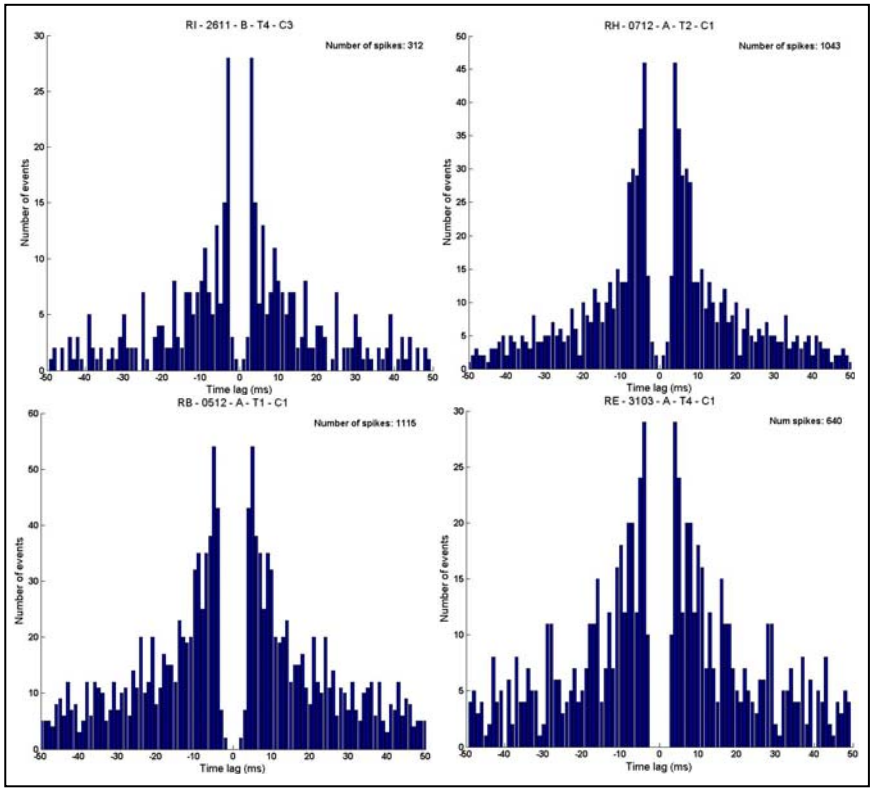


Figure 66: Example of autocorrelogram of pyramid cells.

X-axis represented the time between two spikes in ms and Y-axis represented the number of events. The 4 examples are different cells from 4 different rats.

For these groups of cells, we calculated the mean of several parameters in control condition as maximum frequency of discharge, symmetry of the firing field, the percentage of the area covered by the firing field for a threshold of 20% and 60%, the mean of frequency for all the field, the Skaggs, the volume of the firing field and the compactness of the firing field (see Material and Methods).

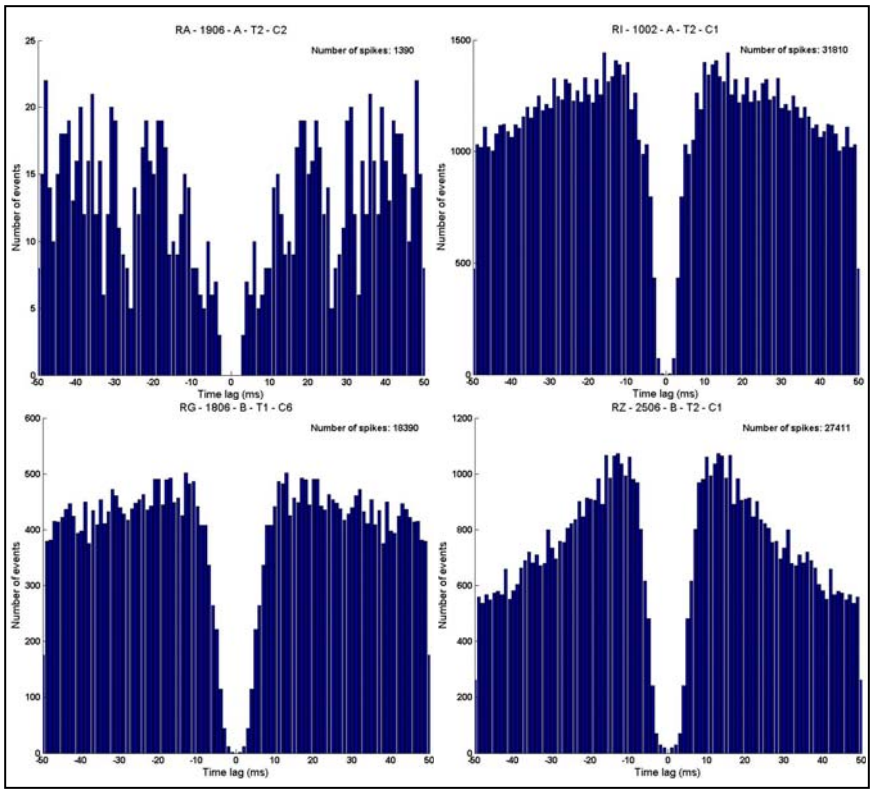


Figure 67: Example of autocorrelogram of interneurons.
X-axis represented the time between two spikes in ms and Y-axis represented the number of events. The 4 examples are different cells from 4 different rats.

In Table 5, all the parameters for all the general groups, as place cells, no place cells, pyramidal cells, interneurons, unclassified cells and all cells are represented.

Table 5: Mean of lidocaine control firing field parameters by groups.

	Total Cell *N = 202*	*Place Cells* *N = 65*	*No Place Cells* *N = 137*	*Pyramid Cells* *N = 127*	*Interneurons* *N = 33*	*Unclassified* *N= 42*
Fmax	14.26±0.66	15.95±0.83	13.45±0.88	12.34±0.60	23.79±2.48	12.09±1.03
Symmetry	0.12±0.006	0.16±0.008	0.10±0.007	0.12± 0.007	0.14±0.01	0.10±0.01
% area 20	33.30±1.72	15.33±1.79	41.83±2.02	24.90±1.77	60.11±3.71	37.63±3.62
% area 60	5.82±0.43	3.41±0.28	6.96±0.60	4.28±0.34	12.42±1.78	5.29±0.67
Fmean	0.91±0.14	0.33±0.02	1.19±0.20	0.33±0.02	3.65±0.67	0.50±0.09
Skaggs	1.36±0.09	1.93±0.18	1.09±0.10	1.60±0.14	0.71±0.12	1.14±0.13
Vol FF	1110±244.71	188.67±20.23	1550±354.95	166.04±13.41	5650±1.22e+3	420.61±128.83
Compct	1.20 ±0.16	2.81±0.35	0.44±0.11	1.71±0.23	0.20±0.05	0.48±0.15

The characteristic parameters of place cells such as Fmax, symmetry, Skaggs and compactness were compared between the group of place cells and no place cells. In Figure 68, some examples are shown as the mean of the Fmax, the % area20, the compactness and the Skaggs for the place cells and no place cells groups. These examples are significantly different between the group of place cells and the one of no place cells. All the place cells factors, Fmax, symmetry, % area20, % area60, fmean, skaggs, volume of FF and compactness were significantly different with a probability of $p=0.078$, $p=4.45*10^{-7}$, $p=1.63*10^{-14}$, $p=1.19*10^{-4}$, $p=0.004$, $p=3.19*10^{-5}$, $p=0.008$, $p=2.212*10^{-11}$. These parameters in the place cells group were higher in comparison to the no place cells group or in comparison to all cells group. In the same way, the % area20 and % area60 and volume of the FF are smaller for the place cells group than for others groups. These differences reflected the specificity of place cells to fire in comparison to no place cells. The difference between the pyramidal cells and the interneurons was also present. The group of unclassified cells was between the pyramidal cells and the interneuron groups.

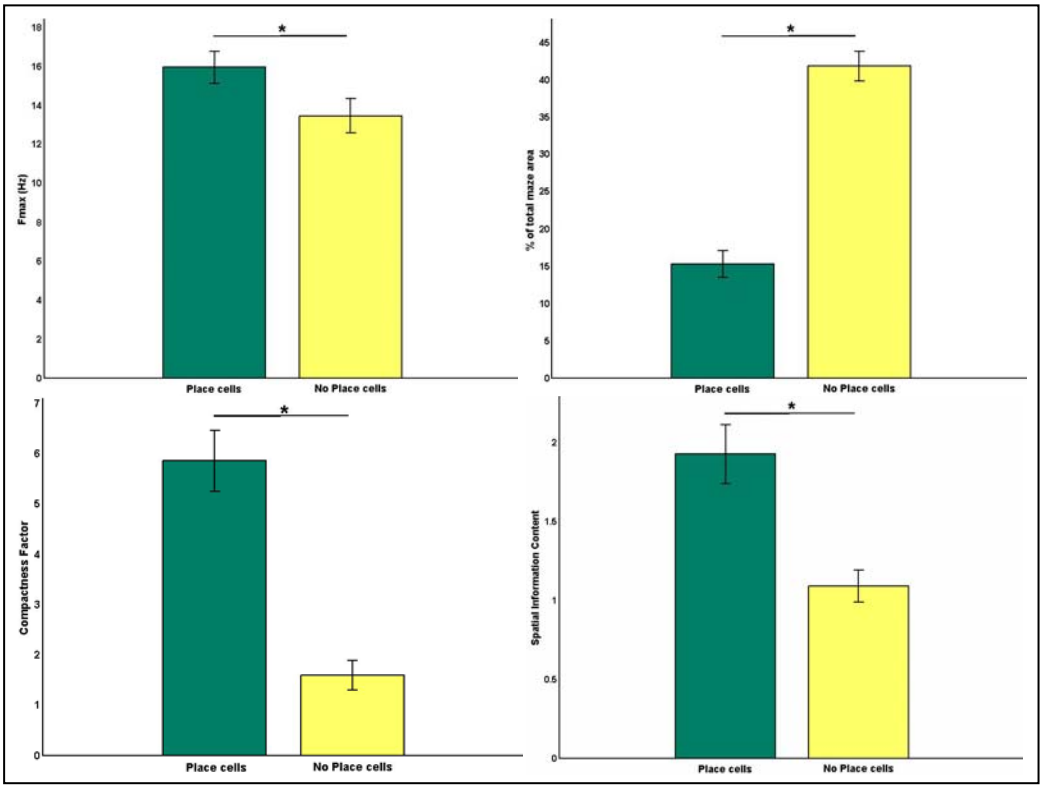


Figure 68: Differences between PCs and no PCs groups in control condition.
We can see four examples (p is the probability to have differences): In green, place cells group, in yellow the no place cells groups. Fmax ($p=0.078$), % area20 ($p=1.63*10^{-14}$), compactness ($2.212*10^{-11}$) and Skaggs ($3.19*10^{-5}$).

We calculated as well the mean of the spike amplitude and the spike width in control condition.

Table 6: Mean of spike parameters by groups.

	Total Cell N = 202	***Place Cells N = 65***	***No Place Cells N = 137***	***Pyramid Cells N = 127***	***Interneurons N = 33***	***Unclassified N= 42***
Amplitude	106.57± 2.80	122.37±5.27	99.08±3.10	110.38±3.67	102.92±5.85	97.94±5.99
Width	412.37±10.95	482.46±12.89	379.12±14.11	481.25±10.19	253.93±20.98	328.5714±24.32

LIDOCAINE EFFECT

All the place cells' study

The main point of this study about the influence of the somatosensory inputs on the generation of the place fields was to record place cells before and after the application of lidocaine on the snout of the rat, in other words with or without the somatosensory inputs. Here, we describe the results obtained by comparing the value of several aforementioned parameters in the control condition, with the application of lidocaine and then after more than two hours after the lidocaine application (recovery). Those results are shown in Table 7, Table 8.

Table 7: Mean firing field parameters of place cell during lidocaine protocol.

Place cells N=65	***Control***	***Lidocaine***	***Recovery***
Fmax	15.95±0.84	13.58±1.01	15.19±1.013
Symmetry	0.16±0.01	0.14±0.01	0.15±0.01
% area 20	15.33±1.78	21.41±2.22	20.08±2.34
% area 60	3.41±0.23	4.59±0.55	4.11±0.43
Fmean	0.33±0.03	0.35±0.04	0.37±0.05
Skaggs	1.93±0.18	1.76±0.14	1.92±0.22
Vol FF	188.67±20.23	243.89±39.16	205.81±23.34
Compct	2.81±0.35	1.70±0.23	2.44±0.29

The spike's amplitude and spike's width were stable. There were no significant differences of those values over the conditions (Table 8). This stability during the protocol even after lidocaine application let us to think that the deprivation of the somatosensory inputs did not alter the cells properties. Spike characteristics stay stable over conditions.

Table 8: Mean of spike parameters of place cell during lidocaine protocol.

Place cells N=65	***Control***	***Lidocaine***	***Recovery***
Amplitude (µV)	106.02±4.57	105.97±4.74	105.21±4.88
Width (ms)	482.46±12.89	476.30±13.78	471.81±14.32

We could observe some significant changes of several place fields parameters calculated comparing between several conditions. Some changes were important as for the compactness which had a reduction of 39.5% with the lidocaine and had a recovery up to 86.8% of the control value. The percentage of the area20 had an increase of 39.7% with the lidocaine but in this case the recovery was only up to 30.9% (Table 7).

In order to have a better understanding of the changes due to the application of lidocaine, we compared several factors over the conditions of the protocol and then between the putative place cell groups. In Figure 69 to Figure 76, examples of place cells could be observed over the three condition of the protocol. Spatial maps are represented in 2D, the colour scale represents the firing rate of the cell, red high rate, and blue low rate. It is also shown the 3D maps in which, we can observe the spatial firing and the rate of discharge of the neuron.

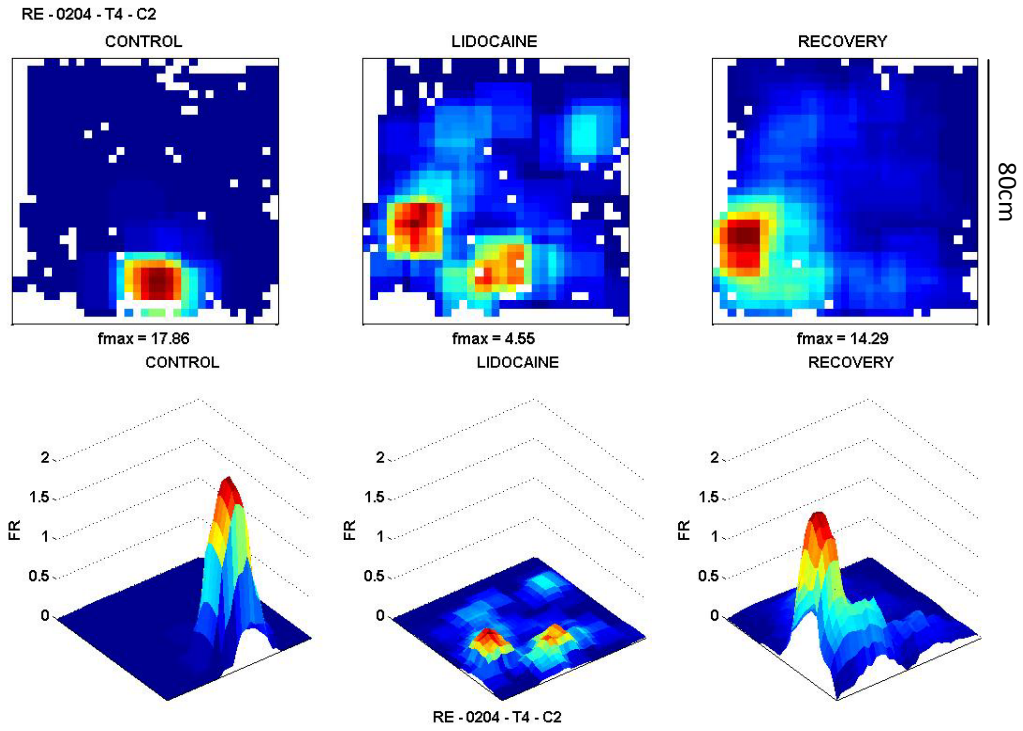


Figure 69: Example of place cell (1).

Place cell from rat E. In the upper part, place cell in control, lidocaine and recovery condition. In the down part, the same cell in a 3D representation.

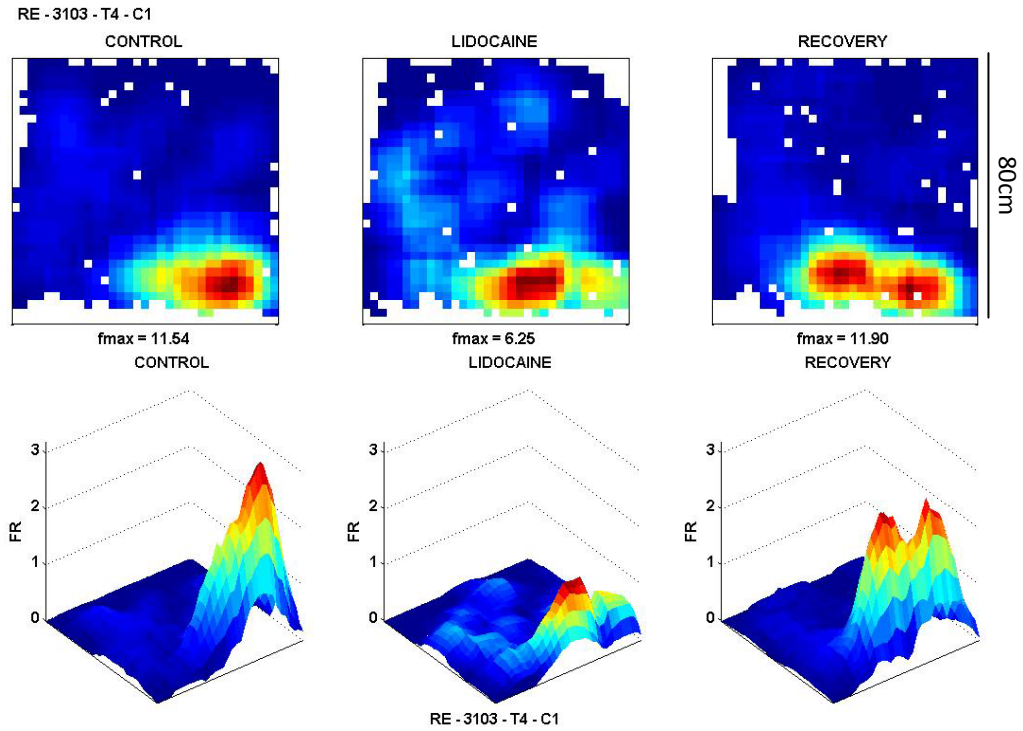


Figure 70: Example of place cell (2).

Place cell from rat E. In the upper part, place cell in control, lidocaine and recovery condition. In the down part, the same cell in a 3D representation.

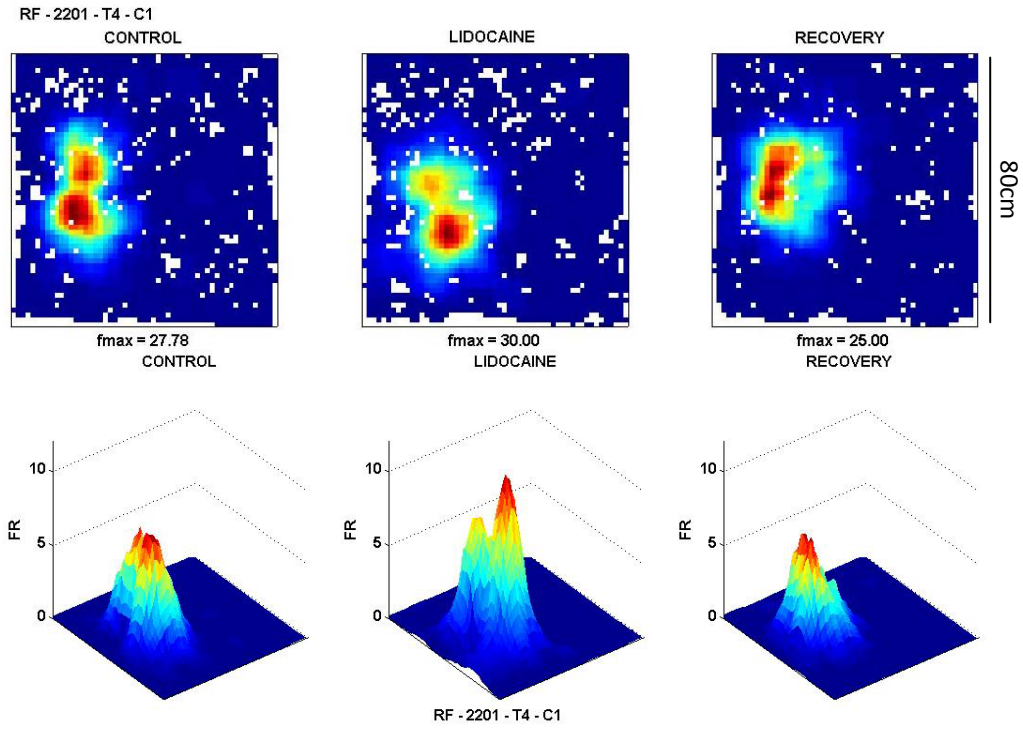


Figure 71: Example of place cell (3).

Place cell from rat F. In the upper part, place cell in control, lidocaine and recovery condition. In the down part, the same cell in a 3D representation.

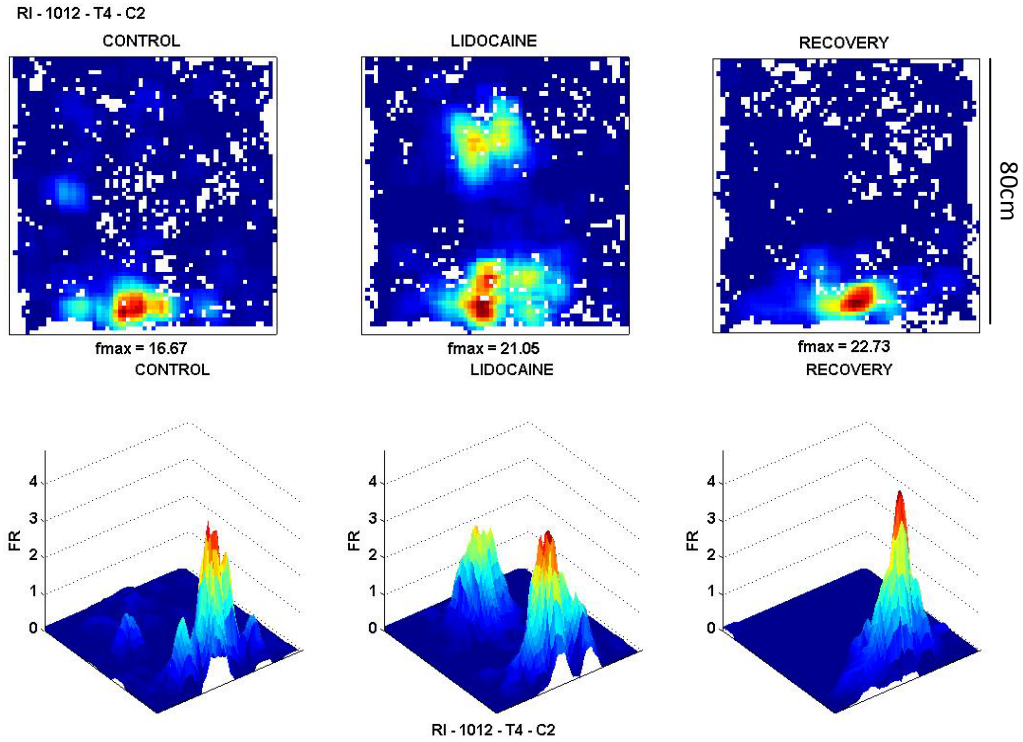


Figure 72: Example of place cell (4).

Place cell from rat I. In the upper part, place cell in control, lidocaine and recovery condition. In the down part, the same cell in a 3D representation.

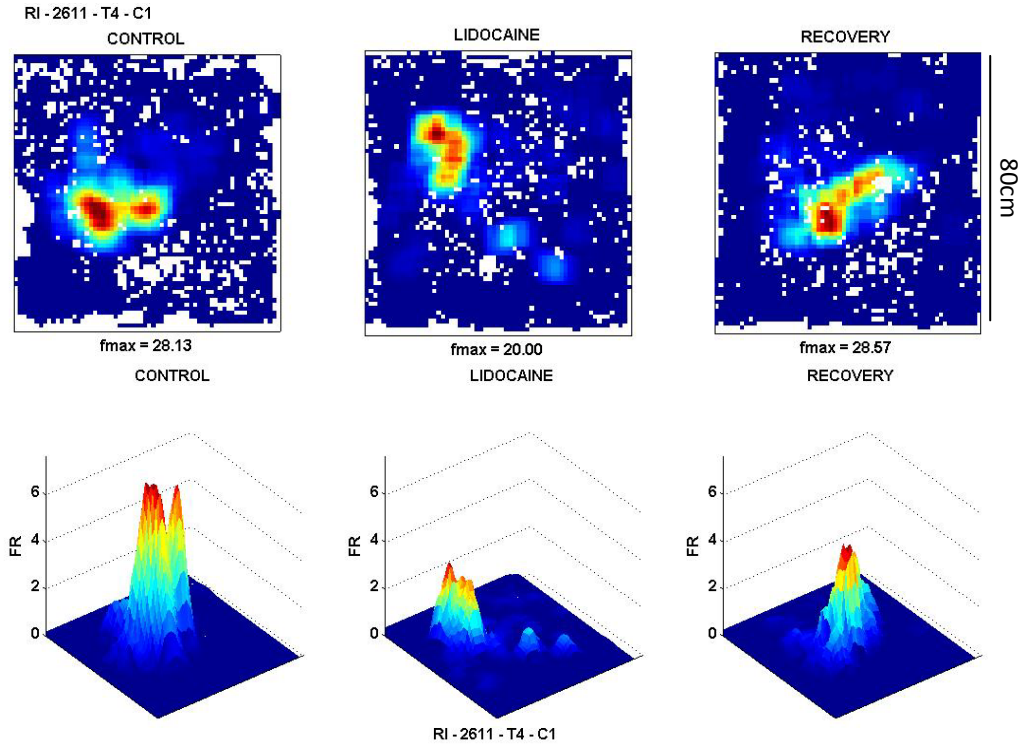


Figure 73: Example of place cell (5).

Place cell from rat I. In the upper part, place cell in control, lidocaine and recovery condition. In the down part, the same cell in a 3D representation.

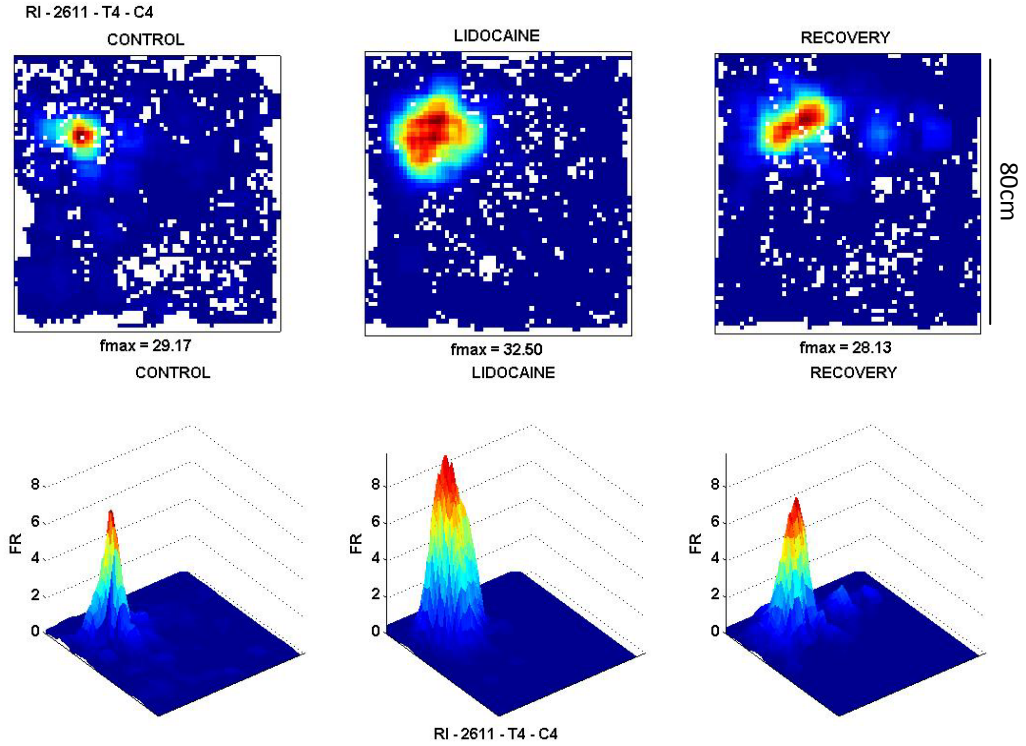


Figure 74: Example of place cell (6).

Place cell from rat I. In the upper part, place cell in control, lidocaine and recovery condition. In the down part, the same cell in a 3D representation.

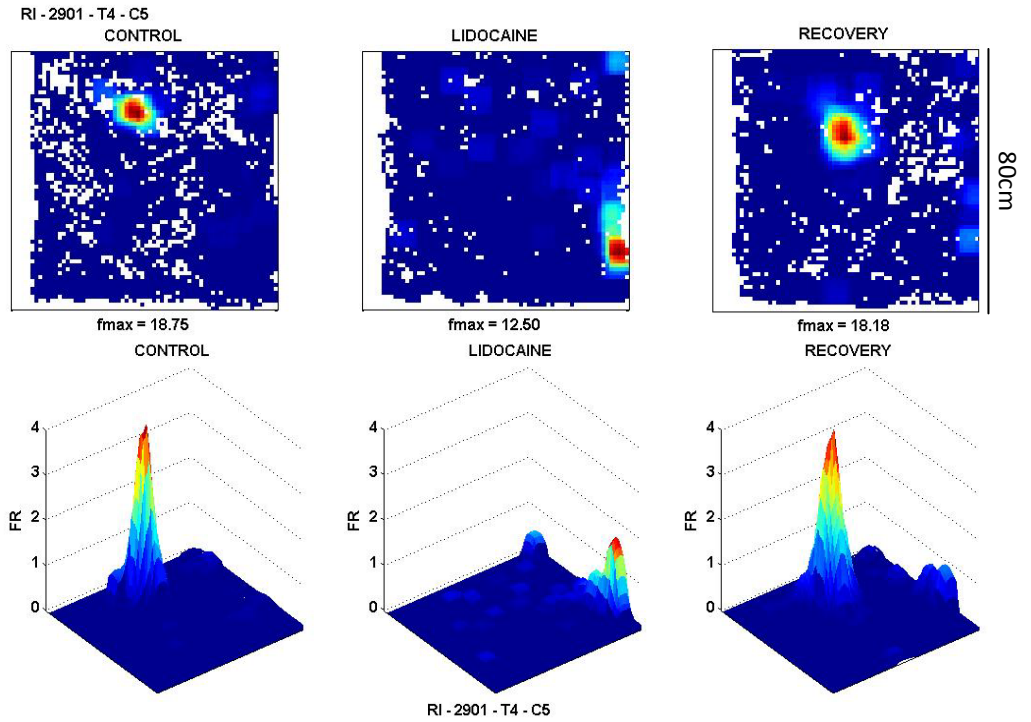


Figure 75: Example of place cell (7).

Place cell from rat I. In the upper part, place cell in control, lidocaine and recovery condition. In the down part, the same cell in a 3D representation.

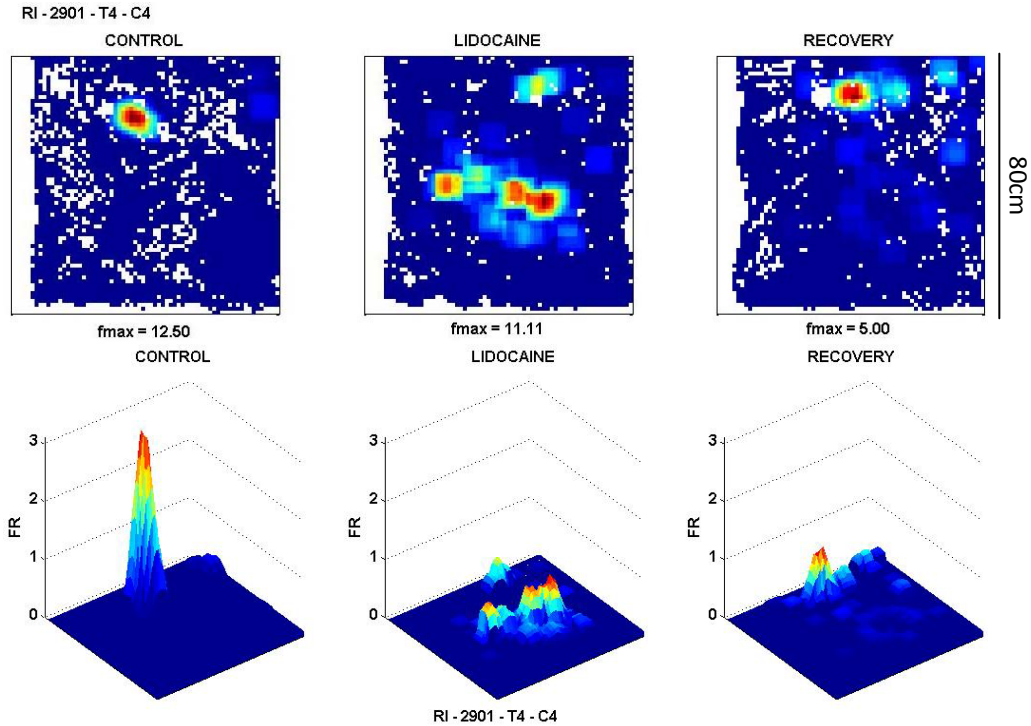


Figure 76: Example of place cell (8).

Place cell from rat I. In the upper part, place cell in control, lidocaine and recovery condition. In the down part, the same cell in a 3D representation.

We wanted to know if some of the place cells properties were affected during the protocol and the somatosensory deprivation. We measure the spike's amplitude and the spike's width during the protocol and comparing it with the no spatially selective group.

Amplitude of the spike:

We could observe that the spike amplitude during the protocol did not change with or without the somatosensory inputs. The amplitude was stable over the condition the place cells group and for the no place cells group. There was difference in the amplitude between the two groups but it is probably due to the cell type and to the natural spike variability. Those results suggested that temporal suppression of the somatosensory inputs did not altered some of the cell properties.

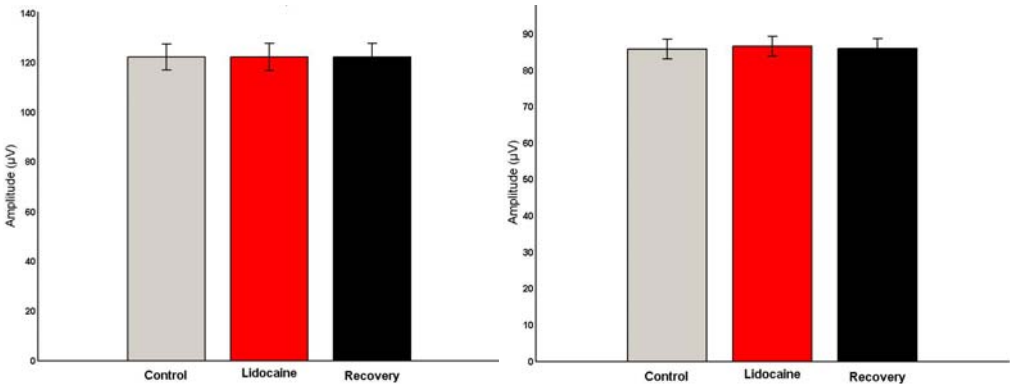


Figure 77: Amplitude of the place cells and no place cells groups.
On the left PC; on the right NO PC.

Width of the spike:

The spike width as the amplitude was one of the property of the cell. We could observe that the spike width during the protocol did not change with or without the somatosensory inputs. The width was stable over the condition for the place cells group and for the no place cells group. These results suggested that temporal suppression of the somatosensory inputs did not alter the cell properties.

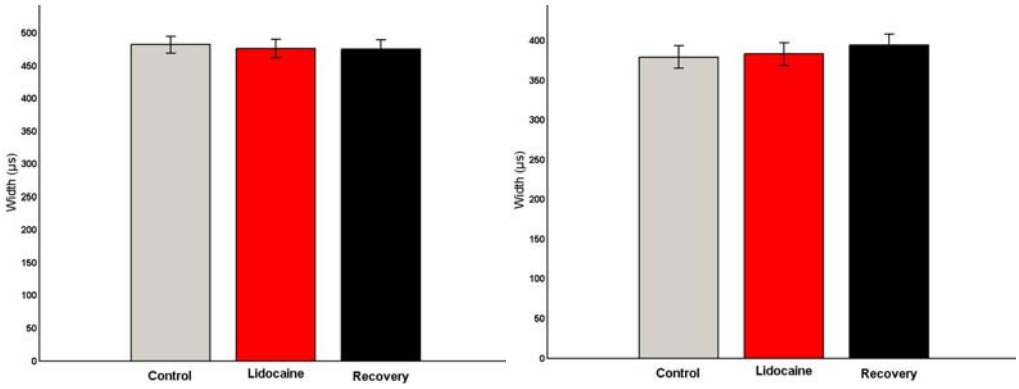


Figure 78: Width of the place cells and no place cells groups.
On the left PC; on the right NO PC.

The spike parameters showed a clear stability over conditions and between groups. We focused then in the place cells parameters aforementioned described for the place group.

% of Area 20:

As mentioned earlier, place cells group was characterised by samller area20 in comparison to the no place cells group (Figure 68). Once the lidocaine was applied and the somatosensory inputs was temporary blocked, the percentage of area20 increased significantly (p=0.0354), reducing but not totally recovering during the "recovery" period (more than 2h after lidocaine application).

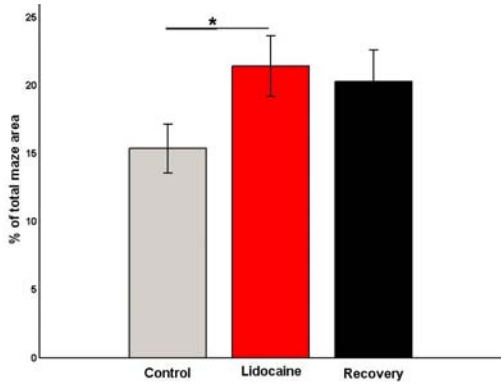


Figure 79: Area20 of the place cells group.
Comparison of control, lidocaine and recovery conditions (*p=0.0354).

% of Area 60:

As for the percentage of area20, the place cells group was characterised by a small percentage of the area60 in comparison to the no place cells group. However, once the lidocaine was applied and the somatosensory inputs were temporary blocked, the percentage of area60 increased but not being significant. In the recovery condition it did not come back to the control level but the trend was that the value of the percentage of

area60 was decreasing (the value was lower than in lidocaine condition) as for the area20.

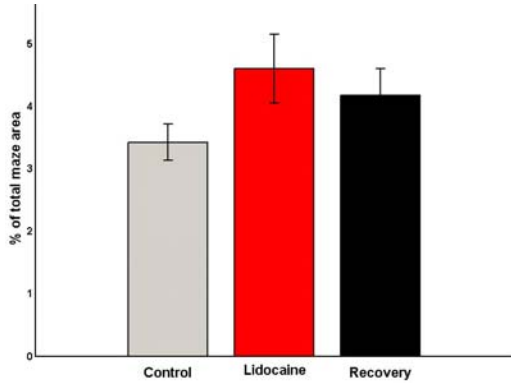


Figure 80: Area60 of the place cells group.
Comparison of control, lidocaine and recovery condition.

Compactness:

Place cells group was defined by a high compactness in comparison to the no place cells group (Figure 68). This factor was important to characterise place cells because it reflected how compact and dense the firing field was. Once the lidocaine was applied and the somatosensory inputs were temporary blocked, the compactness decreased significantly (p=8.2026*10-4). In recovery condition it tended to increase back to the control level (p=0.0445).

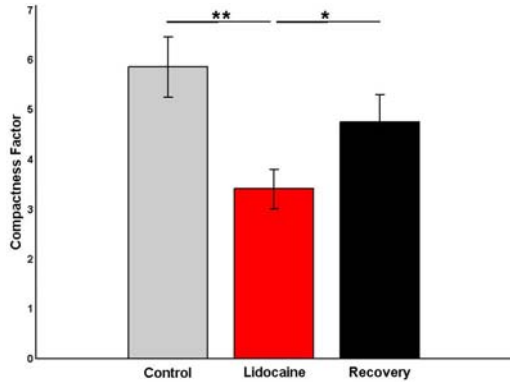


Figure 81: Compactness of the place cells group.
Comparison of control, lidocaine and recovery conditions (p= 8.2026*10^{-4}, * p= 0.0445).**

Maximum frequency:

The maximum frequency was compared during the protocol and with the no place cells group. The maximum frequency of place cells group decreased clearly but not significantly when lidocaine was applied on the snout of the rat. In the recovery

condition a tendency to increase the maximum frequency was presented but the recovery was not complete.

We compared the place group with the no place cells one due to the Fmax was a general parameter not only for place cells. No changes were observed with the lidocaine. In the recovery condition, we noticed a little increase which was not significant. The behaviour in the two groups was different one to each other and lidocaine seemed to have an effect on maximum frequency of the place cell group.

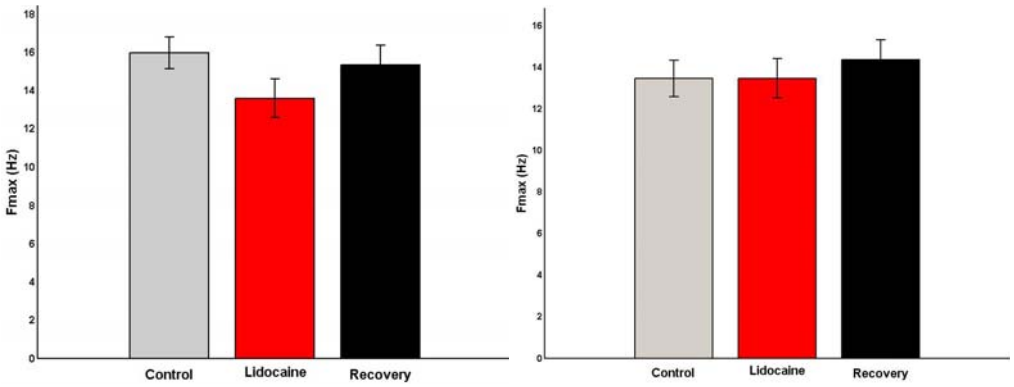


Figure 82: Fmax of the place cells and no place cells groups.
On the left PC; on the right NO PC.

Mean frequency:

The mean frequency was also compared during the protocol and with the no place cells group. For both groups, place cells and no place cells, the behaviour was similar. The mean frequency increased without being significant during all the protocol. The mean frequency for the group of place cells was significantly lower than for the no place cells group (p= 0.0042). This difference was probably due to the cell class. The behaviour of both groups was similar, and lidocaine did not seem to have any effect on the mean frequency.

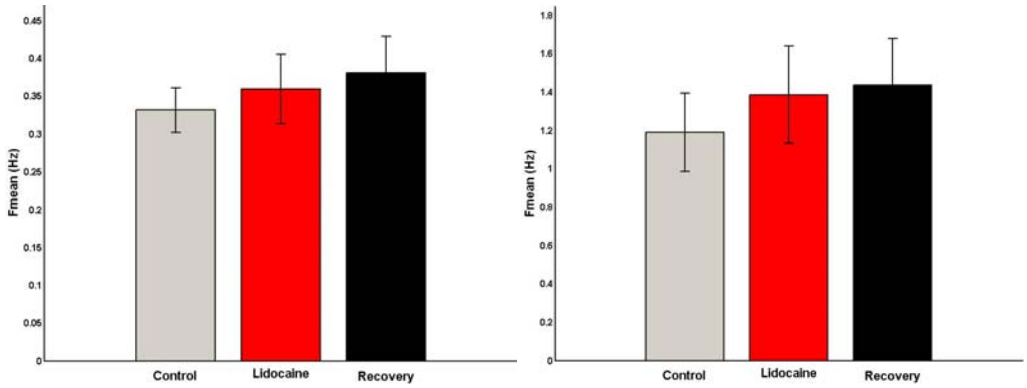


Figure 83: Fmean of the place cells and no place cells groups.
On the left PC; on the right NO PC.

Spatial information content:

Place cells group was defined by high spatial information content (Skaggs) in comparison to the no place cells group (Figure 68). The Skaggs for the place cells groups was significantly different than the one in the no place cells group (it is almost the double, 43.5% higher). The behaviour for the place cell group was comparable with the other significant factors, a decrease with the somatosensory deprivation and an increase in the recovery condition. None of the changes in this group was significant but there was a trend.

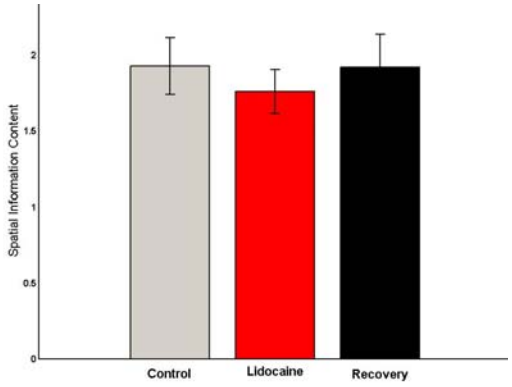


Figure 84: Skaggs of the place cells group.
Comparison of control, lidocaine and recovery conditions.

Symmetry:

Place cells group was characterised by a symmetry factor higher than for the no place cells group. Symmetry was 63% higher for the place cells group than the no place cells group. For this factor also, we could observe a decrease of the symmetry when the somatosensory inputs were temporary blocked. The difference was significant with a p=0.0376. As previously, the recovery had the tendency to come back to the control value but did not reach the level to be significant.

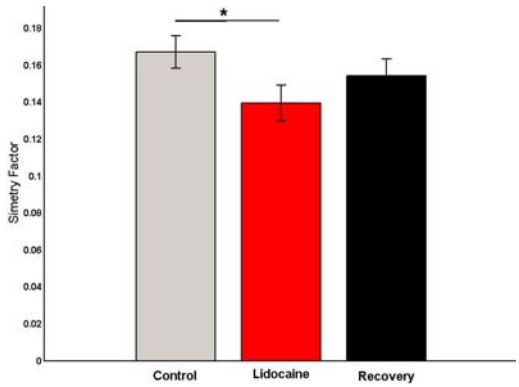


Figure 85: Symmetry of the place cells group.
Comparison of control, lidocaine and recovery conditions (* p=0.0376).

Volume of the Firing Field:

Place cells group was characterised by volume of the firing field higher than for the no place cells group. It was following the same tendency as the % of area20 and the % of area60. It meant an increase during the somatosensory deprivation and a decrease when the deprivation stopped. There was no significant difference, but the general tendency for the place cells group was characteristic.

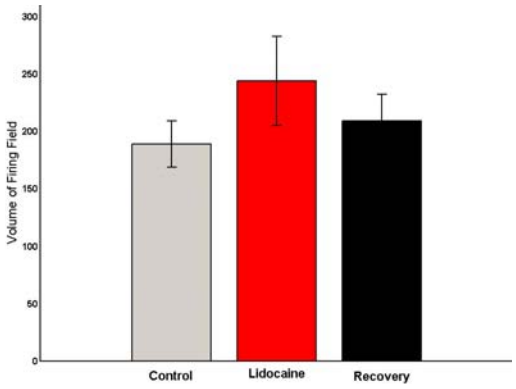


Figure 86: Volume of the place cells group.
Comparison of control, lidocaine and recovery conditions.

For the group of place cells, a clear tendency was characterised with the lidocaine application and for the recovery period. This tendency was significant for some of the place cells parameters and not for others, demonstrating the influence of the somatosensory inputs on the cognitive maps creation.

SEPARATION OF PLACE CELLS

Averaging the whole population may occult opposite changes in different groups. We created two groups using the compactness factor as criteria. If a place cell had it compactness superior in control condition than the one in lidocaine condition, it was part of the group one. The other group, group two, had a compactness superior in lidocaine condition than in control condition. We calculated then all the place cells parameters once again for the two groups. The group one was composed by 51 cells representing a total of 78.5% of the place cells group and the group two was composed by 14 cells representing a total of 21.5% of the place cells group.

Compactness:

The group one showed some high significant differences. The control condition and the lidocaine showed a significant different ($p=1.1326*10^{-5}$). The difference between the lidocaine and the recovery condition was also significant (p=0.0198) and between the control condition and the recovery one it was also significant (p=0.0318).

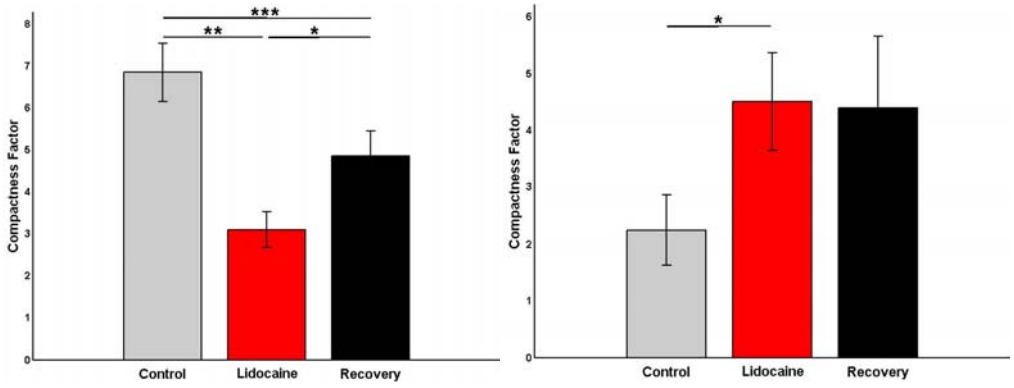


Figure 87: Compactness for the groups across the conditions.
On the left, group 1 (*p=0.0198, **p=$1.1326*10^{-5}$, ***p= 0.0318); On the right, group 2 (*p= 0.0419).

The group two had a different behaviour with a significant one between the control and the lidocaine (p= 0.0419). The behaviour of the two groups was quite different demonstrating that the two groups of place cells had different behaviours when somatosensory inputs were blocked.

Amplitude of the spike:

For both groups, lidocaine did not influence the amplitude of the place cell's spike. No significant differences were found across the condition and between the groups. This confirmed that the cell properties were stable across the protocol.

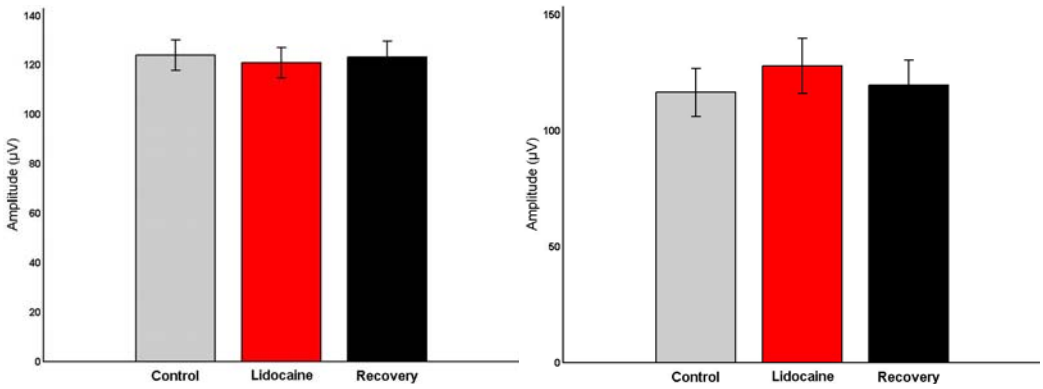


Figure 88: Amplitude for the groups across the conditions.
On the left, group 1; On the right, group 2.

Width of the spike:

For both groups, lidocaine did not influence the width of the place cell spike. No significant differences were found across the condition and between the groups. This confirmed that the cell properties were stable across the protocol.

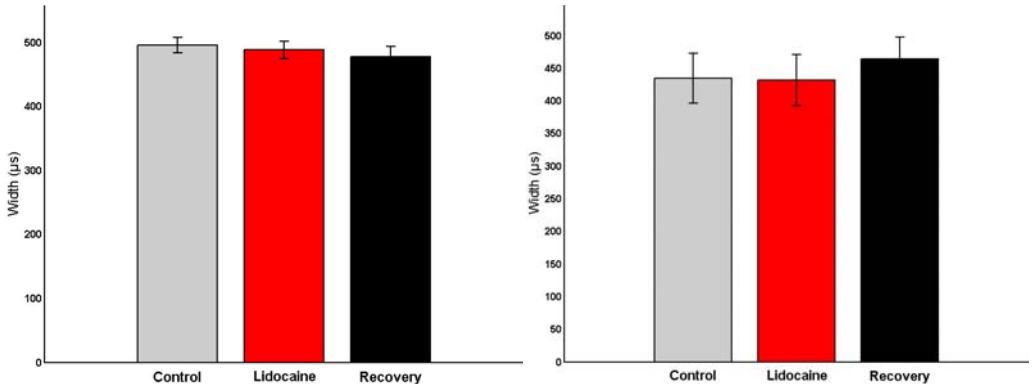


Figure 89: Width for the groups across the conditions.
On the left, group 1; On the right, group 2.

As for the place cells groups, those cells parameters were clearly stable across conditions and between groups showing a non influence of the lidocaine application on the cells properties.

% of Area 20:

The area20 for the group one was significantly different between the condition control and lidocaine (p=9.58*10-4), as well as between the conditions control and recovery (p=0.0248). Between the conditions lidocaine and recovery, even if the difference was not significant, the value of the recovery was inferior of the lidocaine one, showing a clear tendency of recuperation. For the group two, no significant differences were found. The tendency that we could observe was a decrease during the lidocaine condition with a recovery. The behaviour of the two groups was quite different demonstrating that the two groups of place cells had different behaviours when somatosensory inputs were blocked.

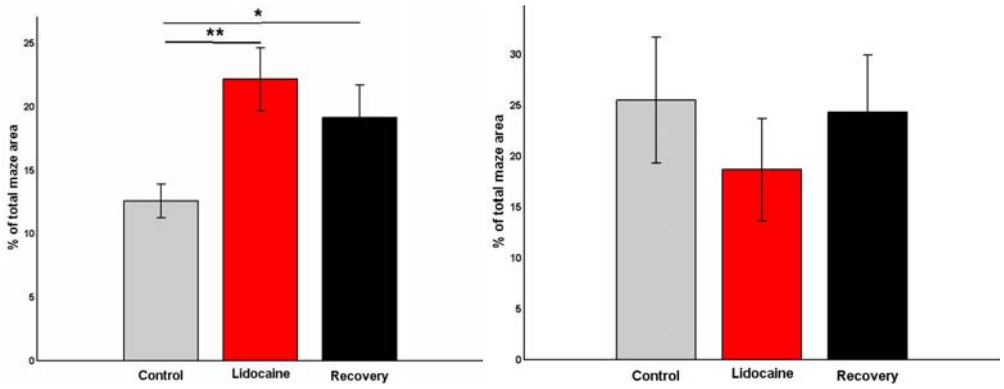


Figure 90: Area20 for the groups across the conditions.
On the left, group 1 (p=9.58*10-4); On the right, group 2.**

% of Area 60:

The area60 for the group one was significantly different between the condition control and lidocaine (p=0.0211).

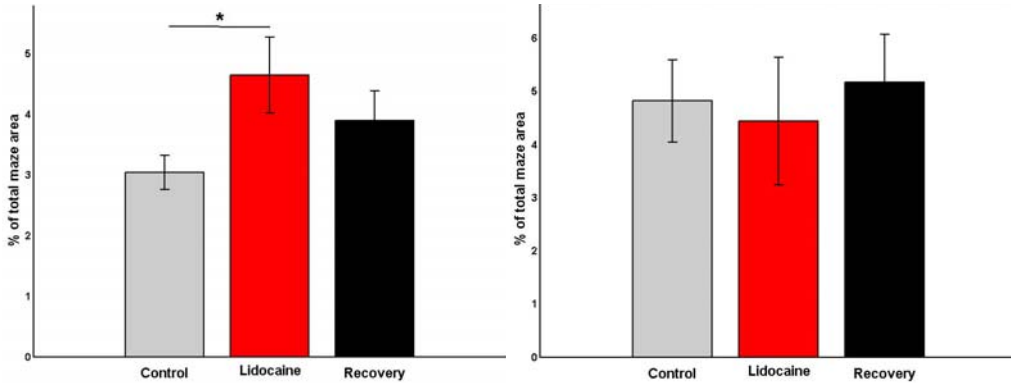


Figure 91: Area60 for the groups across the conditions.
On the left, group 1 (*p=0.0211); On the right, group 2.

Between the conditions lidocaine and recovery, even if the difference was not significant, the value of the recovery was inferior of the lidocaine one, showing a clear tendency of recuperation. The second group had a different behaviour. No significant differences were found but a little tendency of a reduction of the area60 under lidocaine could be observed. The behaviour of the two groups was quite different demonstrating that the two groups of place cells had different behaviours when somatosensory inputs were blocked.

Maximum frequency:

The group one showed a significant difference between the control condition and the lidocaine (p=0.0320). The recovery condition showed a clear tendency to come back to the control condition value. The group two had a different behaviour with no significant differences across the conditions.

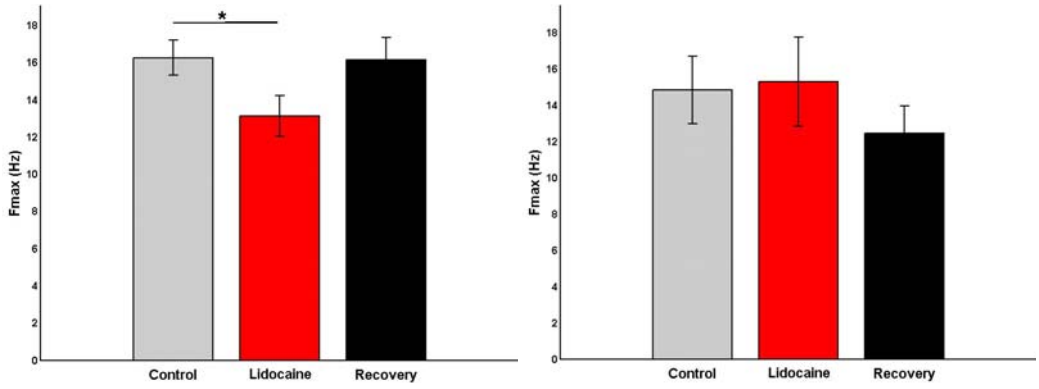


Figure 92: Fmax for the groups across the conditions.
On the left, group 1 (*p=0.032); On the right, group 2.

The behaviour of the two groups was quite different demonstrating that the two groups of place cells had different behaviours when somatosensory inputs were blocked.

Mean frequency:

There were no significant differences for the group one and two. Even if a little tendency for the group one was an increase across the condition and a decrease for the group two, the global behaviour of both group was unaffected by the lidocaine application.

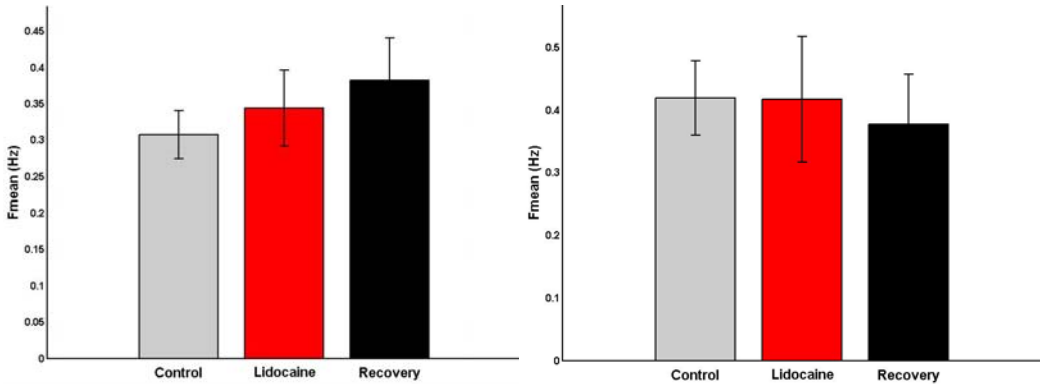


Figure 93: Fmean for the groups across the conditions.
On the left, group 1; On the right, group 2.

Spatial information content:

The group one showed a non significant tendency (clearer than in the global place cells group). The spatial information content decreased with the application of lidocaine and came back to a control level in the recovery condition.

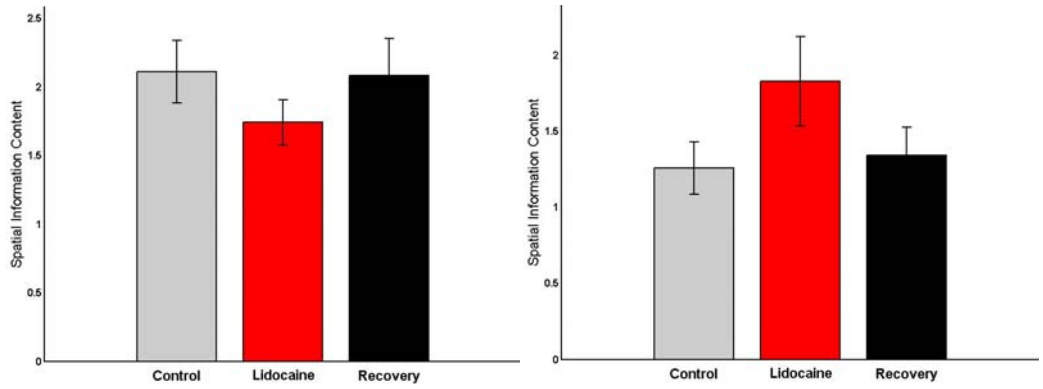


Figure 94: Skaggs for the groups across the conditions.
On the left, group 1; On the right, group 2.

For the group two there were also no significant differences across the condition, but a clear tendency could be observed. The spatial information content increased with the application of lidocaine and came back to a control level in the recovery condition. Note that the Skaggs for the group one is higher than for the group two. The behaviour of the two groups was opposite demonstrating that the two groups of place cells had different behaviours when somatosensory inputs were blocked.

Symmetry:

The group one of the place cells showed only a significant decrease between the control condition and the lidocaine one. The recovery condition showed a tendency of increase but it was not significant.

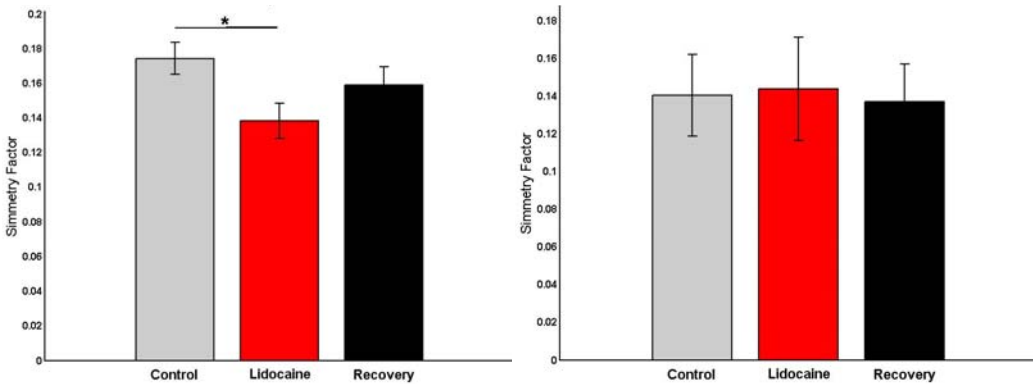


Figure 95: Symmetry for the groups across the conditions.
On the left, group 1 (*p=0.0101); On the right, group 2.

Group two did not shown any differences across the conditions. The behaviour of the two groups was opposite demonstrating that the two groups of place cells had different behaviours when somatosensory inputs were blocked.

Volume of the Firing Field:

No significant differences could be observed in any of the groups. A small tendency to increase could be noted for both of the groups. The group two showed a bigger error, larger variability (larger s.e.m.) under lidocaine than all the others one. The behaviour of volume of the place field did not shown a strong difference between the two groups as the others place cells parameters.

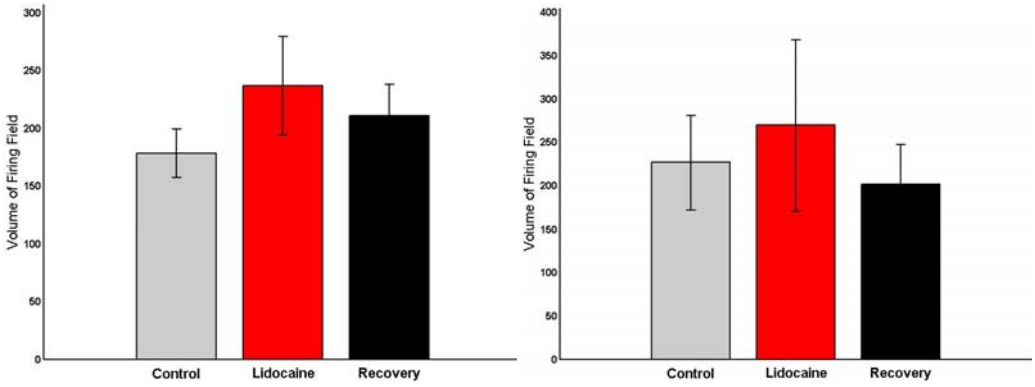


Figure 96: Volume for the groups across the conditions.
On the left, group 1; On the right, group 2.

To conclude, the lidocaine application on the base of the whiskers in order to temporarily deprive the somatosensory inputs seemed to affect significantly to 78.5% of the place cells. The 21.5% of place cells were not significantly affected by the lidocaine application. We demonstrate that the place cells groups shown some evidences that temporary somatosensory deprivation induced changes in the firing pattern. The compactness of the area20 and the symmetry showed significant differences in the population. When the place cells behaviour is carefully studied, two groups could be formed. The compactness, the area20, the area60, and the symmetry showed stronger significant differences. The remaining spatial factors even if not significant showed clearly the same tendency of change during the temporal deprivation. The spatial information content showed stronger difference in place cells group one than in the group of all place cells. The place cells group one confirmed our hypothesis that from the deprivation of whiskers inputs resulted, in the given conditions that animal should preferentially used tactile information to generate cognitive maps, a destruction of the

place fields. We did not observe this on the place cells group two, all the spatial factors showed a relative stability with the lidocaine application.

If the spatial parameters showed differences, it is not the same for the spike amplitude and spike width which in all the condition and groups stayed always stable regarding the control condition.

This temporal deprivation of the somatosensory inputs altered the spatial firing properties of the place cells but not cells properties. These results strongly suggest that temporal deprivation of somatosensory inputs on the whisker do change spatial firing parameters inducing a change or a distortion in the cognitive map.

3.3. HISTOLOGY

As mentioned in materials and methods, after each final recording, animals were sacrificed and histology performed (NISSL staining). In all animals, the position of the implantation was checked. All the implants were successfully performed in the CA1 the hippocampus. Examples showed the NISSL staining focused on the CA1 region to observe the electrode track.

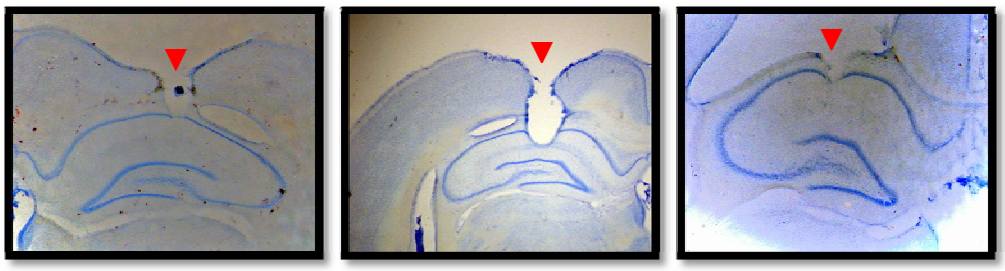


Figure 97: Examples of brain stain with NISSL.
Example of three different rats. Rat D, rat C and Rat B (in this order in the figure). Red arrow indicates the track of the tetrodes.

DISCUSSION

In this work, we develop an original method to temporarily deprive somatosensory inputs; we give behavioural evidence that the tactile deprivation influences animal tactile discrimination and that tactile information participates on the creation of spatial maps. This work gives an overview of the modulation of spatial processing by somatosensory inputs in the rats. Several techniques (from intracellular to single units) and different parts of the brain were recorded (barrel cortex and hippocampus). The discussion will be separated in several parts: acute experiments, behavioural experiments and electrophysiological behavioural experiments.

1. ACUTE EXPERIMENTS DISCUSSION

In this study, we explored a new paradigm that would temporarily deprive whiskers information and would be appropriate to use in an awake animal. This new paradigm consists in applying lidocaine (local anaesthetic) on the snout of the animal. Previous studies in anesthetized animals have used whisker deprivation by subcutaneous injection of lidocaine (Faggin *et al.*, 1997), a method that was found to be effective for blocking the sensory information. However, this procedure is invasive and probably too stressing to deprive somatosensory information from an awake animal. Apart from this method, the most common form of deprivation in awake animals has been the trimming of whiskers (Huston *et al.*, 1986; Steiner *et al.*, 1986; Lebedev *et al.*, 2000; Grigoryan *et al.*, 2005).

We were interested in a method that allowed short term deprivation (hours) and moreover which allowed to monitoring the recovery. Those requirements were taken into account for the first time as far as we know, making this technique new. The vibrissotomia was not possible, since it requires a certain growth time (weeks) to recover given that the whiskers grown about 1mm/days (Ibrahim and Wright, 1975). Given these two constraints, we tested different forms of lidocaine application (injection, aerosol, cream, and liquid drop) to the whisker pad. To characterize this new technique, we recorded *in vivo* intracellularly and extracellularly in barrel cortex (S1) the response evoked by an air puff to the whiskers before and after the application of lidocaine. The different forms of application were all effective to temporarily deprive somatosensory input, but all presented some advantages and disadvantages. The injection technique is an invasive technique which is easily applicable only in anesthetized animal, and too stressing for the awake. Although the EMLA® cream

(Juhlin and Evers, 1990) decreased the response in one out of two cases, the exact dose was difficult to adjust, plus the remaining dried cream could eventually affect whiskers movement. Aerosol application induced temporal tactile deprivation with the inconvenience of being too imprecise in the application area, given that the aerosol drops often stayed on the whisker's surface. We found the topical application of a drop of liquid lidocaine with the help of the syringe to be the method with more advantages to temporarily deprive somatosensory information: easy to apply, not stressing for awake animals, and a course of action that proved to be consistent in 6 cases (Figure 40). Results showed that the drop of lidocaine reduced the cortical sensory-evoked response in a few minutes; it reached a maximum close to 100% blockage after 100 min. The effect of lidocaine was completely reversed after 5–6 h. Lidocaine being a sodium channel blocker (local anaesthetic) prevents any signal to be transmitted. We ruled out the possibility that lidocaine was due to a mechanical effect of the drop by administrating a drop of same or even larger volume of the lidocaine vehicle (32% ethanol in saline). This did not have any effect on the intensity/response curve.

The main goal was to develop a technique useful for awake animal but because of the anaesthesia state, the rat did not whisker, we were not able to test that the effect of the different forms of lidocaine application may have on the awake animal's whisking movements and on the animal's perception. With the experience that we have nowadays, we affirm that this method can be used in awake animals because it is easy to apply and the relative lack of behavioural signs of discomfort in treated animals. In addition, this manipulation offers the possibility to reapply doses of lidocaine to prolong the blockade of somatosensory inputs to the cortex. By means of this manipulation, the role of whisker information in different cognitive tasks, or the plasticity induced by its short term deprivation, can be explored under experimental conditions involving electrophysiological recordings in awake animals and/or behavioural tasks. Temporal sensory deprivation constitutes an interesting and useful strategy for the physiological study of different sensory systems.

For the purpose of this Thesis, the technique was developed to certify that lidocaine application on the snout of the animal temporarily deprive almost totally the somatosensory inputs.

2. BEHAVIOURAL EXPERIMENTS DISCUSSION

In this study, the temporary deprivation technique of somatosensory inputs (Gener *et al.*, 2009) was used in the awake animal. Topical application of lidocaine to the snout of the rat not only disrupted somatosensory inputs responses of whisker tactile stimulation but also was effective to perturb the behavioural performance during a tactile discrimination task of two tactile stimuli. Animals had to correctly discriminate the texture to get a reward. The protocol was tested and improved to reach the optimum level of performance in the shortest time. During the development of the learning protocol, details of the protocols were adjusted or corrected as the delay to get the reward, the time-outs length or the moment of light switch on and off to allow animals to learn faster and performed the task better (see Materials and Methods). The discrimination tasks are commonly used in various investigation fields with several terms of discrimination from visual (Minini and Jeffery, 2006; Jeffery, 2007) to tactile (Diamond *et al.*, 2008).

The protocol was successful and animals achieved a performance of 80% correct discriminations after 18 sessions (less than 2 weeks) of training, in agreement to previous studies (for example, Carvell and Simons, 1990). During the learning phase, time outs and reaction times were measured and remarked when the required threshold was reached. For the several analysed parameters, there were significant differences before and after the 80% level of success showing that the rat behaviour changed when it learnt the task. The originality of this classic discrimination task consists into its association with a local and temporal deprivation. We can test in this way the effect of the deprivation on a behavioural task.

Lidocaine effect on the behaviour

After reaching the 80% of performance, local application of lidocaine on the snout of the animal affected different variables measured during the behavioural task such as: performance, number of time outs, reaction time and fano factor. Those parameters were analysed along time before and after lidocaine application on the whiskers pad and allowed us to characterise the time course of the effect. Lidocaine induced a temporal increase in the time-outs, of the reaction time and of the fano factor and caused a decrease of the percentage of success to random, 45.44%. Those results showed the influence of the whisker inputs information for the rat to discriminate between two

textures and most of all it allowed us to develop the temporary deprivation in awake animals. In summary, animals did not succeed in discriminating the texture during the time of action of lidocaine which was not surprising considering the previous results obtain in the acute experiments (Gener *et al.*, 2009). The time course action of the lidocaine was not the same in the anaesthetised animal and in the awake one. This difference in the maximum action was to 150 min in the anaesthetised animal to few minutes in the awake animals (3-12 min). The differences in time course between temporal deprivation in the anesthetized and awake performing animal are remarkable and deserve to be discussed here. One possible first explanation is the metabolic state, which is slower in the anesthetized animal than in the awake and behaving one, therefore the time course of the drug being longer in the former than in the last. Apart from that difference, it is important to consider that different parameters are being measured in both cases: in the anesthetized animal sensory responses in the primary somatosensory cortex were measured, while in the awake animal was the performance during tactile discrimination. What this suggests is that there could be a first moment of performance drop due to a sudden decrease or loss of tactile information, followed by a certain degree of adaptation to function even with a decreased sensory input. Given that this is a discrimination between two quite different textures, probably a decreased sensory input is still enough to yield correct behavior. Another possibility to be considered is that the stress of the lidocaine application induces per se a decreased performance. This could be quantified by monitoring physiological values such as ECG (i.e. electrocardiogram) that could provide a measurement of stress based on heart rate and heart rate variability. It could be then interesting to measure the stress level by monitoring the heart rate which has demonstrated to be a sensitive parameter to the stress level (Kitney and Rompelman, 1980; Aubert et al., 1999). Doing this, we could better understand the behaviour of animals with temporal somatosensory inputs deprivation. In any event, this striking divergence between sensory information being provided and time course of behavioral sensory discrimination is an issue that deserves in itself further investigation.

Animals, even if trained to discriminate texture, could maybe use other cues to discover in which side the reward will be delivered. To rule out this possibility and to assure that the animal discriminates only texture with its whiskers, we cleaned after each sessions the maze with perfumed alcohol solution. Protocols were performed in the complete darkness (windowless, no light and black walls room), no possible acoustic

cues could be used because the motor for reward delivery was activated after the animal's choice. The last and decisive control was to carry out a tactile discrimination task with the texture/reward side inverted during few sessions with animals which already known the task. The animal would touch the texture and go for the side of reward as it learnt it (learned associated nosepoke). Due to the inverted texture, the animal did not receive any reward but a sound punishment. The result was that animals kept going to the associated nosepoke even if it did not receive any water. Animals even punished had a percentage of success to 78.75% following the learnt association. Animals received reward only in 21.25% of the cases, demonstrating that animals discriminated texture and that the association texture/side was properly done.

One of the remaining problems of the whiskers is to understand how they code for texture and how animals discriminate between textures. Several hypotheses are discussed. The "resonance hypothesis" describe that textures are converted to a spatial code distributed across the whisker pad on the snout (Hartmann *et al.*, 2003; Neimark *et al.*, 2003). "Kinetic signature hypothesis" suggested a conversion of surface shape into precisely timed motion events by individual whiskers (Arabzadeh *et al.*, 2005; Diamond *et al.*, 2008). Experiments of temporal deprivation have the advantage to not block the whisking movement (unpublished observations) but deprive the animal of its somatosensory information by blocking peripheral sodium channels. This could be useful tool for the understanding of whiskers texture coding and from which of the two theories, the "resonance" or the "kinetic signature" hypothesis.

The experiments just discussed had the main goal to validate a behavioural protocol the temporal somatosensory deprivation. This goal was achieved. We used this technique to temporarily deprive whiskers to study the influence of somatosensory input on the cognitive map creation.

3. ELECTROPHYSIOLOGICAL BEHAVIOURAL EXPERIMENTS DISCUSSION

In this study, we carried out two protocols in which single place cells were recorded, clustered and analysed. The first one to demonstrate that the place cells and more generally, the cognitive map creation uses the somatosensory inputs by rotating the tactile cues in a controlled environment. Even if lots of rotation cues are described in the literature (for example, O'Keefe, 1976; Muller and Kubie, 1987; Jeffery *et al.*, 1997; Jeffery and O'Keefe, 1999), none were ever performed (as far as we know) with tactile cues. The second protocol, to explore how somatosensory inputs are involved in the cognitive map creation by temporary depriving those inputs (technique previously describes and validates). Experiments on place cells with sensory deprivation were already carried out (Save *et al.*, 1998) (Visual deprivation) but never on the somatosensory inputs as far as we know. The activity of the denominated "place cells" which we recorded in the area CA1 of the hippocampus have been proposed to encode a spatial representation (cognitive map) (O'Keefe and Nadel, 1978) necessary for spatial navigation. If the place cells received information from the somatosensory inputs, then when tactile cues are rotated, FF should rotate with them and with the lidocaine (deprivation of tactile inputs), FF should be altered.

In all protocols we calculated different place cells parameters (maximum frequency, mean frequency, compactness, symmetry, spatial information content, area of the 20% of fm, area of 60% of the fm, volume of the ff). Equally, spike amplitude and spike width were very stable across conditions. In the rotation protocol, spatial parameters were generally stable across conditions, but in the somatosensory deprivation protocol, most of the spatial factors were affected by the lidocaine application.

Remapping with tactile cues rotation

Place cells were successfully recorded during the specific conditions of the rotation protocol. The place fields recorded were sensitive to the tactile cues rotation in these specific conditions. We observed that 90% of the place fields rotated with the tactile cues and the majority, but not all, rotated back. Pereira describes that 52% of hippocampal cells received somatosensory inputs (Pereira *et al.*, 2007). Ours results show that 90% of place cells recorded in this specific environment are influenced by

somatosensory. This difference is not contradictory since we focused on the place cells which were active in a tactile enriched maze and Pereira *et al.* stimulated electrically all the tract (active cells or not, place cells or not). Our results demonstrated that the cognitive map and more precisely place cells receive and integrate information for somatosensory inputs. Comparing with other rotation protocols (see introduction, (O'Keefe, 1976; Muller and Kubie, 1987; Jeffery *et al.*, 1997)) in which the authors change the place of the visual cue inside of the maze or outside, the behavior of place cells are not always in direct relation with cues.

We observed for the rotation protocol that place cells could be divided in three different groups:"Rotate and return", "Rotate and remap" and "No rotation". Two of these groups ("Rotate and return", "Rotate and remap") were affected by the tactile cues rotation. Only 10% of the recorded place cells were not affected at all suggesting that no somatosensory inputs were part of their system. Data suggest that most of the place cells received inputs from somatosensory cortex. This could be due to the protocol's design focused on the tactile cues. The condition of recording influences importantly the place cells recording. As it has been demonstrated in several previous studies, illumination condition could be crucial for place cells recording. If the recordings were done in the light or in obscurity, the place cells are active or not. In others words, depending the light conditions, a place cell could be recorded or not (Quirk *et al.*, 1990; Markus *et al.*, 1994). With the same approach, place cells in a condition where only tactile cues were relevant, only place cells sensitive to somatosensory inputs were active. There is also a preselection of place cells that fire in the darkness. This does not mean that these place cells were exclusively sensitive to somatosensory inputs.

Temporal changes of place field parameters with tactile deprivation

Place cells were recorded with and without somatosensory inputs during the specific conditions of the lidocaine protocol. It should be noticed that to record place cells in obscurity and with somatosensory deprivation, its already a relevant result similar to the one achieved by E. Save (Save *et al.*, 1998), who described place cells in blind rats.

A majority of place cells showed significant spatial differences when lidocaine was applied on the snout of the rat. The place cells compactness and the place cells symmetry had some significant decrease; the area of 20% of the maximal frequency had

a significant increase under lidocaine condition. However, because it has been described that not all place cells always behave in the same way (Tanila *et al.*, 1997; Skaggs and McNaughton, 1998), we separated place cells regarding to their behaviour in somatosensory deprivation condition. The first group and major population (78.5%) had their spatiality affected by the lidocaine application. An increase in the firing fields' area with respect to the control was observed. A decrease in the spatial parameters such as compactness, symmetry and spatial information content was observed in lidocaine condition. Furthermore, all the spatial parameters had the tendency to come back to the control value after the lidocaine effect. The lidocaine protocol confirmed that tactile information is part of the system and more than that determine that the loss of tactile sense reduces the spatial parameters inducing a destruction of the place cells specificity. All those changes lasted for 120 minutes the somatosensory deprivation. For the second population (21.5%), place cells did not behave at all as the first one. There were no such differences in the results; the only observed change was an increase in the compactness under lidocaine which was the reverse than the first population. The behaviour of the two place cell populations was different regarding the somatosensory inputs deprivation.

Protocol relevance

We took care to avoid too long protocols which will induce animal stress and tiredness. All these precautions were taken in order to assure that all the observations were due to rotation or deprivation itself and not to spurious factors.

The first protocol consisted in a tactile cues rotation in a controlled environment to observe and characterise the place field rotation. The protocol was developed in order to demonstrate that the tactile information from the whiskers of the rat contributes to the flux of information received by the hippocampus (Pereira *et al.*, 2007) and its place cells, as it was demonstrated for other sensory modalities in several rotation experiments since the discovery of the place cells (O'Keefe, 1976). Cues rotation protocol is a common principle in place cells investigation field to investigate the influence of the sensory information on the place field. If one found that the rotation of the referential cues rotated as well the relative firing field one can determine that this firing field was sustained by these set of inputs (Quirk *et al.*, 1992; Knierim *et al.*, 1995; Tanila *et al.*, 1997; Knierim *et al.*, 1998).

The second protocol consisted in a temporary somatosensory deprivation using the validated technique *in vivo* (Gener *et al.*, 2009) and in the behavioural study of lidocaine application developed in our laboratory. The temporal somatosensory deprivation protocol was developed to characterise the influence of the somatosensory inputs on the cognitive map creation. The lidocaine protocol demonstrated and gave some evidence of the place cells behaviour in absence of somatosensory inputs. The deprivation protocol is as the rotation one a common principle in place cells investigation field to demonstrate the influence of a sense on the firing field (Hill and Best, 1981; Shapiro *et al.*, 1997; Save *et al.*, 1998).

Two separated protocols were carried out in order to demonstrate separately the fact that somatosensory inputs were involved in the spatial map creation and that the deprivation of the inputs had an influence on this spatial map representation and characterised it.

It could be objected that the rat is receiving other sensory information. During all the protocols several precautions were taken to let the rat receive only tactile information. The maze was washed after every session with an odorant liquid alcohol to avoid any odorant cues. A uniform white noise (four speakers) was played during all the period of the experiment to avoid any auditory cues. A complete darkness was obtained thanks to black walls and switching off the light in a windowless room to avoid any visual cues. We assumed then that the only relevant information to orientate itself in the environment was provided by the somatosensory inputs.

Interaction between whiskers, somatosensory cortex (S1) and Hippocampus CA1

A possible argument to explain why the place cells did not behave all in the same way could reside in the connectivity of place cells with the somatosensory cortex. As we mentioned in the introduction, the CA1 region of hippocampus receives inputs from CA3 (Andersen *et al.*, 2007), from entorhinal cortex (Steward and Scoville, 1976; Witter and Amaral, 1991; Tamamaki and Nojyo, 1993) and from the perirhinal cortex (Deacon *et al.*, 1983; Suzuki, 1996; Brown and Aggleton, 2001). At the same time the entorhinal cortex integrates information from different areas, in particular sensory areas. Thus, cells from CA1 area could receive then different information from different parts of the brain. This information should influence the CA1 cells and place cells behaviour depending on the type of the relevant information received. In the protocols presented

here, tactile information was the most relevant and was the one participating to spatial representation. If those cells did not receive strong somatosensory inputs it explained why firing fields did not follow the tactile cues rotation and could fire in a different way under lidocaine condition. Our results agree with the results described by Pereira (Pereira *et al.*, 2007) who demonstrated that an electrical stimulation of the infraorbital nerve stimulated not only the somatosensory cortex but also the pyramidal cells of the CA1 in the hippocampus. In their study Pereira *et al.* deducted from the latency that this path result to be a complex multisynaptic and well connected system. The same response type was recorded in the S1 and in the CA1. The somatosensory trigeminal pathway seems to be involved and one of the stronger connexions between the whiskers and the somatosensory cortex (S1), propagating until the CA1 (Diamond *et al.*, 2008). Pereira *et al.* described that 52% of the CA1 cells respond to an electrical stimulation of the infraorbital nerve. Giving this data, it makes sense that not all cells and thus, not all place cells in the hippocampus behave in the same way in relation to the somatosensory inputs changes. We did not observe the same exact percentage of cells (50% of the total cells (PCs and non PCs) recorded in the case of the rotation protocol and 25% of the total cells recorded (PCs and non PCs) in the case of the lidocaine protocol) responding to the somatosensory inputs because in Pereira's study, it is an electrical stimulation (which probably recruits more cells) and in our case it is the natural somatosensory inputs when rat forage which were deprive with lidocaine. Furthermore, in one case it is the cells responding to a stimulus in the other is a place cells recording.

Characterisation of somatosensory influences on CA1 place cells

Deprivation of tactile inputs resulted in a general profound alteration of the spatial maps of place cells. It means a segregation of the firing field in several parts and an effect of decrease in the spatial specificity. This suggested that the deprivation of the somatosensory inputs results in a reduced information from the whiskers being sent to the somatosensory cortex (Gener *et al.*, 2009) as well as the hippocampal place cells. Our results showed that rather than the location of the receptor fields and spike properties of the cells, it was the parameters of the receptor fields that changed when the somatosensory inputs were deprived. The mean frequency did not change but, in contrast, the peak of maximum frequency presents some significant decrease in lidocaine condition. The compactness and symmetry presented as well significant changes in lidocaine condition. These two factors in particularly represents the spatial

distribution of the firing field. What possible explanations to those changes in the spatial firing could be suggested? It is important to consider that the somatosensory information goes through the somatosensory cortex and then to the hippocampus (Pereira *et al.*, 2007). A possible phenomenon is that by changing the inputs, a change between the level of excitatory cells and the inhibitory cells recruited in the somatosensory cortex was induce (Petersen, 2007). This modification between the existed and inhibited cell should change the number of cells and/or which cells of the CA1 hippocampus region were active or not, producing alteration in place cells spatial firing in comparison to control condition. A possible resultant to this mechanism could be that silent cells which did not fired, possibly due to the inhibition received to their adjacent cells start to fired when this neighbour cells begun to not inhibit them anymore because they not received any excitatory inputs from the somatosensory cortex (Thompson and Best, 1989). This possible mechanism remains to be explored.

Not all place cells received somatosensory inputs

There is no anatomical information about the distribution of the somatosensory inputs to hippocampus. The distribution of other inputs for example the visual inputs (Reperant *et al.*, 1987), suggests that there may be different path and organisation, thus being non-homogenous. Then, place fields in the CA1 hippocampus maybe do not behave all in the same way due to the non homogenous cells connectivity. Regarding a recent publication from the Moser laboratory, Henriksen *et al.* demonstrated that place cells and the received inputs from the entorhinal cortex were not homogenously distributed (Henriksen *et al.*, 2010). They showed that the more distal were the place cells in CA1, bigger and more fractionated were the firing fields and other way round, more proximal, more dense and compact. Patricia Sharp also found a similar organisation in the Subiculum (Sharp and Green, 1994). It will then make sense that the organisation of the somatosensory inputs in the CA1 could be also organised non-homogenously as described.

Even if all the evidences that we provided confirmed our hypothesis, some interrogations remain. What about the path integration strategy and the short term plasticity or the context theories?

Somatosensory inputs and the egocentric theory

If all the evidence suggests that without tactile inputs spatial perception changes, why changes were not more important for the firing fields of the place cells during the temporary deprivation? This could be justified by the region in the CA1 where we recorded as we mention previously and also by some compensatory mechanisms. As we mentioned in the introduction, the cognitive map theory is formed by two possible mechanisms. The allocentric one is the influence on the cognitive map of the senses that animal can use, as vision and in our study the tact. The other one is the egocentric mechanism also called path integration (McNaughton *et al.*, 1996; Jeffery, 2007). This mechanism is used in parallel to other spatial strategies. The fact that some place fields remapped in the recovery condition for both protocols (rotation and lidocaine) could mean a reset of the path integration due to the loss of the animal mark inducing a new cognitive map creation (Etienne and Jeffery, 2004). Place cells will then create the cognitive map for the specific environment, as for lidocaine condition, the environment is not perceived identical due to the change of perception. The system re-creates a spatial map. This will append in the most critical case. In our case, the allocentric mechanism even if an anaesthesia is not a surgically alteration (no physical lesion was performed), the deprivation could be not functional 100% even with the several control we performed. Furthermore, we try in every experiment to characterise the action of the lidocaine but in each one, the time of maximum deprivation was different. This could be due to many causes but the most important should be the level of activity of the animal (anesthetised or awake). Another point is that the animal knew the environment due to the consecutive exposure to the maze. We then observed a distortion of place fields and not a complete change. Place cells used then any mechanisms to create its owned cognitive map. If one of the mechanisms is not available then the system compensated it.

Plasticity induced by somatosensory deprivation

The explanation to the observed changes could be the place cells plasticity (Shapiro, 2001; Jeffery and Hayman, 2004; Frank *et al.*, 2006). Plasticity is the ability for the cell to change or adapt their behaviour to changing conditions. This will mean a change in the place cells and not only in the inputs as we suggested previously. It was shown in several studies that the hippocampus CA1 could a place of rapid plasticity (Frank *et al.*, 2004; Frank *et al.*, 2006) or slow plasticity (Shapiro, 2001; Lever *et al.*,

2002). A change in the CA1 inputs as an exposure to an novel environment can produce "rapid plasticity, instability in representations and a dissociation between the stability of a hippocampal representation and the apparent familiarity of a location" (Frank *et al.*, 2006). A change in the tactile inputs to CA1 could then generate plasticity and change the spatial representation of the space. When the somatosensory inputs come back, the place fields characteristic come back to control level. One could understand that the inputs to those neurons are rebalanced. This change as we described was temporal because as soon the somatosensory inputs came back, the place cells behave as in the control condition. Another explanation could be the memory of the place as the subject new it. This phenomenon is highly linked to the episodic memory.

Place cells and contextual cells

Another point which is getting more interest from the scientific community in the last decade is the concept of contextual cells. These cells of the hippocampus would be active not only regarding the space but also in function of the context (nongeometric information). Several studies gave some evidence to support those theories (Sharp, 1999; Anderson and Jeffery, 2003; Hayman *et al.*, 2003; Jeffery *et al.*, 2004; Smith and Mizumori, 2006). These evidences relate the fact that hippocampal cells are place cells but also cells which change their firing according to the context in which the animal is. In other words, the spatiality should be in relation to the memory and principally the episodic memory. Hippocampus will then be a "complex sensory hub associated with the declarative memory" (Pereira *et al.*, 2007). A good example for the study of the context is the study of novelty detection. This phenomenon links the hippocampus to working memory (Sarnthein *et al.*, 1998; Tesche and Karhu, 2000). The somatosensory inputs are also involved in these processes as suggested by Pereira *et al.* (Pereira *et al.*, 2007) and proved in this study. Indeed, the tactile cue rotation or the somatosensory deprivation by altering the perception of the environment will create a new context. Place cells could then generate a new map according information available. The system will actuate as if it will be in a new unknown context. The spatiality is reduced due to the lack of spatial information.

Mechanism of place cells firing

These possible suggested mechanisms lead to an essential question of the place cells research field. How place cells could code for space? What are the mechanisms?

Answering those questions will reveal what is the most adequate interpretation to the observed results. To response properly to these questions it is necessary to understand how place cells fire and how the connection between them is. Recent techniques and experiences gave a start for possible responses. A few groups have demonstrated that stable intracellular recordings of place cells navigating freely in an environment are possible (Harvey *et al.*, 2009; Lee *et al.*, 2009; Epsztein *et al.*, 2010). Harvey *et al.* for example identified three different signatures of subthreshold of place cells. Their data were ”most consistent with a soma-dendritic interference model (Gasparini and Magee, 2006)” which if it will be verified, could be an interaction between inhibitory oscillation near the soma and dendritic excitation. This could be the first step in order to understand the mechanisms. The same research group, Dombeck *et al.*, added to the same technique some high-resolution functional imaging. Their methods allowed to study the spatially modulated activity patterns of pyramidal neuron (Dombeck *et al.*, 2010). That allows now to see which cells are active, when they are active and how they are active at the same time. With these techniques that are the technical frontier, it is now possible to finally understand how place cells spatially fired. Techniques like this will, in a near future, help us to understand the exact mechanistic role of tactile information on place fields.

CONCLUSIONS

I. TEMPORAL SOMATOSENSORY INPUTS DEPRIVATION

- √ Local lidocaine application on the base of the whiskers of anaesthetised rats induces a temporal and reversible deprivation of the tactile inputs to barrel cortex.
- √ Different forms of application were tested and a liquid drop of lidocaine was considered better than others application forms such as injection or cream.
- √ 0.4 ml of local lidocaine reduced to 91.1% the amplitude of the whisker-induced cortical responses, with a peak of effect in 150 min and a recovery in 300 minutes.

II. BEHAVIOURAL TASK UNDER TACTILE DEPRIVATION

- √ We next trained animals to realise a tactile discrimination task.
- √ Liquid lidocaine application at 10% on whiskers' pad in awake animals reduced the percentage of success in the tactile discrimination task from 88% to 48% and came back to 82.5% after the deprivation effect.
- √ The peak action of lidocaine in terms of declined performance was reached in 3 to 12 minutes and the effect was unnoticeable after 20 min of application.
- √ Those results validated the temporal deprivation of somatosensory inputs technique in the awake animals although with a different time course from that in anaesthetised.

III. ELECTROPHYSIOLOGICAL RECORDINGS OF PLACE CELLS

- √ ***Rotation protocol:***
  - ➢ Next, recordings from place cells were successfully obtained in the CA1 of awake rats in a tactile enriched environment without other sensory cues.
  - ➢ When the tactile cues were rotated, place fields rotated in 90% of cases according to the tactile cues.
  - ➢ Those results demonstrate that place cells integrate tactile cues to generate place fields.
- √ ***Lidocaine protocol:***
  - ➢ Finally, place cells were successfully recorded in a tactile enriched environment.
  - ➢ The application of local lidocaine resulted in a transformation of place fields in 78.5% of the cases, either expanding, shrinking or by rate remapping, transformation that was in the majority of cases, reversible.
  - ➢ These findings revealed that somatosensory inputs influence a significant number of the place cells in CA1.

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APPENDICE

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A new paradigm for the reversible blockage of whisker sensory transmission

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Cortical activity

ABSTRACT

The objective of this study was to explore a paradigm that would allow a temporary deprivation of whisker information lasting for a few hours. An additional requirement was to be non-invasive in order to be usable in awake chronically implanted rats without inducing stress. With that aim, electrophysiological recordings from the barrel cortex of anesthetized rats were obtained. The pressure of an air-puff (5–10 ms) delivered to the whiskers was adjusted to evoke a consistent response of around 100 μV (extracellular) or approximately 5 mV (intracellular) in the contralateral cortex. Lidocaine was then locally applied in different forms (cream, local injection, aerosol, drops) and concentrations (2–10%) to the base of the whiskers. The stimulus-induced response was monitored once every 5 s for several hours (3–6 h) in order to characterize its course of action. Local injection of lidocaine induced the fastest and most complete blockage, but was ruled out for being invasive. Out of the remaining forms of application, a lidocaine drop (0.4 ml, 10%) to the base of the whiskers was found to induce a reliable blockage (to an average 9% the original response). The maximum effect was reached after 150–200 min, and the response was totally recovered approximately 300 min after lidocaine application. This characterization should be useful to induce an efficient, short term and reversible blockage of whisker sensory transmission in both anesthetized and awake preparations, while not causing stress in an awake animal.

1. Introduction

Sensory deprivation has been a widely used manipulation to explore plastic changes occurring in sensory pathways either in short or long term. Whisker sensory deprivation has been most commonly achieved by whisker trimming (Diamond et al., 1993; Kossut, 1998; Lebedev et al., 2000; Lee et al., 2007; McRae et al., 2007). Other reversible nerve blocks such as procaine, a Ca^{2+}-induced Ca^{2+}-release (CICR) inhibitor of Merkel cells in the vibrissae in a dose dependent manner (Senok and Baumann, 1997), or nerve pressure have also been used (Decima et al., 2004). Trimming a whisker can induce changes in the corresponding barrel cortex within a few hours (Holtmaat et al., 2006). However, it takes 8–11 days for a new vibrissae to appear (Ibrahim and Wright, 1975), and several days to grow to their normal length at 1 mm/day. Here, we were interested in finding a method that would allow us to deprive rats of tactile information for a few hours, in a way that would cause no stress to an awake rat. This method should be easy to use, reliable, produce the least possible stress and be reversible in few hours. Lidocaine is a local anesthetic which is a potent blocker of sensory and sympathetic transmission, with a large effect on the nerve's refractory period (Scurlock et al., 1978) due to its blockage of sodium channels (Strichartz, 1973; Starmer et al., 1984). In this study we explored different forms of local application of lidocaine on the snout and the intensity and time course of its effect on the sensory-induced responses in barrel cortex.

2. Materials and methods

Eleven adult Wistar rats (250–300 g) were used for recordings in S1 cortex. Anesthesia was induced by intraperitoneal injection of ketamine (100 mg/kg) and xylacine (8–10 mg/kg). The animals were not paralyzed. Maintenance dose of ketamine was 75 mg/kg/h. Deep anesthesia levels were monitored by the recording of low frequency electroencephalogram (EEG) and the absence of reflexes. Rectal temperature was maintained at 37 °C by means of an electric blanket.

Once in the stereotaxic apparatus, a craniotomy (2 mm × 2 mm) was made at coordinates AP −1 to −3 mm from bregma, L 4.5–6.5 mm (Paxinos and Watson, 2005). After opening the dura, extracellular recordings were obtained by means of tungsten electrodes (FHC, Bowdoinham, ME, USA). For stability and to avoid desiccation, agar (4%) was used to cover the area. Sensory responses

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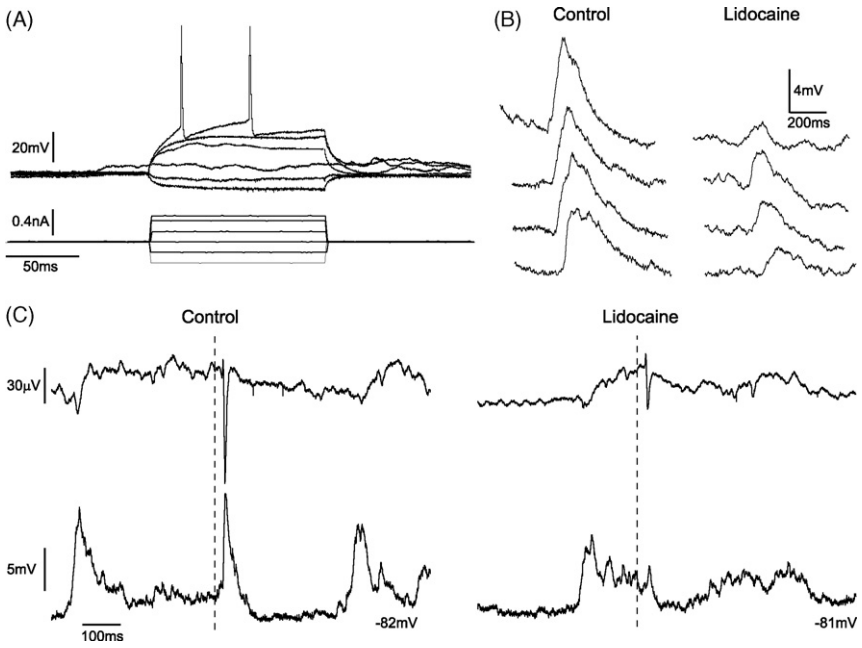

Fig. 1. Blockage of intracellularly recorded whisker-induced synaptic responses by topical application of lidocaine. (A) *I–V* protocol of the recorded neuron. Bottom, intracellularly injected current from −0.4 to 0.5 nA. Top, Vm responses to current injection. (B) Four raw traces of intracellular recordings in response to an air puff stimulation in control (left panel) and after application of topical lidocaine to the whiskers (right panel). Note the decrease of the amplitude to an 81%. (C) Traces of simultaneous LFP (top) and intracellular recording (bottom). The dash line represents the trigger of the air puff in both control (left panel) and in presence of lidocaine (right panel). The parameters of the stimulation were in both cases (control and lidocaine) 10 ms, 5 psi.

evoked in the local field potential (LFP) were used to adjust whisker stimulation (see below). Intracellular electrodes were made with borosilicate glass capillaries 1 mm O.D. × 0.5 I.D., filled with 2 M potassium acetate, and had final resistances of 50–80 mΩ. Intracellular recordings were obtained in close vicinity (<1 mm) from the extracellular recording electrode (Fig. 1A and B). Recordings were digitized, acquired and analyzed using a data acquisition interface and software from Cambridge Electronic Design (Cambridge, UK).

2.1. Whisker stimulation

A puff of air given through a 1 mm diameter tube placed in front of the whiskers (10 mm away) was used for stimulation. The air puff (5–10 ms) was controlled by a stimulator (Master-8, AMPI, Israel) and delivered by a Picopump (WPI, Sarasota, FL). Its pressure was adjusted (5–10 psi) such that it would evoke a response of approximately 100 μV in the LFP and 5 mV in the intracellular recordings (Fig. 1C). The stimulus was directed to the whole whisker area, and particularly focused towards one vibrissae. For the stimulus–response curves the applied pressure was 2–38 psi. Time zero for the stimulus was taken as the time of occurrence of the air puff.

2.2. Lidocaine application

Local application of lidocaine was made at the whisker pad of anesthetized rats (Faggin et al., 1997). Several forms of lidocaine application have been tested in order to find the most efficient and reliable way to block the somatosensory input.

2.2.1. Cream

Lidocaine cream (EMLA® cream, 25 mg/g) was applied to the base of the whiskers (1 g). To improve lidocaine skin absorption, a short massage was done with a Q-tip.

2.2.2. Injection

Liquid lidocaine (Xilonibsa®, 10%) was injected at the whisker base (subcutaneous injection, 4–5 injections, 50 μl each).

2.2.3. Aerosol

Liquid lidocaine (Xilonibsa®, 10%) was vaporized on the snout in the whiskers zone (2 sprays, 0.2 ml).

2.2.4. Liquid drop

A drop of liquid lidocaine (Xilonibsa®, 10%) was applied to the base of the whiskers with a pipette (0.4 ml).

3. Results

In order to characterize a reversible and non-invasive method to block sensory transmission from the whiskers, recordings from barrel cortex of 11 rats were included in this study. In 7 of the rats the complete study was performed in both hemispheres. The number of observations that are given refer to responses in one hemisphere following stimulation of the contralateral whiskers.

Intracellular recordings were obtained from a total of 13 neurons (Fig. 1A) recorded in close vicinity to the LFP recording. We obtained sensory-evoked potentials by an air puff to the whisker (10 ms, 5–10 psi; Fig. 1B) (Reig and Sanchez-Vives, 2007). The average amplitude was 6.1 ± 2.6 mV, their average latency being 11.06 ± 2.49 ms (range 8–15.6 ms). Fig. 1C illustrates simultaneously recorded sensory-evoked synaptic potentials in the LFP and in the intracellular recording. In this particular case the evoked synaptic potential was reduced from 6.4 ± 2.0 mV to an average value of 1.2 ± 1.5 mV (a decrease of 81.3% the control amplitude) following lidocaine application. This decrease was also reflected in the LFP evoked synaptic potential from 58.5 ± 31.37 μV to 7.8 ± 21.0 μV (a decrease of 86.5% the control amplitude). Once it had been proven that the LFP accurately reflected the intracellularly recorded synaptic potentials, the following sensory responses were only evaluated

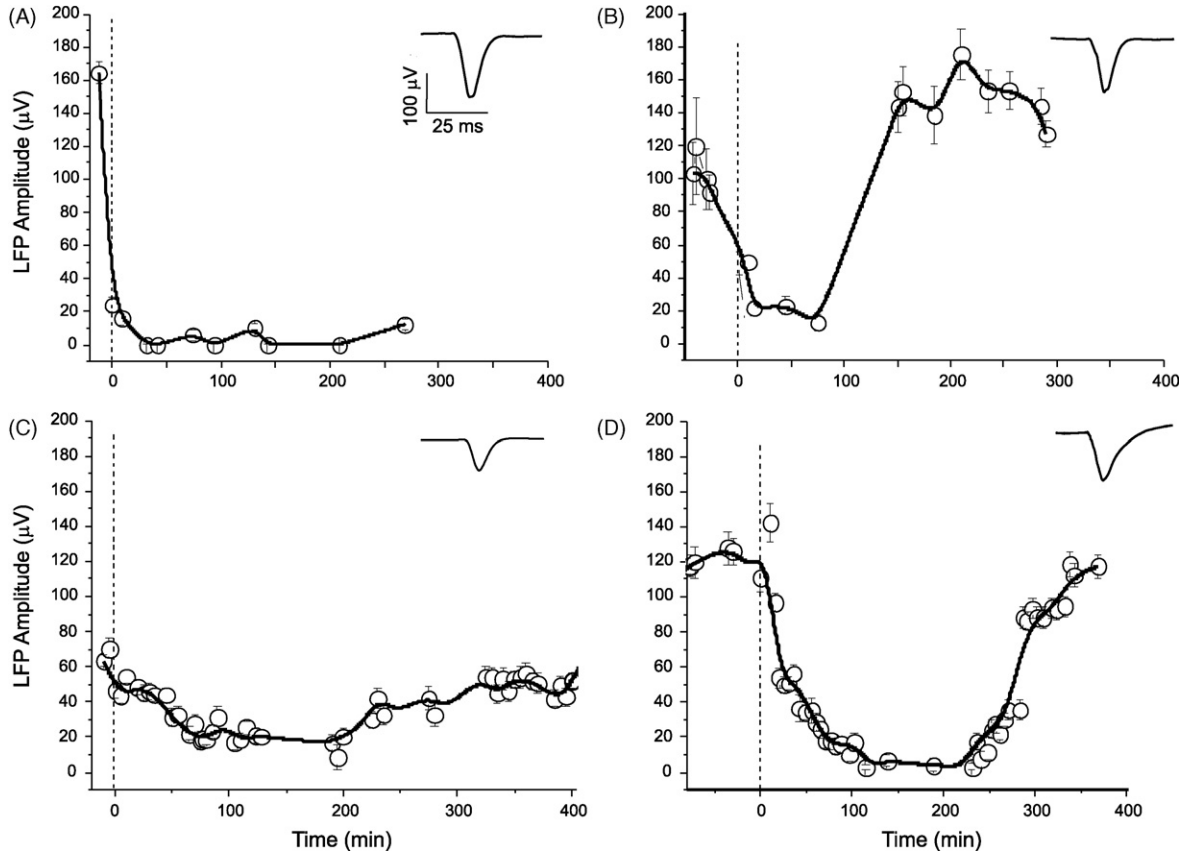

Fig. 2. Time course of action of four different forms of lidocaine application. Zero (dash line) represents the time of lidocaine application. Each circle represents the average amplitude of 60 sensory responses (0.2 Hz) as measured in the LFP in response to the air puff stimuli (5 psi, 5–10 ms duration). (A) Subcutaneous injection of lidocaine (10%, 0.2 ml). (B) Lidocaine in cream, 1 g (25 mg/g). (C) Lidocaine in aerosol application (10%, 2 sprays, 0.2 ml). (D) Liquid drop (10%; 0.4 ml). Insets represent the averaged stimulus-evoked response before lidocaine was applied.

through the LFP. Long (>300 min) and highly stable recordings were needed to monitor the action and recovery of lidocaine application.

Whisker stimulation yielded a sensory-evoked response in the LFP that had an average latency of 13.6 ± 1.07 ms and an average peak amplitude of 77.5 ± 31.2 μV, peak that occurred at a latency of 22.2 ± 1.8 ms ($n = 17$ recordings) in the extracellular recordings. The whisker response evoked in this way was repeated at 0.2 Hz and each of the measured responses were the result of averaging 60 stimuli (300 s). This pattern of stimulation was continued for several hours (3–6 h) in order to monitor the time course of action of lidocaine and its recovery. No significant decays or increases of the sensory-evoked responses were observed over time (Supplementary Material Fig. 1).

Lidocaine was then locally delivered in different forms (local injection, cream, aerosol, drops) and concentrations (2–10%) to the base of the whiskers (Section 2).

Local injection of lidocaine (4–5 injections, 50 μl, 10%) at the base of the whiskers induced the fastest (maximum effect in 30 min) and most complete (97%) blockage ($n = 2$; Fig. 2A). In both cases, 5 h later the response was not yet recovered. This difficult reversibility, together with the fact that it is an invasive technique, suggested that this technique was not ideal for awake behaving animals in spite of inducing an effective blockage. Thus, other forms of application were explored.

In two experiments *in vivo* lidocaine in cream was topically applied to the base of the whiskers (dose 1 g; 25 mg/g) after a sensory-induced response was obtained. We obtained a blockage in one of the two cases 65 min after application, during which it decreased 87% the original amplitude, with a recovery time of 200 min (Fig. 2B). In the second case no effect was observed. Cream application had the advantage of causing less stress than injection to an awake rat, but posed the problem of staying in layers of different thickness and therefore allowing poor the control over the dosage. In an awake animal, the mechanical effect of the cream could also affect the movement of the vibrissae. Overall, cream application did not seem to be a reliable technique to induce temporal blockage.

Aerosol application was carried out in two experiments ($n = 2$). The maximum blockage by aerosol application was of 55% of the control response, with a maximal effect after 105 min and a recovery of the response 320 min after the initial lidocaine application. Even when effective, the aerosol technique presented also a deficit in the control of the applied dose, not so much regarding the amount being sprayed, but rather the distribution of the product over the whisker pad, since small drops remained on the whiskers (Fig. 2C).

A drop of lidocaine (0.4 ml, lidocaine 10%) was found to induce a reliable blockage of the sensory-evoked synaptic potentials (Fig. 2D). Apart from being an easy method to apply to an awake rat (unpublished observations), it seemed to present lesser dosage problems than those reported for the other forms of application. Thus, for a thorough characterization of the lidocaine action, three experiments were performed while doing this form of lidocaine application. In all cases the recordings were carried out in both hemispheres while stimulating whiskers on both sides. The grand average of the effect in 6 cases is represented in Fig. 3B. The average decrease of the response amplitude was of 91.1% the control response in 150 min and with a recovery time of 300 min. Not only

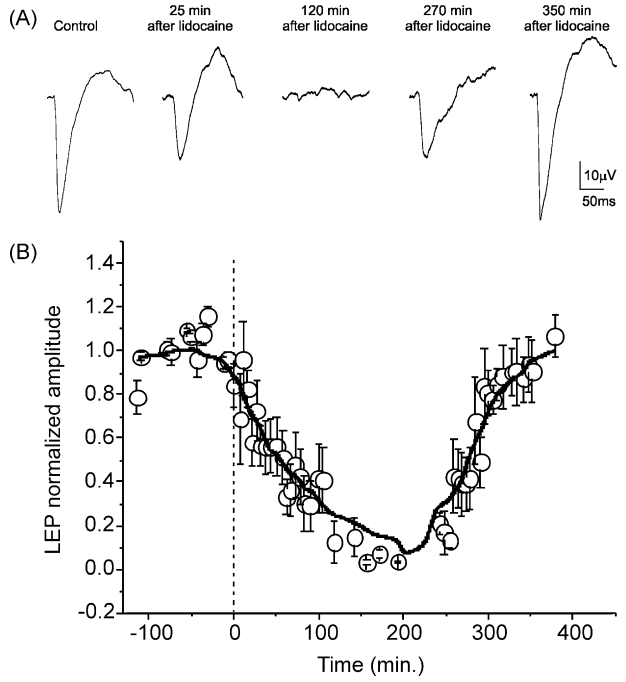

Fig. 3. Effect of topical application of a drop of 10% lidocaine (0.4 ml) to the whisker pad. (A) Waveform average of extracellularly recorded evoked response in control condition, after lidocaine application and during recovery. The stimulus parameters were constant and consisted of 5 ms duration and 5 psi pressure. Each trace represents the average of 60 stimuli occurring at 0.2 Hz. (B) Grand average of the time course of action of topic lidocaine ($n = 6$). Each point represents the average peak amplitude evoked by 60 stimuli per recording, therefore 360 responses in grand average. The sensory-evoked responses were normalized with respect to the average of the control response. The parameters of the air puff stimulation were the same in all cases (5 ms duration, 5 psi). The maximum blockage was achieved in approximately 150 min.

the peak amplitude decreased but also the downward slope of the evoked potential, that was of 2.1 ± 0.8 mV/s in control and slowed down to 0.9 ± 0.6 mV/s in lidocaine, showing afterwards a complete recovery (Fig. 3A).

Intensity of the stimulus *versus* amplitude of the stimulus-evoked response relationship was generated by random modifications of the air puff pressure delivered to the whiskers (5 ms duration; Fig. 4A). Such stimulus–response curve was carried out before and after application of 0.4 ml 10% lidocaine ($n = 6$). The amplitude of the sensory response was represented against the intensity of the stimulation. While the responses were noticeably reduced by this dose of lidocaine, the stimulus–response relationship was maintained in all cases at this dosage. Following the end of lidocaine action (>300 min), the stimulus–response relationship returned to control levels (Fig. 4A).

In order to rule out the possibility that the mechanical effect of a drop of lidocaine on the whisker's base could have an effect decreasing the sensory-evoked response, a drop of the vehicle of lidocaine, 32% ethanol, was applied ($n = 4$). Fig. 4B illustrates the average response ($n = 4$) to different intensities of a 5 ms air puff (0–38 psi). The stimulus–response curve was first obtained in control conditions (black squares), and then obtained again after the topical application of 0.5 ml of 32% ethanol (white circles). No significant difference was found subsequent to the application of a drop without lidocaine, therefore ruling out a mechanical effect.

In order to further test the effect of topical application of liquid lidocaine, we explored the response to one single whisker stimulation (5 ms, 10 psi air puffs) by trimming all the remaining ones ($n = 4$). The rest of the protocol was identical to the one described before. In all cases, 10% lidocaine (0.4 ml) was effective to block the stimulus-induced response. The average response to one whisker stimulation in control condition was 108.5 ± 12.9 μV. Following lidocaine application the response was reduced to an average value of 28.6 ± 4.9 μV, therefore a reduction of 73.68%.

To conclude, topical application of liquid lidocaine applied at the base of the whiskers induces a reversible blockage in barrel cortex sensory responses, and the recovery time can be achieved in approximately 3 h after the maximum effect. This characterization could be useful to induce a reversible blockage of whisker information in both anesthetized and awake preparations in order to explore changes derived from a short term somatosensory deprivation. Longer blockages would require repeated lidocaine applications.

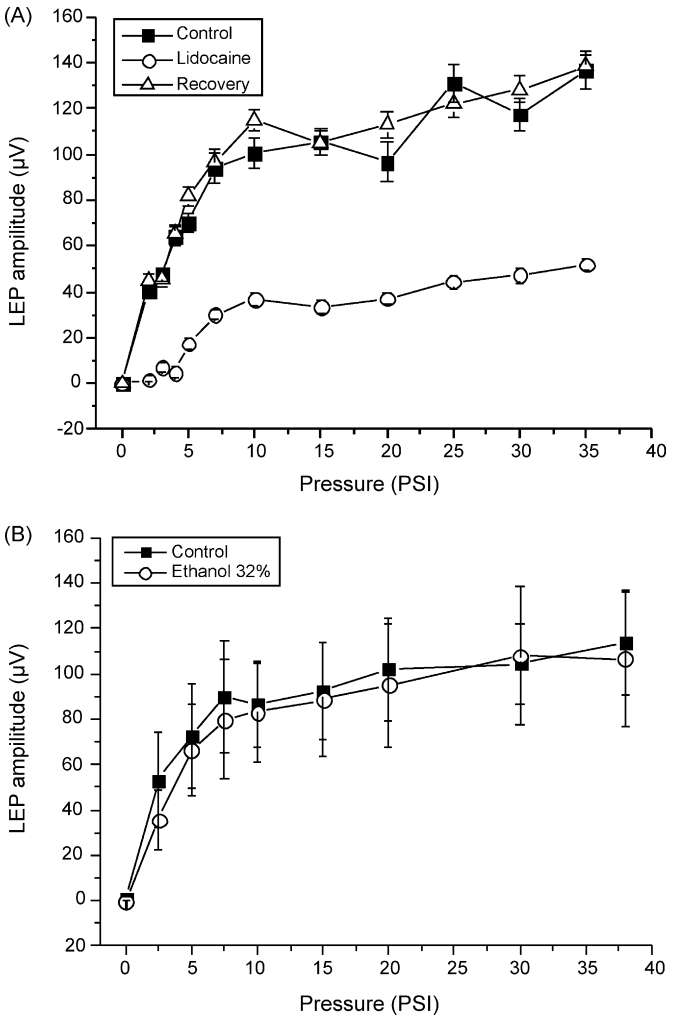

Fig. 4. Stimulus–response relationship in control and lidocaine. (A) Stimulus–response graph to an air puff of 5 ms and randomly varied pressure (0–38 psi). In this case the responses have been studied in control condition, following topical application of 0.4 ml 10% lidocaine on the whisker pad and again after recovery from lidocaine blockade (246 min later). Each point is the average of 60 stimulus that occurred with a frequency of 0.2 Hz. The error bars correspond to S.E.M. Similar results were obtained 6 cases. Notice that lidocaine effect is totally reversible, although it takes 4 h to recover. (B) Stimulus–response relationship in control and lidocaine vehicle (32% ethanol). An air puff of 5 ms duration and randomly varied pressure (0–40 psi) was tested first (control). Next, 32% ethanol was applied in a 0.5 ml drop over the whisker pad, while no significant difference in the response to the same stimulus was observed. This graph is the result of averaging the responses to the same stimulus and conditions in two different rats. Each point is the average of 60 stimulus that occurred with a frequency of 0.2 Hz. The error bars correspond to S.E.M.

4. Discussion

In this study, we explored a new paradigm that would temporarily deprive whisker information and would be appropriate for use in an awake animal. Previous studies in anesthetized animals have used whisker deprivation by subcutaneous injection of lidocaine (Faggin et al., 1997), a method that was found to be efficient. However, this procedure is invasive and probably too stressing to deprive somatosensory information from an awake animal. Apart from this method, the most common form of deprivation in awake animals has been the trimming of whiskers (e.g. Huston et al., 1986; Steiner et al., 1986; Lebedev et al., 2000; Grigoryan et al., 2005). Given that we were interested in a method that allows a short term deprivation (of hours) the vibrissotomia was not ideal, since it requires a certain growth time to recover.

Given these two constraints, we tested different forms of lidocaine application (injection, aerosol, cream, and liquid drop) to the whisker pad. To characterize this new technique, we recorded *in vivo* intracellularly and extracellularly in barrel cortex (S1) the response evoked by an air puff to the whiskers before and after the application of lidocaine. The different forms of application were all effective to temporally deprive somatosensory input, but presented some advantages and disadvantages. The injection technique is an invasive technique which is applicable only in anesthetized animal, but too stressing for the awake. Although the EMLA® cream (Juhlin and Evers, 1990) decreased the response in one out of two cases, the dose was not easy to adjust, plus the remaining dried cream could eventually affect whiskers movement. Aerosol application induced temporal tactile deprivation with the inconvenience of being too imprecise in the application area, given that the aerosol drops often stayed on the whisker's surface.

We found the application of a drop of liquid lidocaine to be the method with more advantages to temporally deprive somatosensory information: easy to apply, not stressing for awake animals, and a course of action that proved to be consistent in 6 cases (Fig. 3B). As we demonstrate in the results, the drop of lidocaine started to reduce the sensory-evoked response in a few minutes, it reached a maximum close to 100% blockage between 100 and 200 min and it was totally reversible after 5–6 h.

We ruled out the possibility that the effect of the lidocaine was due to a mechanical effect of the drop, since a drop of same or even larger volume of the lidocaine vehicle (32% ethanol) did not have an effect on the intensity/response curve. However, in the anesthetized animal we were not able to test what the effect of the different forms of lidocaine may have on the awake animal's whisking movements.

Interestingly, this method can be used in awake animals because it is easy to apply and it is not stressful for the animals (unpublished observations). Repetitive applications over time could provide longer blockages if necessary. By means of this manipulation, the role of whisker information in different cognitive tasks, or the plasticity induced by its short term deprivation, can be explored under experimental conditions involving electrophysiological recordings in awake animals and/or behavioural tasks.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jneumeth.2008.08.012.

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