Intracranial EEG analysis during spatial memory tasks: the role of high frequency oscillations

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Curs 2015-2016

Director: ADRIÀ TAUSTE CAMPO
GRAU EN ENGINYERIA BIOMÈDICA
Intracranial EEG signal analysis during spatial memory tasks: the role of high frequency oscillations

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Summary | Historically, declarative memory has been indirectly studied using spatial navigation experiments in rodents. Theories of hippocampal function expanded with the discovery of neural ensembles (place cells, grid cells) able to depict current spatial representations of the physical world. Place cell coupling to theta rhythm, or theta phase precession, first related neural firing to brain oscillations. Replay of past place cell sequences during short brain oscillations (sharp waves) established a basis for memory encoding after learning, but the involvement of grid cells in replay is unknown. We hereby present evidence that replay of rodent grid cells during the sharp-wave ripple complex in awake spatial navigation tasks is fairly low, contrarily to replay in place cells. Overcoming the technical limitations faced in this study will help to unveil part of the hippocampal contribution to memory processes. We also present a pilot study with an epilepsy patient, one of the first attempting detection of high frequency oscillations during cognitive processes. Future more task-design controlled research might help to identify behavioral correlates of high frequency oscillations and might possibly unveil their role in cognitive function and memory processes.

“Every day is alone in itself” – H.M.

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1 Introduction

1.1 Concept of memory

Memory, defined as “the mental capacity or faculty of retaining and reviving facts, events, impressions, etc., or of recalling or recognizing previous experiences”, is one of the most important and optimized abilities of the brain. The remarkable ability to selectively remember helps animals adapt to different environments and encode higher order abstract concepts. Memory influences our daily behavior and ultimately defines us as individuals. Nevertheless, the exact mechanisms underlying memory function are not well understood and memory remains one of the most intriguing areas of investigation in neuroscience.

The neural computations underlying remembering stimuli likely follow a general sequential process. First, one needs to encode the sensory information and place it in the memory space. Second, memory storage must reflect the amount of time the information is relevant. Third, one must then be able to access and retrieve the stored information. Classically, memory has been divided into three categories with regards to the retention time of information: sensory memory, short-term/working memory, and long-term memory (Fig. 1). Within long-term memory, where information can be retained up to a lifetime, there is generally a division between explicit/declarative memory, which is conscious (e.g. remembering your mom’s birthday, knowing what a mom is), and implicit/nondeclarative memory, which is unconscious (e.g. knowing how to drive your car or feeling anxiety when asked to present a seminar). Declarative memory, at the same time, can either involve episodic memory and semantic memory. Episodic memory is associated with individual experiences (e.g. seeing a dog for the first time), while semantic memory forms abstract concepts as a result of recurrently experiencing similar episodes (e.g. knowing what a dog is). Unsurprisingly, episodic and semantic memory have been proved to be intrinsically related\(^1\).

![Figure 1: Types of memory.](image-url)
The study of memory dates back to ancient Greece with Aristotle’s *Tabula rasa* concept. *Tabula rasa* refers to the epistemological idea that humans are born without mental content but that knowledge is the sum of our experiences. Philosophy governed the study of memory until the mid 1880s, when psychologist Hermann Ebbinghaus first developed a scientific experiment to test memory. The influence of behaviorist Karl Lashley’s experiments in the 1930s and 1940s contributed to the popular misconception that memory was a brain function widely distributed among brain areas. It was not until 1953 after neurosurgeon William Scoville performed a bilateral medial temporal lobe surgical resection on an epilepsy patient, H.M., that specific brain regions were implicated in mnemonic function\(^2\). Scoville caught the attention of the scientific community by reporting “a grave loss of recent memory”. The finding that H.M. could perfectly recall events before the surgical procedure, together with his intact ability to learn new skills (despite forgetting the actual learning episodes) and an excellent sustained or working memory, concluded that only his declarative memory had been impaired\(^3\). Subsequent research in both humans and H.M.-inspired animal lesion models implicated the hippocampal formation in normal declarative memory function and helped delineate the medial temporal lobe (MTL) memory system\(^4\). Furthermore, it refuted the contemporary Lashley’s ideas and established the theory of memory as we know it today: an organized and independent brain function. However, the neural mechanisms of memory encoding and storage were still not understood.

### 1.2 MTL memory system in spatial learning

MTL structures relevant for memory processes in the mammalian brain were first gathered together by Larry Squire and Stuart Zola-Morgan\(^4\). Later studies implicated the communication between regions based on both functional and anatomical links, which helped define the overall circuitry\(^5,6\) (Fig. 2).

To summarize, the hippocampal formation or hippocampus can be divided into dentate gyrus (DG), CA1-CA3 regions, and subiculum. The parahippocampal region, a transition area between the hippocampus and neocortex, contains the medial entorhinal cortex (MEC), the lateral entorhinal cortex (LEC), the presubiculum (PrS), and the parasubiculum (PaS)\(^6\). Bilateral projections between the hippocampus and the parahippocampal region are also present.

#### 1.2.1 Brain’s positioning system

The rapid growth of electrophysiological techniques and in particular single-unit (i.e. single neuron) recordings provided a unique opportunity to study memory function. With direct neurophysiological recordings, experiments no longer had to be based purely on cognitive function and behavioral responses. Researchers could now quantitatively study brain electrical activity through scalp or intracranial electrodes, and correlate such recordings with behavioral activity.

Beyond the clinical studies of H.M., MTL theories of mnemonic function expanded in 1971, when John O’Keefe and his colleagues designed a setup where intracranial recording micro-
Figure 2: MTL memory system in the rat brain. (a) Anatomical representation of the parahippocampal cortex and hippocampal formation and (b) circuitry model of their communication. Notably, there is a relative preservation of circuitry from rodent to human. Extracted from Moser, Edvard I and Roudi, Yasser and Witter, Menno P and Kentros, Clifford and Bonhoeffer, Tobias and Moser, May-Britt.6

Electrodes were placed in freely moving rodents. They noticed single neuronal units in the hippocampal CA1 subregion whose firing response depended on the animal's position in space, and were independent from any sensory stimulus7. Concretely, a single one of these 'place cells' fired only if the animal was at a specific position in space, known as a place field8 (Fig. 3a). Different place fields from different place cells covered the exploratory space, providing a neural basis for spatial orientation. Later, other cell types such as head direction cells9, 10 (whose firing depends on the head orientation; discovered by Jim Ranck and Jeffery Taube) and border cells11, 12 (firing along specific borders of the physical space) were reported, suggesting a high-level integrated network for spatial navigation.

Notably, a relation between place cell firing and hippocampal local field potential (LFP), specifically with the theta rhythm (~4-12 Hz), was first pointed out by John O'Keefe and Michael Recce in a 1993 paper13. Theta rhythm has a role in spatial navigation, through a mechanism called 'theta phase precession': place cell spikes at early phases of the theta oscillation represent earlier locations in space, and place cell spikes at later phases of theta represent future locations. Theta rhythmicity supplies a temporal code, a timing signal against which firing is measured. In sum, it is a robust example of coupling between a brain oscillation and neuron firing. Very recently, place cells have been reported to also couple to gamma oscillations at different frequencies (~25-150 Hz) (Lasztóczi & Klausberger, 2016, Neuron).

These important revelations motivated many spatial learning experiments aimed to find the
origin of the place cell signal. In one of these attempts, May-Britt Moser and Edvard I Moser’s laboratory isolated the CA1 subregion from the rest of a rodent hippocampus and observed the conservation of place cell firing, suggesting its signal was modulated by efferences outside of the hippocampus, in the entorhinal cortex\(^\text{14}\). After inserting recording electrodes in the medial entorhinal cortex (MEC), they localized a type of cell that also fired very regularly in dependence of the animal’s current spatial position\(^\text{15}\). Concretely, the response of those cells had a grid-like pattern, so that their firing occurred whenever the animal was placed on a vertex of the grid (Fig. 3b). They called these neurons ‘grid cells’.

![Figure 3: Place cells and grid cells’ firing responses. (a) Place cells have a single location of response (place field). (b) Grid cells have multiple firing fields organized in a grid-like shape.](image)

Recently, place and grid cell related activity has been identified in humans\(^\text{16,17}\). John O’Keefe, May-Britt Moser and Edvard I. Moser shared the 2014 Nobel Prize in Physiology or Medicine “for their discoveries of cells that constitute a positioning system in the brain”.

**1.2.2 Spatial navigation mechanisms**

The fact that the hippocampus had such an important contribution to spatial representations of the physical world called into question the relevance of the hippocampus in declarative memory processes. This gap between research inspired by patient H.M. experiments and studies motivated by spatial cognition was originally argued by John O’Keefe\(^\text{18}\), but general theories about hippocampus function did not account for a coexistence until recently. Based on the fact that the anatomical structures that allow declarative memories and spatial navigation are fundamentally the same (hippocampus and entorhinal system), it is now commonly argued that declarative memory evolved from primitive spatial navigation networks\(^\text{19}\).

Navigation involves two intricate processes working together to produce an accurate spatial representation: allocentric navigation and egocentric navigation. In short, allocentric navigation refers to a spatial map, which is context independent, where context means current
position or time. On the other hand, egocentric navigation depends on current position from a first-person viewpoint. An analogous division can be made in declarative memory, since semantic memory refers to context independent (or abstract) concepts and episodic memory contains the accumulation of subjective experiences in which time and space are relevant.

Furthermore, one can think that recurrent subjective exploration of a novel environment, and thus the accumulation of egocentric navigation episodes, can be then translated into a context independent (or allocentric) map. Similarly, declarative memory uses multiple episodic memory events to build a more abstract concept in the semantic space. Therefore, it is plausible to hypothesize that spatial navigation was the evolutionary basis of declarative memory, in which spatial memory is a subset.

Spatial navigation experiments have served as an indirect method to study declarative memory processes, such as encoding or retrieval. Spatial navigation experiments have multiple advantages: feasible experiment design for rodents, backing from many research validation studies and, since they treat the mammalian brain, conclusions may be later translated across species to models of human mnemonic function.

1.3 High frequency oscillations

Even though single neurons work with binary signals called action potentials or ‘spikes’, associated with sudden ionic current fluctuations, the sum of co-occurrent action potentials together with synaptic potentials and glial potentials can be recorded as continuous brain signals called extracellular field potentials or, more commonly, local field potentials (LFPs). The synchronous activity of spatially proximal units gives rise to brain oscillations (also called brain rhythms). Brain oscillations coming from large enough neural ensembles exhibiting simultaneous activity may be observed at a macroscopic scale with electroencephalography (EEG) techniques.

EEG was invented by a German neurologist in 1924, Hans Berger, when he used his ordinary radio equipment to amplify the brain’s electrical activity from the scalp. Berger was also the first one to depict brain waves and described “a rhythmic oscillation of potential at frequency of 10 cycles per second”. In 1934, Adrian and Matthews confirmed his experiments and together with Berger named the 8-12 Hz paradigmatic oscillation “alpha wave”. Since then, many experiments have supported Berger’s initial hypothesis that brain oscillations would change depending on the current cognitive states of the person. Generally, oscillations are characterized by their amplitude, frequency and phase. Concretely, different cognitive states are thought to generate oscillations of different frequencies. Traditionally, several brain oscillations have been commonly described in humans depending on their working frequency range: delta (0.1-3 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (>30 Hz). Brain signals with high amount of low frequency components are associated with a more relaxed or sleep state, whereas signals with high frequency components indicate alert or information-rich task processing states. Hierarchical cross-frequency coupling between brain oscillations provides a basis for complex processes and behaviors.
Initially, little attention was paid to components in higher frequency bands. Over time, every rhythm above 30 Hz has been referred to as high frequency oscillation. However, both the advent of digital EEG allowing broad-band frequency recordings (beyond 70 Hz that analog EEG gave) and findings in animal neurophysiology showing the existence of oscillations in high frequency ranges during cognitive tasks have resulted in growing interest about HFOs. New frequency bands have been described in the high frequency range, especially in rodents, although there's no common consensus about the cutoff frequencies of each band. The gamma band has been split into ‘slow gamma’ (∼30-90 Hz), ‘high gamma’ (∼90-140 Hz), ‘ripple’ (∼100-300 Hz) and ‘fast ripple’ (∼250-500) bands.

In order to study high frequency bands, intracranial EEG (iEEG) electrodes are normally used over scalp EEG electrodes. The scalp and the skull act as low-pass filters that attenuate frequencies over the beta and gamma ranges, prominently over 150 Hz. Furthermore, scalp electrodes provide poor spatial information, which is certainly an issue when studying deep structures such as the hippocampus. The increase in spatial resolution, added to the very good temporal resolution provided by EEG (up to MHz), comes with an increased invasiveness of the recording procedure.

1.3.1 HFOs in the rodent hippocampus: the sharp wave-ripple complex

In rodent hippocampal research, characteristic oscillations are described to be different from those in human scalp EEG. Low frequency bands theta and alpha are combined into rhythmic slow activity (RSA or simply theta, ∼4-12 Hz). In the high frequency range, the mechanisms of HFOs in the hippocampus are perhaps the best understood ones, and thus have grown in importance during the last decades. The hippocampus LFP of conscious rodents shows three main types of rhythms: theta rhythms, sharp wave-ripple (SPW-R) complexes and gamma rhythms. Since different behaviors generate a certain rhythm, each type of rhythm has different generation mechanisms and they are associated with characteristic neuron firing, they are thought to have different functions. Theta rhythm has been associated with several functions, from sensory processing to locomotion control, and has a very notable role in spatial navigation with the ‘theta phase precession’ mechanism. Gamma oscillations’ function is still unclear, but they have been proved to influence information processing flows. Sharp waves are thought to play an important role in memory consolidation, and will be hereby studied in more detail.

Sharp waves, previously thought to be artifactual, were formally characterized by György Buzsáki in 1982. They are large amplitude (1-3 mV), transient (40-100 ms) aperiodic waves of the LFP observed in the dendritic layer of the CA1 region during awake immobility, ‘consummatory’ (finished task) behaviors and slow wave sleep. Self-organized bursts of CA3 pyramidal cells’ population activity produce excitatory postsynaptic potentials (EPSPs) in the form of sharp waves that depolarize the CA1 region. The depolarization of the CA1 region commonly causes a short-lived ripple potential and discharge of the CA1 region. These SPW-
R complexes are propagated through the output networks of the hippocampus (subiculum, parasubiculum, and deep layers of the entorhinal cortex), where similar complexes may be observed (Fig. 4)\textsuperscript{37,40}.

![Figure 4: Bursts of activity in the CA3 region produce a sharp wave-ripple complex in the CA1 region of the rodent hippocampus. These sharp wave-ripple complexes may be observed in the output networks: subiculum ('Sub'), parasubiculum ('Para') and deep layers of the entorhinal cortex ('EC'). Reproduced from Buzsáki, György and Chrobak, James J, 2005\textsuperscript{40}.](image)

Different rhythms in the rodent hippocampal LFP have been shown to correlate with distinct behavioral states. During exploratory behaviors and rapid eye movement (REM) sleep, theta oscillations predominate in the LFP of the CA1 region. On the other hand, during consummatory behaviors theta activity is replaced by the so-called irregular large-amplitude activity (ILA), where SPW-R events occur together with sleep spindles and slow oscillations\textsuperscript{41,37}. Gamma oscillations occur during theta and ILA, but their amplitude is much more variable in non-theta states\textsuperscript{42}.

1.3.2 Replay and SPW-R complexes in memory consolidation

Related to the theta-SPW-R dichotomy, Buzsáki outlined a “two-stage model of memory trace formation”\textsuperscript{43} in 1989, thereby providing an influential theory about memory consolidation. In the first stage of memory formation or active learning, hippocampal circuits are thought to receive theta-synchronized input from the entorhinal cortex and their neurons are weakly potentiated during a transient period. In the second stage or memory consolidation, occurring during quiescent states, those previously stimulated neurons initiate the bursting that causes the sharp waves. Those neurons provide the output synchronization from the hippocampus to neocortical structures, where hippocampus-independent memories may be formed\textsuperscript{38}. Also, the synchronized bursting occurs at an optimal frequency for long-term potentiation mechanisms\textsuperscript{44,45}.

Crucially, sharp-wave events to be part of a memory consolidation process, they may need to carry encoded information from previously experienced events. Indeed, ‘replay’ events occur...
during SPW-R complexes\textsuperscript{46}. The basis of replay was established by Matthew Wilson and Bruce McNaughton in 1994, when they observed that ensembles of neurons that fired together during a spatial memory task were more prone to fire together during sleep epochs\textsuperscript{47}. Replay refers to the episode in which place cell firing sequences during theta activity or active exploration are reproduced during SPW-R activity or quiescent states (not only sleep). It was subsequently discovered that neural ensembles not only fire together, but also fire in the same order as they fired during the actual event\textsuperscript{48}. It has also been observed that reverse replay (sequence in reverse order) occurs during SPW-R activity after initial spatial learning, and forward replay (sequence in forward order) occurs in anticipation of reintroduction to a previously learned maze\textsuperscript{46} (Fig. 5). Reverse replay might occur because the most recent memories are the most spatially relevant when it comes to decision-making. Nonetheless, it showed that place cells not only can encode current spatial representations, but that they can also represent past and future positions in space for memory consolidation processes.

During SPW-R complexes, lasting about 40-100 ms, place cell replayed spike sequences are time-compressed by a factor of 10, meaning that they are reproduced at a faster timescale during SPW-R events than during the actual tasks\textsuperscript{37,49}. It has been also observed that interrupting SPW-R complexes by electrical stimulation after a spatial memory task impairs spatial learning\textsuperscript{50}, suggesting a causal role for SPW-R complexes in memory consolidation.

![Figure 5: Replay phenomenon of place cells during a running experiment in rodents. Reverse replay (sequence in reverse order) occurs during SPW-R activity after initial spatial learning (purple box), and forward replay (sequence in forward order) occurs in anticipation of reintroduction to a previously learned maze (red box)\textsuperscript{46}. Reproduced from Kamran Diba and György Buzsáki, 2007.](image)

Despite the numerous amount of studies investigating place cell replay, the role of grid cells in replay events and consequently in memory consolidation processes remains unclear\textsuperscript{51}. Studying the involvement of grid cells in replay events could also help unveil the origin of the place cell signal, which has been hypothesized to be created by linear combinations of different grid cells activity inputs, together with other spatially relevant cells (i.e. head direction cells or border cells)\textsuperscript{44}. In this project, we studied rodent grid cell firing during spatial memory experiments and resting periods, and developed a computational methodology to analytically characterize this process and classify grid cell firing during these periods.
1.3.3 HFO studies in humans

The preservation of SPW-R complex across mammalian species (Fig. 6)\textsuperscript{52} suggests a similar relationship between memory consolidation and SPW-R complexes in humans. However, both the novelty of HFO research and the invasiveness of iEEG recording electrodes have limited the amount of these studies. Nevertheless, epilepsy patients provide a unique opportunity to study HFOs during the iEEG monitoring phase they undergo for pre-surgical diagnostic assessment. Such monitoring phase (10-14 days) allows to better localize the epileptic focus and thus guide any potential future clinical intervention (e.g., resectomy, electrothermocoagulation)\textsuperscript{53}. In those cases, depth electrodes are only implanted to record clinically relevant brain sites within the hemisphere where the epileptic focus can possibly be found. Since most of the patients that are suitable candidates for surgery suffer from temporal lobe epilepsy, which involves the necessity to record hippocampal and parahippocampal regions, cognitive experiments during the monitoring phase might provide insight on the role of HFOs.

![Figure 6: Representative traces of SPW-R complexes that show conservation among species. Reproduced from Buzsáki, György, 2015\textsuperscript{41}, adapted from Buzsáki, György and Logothetis, Nikos and Singer, Wolf, 2013\textsuperscript{52}.](image)

Notably, since the beginning of HFO research in humans, two types of HFOs have been distinguished: physiological HFOs, which are thought to be the equivalent of those found in rodents\textsuperscript{30,54}, and pathological HFOs (pHFOs), which have been proposed as a biomarker for epilepsy\textsuperscript{55}. While pHFOs were first thought to cover the fast ripple frequency band (250-500 Hz)\textsuperscript{30}, it has been shown they also appear in lower frequency bands\textsuperscript{56}. In general, discriminating physiological from pathological HFOs is a challenging problem. However, it is commonly assumed HFOs occurring immediately after a stimulus presentation are physiological\textsuperscript{57}.

Recent studies have pointed out the relevance of HFOs in active memory processing both in non-human primates\textsuperscript{58,59,60,61} and humans\textsuperscript{62,63}. In this project, we further studied the involvement of HFO events in cognitive processing during spatial cognition tasks, and developed a methodology to quantify the involvement and modulation of human HFOs in a cognitive task.
1.4 Motivation and Thesis Objectives

The work presented was motivated from the aim to better understand the role of the hippocampus in spatial processing, and declarative memory processing in general, by developing and applying signal analysis techniques to intracranial recordings of both rodents and humans. The main goals, together with concrete objectives of this project, can be split in two:

1. Studying MEC grid cells of the rodent's MTL memory system in replay processes through the analysis of SPW-R events and neural firing in the awake state during spatial learning tasks. Our hypothesis was that grid cell activity would be increased during SPW-R events compared to general ILA activity. To better characterize this relationship, an algorithm to detect SPW-R events had to be created and post-processing computational methods had to be used to relate grid cell firing with replay events.

2. Studying the association of HFO events in humans during active cognitive processing and specifically during spatial decision-making experiments. Our hypothesis was that the number of HFO events would be increased during spatial planning tasks when compared to baseline (pre-task) activity. A detection algorithm was used to infer HFO events and post-processing computational methods were used to extract more specific task-related information.

A complete understanding of the mechanisms by which MTL oscillations underlie spatial cognition, together with the distinction of HFOs and pHFOs, may ultimately help people with neurological disorders or memory deficiencies such as epilepsy, amnesia or Alzheimer's.

1.5 Project

The project hereby presented is the result of two different workloads within a common field of research. The first project emerged from a collaboration between the Epilepsy Unit at Hospital del Mar and the Departament de les Tecnologies de Comunicació i Informació (DTIC) from Pompeu Fabra University, both placed in Barcelona, Spain. They were coordinated respectively by Alessandro Principe at Hospital del Mar and Adrià Tauste and Raphael Kaplan at DTIC. The second project was carried out during a full semester internship at the Center for Learning and Memory (CLM) at the Department of Neuroscience, part of the University of Texas at Austin, United States. This second project stems from a research line leaded by Prof. Laura Lee Colgin, and was coordinated by Prof. Colgin herself.

The author declares no external personal funding for the accomplishment of the project.

1.6 Acknowledgements

I would like to thank everyone who helped in the completion of this work either by direct counseling, material facilitation or emotional support.
2 Methods

2.1 Replay in grid cells of rodents

Animals and surgery. Three male Long Evans rats weighing approximately 350-500 g had been implanted for this study. Rats were labeled Rat-20, Rat-26 and Rat-29. All rats had been implanted 'hyperdrives' in superficial layers of MEC (Fig. 7A, B, C). The implanted device had 16 movable polimide-coated platinum-iridium (90–10%) tetrodes† made from 17 mm wire (California Fine Wire). Each tetrode recording channel was labeled with a number. Input impedances at the electrode tips had been reduced to ∼300 kΩ at 1kHz by using platinum coating. The screws fixing the skull had been secured with cement.

After surgery, rats were housed individually in custom-built acrylic cages (∼40 cm × 40 cm × 40 cm)64. Cages contained enrichment materials such as plastic balls and cardboard tubes to ensure fast surgical recovery. Rats were let to fully recover from the surgical procedure before running the experiments. During this period, tetrodes were gradually lowered until they achieved the their target positions at superficial layers of MEC. Histological images post-surgery confirmed target locations of the electrodes (Fig. 8).

All experiments were conducted according to the guidelines of the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals under a protocol approved by the University of Texas at Austin Institutional Animal Care and Use Committee.

Recording. The implantation methodology used allowed in vivo chronic neural probing. Integrated Neuralynx data acquisition system together with Cheetah software (Neuralynx, Bozeman, MT) were used to obtain 0.1-500 Hz band-pass filtered continuous iEEG recordings during experiments at a sampling frequency of 2,000 Hz. Single-neuron activity was recorded at 30 kHz, then band-pass filtered between 600-6,000 Hz and stored as a spiking times vector. Light-emitting diodes (LEDs) were used to track the animal’s movements and positions at 30 Hz during the tasks65.

Experimental design and protocol. Rats were housed on a reverse light-dark cycle (lights off from 8 a.m. to 8 p.m. and lights on from 8 p.m. to 8 a.m.); behavioral sessions took place during the dark phase. During the data collection period, rats were food-deprived to 90% of their free-feeding weight64. Rats were pre-trained to run on an elevated 2 m linear track before surgical implantation.

After recovering from surgery, rats resumed behavioral training, which consisted of three 10-min sessions per day on a linear track (2 m long, 10 cm wide, and 64 cm above the floor). Rats were trained to run back and forth on the track, as described previously67. Rats were rewarded with small pieces of sweet cereal or cookies at both ends of the track. Before data acquisition began, rats were trained on the track for at least 3 days to ensure environmental familiarity. Each recording session, of 10 min duration, was followed by a 10 min rest session.

†Tetrode: set of four small (< 30 µm ø) electrode wires bundled together used in intracranial electrophysiological recordings. Tetrode recording turns out to be helpful for spike sorting and clustering tools.
During each rest session, rats were placed in a towel-lined, elevated flower pot. Each day, three running sessions and three resting sessions were recorded. Rat-20 had 7 recording days in total, while Rat-26 had been recorded 8 days and Rat-29 had been recorded 3 days in total.

Data Conditioning. All neuron spikes retrieved from the continuous iEEG signal do not come from the same cell. Spike sorting and clustering techniques (Fig. 7D) help to associate recorded action potentials to one neuron or another based on several parameters of the action potential waveform. A graphical classification software, together with autocorrelation and cross-correlation measures had been used in this case (MClust; A.D. Redish, University of Minnesota, Minneapolis). The parameters used, namely waveform amplitudes, peak-to-valley ratios and energies, created a multi-dimensional space where the graphical software could operate. Some other additional restrictions were applied to the action potential waveform to
Figure 8: Post-implantation histology image of Rat-26 MTL memory system. The tetrode insertion at the medial entorhinal cortex (MEC) was marked for clarification.

ensure its authenticity\textsuperscript{65}.

Among the found neurons in the recording space and their associated spikes, provided within a spiking times vector, grid cells needed to be detected. These neurons had already been detected for the project using gridness scoring and bursting properties, among others, but can be easily distinguished based on average firing rate and its dependency on position. By filtering velocities under 5 cm/s, and removing the extremes of the linear track (i.e. ensuring the animal is in non-resting state and there is no chewing interference), clear grid cell fields appear (Fig. 9). It is clearer when the animal moves through an open field, but we could still see them. Another interesting feature of grid cells is they are highly directional, so that they only fire when the animal is moving in a certain direction in space. Grid cells were named after its associated continuous recording tetrode channel number and then they were given a unique number identifier (e.g. ‘TT12-1’ referred to the first grid cell of tetrode channel 12).

Data was given as the clustering software output it. Only linear track running sessions were relevant for the posterior analysis; resting sessions were not used. Inside every animal’s folder, inside every date folder there was a text formatted file (.txt) with the names of the grid cells found for that specific date. From grid cells’ names, for every session on that date, one had to load the associated continuous recording (.ncs type of file named after the associated tetrode channel, e.g. ‘CSC12’) and the spiking times vectors of the nearby cells. Also, every session had the position of the animal recorded in a .nvt file, that also was relevant to us. All these were loaded to MATLAB variables by using Neuralynx-MATLAB interfacing scripts provided by CLM. The loading was made semi-automatic by making the script capable of reading grid cells’ names on the text file and loading the respective data. Continuous iEEG data was con-
Figure 9: Firing rate over time and distance of the recorded neurons reveals grid cell fields. (Above) Position of the animal in the linear track over time (sampled at 30 Hz; black dots); spiking times of recorded MEC grid cells have been superposed onto the trajectory. Directionality of cells may be noticed. (Below) Grid cells’ frequency of firing depends on the position in the linear track. Grid fields can be appreciated in 1-D.

Data Analysis. Data was analyzed using custom software written in MATLAB (MathWorks, MA), unless indicated otherwise. Specific analysis methods are described in detail below.

The continuous wide-band iEEG signal was band-pass filtered in the ripple band (100-250 Hz) using a 4th order Butterworth filter. A root mean square (RMS) power vector of the band-pass filtered signal was computed using an overlapping moving window of 10 ms duration and 5 ms step. One RMS value was obtained at each window step. The RMS value of a discrete signal vector \( \mathbf{x} \) of length \( N \), \( \mathbf{x} = (x_1, x_2, x_3, \ldots, x_N) \), is defined as

\[
\mathbf{x}_{\text{rms}} = \sqrt{\frac{1}{N} \left( x_1^2 + x_2^2 + x_3^2 + \ldots + x_N^2 \right)}.
\]

(1)

SPW-R events were detected by means of an automatic thresholding algorithm that marked a sharp wave at the MEC whenever the RMS power exceeded 3 standard deviations above the RMS mean power\(^{68}\). Events with interevent distance of less than 20 ms were joined together.
Events with overall duration of less than 20 ms were discarded.

To ensure SPW-R complexes were detected during consummatory behaviors (non-theta periods), several layers of robustness were applied to the algorithm: (1) only selecting sharp-wave epochs at the extremes of the linear track, (2) filtering out intervals considerably high velocities of the animal, and (3) filtering out intervals with high theta-delta ratio.

First, only SPW-R detections at the extremes of the linear track were selected. The bi-dimensional position vector \((x,y)\) was projected onto a single-dimension vector that represented the 1-D position on the linear track. Only linearized positions below 30 cm and above 150 cm, the extremes of the running track, were relevant for the analysis. A velocity vector was then derived by differentiating the position vector over the time vector. Since during-task or theta periods are incompatible with SPW-R activity, data recorded when the animal had a speed of above 5 cm/s was also discarded.

Theta activity intervals were also automatically detected by computing the theta-delta ratio \((\theta / \delta)^{69}\). A non-overlapping Hamming window of duration 2.0 s was used to compute the spectrogram of the wide-band iEEG signal, and then the ratio between the theta (5-10 Hz) and delta (2-4 Hz) power bands was computed at every window step. Only epochs where the theta-delta ratio was 1 standard deviation below the mean were considered non-theta or SPW-R activity, and thus consequently kept for the analysis. Visual inspection was used to determine the beginning and end times of the SPW-R complexes. Visual inspection was also needed in some cases to discard remaining theta epochs. An algorithm using Wavelet Analysis was implemented to detect the sharp wave deflection in the wide-band iEEG trace derivatives, but was later suppressed as its contribution to robustness was fairly low. The continuous iEEG trace was 4th order Butterworth band-pass filtered between 0.01 and 500 Hz for visualization purposes.

Firing of grid cells was compared between periods of running, that were assumed as theta activity, and during SPW-R events. Firing rate is defined as

\[
v = \frac{n_{sp}}{T}
\]  

(2)

where \(n_{sp}\) is the spike count in a window of time and \(T\) is the duration of the window. Running epochs were detected when the animal was located at the center of the linear track (i.e. linearized distance above 30 cm and below 150 cm) and had a speed above 10 cm/s. Running epochs of 1 s duration were selected, and the firing rate of the grid cells during these epochs was computed by simply counting the spiking events during the epoch. SPW-R epochs were taken from the previously described automatic thresholding algorithm, so that the onset of the ripple fell just in the middle of the epoch and those lasted for 1 s as well. The firing rate of the grid cells during SPW-R epochs was also computed by counting the spiking events in those time windows. The maximum number of theta epochs and SPW-R epochs were selected for comparison. A box plot was used to illustrate the firing rate comparison between periods of theta activity and periods of ILA or non-theta activity, as well as concrete periods of SPW-R
within ILA.

**Code availability.** Neuralynx interface for MATLAB may not be provided. MATLAB code for the detection of SPW-R events and replay analysis is available upon request.

## 2.2 HFOs in the human brain

**Patient and surgery.** Patient with initials B.M., C. underwent an implantation surgery procedure for chronic epilepsy monitoring and eventual diagnosis on July 7, 2015 at the Epilepsy Unit of Hospital del Mar in Barcelona. Only the right hemisphere, responsible for the epilepsy focus, was implanted. A total of 14 electrodes were inserted at different depths, and they overall covered the MTL memory system, including the hippocampal complex, as well as other regions at the occipital, parietal and frontal lobes (Fig. 10).

![Figure 10: Implantation three-dimensional model (left) and scheme (right) for patient BMC. All electrodes were placed at the right hemisphere, and covered the MTL memory system, including the hippocampal complex, as well as the occipital, parietal and frontal lobes. Electrodes ‘D’ and ‘H’ were more superficial and thus are not shown in this section image.](image)

A virtual three-dimensional model of the implantation scheme was created using the post-surgical MRI data and a modeling software (3D Slicer, [www.slicer.org](http://www.slicer.org))\(^7\). Each electrode was labeled with letters, namely A, B, C, D, E, F, G, H, K, L, O, P, Q and R. Each electrode had 15 recording contact channels numbered 1 to 15 from the deepest inserted to the most superficial
one, respectively. Contacts were labeled with the electrode name and the contact number, e.g. A1 for the deepest contact of electrode A. Contacts were equally spaced 3.5 mm between one another.

**Recording.** The recording session to be analyzed, comprising the cognitive experiment, started on 07/30/2015 at 06:58:16 and lasted until 07/31/2015 at 07:36:27. The cognitive experiment itself started on 07/30/2015 at 15:53:59 and lasted until 07/30/2015 at 16:25:59, with an approximate duration of 32 minutes. iEEG data were recorded using NeuroWorks software (Natus Medical, San Carlos, CA, USA) at a sampling rate of 500 Hz and subsequently stored at the hospital file system. The task was performed using a standard computer screen for images input and three keys from a standard computer keyboard for user interfacing and response. The image presentation onset at the computer screen was given by a Transistor-Transistor Logic (TTL) electrical signal of an Arduino system. This so-called trigger signal was at the same time recorded by the EEG amplifier as if it were an extra contact (at channel R10), so as to synchronize iEEG and the cognitive experiment. The trigger signal had a DC voltage component and marked trial events by a deflection in amplitude of roughly 1.5 mV. From a total of 209 available channels, only 125 and the trigger channel were set for actual recordings.

**Experimental design and protocol.** The decision-making task hereby used was a very pilot study with the purpose of inducing HFOs through maze presentation trials. Despite the participant responding most of the time (85.31% of the time), the fact that this task turned out to be too difficult (46.72% correct when answering, 39.86% overall correct), together with the limited amount of trials after artifact removal, impaired the possibility of studying behavioral correlates beyond performance (correct/incorrect). Nonetheless, it set a basis for future more task-design oriented research addressing behavior.

The participant was asked to perform 143 trials of a spatial decision-making task in the same day (07/30/2015, session lasted from 15:53:59 to 16:25:59). Those trials had a total maximum duration of 6 s and came up joined in several blocks of variable amount of trials (1 block of 10 trials, 1 block of 53 trials, 4 blocks of 20 trials); the time distance between blocks of trials ranged from 0 s to 0.5 s. Each trial was designed by Raphael Kaplan and consisted of an ordered sequence of image presentations, marked by the trigger signal, hereby detailed and graphically represented in Fig. 11 and Fig. 12:

1. **Baseline image (0s-1.0s).** An image consisting of a small centered orange cross on top of a dark background was presented to the participant, in order to record baseline iEEG activity. This fixed image had a duration of 1.0 s and marked the onset of the trial (0 s).

2. **Allocentric view of maze image (1.0s-4.0s).** Just after the baseline image, a top view image of a virtually designed maze was presented. The maze had been designed on a black and white grid and had a circuit enclosed by blue walls. Additionally, two squares

† Allocentric: linked to a reference frame based on the external environment and independent of one’s current location in it.
(red and green) were present inside the maze. The red square represented the position of the user inside the virtual world; the green square symbolized the maze goal. The position of the squares, as well as the maze design, changed from trial to trial. The height of the objects, given it was a top view, could not be distinguished from this image. This image stayed fixed from +1.0 s to +4.0 s from the baseline image presentation time.

3. **Orange circle on allocentric view (4.0s-4.5s).** Immediately following the allocentric view of the maze, another allocentric view of the same maze was presented. This time, the view was from the same side but had been tilted so that the three-dimensional perspective of the maze could be appreciated. On top of this image, an orange circled appeared. The orange circle determined the following image, and could either appear directly on top of the user virtual position (Fig. 11) or at another place of the maze in between the user virtual position and the maze goal (Fig. 11). This tilted image together with the orange circle lasted for 0.5 s and stayed fixed from +4.0 s to +4.5 s from the baseline image presentation time.

![Image 1](image1.png)

![Image 2](image2.png)

**Figure 11**: Single trial sequence: (1) Baseline image (0s-1.0s), (2) Allocentric view of maze image (1.0s-4.0s), (3) Orange circle on allocentric view (4.0s-4.5s), (4) Egocentric view of maze image and response (4.5s-6.0s). In this example, the orange circle appeared directly on top of the user position in the virtual maze (red square). The correct response in this case is “Left”.

4. **Egocentric view of maze image and response (4.5s-6.0s).** An egocentric view of the maze was shown in dependence of the orange circle that had appeared during the +4.0 s to +4.5 s time frame; this meaning that the egocentric view was shown wherever the orange circle had appeared in the previous tilted maze image. From the time the egocentric view

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†Egocentric: linked to a reference frame based on one’s own location within the environment.
Figure 12: Single trial sequence: (1) Baseline image (0s-1.0s), (2) Allocentric view of maze image (1.0s-4.0s), (3) Orange circle on allocentric view (4.0s-4.5s), (4) Egocentric view of maze image and response (4.5s-6.0s). In this example, the orange circle did not appear on top of the user position in the virtual maze (red square), but in between the user position and the maze goal (green square). The correct response in this case is “Right”.

of the maze appeared on the screen, the participant was able to give a response using the keyboard. While the duration of the previous image presentations (steps 1-3) was fixed (1 s, 3 s and 0.5 s, respectively), the duration of this last image depended on the response time of the participant. When the participant responded, the egocentric view image of the maze disappeared, the trial was considered over and the baseline image from the next trial appeared on the screen. If there was no response from the participant in the +4.5 s to +6.0 s time window from the baseline image presentation, then the trial ended and the baseline image from the next trial appeared on the screen.

With this experimental setting, the participant had to respond which was the optimal direction to follow, this is, the direction that would require less distance to get to the maze goal. There were three possible balanced responses, “Forward”, “Left” and “Right”, that the participant had to indicate using a single keyboard key previously learned. The trials in which the participant did not respond were marked “NaN”. Then, the responses were compared to ground truth data and a binary vector was constructed so that “0” referred to an incorrect response and “1” indicated a correct response. A part from the response vector, other variables were collected for each trial. Among these variables we had response time (measured in ms from the egocentric maze view presentation (+4.5s), maze novelty (measured with whether it was the 1st, 2nd, ..., n-th time the maze had appeared) or maze difficulty (measured in different ways, but for example whether the orange circle appeared at the red square, ‘1’, or further in the maze, ‘2’). A total of 53 different mazes were presented during the experiment.
Data Conditioning. One of the most challenging parts of the project was creating an integrated, automatized data conditioning pipeline for the occasion that may serve in future experiments. The cognitive task left multiple sets of data (iEEG with trigger signal, contact labels, collected trial variables) that needed to be read and joined altogether for posterior data analysis. The collected trial variables from the experiment were provided in a 143 row spreadsheet, with one row per presented trial. The intracranial EEG data was exported from NeuroWorks to a text file format (Fig. 13). The associated recording contact labels had been written in a spreadsheet. Custom MATLAB scripts able to interpret these data were written for every case. A complete MATLAB dataset (.mat file), compatible with both EEGLAB\textsuperscript{71} and Chronux\textsuperscript{72} toolboxes was obtained in the end.

Once the iEEG data was properly stored, bipolar difference signals were computed by subtracting neighboring electrodes, this is, differentiating the iEEG matrix over the channels dimension. Non-relevant subtractions such as ‘B1-A15’ were removed from the matrix, leaving 106 significant channels from the first 125 recording ones. After the bipolar signals were obtained, they were normalized by subtracting their mean and dividing them by their standard deviation. Then, they were high-pass filtered at 1 Hz and low-pass filtered at 245 Hz using Butterworth 4-th order filters. Also, power line interference at 50 Hz and its harmonics were great contributors to signal noise. An Infinite Impulse Response (IIR) band-stop notch filter of 40-fold attenuation at the -3 dB frequency was designed. Signals were notch-filtered three times at 50, 100, 150 and 200 Hz.

Continuous iEEG signals for every bipolar channel were automatically split, using several scripts, into epochs marked by the trigger channel. Deep channels falling nearby the MTL...
memory system (namely 'B2-B1', 'B3-B2', 'B4-B3', 'C2-C1', 'C3-C2', 'C4-C3') were paid special attention. First, trigger events were detected using a thresholding algorithm. Then, those events were labeled as ‘TTL+Start’, ‘Allocentric’, ‘OrangeCircle’, ‘Egocentric’ sequentially, referring to those relevant presentation times of each trial. Then, collected trial variables were loaded and only certain blocks of trials were selected for the posterior analysis (making a distinction between correct and non-correct, for example).

**Data Analysis.** Data was analyzed using custom software written in MATLAB (MathWorks, MA), unless indicated otherwise. Specific analysis methods are described in detail below.

Chronux toolbox was used to compute trial-averaged time-frequency spectrograms of the previously selected trial blocks saved into a data set. Chronux uses the multi-taper method from David J Thomson that overcomes some of the limitations of the Fourier Transform for small number of samples (trials in our case). It computes the power spectral density using a number of tapers and a moving window. The parameters used were: moving window of 0.25 s duration and 0.02 s step, 4 Hz spectrogram bandwidth, 2 tapers, no padding and trial-averaged (movingwin = [0.25 0.02], params.tapers = [movingwin(1)*4 2], params.pad = -1, params.trialave = 1). The mean frequencies along time were computed from the Chronux output spectrogram matrix. Then, this spectrogram matrix was divided at every time point by the mean frequency vector, to see relative deviations from the mean along time. The common logarithm of the resulting spectrogram was computed to reduce power difference changes and see spectral changes at high frequency bands.

This first result was compared with an HFO detection method based on Hilbert transform applied on independently filtered frequency bands and developed by Mayo Clinic researchers in Kucewicz et al., 2014. Following that method, each individual trial was Butterworth 4-th order bandpass filtered for every 1 Hz bands between 50 and 500 Hz. These filtered bands were then z-scored individually and joined in a matrix, to be graphically represented in sort of a time-frequency spectrogram, where at each frequency $f$ [Hz] column there was a time series filtered between $f-0.5$ Hz and $f+0.5$ Hz. These time-frequency spectrograms obtained for every trial were binarized by thresholding at a z-score of 3.0 to detect significant signal changes, assumed HFOs, and joined together in a cumulative HFO detection plot along previously selected blocks of trials.

**Code availability.** DARPA function for HFO detection from Mayo Clinic cannot be provided. The MATLAB code for data conditioning and alternative HFO detection method, as well as post-processing code are available upon request.
3 Results

3.1 Replay in grid cells of rodents

Results shown here were from Rat-26, day 2013-08-01, all activity sessions. Tetrode 12, which presented two different grid cells with similar firing fields (‘TT12-1’ and ‘TT12-2’, see Fig. 9) was paid special attention to assess firing between grid cells.

The SPW-R detection algorithm hereby used has been widely validated through literature, but a detection example is shown in Fig. 14 and compared with a non-detection theta rhythm example in Fig. 15. afterwards.

Figure 14: SPW-R events detection. Continuous iEEG trace showing a sharp wave (middle) and bandpass filtered trace in the ripple band (100-250 Hz, bottom). Activity in the ripple band above the RMS adaptive threshold (black dots) marked the occurrence of a SPW-R event. No grid cell firing (top, blank) was present during the event. Amplitude is in arbitrary units.

Although ripple events are detected at 3 standard deviations from RMS mean, the onset of sharp waves is considered to be at the immediately previous 1 standard deviation crossing, and
thus earlier in time. They can be easily characterized by a fast negative deflection and ripple activity.

The SPW-R event detection algorithm was relevant to compare periods of sharp waves activity and periods of theta activity in terms of grid cell firing. The paradigmatic case, illustrated in Fig. 14 and Fig. 15, grid cell firing was low or non-existent during periods of sharp wave activity and relatively high during periods of running or theta activity.

Figure 15: Theta activity during running. Continuous iEEG trace (middle blue) with overlapped low-frequency activity showing a clear theta rhythm (1-15 Hz, middle black). Ripple activity (bottom) was consistently not detected as a SPW-R event. Grid cell firing was highly present during this time interval (refer to same cell labeling as Fig. 9). Amplitude is in arbitrary units.

The proper quantification of these results was done using box plots, shown in Fig. 16. In order to have more data in the comparison, specially SPW-R event detections, we joined data from all three activity sessions during the same day, overall considered invariant based on individual box plots of the activity sessions. Measures such as the Difference Betweenen
Figure 16: Each plot shows the firing rate comparison between theta (left), irregular large activity (ILA) or non-theta (middle) and periods of SPW-R events (right) of two near grid cells along the three sessions. Similar number of epochs for each type of activity were selected to enable comparison.

Medians (DBM) as a percentage of the Overall Visible Spread (OVS) were used to determine the possibility of a significant difference of firing rates between periods of theta activity, periods of non-theta activity (ILA) and SPW-R epochs. DBM is computed by subtracting medians of two distributions or boxes and OVS is computed by subtracting the upper limit of the highest distribution and the lower limit of the lowest distribution. Given our sample size (∼50 epochs), we can say there ‘tends to be’ a difference between overlapping distributions with overlap between medians from DBM/OVS >33%. Also, we can affirm there is a difference if spreads of the distributions do not overlap.

Box plots show three things. First, although we cannot completely affirm there is a difference in firing between theta activity and ILA, there tends to be a difference in firing between theta activity and ILA in both grid cells (TT12-1, DBM/OVS = 44.4%; TT12-2, DBM/OVS = 33.3%). Second, there certainly is a difference in firing between theta activity and periods of sharp waves in the first grid cell (no overlapping distributions) and it is likely there is a difference in firing between theta activity and periods of sharp waves in the second grid cell (DBM/OVS = 66.7%). Third, there is a significant difference in firing between ILA and concrete periods of SPW-R events in the first grid cell, whereas it is likely possible that there is a difference in firing between ILA and periods of SPW-R events in the second grid cell (DBM/OVS = 66.7%).
3.2 HFOs in the human brain

Results are shown in Fig. 17 and Fig. 18. Results from the Chronux analysis were plotted together with results from the Hilbert transform-based HFO detection algorithm. Then, cumulative HFO counts along trials were binned along time independently of frequency.

Figure 17: HFO detection scheme in humans using correct trials. (Top) Results of the Chronux toolbox spectrogram method averaged along trials. Maze presentation is marked with a black vertical line at 1 s. (Middle) HFO cumulative counts along trials using the Hilbert transform-based method. (Bottom) Cumulative counts were binned independently of frequency band and plotted as a histogram along time. Significance line was placed 3 std above HFO count before maze presentation. Maze presentation is marked with a red line.

Representative results for the block of correct answers (Fig. 17) show a significance line crossing after the maze image presentation, suggesting HFO inducement by the stimulus onset. The significance line represented 3 standard deviations above the HFO count mean in the baseline (fixation cross) period. Contrarily, representative results for the block of non-correct
trials (both not responded and incorrect, Fig. 18) do not show the same significance line crossing but the HFO count was maintained at the baseline count level along the trial. Four out of the six analyzed channels (66.67% of the channels) showed this same situation ('B2-B1', 'B3-B2', 'C3-C2', 'C4-C3').

Figure 18: HFO detection scheme in humans using noncorrect trials. (Top) Results of the Chronux toolbox spectrogram method averaged along trials. Maze presentation is marked with a black vertical line at 1 s. (Middle) HFO cumulative counts along trials using the Hilbert transform-based method. (Bottom) Cumulative counts were binned independently of frequency band and plotted as a histogram along time. Significance line was placed 3 std above HFO count before maze presentation. Maze presentation is marked with a red line.
4 Discussion and conclusions

4.1 Replay in grid cells of rodents

We hereby assessed grid cell firing during SPW-R events as an indirect measure of grid cell involvement in replay. Results show, not accordingly with first studies in literature about the topic (H Freyja Ólafsdóttir, Francis Carpenter and Caswell Barry, 2016 \(^{51}\)), that superficial layers’ grid cells are probably not driving sharp waves or the origin of place cell signal, since its firing is significantly lower (almost non-existent) during periods of SPW-R events. This is consistent with the current thought that sharp waves are instead generated in the CA3 hippocampal region\(^{37}\). Nevertheless, the hereby presented results were considered just an additional task, focusing more on the robust and semi-automatized development of the proper data conditioning and analysis methodology.

The fact that only a few grid cells were analyzed in these first studies, including this work, creates a need for more similar research to obtain a complete image of the underlying mechanisms of replay. Now, as said before, replay detection was done in an indirect way, by detecting SPW-R events. Since it can not be stated that whenever there is a SPW-R event there is evidence of replay, a method to distinguish both cases is needed. A common method to detect replay is to evaluate the place cell signal. However, the low density of grid cells in the MEC (\(\sim 20\%\) of cells), in contrast with the high density of place cells in the CA1 area (>50\% of cells), causes the experimenter to direct all the tetrodes to MEC, and thus leaves the follow-up analyses unable to detect place cell replay. Thus, perhaps the most important limitation of these and future studies is technical, and refers to the inability to simultaneously record iEEG signal from both place cell and grid cell regions.

Also, another limitation would be the amount of available grid cells per session to analyze. Having more simultaneously recorded grid cells would provide a more feasible scenario to assess replay, by possibly finding firing sequences in a significant order and evaluate their significance using shuffling methods. The fact that very few grid cells are detected in each session makes the analysis of replay even more complicated.

4.2 HFOs in the human brain

The most importance of HFO detection in the human brain workload has been put into the methodology development (data conditioning, data analysis) and not so much focused on results.

Perhaps the most important limitation of this study was the presence of external artifacts, that left us with few trials after artifact removal techniques. ICA algorithms of EEGLAB were used firstly but didn't result effective since they filtered out most of the trials. Visual inspection was finally used to filter out spurious trials.

Another limitation of this study, replicated among other human studies, is the fact that interictal pHFOs might be present during the experiments, since the implanted electrodes have been placed in the epileptic hemisphere.
An interesting area of future investigation would be to evaluate the inter-patient variability in terms of HFO both number and occurrences in time, but the fact that epilepsy monitoring and thus electrode implantation schemes are different in each patient make it difficult for an inter-patient study to succeed at this point.

4.3 Conclusions

The role of the hippocampus in memory consolidation during the awake state has been increasingly studied in recent years. Replay within SPW-R events of place cells has been established as a fundamental and robust pattern of spatial memory encoding, but the role of grid cells in place cell signal generation and replay is not yet understood. Here, we present evidence that grid cells are not involved in SPW-R events and thus they are probably not driving the sharp wave signals. Future analysis of grid cells during awake replay, overcoming the presented limitations, might unveil part of the hippocampal contribution to memory processes.

Human studies consistently show the involvement of HFOs in cognitive processing during spatial decision-making tasks. In this pilot study, which was one of the first attempting HFO detection in humans, spatial decision-making task was used to detect HFOs after a cognitively demanding image presentation. We present evidence of increased HFO count at the onset of trials at MTL memory system relevant channels in deep contacts targeting the hippocampal region. Future more task-design controlled research will provide enough data to identify behavioral correlates of HFOs and might possibly unveil the role of HFOs in cognitive function and, ultimately, memory processes.
References


