With the following project proposal, “Neuronal mechanisms of associative memory formation in the human medial temporal lobe”, I applied for the Walter Benjamin Programme of the German Research Foundation on 2020/02/01. Information about this program can be found at this website.

The application was approved on 2020/06/19.

Project Description – Walter Benjamin Programme

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Neuronal mechanisms of associative memory formation in human medial temporal lobe

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1 Project Description

1. Starting Point

1.1 State of the art and any preliminary work

1.1.1 Overview and broader impact

Associative memory is the ability to learn and remember novel associations between unrelated items[1]. Humans are adept in forming and retrieving associative memories. For example, when exploring an unfamiliar city, we are able to quickly interconnect objects (such as a famous sight) with their local spatial environment (such as a park).

In search of the neuronal mechanisms underlying the establishment of associative memories, this research project will test the key hypothesis that associative object-location memories are created by simultaneous reactivation of object-responsive cells (coding for specific objects) and spatially-modulated cells (representing the corresponding spatial context) during sharp wave-ripples[2] (SPW-Rs). SPW-Rs are a neural network phenomenon associated with enhanced transient excitability in the hippocampus and connected brain regions. Given that SPW-Rs orchestrate the induction of synaptic plasticity[3], such synchronous reactivation of object-responsive and spatially-modulated cells during SWP-Rs may lead to the establishment of neural circuits encoding the entire associative object-location memory.

I will test this key hypothesis by means of direct electrophysiological recordings from the human medial temporal lobe (MTL) of epilepsy patients who are implanted with intracranial depth electrodes for diagnostic purposes. These recordings provide the unique opportunity to investigate the neural foundations of human cognitive functioning at a temporal and spatial resolution that is comparable to rodent electrophysiological studies[4]. Patients will perform a virtual spatial navigation task that requires them to form associations between different objects and their corresponding locations in a virtual environment[5,6], while single-neuron activity and local field potentials (LFPs) are recorded via intracranially implanted electrodes.

The project will lead to novel and fundamental insights into the formation of associative memories in the human brain that go beyond the identification of (sub)cellular mechanisms in rodents[7] and the elucidation of involved brain regions in human neuroimaging studies[8]. Based on the findings, mechanistic explanations for impaired associative memory performance in various neurological and psychiatric diseases (such as Alzheimer's disease) may be derived.

1.1.2 Neural mechanisms of associative memory formation

Synaptic plasticity has been identified as the leading (sub)cellular mechanism for associative memory formation in animal studies: if neurons that encode different types of information co-activate within several tens of milliseconds, modifications of the synapses between the neurons occur[7]. Specifically, neural circuits that interconnect the different types of information may emerge by means of long-term potentiation (LTP), which strengthens synaptic transmission[9,10] and is facilitated by excitatory network states such as SPW-Rs[3].

Synaptic plasticity supporting associative memory formation may particularly occur in the hippocampus and surrounding MTL structures that have been identified as key regions for this cognitive function[11]: Patients with damage to the hippocampus exhibit impaired performance in multiple types of associative memory[12]; hippocampal inactivation in rodents similarly leads to deficits in the encoding and retrieval of associative memories[13,14]; and neurons in monkey hippocampus are involved in the formation of associative memories by increasing or decreasing their firing rate in parallel to the acquisition of new scene-location memories[1].

In this research project, objects and their corresponding spatial contexts will constitute the separate elements that are combined into the associative memories. The neural codes representing these stimuli are thus described in the following.

1.1.3 Single-neuron codes of space

In recent decades, multiple cell types involved in spatial navigation have been identified in both animals and humans (Fig. 1): ‘place cells’ encode a specific location in space[15,16]; ‘head-direction cells’ provide an internal compass by activating whenever the subject is moving in a given direction[17,18]; ‘border cells’ increase their firing rates in proximity to spatial boundaries[19]; and ‘grid cells’ exhibit firing fields arranged in a regular grid tiling the entire environment into equilateral traingles[20,21]. These allocentric representations of space (i.e., neurons encoding spatial information in relation to the external world) are complemented by neural codes of egocentric space (i.e., neurons that encode spatial information in relation to the subject): 'center-bearing cells’ activate whenever the center of the environment is in a given egocentric direction and distance from the subject[22]; ‘item-bearing cells’ increase their firing rate whenever an external object is in a specific egocentric direction from the subject[23]; and ‘egocentric boundary cells’ encode the egocentric direction and distance towards a spatial boundary[24].

How single-neuron representations of space participate in the formation of associative object-location memories remains unknown, however.

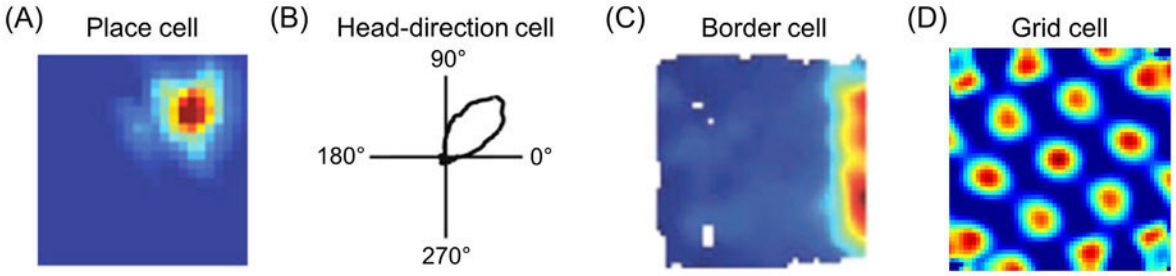


Fig. 1. Spatially-modulated neurons in the MTL. (**A**) Place cell[25]. (**B**) Head-direction cell activating whenever the subject moves in a specific allocentric direction[17]. 0° points ‘east’. (**C**) Border cell that activates whenever the subject is close to an environmental boundary[19]. (**D**) Grid cell exhibiting multiple firing fields arranged in a regular grid[20].

1.1.4 Single-neuron codes of objects

Object-responsive cells encode the identity of specific objects and may thus provide relevant object information during the formation of associative object-location memories. In human

hippocampus and amygdala, about 20% of the neurons were shown to activate in response to one or more visual objects[26]. In some cases, object-responsive cells may constitute 'concept cells', which are neurons in the human MTL that respond in a remarkably selective and abstract manner to particular objects or persons[27].

Object-responsive cells will be identified in this project in order to study whether their reactivation during SWP-Rs occurs in parallel with the reactivation of spatially-modulated cells.

1.1.5 Sharp wave-ripples (SPW-Rs) and their relevance for memory formation

SPW-Rs are one of the most synchronized activation events in the brain and associated with an enhanced transient excitability in the hippocampus and connected brain regions[2]. SPW-Rs consist of the sharp wave, which is a negative deflection in the local field potential (LFP), and the ripple, which is a fast oscillation (Fig. 2). SPW-Rs occur during "off-line" states of the brain, associated with consummatory behaviors such as immobility or sleep[2]. In task situations with increasing levels of memory demands, properties of SPW-Rs adapt accordingly[28] and the suppression of hippocampal SPW-Rs during post-training consolidation periods impairs spatial memory[29]. Therefore, SPW-Rs have been suggested as a mechanism to create new memories – during initial formation and during later consolidation.

1.1.6 Neuronal reactivation during sharp wave-ripples (SPW-Rs)

An intriguing feature of SPW-Rs is their spike content: neurons activating during SPW-Rs are part of neural substrates that encode specific events experienced by the awake animal during prior behavior[2]. For example, place cells that activate sequentially during navigation on a linear track reactivate in the same sequence during rest periods (Fig. 2).

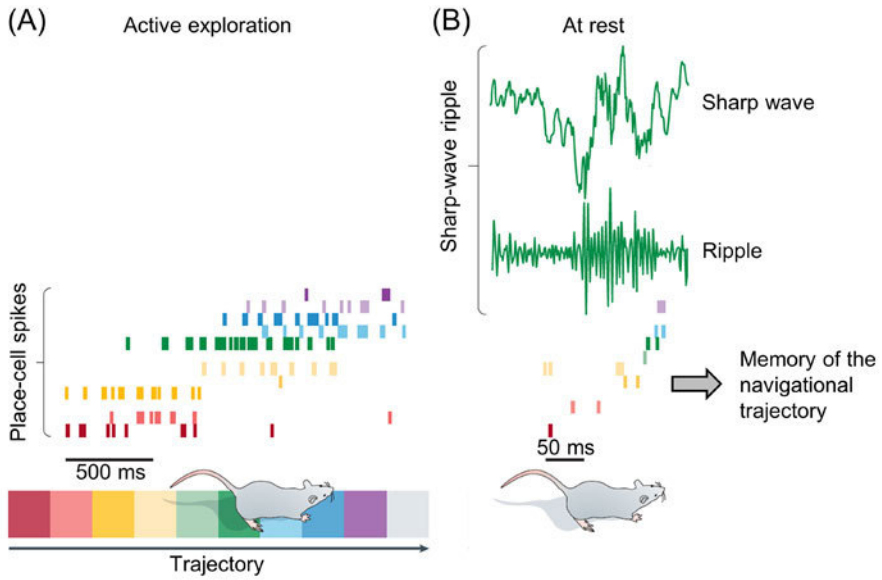


Fig. 2. Place-cell reactivation ('replay') during SWP-Rs. (**A**) During active exploration of an environment, place cells fire whenever the rodent moves across their receptive field (the 'place field'). As a population, the activated place cells encode the entire navigation trajectory. (**B**) At rest, the place-cell population 'replays' the trajectory during SWP-Rs in a time-compressed format. This mechanism may form a memory of the navigation trajectory. Modified from ref. [30].

The reactivation of neurons during SPW-Rs is temporally compressed so that reactivation takes place within a time window short enough to enable LTP between neurons encoding different aspects of the behavioral event[31]. Via this mechanism, connectivity between these neurons may be strengthened[32,33]. When occurring during brief pauses of exploration[31], SWP-R-triggered reactivation may lead to a transient memory representation, whereas SPW-R-triggered reactivation during slow-wave sleep may transform the transient memory representation into a long-lasting memory trace. The latter may include the transfer of hippocampus-dependent representations to neocortical storage sites, because SPW-Rs lead to a transient network synchronization between the hippocampus and downstream areas[2,34]. Such a function of SPW-R-triggered reactivation would be in line with the two-stage model of memory consolidation[35]:

during learning the neocortex provides the hippocampus with new information that induces a transient synaptic reorganization of its circuits, followed by the transfer of the modified hippocampal content to neocortical circuits[36].

It is thus the hypothesis of this project proposal that SPW-R-triggered reactivation of stimulus-specific neurons (i.e., object-responsive versus spatially-modulated neurons) is a key mechanism in the formation of associative memories: SPW-Rs may bind neurons coding for different types of information (i.e., objects versus spatial contexts) into a coherent associative memory and transfer the associative memory to the neocortex.

1.1.7 SPW-Rs in humans

SWP-Rs do not only occur in rodents, but also in many other species including humans[2]. Several prior studies have begun to unravel the function of SWP-Rs in humans using direct electrophysiological recordings from the MTL of epilepsy patients (Fig. 3). These studies found that SWP-Rs are presumably slower in humans than in rodents and that they occur frequently during awake immobility[37]. In a free recall task, SWP-R rate during encoding of visual stimuli predicted subsequent free-recall performance of the visual stimuli[38]. In another recent study, iEEG was employed to show that SWP-Rs during non-REM sleep trigger the reactivation of stimulus-specific gamma activity identified during the encoding of visual stimuli[39].

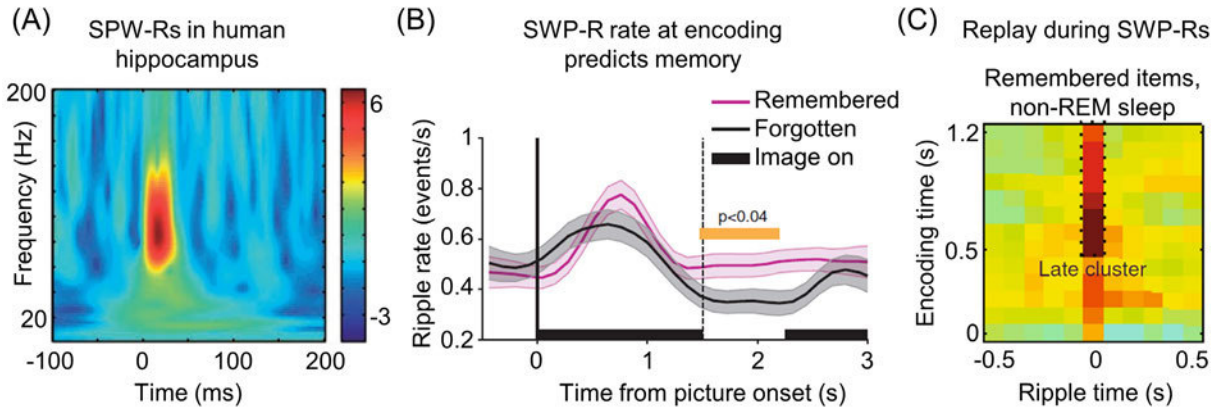
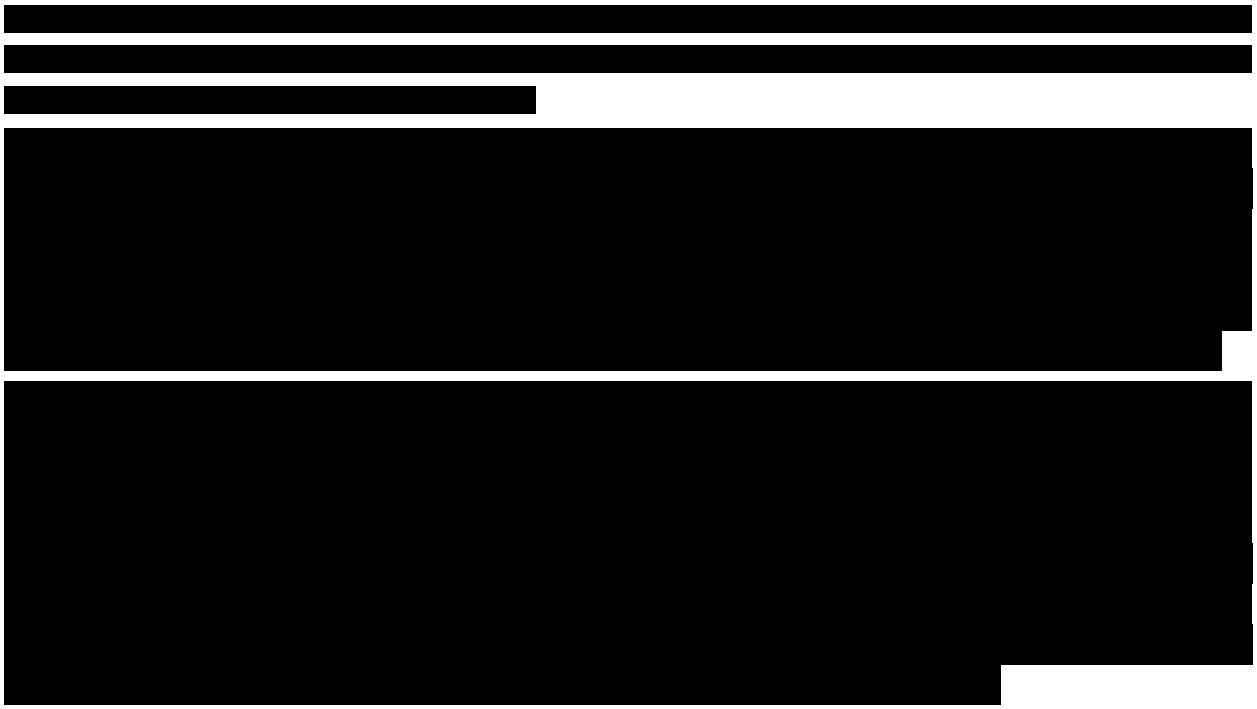


Fig. 3. SPW-Rs in human MTL. (**A**) Time-frequency power spectrum of SPW-Rs recorded from human hippocampus. Adapted from ref. [37]. (**B**) During encoding of visual stimuli, SPW-R rate is higher for remembered as compared to forgotten stimuli. Adapted from ref. [38]. (**C**) During non-REM sleep, SWP-Rs are accompanied by replay of stimulus-specific gamma-power activity, specifically for remembered visual stimuli. Adapted from ref. [39].

However, no study has examined the interplay between single-neuron activity and SWP-Rs in humans to date. Particularly, it has not been tested whether content-specific single-neuron activity reactivates during SPW-Rs to enable the formation of associative (object-location) memories. Answering this question would provide completely novel and fundamental insights into the mechanistic role of SWP-Rs for the formation of declarative memories in humans.

1.1.8 Preliminary own work

[redacted]



1.2 Project-related publications

Sections 1.2.1 and 1.2.2 together must not exceed 10 publications; please number them consecutively.

1.2.1 Articles published by outlets with scientific quality assurance, book publications, and works accepted for publication but not yet published

(1) Kunz L, *et al*. Hippocampal theta phases organize the reactivation of large-scale electrophysiological representations during goal-directed navigation. **Science Advances**; *5*, eaav8192 (2019).

(2) Kunz L*, Maidenbaum S*, Chen D*, *et al*. Mesoscopic neural representations in spatial navigation. **Trends in Cognitive Sciences**; *23*, 615-630 (2019). (*shared first-author)

(3) Chen D*, Kunz L*, *et al*. Hexadirectional modulation of theta power in human entorhinal cortex during spatial navigation. **Current Biology**; *28*, 3310-3315 (2018). (*shared first-author)

(4) Kunz L, *et al*. Reduced grid-cell-like representations in adults at genetic risk for Alzheimer's disease. **Science**; *350*, 430-433 (2015).

1.2.2 Other publications

(5) Bierbrauer A*, Kunz L*, Gomes CA*, *et al*. Unmasking selective path integration deficits in Alzheimer's disease risk carriers. **medRxiv**; *19009662* (2019). (*shared first-author)

(6) Kunz L. Untersuchung von "grid cell"-basierten Repräsentationen des entorhinalen Kortex in Erwachsenen mit genetisch erhöhtem Risiko für Morbus Alzheimer. Doctoral dissertation, **Universitäts-und Landesbibliothek Bonn** (2017).

1.2.3 Patents

1.2.3.1 Pending

None.

1.2.3.2 Issued

None.

2 Objectives and work programme

2.1 Anticipated total duration of the project

The anticipated total duration of the project is two years (01/01/2021-12/31/2022).

2.2 Objectives

2.2.1 Overview

The suggested research project targets the fundamental question how the human brain creates associative (object-location) memories. I hypothesize that the simultaneous reactivation of spatially-modulated and object-responsive neurons during SPW-Rs constitutes a key mechanism in this process by enabling LTP between the participating neurons (Fig. 4).

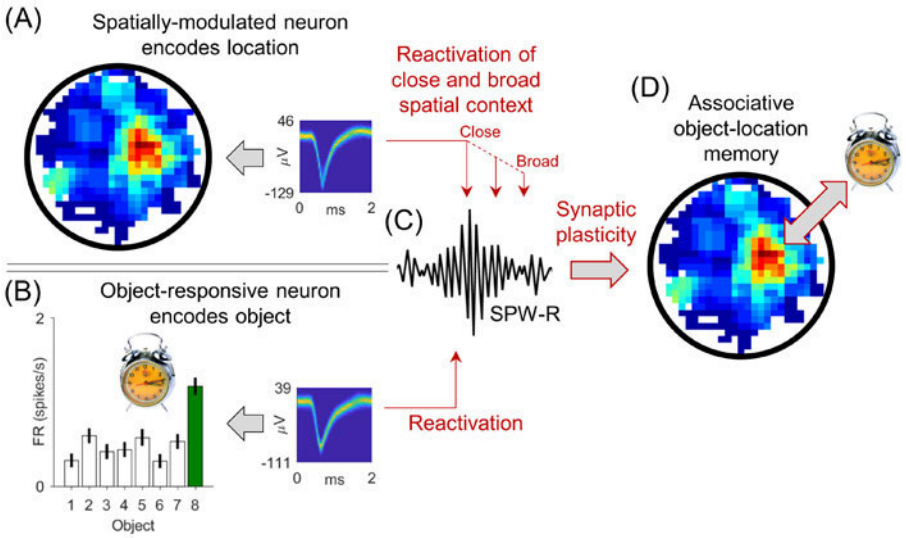


Fig. 4. Key hypothesis. Brain mechanisms of associative memory formation may include a synchronous reactivation of spatially-modulated neurons (**A**) and object-responsive neurons (**B**) during SPW-Rs (**C**) to create associative object-location memories (**D**). Spatially-modulated neurons representing the close and broad spatial context of the object may unfold sequentially during SPW-R-triggered reactivation.

I will test this hypothesis by means of single-neuron and LFP recordings in neurosurgical epilepsy patients who perform a virtual navigation task in which associative memories between objects and their corresponding spatial contexts have to be formed (see section 2.3 for details). Single-neuron and LFP recordings will be performed before the task ('pre-task period'; duration of 60 minutes), during the task ('task period'; duration of 30-60 minutes), and after the task ('post-task period'; duration of 60 minutes). [redacted] Spatially-modulated cells and object-responsive cells will be identified by analyzing the data acquired during the task period. SWP-Rs will be detected during all three task periods. The activity of spatially-modulated neurons and object-responsive neurons will then be studied during all three task periods and it will be tested whether reactivation occurs preferentially and synchronously during SPW-Rs. I hypothesize that reactivation during SPW-R occurs during the task period and the post-task period, providing a mechanistic explanation for the formation of associative object-location memories during the task and the strengthening of these object-location memories after the task.

2.2.2 Aim 1: Single-neuron representations of space and objects in human MTL

In the virtual navigation task employed for this project, patients form associative memories between objects (e.g., the alarm clock) and locations within the virtual environment (e.g., the central area of the environment). To understand the formation of these associative object-location memories, I will first identify single-neuron representations of the different aspects of the memories – i.e., single-neuron codes of space and single-neuron codes of objects.

Single-neuron codes of space include place cells, direction cells, border cells, grid cells, center-bearing cells, and egocentric boundary cells. Place cells may exhibit place fields exactly where the objects are located (encoding the narrow spatial context of the objects) and may therefore contribute the most relevant and precise spatial information to the associative memories. However, other types of spatially-modulated neurons are, presumably, recruited into the neural substrate of the object-location memories as well, because they encode the broader spatial context in which the objects are placed (i.e., the entire virtual environment). During SPW-R-triggered neuronal reactivation, spatially-tuned cells encoding the narrow spatial context may immediately follow the reactivation of object-responsive cells, whereas spatially-tuned cells encoding the broader spatial context may reactivate with a slight temporal delay.

Object-responsive cells encoding one of the objects placed within the virtual environment will be identified as the second neural building block of the associative memories. Preliminary analyses show that about 15% of the recorded neurons modulate their firing rate as a function of object (Fig. 5), in line with a previous study[26].

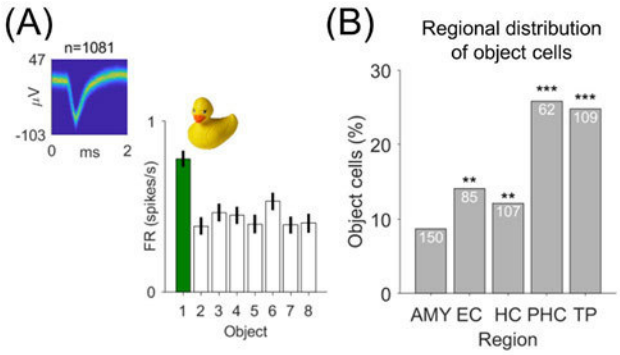


Fig. 5. Object-responsive cells in human MTL (preliminary results). (**A**) Exemplary neuron coding for object 1 (the rubber duck in this case). Error bars represent SEM. Left upper subplot depicts spike-density plot[41]. FR, firing rate. (**B**) Significant proportions of object-responsive cells are found in entorhinal cortex (EC), hippocampus (HC), parahippocampal cortex (PHC), and temporal pole (TP). AMY, amygdala. $^{**}P < 0.01$; $^{***}P < 0.001$.

The successful completion of aim 1 (i.e., the identification of the cell types outlined above) will allow me to examine the reactivation of spatially-modulated and object-responsive neurons during SPW-Rs in later stages of the project. It will also be highly informative about the neural mechanisms of human memory and spatial navigation in general [redacted], because the investigation of single-neuron codes of space and memory processes in the human brain is only rarely possible.

2.2.3 Aim 2: Characterization of human SPW-Rs during virtual spatial navigation

SPW-Rs may facilitate the formation of associative memories, because they are related to increased excitability in the hippocampus and connected brain regions and may thus trigger the simultaneous reactivation of stimulus-specific neurons. SPW-Rs have been extensively investigated in rodent spatial navigation studies[2], but evidence for SPW-Rs during virtual navigation in humans is missing so far. Hence, I will examine whether and to what extent the human hippocampal formation exhibits SWP-Rs during virtual navigation. Fig. 6 shows preliminary results on SPW-Rs during virtual navigation, recorded from hippocampal depth electrodes in a different dataset[6].

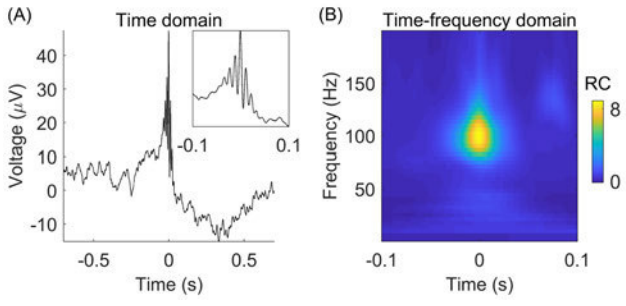


Fig. 6. SPW-Rs recorded from the hippocampus of presurgical epilepsy patients during virtual navigation (preliminary results from a different dataset). (**A**) Grand average of all SPW-Rs (688 SPW-Rs recorded from 10 bipolar channels in 7 patients) in the time domain. Inset magnifies the time period around the ripple peak to show the fast oscillation. (**B**) Grand average power-spectrum of all SPW-Rs in the time-frequency domain. Peak frequency is at about 100 Hz. RC, relative change.

After a general description of SPW-Rs, I will test whether characteristics of SPW-Rs vary as a function of task state, learning, and memory, because SPW-Rs have been identified as a biomarker for mnemonic operations[2,28,37,39,42]. Positive results would provide first evidence that SPW-Rs are relevant for spatial memory in humans [redacted]. Specifically, I will test (i) whether SPW-Rs are longer in a first session (novel) as compared to a second session (familiar) of the same task; (ii) whether SPW-Rs are longer during the task (high memory demand) as compared to a pre-task period (no memory demand); (iii) whether SPW-Rs are longer during good as compared to bad trials; (iv) whether SPW-R duration decreases as learning progresses; and (v) whether SPW-Rs occur particularly during consummatory periods of the task in comparison with preparatory periods (Fig. 7).

Recent studies in rodents recorded SPW-Rs both in hippocampus and in neocortical regions, and revealed that coupling between hippocampal and neocortical SPW-Rs was strengthened during sleep following learning. SPW-Rs may thus enable the transfer of initially hippocampus-dependent memories to the neocortex[42]. Hence, I will examine whether and to which extent extrahippocampal SPW-Rs can be observed in the virtual navigation task employed for this project and whether they are temporally synchronized with hippocampal SPW-Rs.

2.2.4 Aim 3: Human brain mechanisms of associative memory formation

Aim 3 will interconnect the results obtained from aims 1 and 2 in order to target the key question of the proposed research project: what are the neural mechanisms underlying associative memory formation in humans? To answer this question, I will test whether SPW-Rs in human MTL are accompanied by a coordinated reactivation of spatially-modulated neurons and object-responsive neurons.

For the pre-task period, I assume no coordinated interplay between the activity of stimulus-specific cell types and SPW-Rs (Fig. 7A). By contrast, I hypothesize that SPW-Rs during the task are accompanied by increased activity of spatially-modulated neurons and object-responsive neurons (Fig. 7B). This finding would provide first empirical evidence that the formation of associative memories in humans relies on a SPW-R-triggered reactivation of content-specific single-neuron activity [redacted] In particular, I will test whether high activity of object-responsive neurons during SPW-Rs is followed by increased activity of spatially-modulated neurons and vice versa. A directed association would support the interpretation that one type of information cues the recall of the other type of information. Additionally, I will examine whether spatially-modulated neurons encoding the narrow spatial context are reactivated in closer temporal association with object-responsive cells as compared to spatially-modulated neurons encoding the broader spatial context. Over the course of the task (accompanied by learning), I hypothesize an increased content of SPW-Rs, meaning that reactivation of spatially-modulated neurons and object-responsive neurons becomes more likely and more selective. Because SPW-Rs in rodents preferentially occur during consummatory behavior, I expect a higher incidence of SPW-R-triggered reactivation during task periods of ‘re-encoding’ and ‘inter-trial interval’ (ITI), in which the subject collects a given object from its correct location and is presented with a blank screen, respectively (Fig. 7B). Across patients, I will furthermore test whether stronger SPW-R-locked reactivation is associated with steeper learning curves. For the post-task period, I hypothesize a similar degree of SPW-R-triggered reactivation of spatially-modulated and object-responsive neurons as compared to during the task – decaying with increasing temporal distance to the task (Fig. 7C).

2.2.5 Aim 4: Relevance of SPW-Rs during sleep for memory consolidation

What are the neural mechanisms that support the transformation of initial, labile memory representations into long-term, stable memory traces? To examine this question, a subgroup of patients will perform the virtual navigation task on two consecutive days. Based on predictions from the two-stage model of memory consolidation[35], I will test whether the rate of SPW-Rs during sleep predicts spatial memory performance on the second day. This analysis will provide crucial insights into the relevance of SPW-Rs for long-term memory consolidation in humans.

2.2.6 Summary

The proposed project “Neuronal mechanisms of associative memory formation in human medial temporal lobe” will take advantage of the rare opportunity to record single-neuron activity from the human brain in order to provide new mechanistic insights into the neural foundations of associative memory in humans. Analyses will identify stimulus-specific single-neuron representations of space and objects and study their reactivation during hippocampal and extrahippocampal SPW-Rs. The findings will result in novel and fundamental insights into the formation of associative memories in humans, being relevant both from a basic science perspective and a clinical perspective, because impairments in associative memory are observed in neurological and psychiatric diseases such as Alzheimer’s disease[43]. The main research aims are illustrated in Fig. 7.

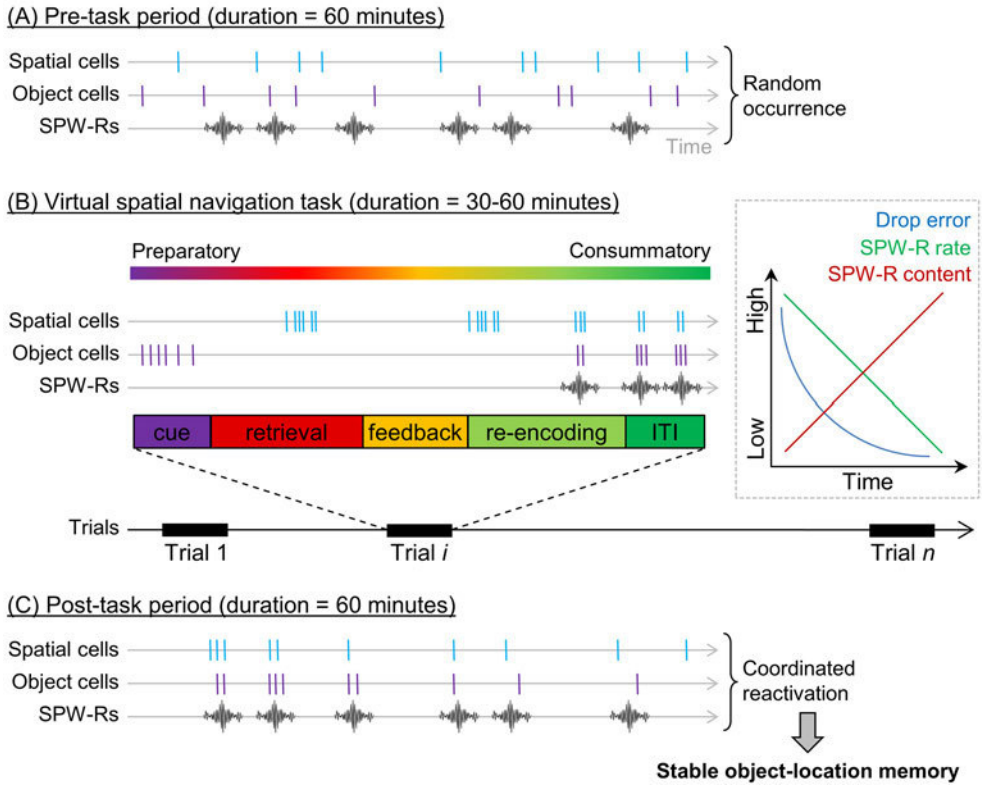


Fig. 7. Summary of the research aims. (**A**) For the pre-task period, in which the patients rest in their beds, I hypothesize a random association between single-neuron activity (identified as spatially-modulated cells or object cells during the subsequent task) and SWP-Rs. (**B**) For the virtual spatial navigation task, I hypothesize (i) that spatially-modulated cells and object cells can be identified; (ii) that SWP-Rs occur particularly during consummatory task periods (re-encoding and ITI); and (iii) that spatially-modulated cells and object cells reactivate simultaneously during SWP-Rs. ITI, inter-trial interval during which patients are presented with a blank screen. Spatial cells activate when the subject is in a particular location or moving in a specific direction; object cells activate when a specific object is presented. Furthermore (gray box), I hypothesize that the drop error decreases over time due to learning, accompanied by a decreasing SPW-R rate and increasing SPW-R content. (**C**) For the post-task period, in which the patients rest in their beds, I hypothesize that single neurons identified as spatially-modulated or object-responsive cells during the task exhibit reactivation during SWP-Rs (which decays over time). The coordinated reactivation of spatially-modulated cells and object cells during SPW-Rs may provide an explanation for the formation of stable object-location memories.

2.3 Work programme incl. proposed research methods

In the following, I will describe the behavioral task, methods for intracranial electrophysiology recordings in epilepsy patients, planned analyses, and a timeline for milestones of the project.

2.3.1 Virtual spatial navigation task

During intracranial electrophysiology recordings, patients will perform a virtual spatial navigation task[5,6] requiring them to form eight associative memories between different objects and their corresponding spatial contexts (Fig. 8). Similar tasks in rodents have been used to show that the formation of associative food-location memories are hippocampus-dependent[14]. Patients initially learn the locations of the eight objects by collecting each object from its location once. Patients then complete variable numbers of test trials. In each trial, patients are first presented with one of the eight objects. During the subsequent retrieval period, patients navigate to the assumed object location and indicate their decision via a button press. Next, patients receive feedback on the accuracy of their response, which is measured as the distance between the response location and the correct location ('drop error'). The retrieved object then appears in its correct location and patients collect it from there to further improve their associative object-location memories. Between trials, patients are presented with a fixation crosshair on a blank screen (ITI). The virtual environment consists of a grassy plain, surrounded by a cylindrical cliff, with distal cues such as mountains for orientation. Patients navigate the virtual environment using the laptop keyboard. Instantaneous virtual locations and heading directions are sampled at 50 Hz. Behavioral data is aligned to the electrophysiological data via simultaneously recorded trigger signals.

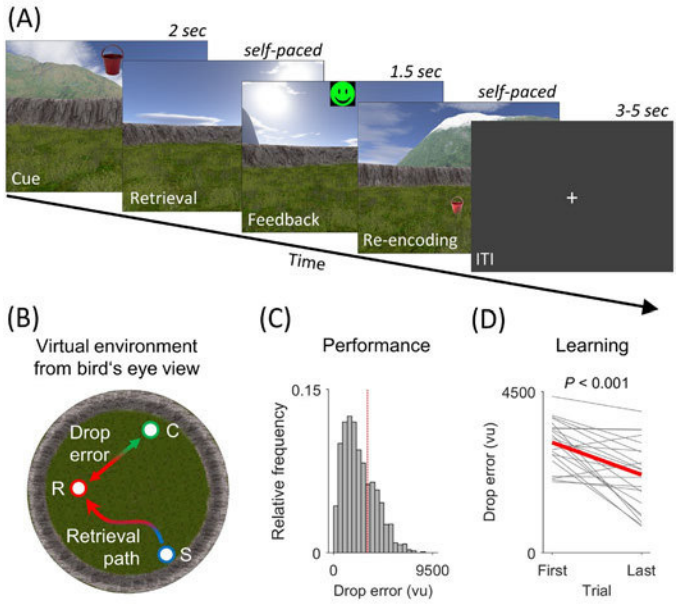


Fig. 8. Virtual navigation task and behavior in a cohort of epilepsy patients from a previous study[6]. (**A**) At the beginning of the experiment (not shown), patients collect eight different objects from different locations within the virtual environment. Afterwards, patients complete up to 160 test trials, in which they are first presented with one of the eight objects ('cue'). Patients then have to navigate to the remembered location of that object and give a response ('retrieval'). Next, patients receive feedback depending on the accuracy of their response ('feedback') and have to collect the object from its correct location ('re-encoding'). ITI, inter-trial interval between consecutive trials. (**B**) Overhead view of the virtual environment that consists of a grassy plain and is surrounded by a cylindrical cliff. The trial-wise drop error is calculated as the Euclidean distance between the response location ('R') and the correct location ('C') and quantifies spatial memory performance. (**C**) Histogram of drop errors across all trials and all patients. Red dashed line indicates overall chance performance. About 70% of trials were above chance level. (**D**) Change in mean drop error between the first and the last trial. Gray lines, patient-wise data; thick red line, average across patients.

2.3.2 Human intracranial electrophysiology

[redacted]

[redacted]

IEEG will be performed via standard depth electrodes provided by AD-TECH (AD-TECH, Racine, WI, USA) at a sampling rate of 2 kHz using a Compumedics system (Compumedics, Abbotsford, Victoria, Australia). Patients are implanted with multiple depth electrodes covering temporal, frontal, parietal, and (less often) occipital regions. These macroelectrodes record LFPs within a radius of several millimeters[25]. For analyses, signals will be re-referenced in a bipolar scheme. To assign electrode channels to brain regions, channel locations will be identified manually in post-implantation MRI scans transformed into MNI space using PyLocator (http://pylocator.thorstenkranz.de/). MNI coordinates of channel locations will then be assigned to brain regions according to standard atlases as implemented in FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL) and FreeSurfer (https://surfer.nmr.mgh.harvard.edu/).

Single-neuron activity will be recorded using microelectrodes that are part of Behnke-Fried electrodes[45] provided by AD-TECH: Each Behnke-Fried electrode contains a bundle of nine platinum-iridium microelectrodes that protrude from the tip of the depth electrode[46] (Fig. 9). The first eight microelectrodes are used to record action potentials and LFPs. The ninth microelectrode serves as reference. Patients are implanted with multiple microelectrode bundles covering MTL regions such as amygdala, entorhinal cortex, fusiform gyrus, hippocampus, and parahippocampal cortex. Microwire data is recorded at 30 kHz using NeuroPort (Blackrock Microsystems, Salt Lake City, UT). Neuronal spikes will be detected and sorted using WaveClus[47] running under MATLAB (The MathWorks). All clusters will be visually inspected to ensure high quality. If necessary, clusters will be manually adjusted or excluded. To identify the recording sites in the MTL, the tips of the Behnke-Fried electrodes will be assigned to brain regions based on post-implantation MRI scans so that neurons recorded from the corresponding microelectrodes can be assigned to these regions.

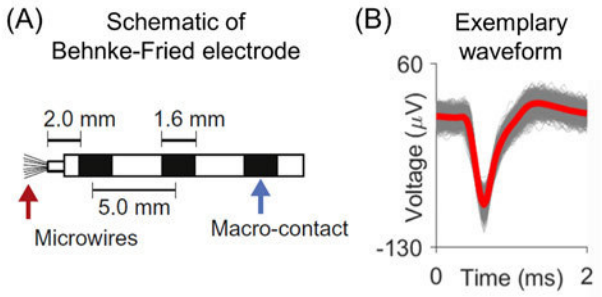


Fig. 9. Single-neuron recordings. (**A**) Schematic of a Behnke-Fried electrode: a bundle of microelectrodes (diameter of 40 µm) protrudes from the tip of the depth electrode. Reprinted from ref. [46]. (**B**) Exemplary neuron recorded from the right amygdala of a patient at the Freiburg Epilepsy Center. Gray lines, single spikes; red line, mean waveform; ms, milliseconds.

Epileptologists will provide information about electrode channels located in areas that exhibit epileptic activity, which will be excluded from analyses. All research will be performed in accordance with the declaration of Helsinki.

2.3.3 Identify single-neuron representations of space and objects in human MTL (aim 1)

The key hypothesis of this research project requires the identification of single-neuron representations of space and objects, which will be the task of aim 1. Because the vast majority of our knowledge on single-neuron codes of space comes from rodent electrophysiology, the investigation of spatially-tuned cells in the human MTL itself is highly valuable. Some of the known spatially-modulated cells have not even been described in humans before (such as border cells or center-bearing cells).

To identify spatially-modulated and object-responsive cells, neuronal firing rates will be examined as a function of different behavioral variables using an ANOVA framework (following for example ref. [48]). In this framework, instantaneous neuronal firing rates are modelled via different predictors such as ‘location’ (to identify place cells), ‘direction’ (to identify direction cells), ‘distance to the boundary’ (to identify border cells), ‘egocentric direction towards the environmental center’ (to identify center-bearing cells), and ‘object’ (to identify object cells). Significance of a given predictor will be assessed via conservative surrogate statistics (following for example ref. [21]).

The different cell types will be identified, their distribution across different regions of the MTL will be examined, and response properties (including phase locking, phase precession, and theta modulation[25]) will be compared to findings in rodents. The relationship between their tuning strength and spatial memory performance will be scrutinized in order to understand their relevance for human behavior. [redacted]

2.3.4 Describe human SPW-Rs during virtual spatial navigation (aim 2)

Despite an extensive literature on SPW-Rs during spatial navigation in rodents [e.g., refs. [28,29,49]] and first human iEEG studies on SPW-Rs during non-spatial memory tasks [e.g., refs. [37–39]], no study to date has examined the occurrence of human SPW-Rs during virtual spatial navigation. Hence, aim 2 of this research project targets an extensive description of human SPW-Rs during virtual spatial navigation.

I will identify SPW-Rs in the LFPs recorded from both micro- and macroelectrodes. SPW-Rs will be detected following previously established methods and after excluding epileptic activity from recording channels based on visual inspection and automatic artifact rejection techniques[50]. Furthermore, physiological SPW-Rs will be carefully distinguished from pathological high-frequency oscillations (HFOs) associated with epileptogenic processes. SPW-Rs obtained from microelectrodes will be compared to SPW-Rs from macroelectrodes to ensure similar quality. SPW-Rs will be analyzed in both hippocampal and extrahippocampal electrode channels (including temporal, frontal, parietal, and occipital regions) and will be characterized with respect to criteria outlined before[2]. The temporal synchrony between hippocampal and extrahippocampal SPW-Rs will be evaluated using cross-correlation analyses[42]. [redacted] Furthermore, this work will lay the foundation of being able to examine whether SPW-Rs trigger the reactivation of stimulus-specific neurons (aim 3).

2.3.5 Identify human brain mechanisms of associative memory formation (aims 3 and 4)

After having provided separate descriptions of single-neuron representations of space/objects and SPW-Rs (aim 1 and 2, respectively), I will interconnect the different phenomena to answer the question whether SPW-Rs trigger the reactivation of stimulus-specific single neurons. To this end, I will track the activity of spatially-modulated neurons and object-responsive neurons

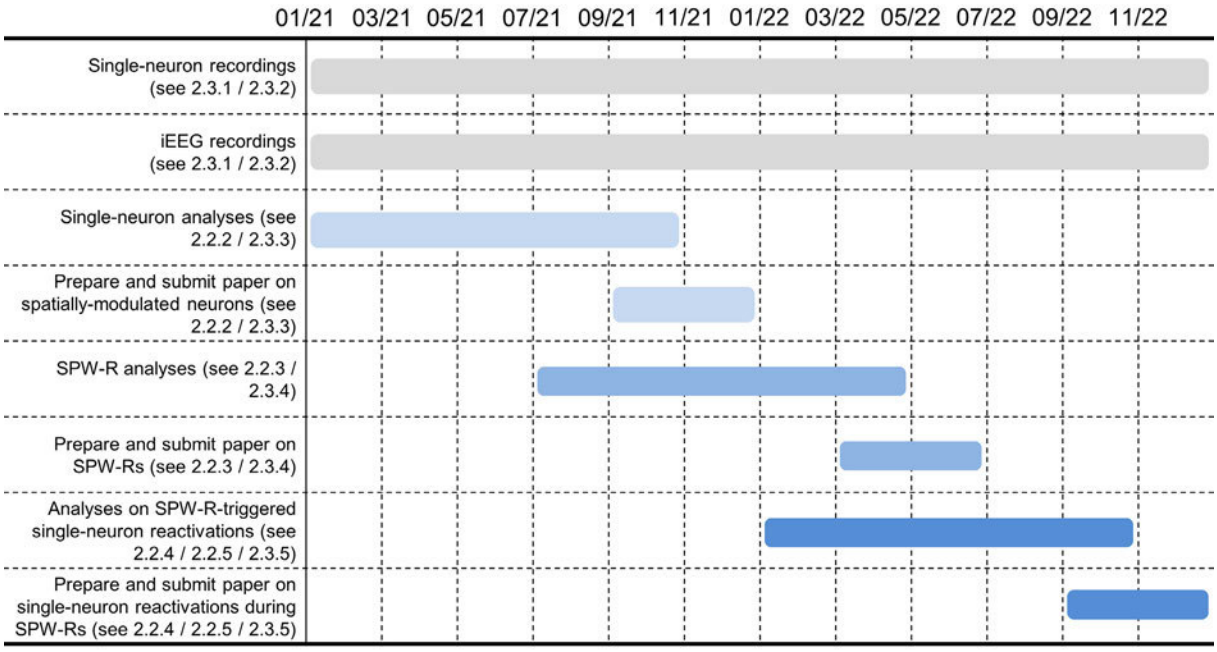
throughout the three time periods of interest: during the pre-task period, during the task period, and during the post-task period. In all three periods, I will assess whether the activity of these cell types increases during SPW-Rs. Specifically, I will study whether increases in firing rate of one cell type is accompanied by firing rate increases of the other cell type during exactly the same SPW-Rs. Such a SPW-R-triggered synchronous activation would enable LTP between the participating neurons in order to recruit them into a common neural circuit. Furthermore, I will elucidate whether firing rate increases of one cell type consistently precede the firing rate increases of the other cell type during SPW-Rs, being indicative of a specific directionality in the association. The content of SPW-Rs will be quantified as the strength of the reactivation of both cell types (i.e., percent increase in firing rate) and how temporally confined the reactivation is. Moreover, SPW-R-triggered reactivation will be analyzed as a function of task phase and patient-wise spatial memory performance to assess its behavioral relevance.

Finally, to further extend the importance of SPW-Rs for associative memory formation, I will acquire a group of patients completing the virtual navigation task on two consecutive days. On both days, these patients will perform the task with exactly the same object-location associations. SPW-Rs will then be detected throughout the night [following ref. [50]] to correlate their incidence with (i) spatial memory performance on the second day and (ii) the amount of performance improvement from the first to the second day. A positive relationship would provide evidence for the importance of SPW-Rs in long-term memory consolidation (aim 4).

[redacted]

2.3.6 Timeline

[redacted]



3 Bibliography concerning the state of the art, the research objectives, and the work programme

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4 Relevance of the project to research career objectives

[redacted]

5 Reasons for selecting the host institution(s)

[redacted]

6 Supplementary information on the research context

6.1 Descriptions of proposed investigations involving experiments on humans or human materials

The proposed project makes use of intracranial electrophysiology recordings in neurosurgical epilepsy patients, while the patients perform an object-location memory task in a virtual environment. Recordings and the task are outlined in section 2.3.1 (“Virtual spatial navigation task”) and 2.3.2 (“Human intracranial electrophysiology”). In the following, I will describe the ethical and legal aspects of the project. The research has been approved by the Ethics committee of the University Hospital Freiburg, Freiburg im Breisgau, Germany.

6.1.1 Treatment or experiment

Epilepsy patients will perform an object-location memory task in a virtual environment (see section 2.3.1). Patients complete the task on a laptop computer during the continuous invasive video-EEG-monitoring (see below) while sitting in their hospital bed. The task lasts between 30 and 60 minutes, depending on each patient’s compliance. Patients are free to pause or quit the task whenever they want. The task is already well-established and has been used in several previous studies, both with healthy participants and epilepsy patients[5,6,40]. Participation in the task will not interfere with any clinical procedure.

6.1.2 Criteria for selecting test subjects

Participants are neurosurgical epilepsy patients undergoing invasive video-EEG-monitoring at the Freiburg Epilepsy Center for diagnostic purposes in order to identify the anatomical origin of their epileptic seizures. This procedure involves the implantation of intracranial depth electrodes, which are stereotactically inserted by neurosurgeons of the Department of Stereotactic and Functional Neurosurgery of the University Medical Center Freiburg. Electrodes are implanted in various regions (including temporal, frontal, parietal, and occipital regions). The number of implanted electrodes and the anatomical electrode locations are solely determined by clinical needs. Select intracranial depth electrodes are outfitted with microelectrodes to perform single-neuron recordings (so-called Behnke-Fried electrodes[45]), given that patients have agreed to the implantation of microelectrodes and have provided written informed consent. After electrode implantation, patients are continuously monitored for a time period of about one to three weeks to record epileptic seizures and to detect associated epileptic brain activity. Intracranial recordings are then clinically analyzed by epileptologists of the Freiburg Epilepsy Center.

[redacted]

[redacted]

6.1.3 Description of potential risks and precautions taken

Participation in the virtual spatial navigation task does not involve any potential risks for the patients. The task has been used in multiple previous studies (e.g., refs. [5,6,40]) and is well-tolerated by both epilepsy patients and healthy participants.

[redacted]

6.1.4 Method of informed consent

On a preoperative visit, epileptologists, neurosurgeons, and research staff members will discuss the research protocol with the patients. Written informed consent regarding microelectrode implantation and participation in the virtual navigation task will then be obtained prior to implantation. Written informed consent regarding the implantation of intracranial depth electrodes will be obtained as part of the standard clinical procedure.

6.2 Descriptions of proposed investigations involving experiments on animals

None.

6.3 Descriptions of proposed investigations involving dual use research of concern

None.

6.4 Data handling

Human intracranial electrophysiological data and behavioral data will be recorded during the time periods of interest (pre-task period, task period, post-task period) and stored on hardware of the Freiburg Epilepsy Center, Germany, for use in this research project. A copy of the data will also be archived for long-term use (>10 years) in order to employ the data for other research

questions. Data will be stored and processed inside the secure firewall of the Epilepsy Center Freiburg.

[redacted]

6.5 Other information

Please use this section for any additional information you feel is relevant which has not been provided elsewhere.

None.

7 People / collaborations / funding

7.1 Employment status information

Name and location of current institution

Freiburg Epilepsy Center

Medical Center – University of Freiburg

Faculty of Medicine

University of Freiburg

Breisacher Str. 64

79106, Freiburg im Breisgau

Germany

7.2 Researchers in Germany and abroad with whom you have agreed to cooperate on this project

(1) Prof. Dr. Andreas Schulze-Bonhage, Freiburg Epilepsy Center, Germany.

7.3 Researchers with whom you have collaborated scientifically within the past three years

This information will help avoid potential conflicts of interest.

(1) Prof. Dr. Nikolai Axmacher, Ruhr-University Bochum, Germany.

(2) Prof. Dr. Andreas Schulze-Bonhage, University Hospital Freiburg, Germany.

(3) Prof. Dr. Liang Wang, Chinese Academy of Sciences, China.

(4) Prof. Dr. Joshua Jacobs, Columbia University in the City of New York, USA.

(5) Prof. Dr. Michael Kahana, University of Pennsylvania, USA.

(6) Prof. Dr. Bryan Strange, Alzheimer's Disease Research Centre, Spain.

(7) Prof. Dr. Volker Coenen, University Hospital Freiburg, Germany.

(8) Prof. Dr. Christian Bien, Bethel Epilepsy Centre, Germany.

(9) Prof. Dr. Martin Reuter, University of Bonn, Germany.

(10) Prof. Dr. Christian Montag, Ulm University, Germany.

7.4 Project-relevant cooperation with commercial enterprises

If applicable, please note the EU guidelines on state aid or contact your research institution in this regard.

None.

7.5 Relevant participation in commercial enterprises

Information on connections between the project and the production branch of the enterprise

None.

7.6 Additional information

If applicable, please list proposals for this project previously submitted to a third party.

None.

8 Requested modules/funds

8.1 Walter Benjamin fellowship

Indicate the period and the institution(s) for which you are applying for the Walter Benjamin fellowship.

Period:

01/01/2021 – 12/31/2022

Institution:

Electrophysiology, Memory & Navigation Laboratory (Dr. Joshua Jacobs)

Department of Biomedical Engineering

Columbia University in the City of New York, New York, USA

8.2 Walter Benjamin position

Indicate the period and the institution(s) for which you are applying for the Walter Benjamin position.

None.

8.3 Temporary substitute clinician under the Walter Benjamin Programme

Indicate the period and the institution(s) for which you are applying for a temporary substitute.

None.

8.4 High publication costs

Publication costs of up to €5,000 per year can be requested if the appropriate publication of your project findings calls for a book format involving high publication costs. Please justify your request accordingly.

[redacted]