Thesis title: The hippocampus as an indexing machine of episodic memory

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At the very beginning of my PhD Simon Hanslmayr and Bernhard Staresina invited me to a meeting discussing which dataset should be used to investigate “Index Neurons”. I was unrecoverably lost which found its peak when Bernhard understood some implications before Simon finished his sentence. That was the only time I doubted myself.

I soon recovered my excitement for research although often stumbled on unseen ground. I am very grateful to Simon, my *Doktorvater* for his guidance and his trust in me even when I was hard stuck on a particular problem for weeks or head over heels down a rabbit hole. For me, Simon embodies coolness and a keen mind in equal parts. I would also like to thank my second supervisor Howard Bowman. I find your intuitive grasp on mathematics inspiring and thoroughly enjoy our long conversations about neuroscience and all other things.

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Table of content

General Introduction

The purpose of this work: finding the single neuron underpinnings of episodic memories in humans (do we have an ESN code independent of CN?)

# Abstract

# I will begin at the beginning; briefly touch upon the different forms of memory, we here are interested in episodic memory; discuss how epilepsy allows us to drill holes in peoples brain and what electrodes we use now, and will use in the future; I will continue to describe what kind of signal we record with these wires: the local field potential and the action potentials of neurons; I will talk about the hippocampus as a central brain structure involved in memory processing, how information flows into and out of the structure and introduce a prominent theory how cortical representations are coded in the hippocampus for later retrieval (called the Indexing Theory); there are different theories that propose different roles to the hippocampus and the cortex over time, so I shall describe them here (maybe better before the indexing theory?); in rodents a memory trace is referred to as the “engram” and I will briefly outline research relevant research that can inform how index neurons might be allocated; lastly I will describe the most common type of neuron in the human brain: the concept neuron. Maybe I should also mention the other types?

# The shocking origin of neuroscience

If one were to set out in search for the earliest scientific breakthrough that led to this work an educated guess would land on Luigi Galvani. The legacy of the Italian polymath is grounded in his discovery that the muscles of frogs twitch when electrically stimulated

(Galvani, 1791). He thereby refuted the contemporary belief that animal spirits inside hollow nerves drive movement and sensation. Although he was wrong in attributing the muscle twitches to an innate force he called “animal electricity”, he still managed to demonstrate the electrical nature of nerve impulses, thereby laying the foundation of electrophysiology and modern neuroscience (Piccolino, 1998)

The impact of Galvani's work was so immense that it has been likened to the French Revolution (Piccolino, 1998). It ultimately led Alessandro Volta to invent the electrical battery and inspired Mary Shelley to write the classic horror story *Frankenstein* (Piccolino, 1998). Galvani's name survives until today in the verb *galvanize* and still has a place in popular culture through songs such as Galvanize (curiously by The Chemical Brothers).

# A walk down memory lane

We experience the world around us filtered through the lens of our experiences. Without memories we could not hold on to these experiences locking us perpetually in the present. It follows that memories are at the core of what makes us humans.

Episodic memory, a term coined by Endel Tulving in 1972 (Tulving, 1972), is the ability to encode and later recollect experiences that contain a what, where and when. They are rich in detail, integrating information from multiple modalities, they are encoded automatically, require no repetitions, and can last an entire life (Teyler & Rudy, 2007; Tulving, 2002). By remembering these episodic memories, it is as if we were mentally transported back to that time, re-experiencing them anew (Tulving, 2002).

An example for an episodic memory is when I was sitting in a small coffee shop in Sevilla in the company of my loved one. The sun had not yet reached its peak and was pleasantly warm. A mild breeze carried over the smell of freshly brewed coffee and bits of conversations from other patrons. It was a satisfying way to start the day, my body still exhilarated from the workout we just finished. The waiter brought over two coffees. On the way back to the kitchen he hesitated, turned on his heel and walked back to our table. "Your PhD thesis was a fantastic read", he said with a slight Spanish accent, adding "but why was your example for episodic memories so long?".

Semantic memories on the other hand refer to factual knowledge and understanding of concepts (such as knowing that the very real coffee shop in the above story was called "La Nueva Peseta"; (Squire, 1987)). Together with episodic memories they belong to the subgroup of declarative memories (Squire, 1987). Declarative memories, sometimes also called explicit memories, can be expressed (i.e., declared) overtly and form the basis for conscious recollection (Squire, 1987, 1992). In reality the line separating semantic and episodic memories can get blurry. For example, if you were asked how old you were when you received your childhood pet, the retrieved memory would have semantic (your age) and episodic aspects (the experience itself).

Declarative memories can in turn be distinguished from non-declarative memories (Squire, 1992). This category contains procedural memory (e.g., knowing how to make a coffee) and priming, which refers to the phenomena that exposure to a stimulus influences the behaviour or response to a later stimulus (e.g., judging someone’s character as "warmer" after holding a warm coffee; (Williams & Bargh, 2008)). These memories do not require conscious perception which is why they are also referred to as implicit memories (Squire, 1992).

# Intracranial EEG (iEEG) and epilepsy

Ward and Thomas (1955) were the first to successfully record human single neurons. They did so in the posterior temporal lobe using glass micropipettes while surgeons tried to localize the epileptic focus and repair a bone defect in the patient’s skull.

Roughly 1% of the population suffers from epilepsy, and in one-third of these cases treatment and medication provide no remedy from seizures (Kwan et al., 2011) If the seizure onset is focal, i.e., spatially confined it is sometimes possible to resect the epileptic tissue which effectively cures the patient (Engel, 1996).

Henry Molaison, also known as Patient H.M., was the most prominent epilepsy patient. He underwent a resection of both hippocampi and large parts of his MTL, which led to a seizure-free life (Corkin, 1984; Scoville & Milner, 1957; Squire, 2009). As a side effect of the surgery, he developed a graded retrograde amnesia and a complete anterograde amnesia, meaning that he retained some distant memories, but could neither remember recent memories nor create new ones (Corkin, 1984; Scoville & Milner, 1957; Squire, 2009). This inspired a new wave of research implicating the hippocampus and neighbouring structures in episodic memory processing (Corkin, 1984; Scoville & Milner, 1957; Squire, 2009). Nowadays, an extensive battery of tests is administered prior to resection, with the aim to exclude as much healthy tissue as possible (Parvizi & Kastner, 2018). One important procedure is the intracranial implantation of depth electrodes at suspected seizure onset zones, based on seizure characteristics, anatomical scans, and long-term surface EEG recordings (Parvizi & Kastner, 2018). Once implanted these electrodes typically remain in place for 1-2 weeks to gain an understanding which brain regions are responsible for the generation of epileptic seizures and will later be resected (Parvizi & Kastner, 2018; Quian Quiroga, 2019). While these electrodes are implanted, researchers perform experiments with willing patients granting insight into the neurophysiological underpinnings of various brain functions.

The clear advantage of intracranial electrophysiological recordings over traditionally used non-invasive methods is a spatially confined and well localized signal (vs. surface EEG or MEG) with a high temporal resolution (vs. fMRI) (Quian Quiroga, 2019). In contrast to invasive recordings in animals, humans can typically perform a task after minimal instructions and can provide comprehensible verbal feedback when prompted. A severe disadvantage of intracranial recordings is a relatively limited coverage of the brain compared to traditionally used brain recording methods. This downside is exacerbated by the fact that the spatial positions of the intracranial electrodes are determined by clinical need and not scientific experimentation (Parvizi & Kastner, 2018; Quian Quiroga, 2019). Furthermore, access to epileptic patients that are willing to participate in scientific research is limited. Finally, even if these hurdles are overcome, it is important to ascertain that pathologic epileptic activity does not influence the obtained results (Parvizi & Kastner, 2018; Quian Quiroga, 2019).

The type of microwire electrodes that are still in use today (Fried et al., 1999) have been described in the early 70s by Babb and colleagues (Babb et al., 1973). These so-called Behnke-Fried electrodes are single-use intracranial depth electrodes that consist of a 1.3 mm hollow macroelectrode through which a bundle of eight high-impedance microelectrodes and one low-impedance microwire is inserted. By default, the low-impedance wire is used as a reference for the high-impedance wires. Microwires have a width of ~40 µm and radially protrude 4-5 mm past the end point of the macro depth electrode. They are made from platinum, which has a high impedance for lower frequencies and a low impedance for higher frequency bands. This allows the recordings of action potentials of multiple local single neurons superimposed on local field potentials. Each microwire bundle typically yields around a dozen separate neurons. Usually, fewer single neurons can be recorded at the end of the first recording week, which is likely due to gliosis at the microwire tip (Fried et al., 1999).

Newer probes such as the Neuropixels 1.0 probe contain 384 channels across a 20 µm × 70 µm × 10 mm shank (Dutta et al., 2019; Jun et al., 2017), providing an advantage over conventionally used microwires which can spread out during electrode implantation in an unpredictable way. ~~As a consequence, spike detection and clustering in conventionally used electrodes cannot rely on local similarities between microwires~~. Not only does this new probe allow for a higher quantity of recorded neurons, but the rigid distance between neighbouring channels (20 µm) allow a higher quality spike sorting as spikes are propagated across contacts. Using a Neuropixels probe Durand and colleagues (Durand et al., 2022) recorded almost 600 neurons across 13 different brain regions using six different Neuropixels probes in a mouse. In the first reported use of this novel probe in humans, Paulk and colleagues recorded upwards of 300 cortical single neurons in two patients awaiting DBS implantation for movement disorder. However, in one epilepsy patient awaiting tissue resection, the probe in the lateral temporal lobe only recorded the activity of 29 neurons (Paulk et al., 2022). Of note, the entire recording was conducted within the confines of the operating room for just 15 minutes, so no experimental intervention was possible (Paulk et al., 2022). Compared to commonly used electrodes in humans, the higher yield of neurons with newer probes will facilitate analyses of assemblies of neurons and their interactions with different brain regions (Durand et al., 2022).

# Microwire recording – LFP and Single Units

The recorded signal from the microwires can be divided into two components depending on their frequency. The first component is the local field potential (LFP), which reflects changes in the extracellular membrane potential and ranges until 300 Hz. Superimposed onto the LFP is the activity of individual neurons and multiunits in close proximity to the microwire.

Action potentials (also called *spikes*) are characterized by a steep and transient amplitude increase in the signal. Spike detection and sorting can be implemented using a variety of existing toolboxes, with new ones being developed continuously that demonstrate promising results (Pachitariu et al., 2023). Here, we used the wave\_clus algorithm, which is described in detail in Chaure and colleagues (Chaure et al., 2018). The following is a brief synopsis of the processing steps performed by this algorithm. The first step to detect these neural spikes is to filter the data so it only contains the spike-band which ranges from 300 Hz to 3000 Hz. Next, the data is segmented into smaller epochs of typically five minutes each, so artefacts occurring in one segment do not increase the threshold across the entire recording. Each one of these epochs is then individually thresholded using some form of deviation to a measure of central tendency (such as the mean or median). Points where the threshold is surpassed are stored as putative spikes. This spike detection is done separately for positive and negative deflections. Once a spike is detected the features of each spike-waveform are computed using a Haar wavelet and the most significant coefficients are identified using a Lilliefors test (Chaure et al., 2018). Next, nonparametric clustering is performed in the feature space using superparamagnetic clustering. Superparamagnetic clustering groups spike waves into clusters based on nearest-neighbour interactions (Blatt et al., 1996). Through template-matching in Euclidian space unclassified waveforms are assigned to one of the identified clusters. The resulting clustering solution is then manually inspected and further optimized by rejecting artefact cluster, splitting clusters that represent multi-unit activity and merging clusters that likely stem from the same neural source (Chaure et al., 2018).

Text

Description automatically generated with low confidence **Figure XX.** Example schematic of a power spectrum visualising the 1/f relation between power and frequency. The black line represents the aperiodic component of the signal, and the grey line reflects the superimposed periodic activity. The x-axis shows the frequency, and the y-axis displays the power. Both axes are in log-space.

The extracellularly recorded local field potential (LFP) represents synchronously active neurons that are spatially aligned. Synaptic activity is the largest contributor to the LFP, but transmembrane currents from soma, dendrites, spikes, and spike afterpotentials also impact the LFP (Buzsaki et al., 2012). The LFP can be divided into periodic (oscillatory) and aperiodic (fractal, non-oscillatory) components (see Figure XX). Aperiodic power is inversely related to the frequency and roughly follows a 1/f relationship (where f is the temporal frequency). This power-frequency relationship is likely due to dendrites acting as a low-pass filter (Linden et al., 2010) (xx re-read abstract) and because fewer neurons can be active in shorter cycle lengths of higher frequencies (Buzsaki et al., 2012). In the past, the aperiodic part of the signal was often ignored or considered background noise (Donoghue et al., 2020). However, more recent research has pointed to the steepness or tilt as well as the offset of the 1/f aperiodic component as an indicator for excitation (xx) and a proxy for neural firing (Manning et al., 2009). The periodic part reflects true oscillatory activity (i.e., rhythmic activity in a circumscribed frequency range). Activity in these narrowband frequencies have been associated with a wide range of cognitive processes (xx) and states (xx). Analysing this oscillatory activity without consideration of the 1/f shape can be problematic (Herweg et al., 2020; Samaha & Cohen, 2022) as the shape of the 1/f can bias the oscillatory activity. Moreover, a tilt or change in offset can be erroneously interpreted as a change in oscillatory activity (Herweg et al., 2020). There are multiple methods to separate the signal into periodic and aperiodic parts such as Irregular Resampling Auto-Spectral Analysis (IRASA; Gao et al. (2011)) and Fitting Oscillations and One Over F (FOOOF; Donoghue et al. (2020)).

# The hippocampus

The etymological root of *hippocampus* comes from the Greek words "hippos" (horse) and "kampos" (sea monster) and trace back to the anatomist Julius Caesar Aranzi, who compared the shape of the hippocampus to that of a sea horse (Bir et al., 2015). Although the term hippocampus prevailed, different names have been proposed in the past, such as “silkworm” or “Ram’s horn” (Bir et al., 2015). Humans have two mirrored hippocampi, one in each hemisphere. These hippocampi are located beneath the neocortex within the medial temporal lobe (MTL). The hippocampus can be divided into the dentate gyrus, hippocampus proper (CA1-CA3) and the subiculum. Highly processed information flows from prefrontal neocortex, perihinal cortical areas and association cortices through the EC to the hippocampus (Teyler & Rudy, 2007). This cortical information is integrated with subcortical input from the amygdala and thalamus (Teyler & Rudy, 2007). This positions the hippocampus uniquely to integrate cortical and subcortical information streams (Swanson & Mogenson, 1981 xx).

# Information flow during memory processing

According to a model by O’Reilly and Rudy (O'Reilly & Rudy, 2001), during memory encoding information from the cortex reaches the entorhinal cortex (EC) where two representations are generated. One representation is projected via the broad and diffuse perforant path to the dentate gyrus (DG), forming a sparse rendering of the cortical activity pattern. The DG then connects to CA3 through the sparse, focused and topographically arranged mossy fibre pathway, with approximately 70 synapses linking to each CA3 neuron in rats (O'Reilly & Rudy, 2001). At the same time, the other representation bilaterally xx projects from the EC to CA1 and back xx. This connection is point-to-point and not diffuse like the perforant path (Tamamaki, 1991 xx). Due to the coactivity of neurons in CA3 and CA1, their diffuse and widespread synaptic connections through the Schaffer Collaterals are strengthened. During retrieval, a partial input of the original representation is sufficient to reactivate the representation in CA3, where the entire representation is pattern completed. This in turn reinstates the appropriate CA1 representation that can project back to the EC because of the bidirectional connection between EC and CA1 (O'Reilly & Rudy, 2001).

# The Indexing Theory of the human hippocampus

More than three decades ago, Teyler and DiScenna proposed the Indexing Theory as a framework to explain hippocampal function during the encoding and retrieval of episodic (at the time called experiential) memory in humans (Teyler & DiScenna, 1986). According to the Indexing Theory, during initial encoding the various multimodal elements that make up an episode instate a cortical activity pattern that is projected to an assembly of neurons in the hippocampus. Subsequently, a partial input of the initial experience is sufficient to reactivate the entire assembly of associated hippocampal neurons, a process known as pattern completion. These neurons then project back to the neocortex, reinstating the entire experience (Teyler & DiScenna, 1986; Teyler & Rudy, 2007). Pattern separation refers to the complementary ability to distinguish between distinct episodes. Because each experience is uniquely indexed, even the highly overlapping cortical representations of two similar episodes can be separated in the hippocampus (Teyler & DiScenna, 1986; Teyler & Rudy, 2007). This hippocampal index allows a flexible way to quickly store the cortical representation of an episodic memory. Over time, the initially strengthened synaptic connections for unimportant memories either decay (xx) or fall victim to interference (xx). Within this framework the function of the hippocampus can be likened to a librarian: it can direct one to the necessary information within the library (the neocortex) but does not possess the knowledge itself. This implies that the hippocampus is content-free and does not contain/store <-xx semantic information. This is because hippocampal neurons arbitrarily bind concurrent cortical activity irrespective of what (content xx) it represents.

# Different types of memory

The Standard Model of Systems Consolidation (Squire & Alvarez, 1995) proposes that a memory trace is initially encoded in the hippocampus and only weakly encoded in the cortex. Over time the hippocampus reactivates the cortical pattern thereby gradually strengthening the synaptic connections that formed the initial memory trace in the cortex - a concept that dates back to Marr (Marr, 1971). As a result, the hippocampus eventually becomes redundant. This is in line with the graded retrograde amnesia observed in patient H.M. (Scoville & Milner, 1957), whose hippocampus and extensive parts of the medial temporal lobe (MTL) had been removed. The forgetting of more recent memories can be attributed to their incomplete consolidation.

McClelland and colleagues (McClelland et al., 1995) extended the Standard Model of Systems Consolidation and developed a computational theory wherein the hippocampus is responsible for rapid learning of new information that could then be integrated in the neocortex over longer time periods. This would prevent catastrophic interference between older and newer memories in the cortex. The hippocampus separates experiences and avoids interference through the implementation of a sparse and orthogonal code where each event is represented by a distinct assembly of neurons. The neocortex and hippocampus as Complementary Learning Systems provide a solution to the challenge that the brain needs to both recognize general patterns in the environment and capture the details of a particular episode (O'Reilly & Rudy, 2001). In contrast, the Multiple Trace Theory (Nadel & Moscovitch, 1997) proposed that the hippocampus remains essential for episodic memory even for remote memories. However, similar to the Systems Consolidation account, the hippocampus aids in the stabilisation of semantic memories in the neocortex. In support of this Corkin (Corkin, 2002) argued that remote memories of H.M. were semanticized and thus did not reflect retrieval of true episodic memories.

Importantly, whether the Systems Consolidation or the Multiple Trace Theory prevails has no bearing on the concept of a hippocampal index assembly which is compatible with either framework.

# How are neurons allocated to a memory trace?

Over one hundred years ago Richard Semon proposed that a memory is represented by the long lasting physical changes in neural assemblies that encoded the initial experience (Semon, 1904). This memory trace is termed “engram” in the animal literature (Josselyn et al., 2015; Park et al., 2016; Semon, 1904). Unlike Index Neurons, which are assumed to be in the hippocampus, the entire engram representing an experience spans multiple assemblies in various brain regions that are functionally connected (Roy et al., 2019). Optogenetics and chemogenetics (used in humans? xx), which are not available in human research, have been especially beneficial to memory research in animals. Experiments conducted on rodents revealed that neurons are allocated to an engram based on their excitability, with those having higher excitability more likely to be included (Frankland & Josselyn, 2015; Josselyn, 2010). Excitability is defined as the inclination of a neuron to fire an action potential in response to a signal (Dong et al., 2006). Rashid and colleagues (Rashid et al., 2016) showed that neurons assigned to an engram inhibit neighbouring neurons for about six hours through GABAergic interneurons. Without this inhibition, memories that occur close in time might be encoded by non-overlapping neurons.

After being allocated to an engram, neurons representing an event remain in a state of elevated excitability for over six hours. Consequently, some of the initial engram neurons are likely to be coallocated to events that occur within this timeframe (Cai et al., 2016; Rashid et al., 2016). After this period, excitability drops making it less likely that the same engram neurons represent temporally distant events (Frankland & Josselyn, 2015; Silva et al., 2009)). Cai and colleagues (Cai et al., 2016) found evidence for this in CA1 of mice, that were presented with context A, followed by context B seven days later and then context C five hours later. Engrams representing the contexts separated by a shorter temporal gap were largely overlapping, while those with a larger time delay showed no such overlap. Rashid and colleagues (Rashid et al., 2016) extended these findings by optogenetically stimulating neurons in the lateral nucleus of the amygdala that were allocated to an event 24h before a second event took place (i.e., outside of the 6 hour window of increased excitability). Due to this artificially induced excitability the second event was coallocated to the same subset of neurons. A similar result was obtained when the remote memory was retrieved prior to acquisition of a related memory, suggesting a mechanism for integrating newer memories with relevant older memories ((Rashid et al., 2016; Yokose et al., 2017) Yokose et al., 2017: two distant memories show an overlap if they are co-retrieved). This mechanism of coallocation is suspected to be responsible for false memories: engram cells in the dentate gyrus active during the exploration of context A were optogenetically reactivated in context B, where the mice also received footshocks. Mice then showed fear reinstatement in context A (artificial fear memory) and B (natural fear memory), but not in a third neutral context (Ramirez et al., 2013). Similarly, Vetere and colleagues (Vetere et al., 2019) tagged neurons in the olfactory bulb and synchronized it with either appetitive or aversive neural pathways. Subsequently mice showed attraction or aversion to the real odour giving credence to the idea that an artificial memory was created the absence of a real experience. Engram neurons are necessary and sufficient for memory retrieval. After destroying a subset of neurons that were initially allocated to a fear memory mice suffered from a profound memory loss (Han et al., 2009). Importantly this loss-of-function was specific to the fear memory and new fear conditioning was possible. Ablating other neurons did not lead to a disruption in memory. Conversely, artificial reactivation of engram cells in the dentate gyrus reliably led to the retrieval of the memory even in the absence of external retrieval cues (Liu et al., 2012). In a neutral context mice did not freeze until the engram representing the fear memory was optogenetically reactivated. This represents a gain-of-function and cements engram cells as causally relevant for memory processing.

Although findings from rodent brains do not by default translate to the human brain, there is enough overlap that non-human animal work can inform human research and provide useful hypotheses. For instance, it is unknown how neurons become assigned to a memory in humans, but it is possible that excitability determines this allocation process as well.

# Neurons coding content: Concept Cells

Concept cells are neurons in the human MTL that fire in response to specific concepts in an all-or-none way (Rey et al., 2018). They exhibit a high degree of multimodal invariance (i.e., they respond to Jennifer Aniston as an image or her spoken name) and context invariance (i.e., a concept neuron tuned to Jennifer Aniston would activate regardless of whether you see her in a movie, a park or in a café) (xx).

Curiously, the latency of their firing rate is much later than would be required by simple sensory processing and object recognition, which is an indication of their involvement in memory processing (Mormann et al., 2008). This lines up with the observation that most concept neurons are tuned to personally relevant concepts and depend on the subjective and conscious perception rather than objective sensory properties (Quiroga et al., 2014, 2008).

These concept neurons are not topographically organized, i.e., spatially close concept neurons might code for vastly different concepts (Quiroga 2016?). This spatial organization benefits episodic memory processing as it allows association between any two concepts without connecting distant areas (Quian Quiroga, 2019). According to Quian Quiroga (Quian Quiroga, 2019; Rodrigo Quian Quiroga, 2012; Quiroga, 2020) these CN are the building blocks of episodic memory formation and retrieval. If you met your best friend in your favourite café the concurrent activation of two assemblies of CN (one for your friend and one for the café) would represent the episode in the hippocampus. These assemblies would then project back to the neocortex reinstating the sensory activity pattern first induced during the formation of the episode. This back-projection parallels the one described in the Indexing Theory (Teyler & DiScenna, 1986; Teyler & Rudy, 2007) with the important difference that the hippocampal representation consists of previously existing concept specific representations/assemblies.

A separate memory of the same friend in a park would in turn be represented by the simultaneous activity of the same assembly coding for your friend and another assembly representing the park.

GOAL OF THIS THESIS

# Additional notes:

Subfields and how they are connected add citation:(doi.org/10.1016/j.cub.2015.10.049).

CA3 has a vast autoassociative network with synapses onto other CA3 neurons. This physioologiy inspired many impoortant bla bla (already in marr)

Medial septum makes theta in hippocampus

Research into different kind of neurons has led to a veritable embarasse de richesse

Episodic memories although originally defined as such, can implicitly considered conjunctive codes (Oreily & rudy paper). Complementary learning systems: O’Keefe & Nadel (1978): taxon local | hirsh 1974 | McClelland et al. (1995)

The hippocampus automatically binds sensory elements into a conjunctive code which corresponds well with the definition of episodic memory (O’Reilly & Rudy 2001).

In 1929 Hans Berger published his seminal work where he recorded electric potentials on the human scalp using an electroencephalopgraph (german: Elektroenkephalogramm; Berger, 1929). He mostly observed oscillations around 10 Hz, which he therefore termed alpha oscillations.

Title: High frequency power reinstatement in the human hippocampus during episodic memory

Abstract

Previous work has identified single neurons in the human hippocampus that significantly increase their firing rate during the encoding and retrieval of specific episodic memories (Episode Specific Neurons; ESNs). High frequency power (40-200 Hz; HFP) in the local field potential has been used as a proxy for multi-unit activity. We here studied the reinstatement of HFP in the hippocampus of patients while they completed a memory association task. Consistent with earlier observations we find a significant number of microwires that show a reinstatement of HFP from encoding to retrieval in individual episodes. Importantly, this reinstatement is not driven by a content-specific code (i.e., population activity of Concept Neurons). This effect is limited to later remembered episodes and not present for later forgotten episodes. These findings extend the discoveries of the previous chapters from the single neuron level to the population activity reflected in the local field potential.

Introduction

Episodic memories refer to distinctive events that occurred at a specific time and space. These memories are composed of multiple multimodal elements.

In the previous chapter we investigated the formation and retrieval of these episodic memories on the level of single neurons in the human hippocampus. These neurons (called Episode Specific Neurons; ESNs) increase their firing rate during encoding and retrieval of specific episodic memories. We provided compelling evidence that this episode specific code is separate from Concept Neurons. In this chapter we will delve into the neurophysiological substrates of memory processing that is one level above individual neurons: the local field potential (LFP). In contrast to local neural firing, LFPs reflect the aggregate of a myriad of local and distant transmembrane currents (xx). We will focus on the role of high frequency power (HFP; 40-200 Hz) as a proxy of local synchronous spiking activity (xx). Most of the literature examining the relation of spiking activity and HFP is based on studies in monkeys in early sensory cortical areas that have a topographic structure (Ray et al., 2008) (likely due to synchronisation), (Buzsaki et al., 2012; Leszczynski et al., 2020; Ray & Maunsell, 2011; Whittingstall & Logothetis, 2009), but some evidence has been reported in humans (Kucewicz et al., 2014; Manning et al., 2009; Miller et al., 2009; Nir et al., 2007). Although neighbouring neurons in the hippocampus are not structured topographically and often represent very different concepts (Redish et al., 2001)rodrigo xx) there is some evidence that the HFP-spiking relationship remains intact (Kucewicz et al., 2014; Manning et al., 2009).

It is unclear if enough neurons are part of one assembly of ESNs (see Chapter 1) to increase HFP, and further if these neurons are close enough in space and fire in synchrony. Preliminary evidence comes from Rutishauser and colleagues who reported that roughly 20% of all neurons in the hippocampus and amygdala respond to novel stimuli (xx), which is likely enough to elicit HFA. However, the authors do not report whether these neurons respond to specific new episodes or new episodes in general and how many of them reinstate their firing rate during retrieval. Based on the average number of identified Concept Neurons, recorded neurons, and presented images, it is estimated that approximately one million neurons within the medial temporal lobe code for a given concept. This represents only 0.1% of the total number of neurons in the MTL (R. Q. Quiroga (2012)), which likely does not impact HFP.

In conclusion, we postulate a reinstatement of power in the high frequency band from encoding of specific trials to their reinstatement during an episodic memory task. As Concept Neurons are thought to be part of smaller assemblies (R. Q. Quiroga, 2012) we expect not to find changes in high frequency power induced by specific concepts.

Materials and Methods

Procedure of memory experiment 1 and experiment 2

See above.

Participants

See above.

Ethical approval

See above.

Behavioural analysis

See Above.

Co-Registering

See Above.

Recording System and Electrodes

See above.

Statistical analysis

All statistical analyses were conducted using MATLAB R2020a on a computer running Windows 10 Enterprise. The significance threshold for all statistical tests was set at 0.05. Unless specified otherwise, all permutation tests were implemented with *N* = 1,000 random draws.

Identification of Episode Specific Neurons (ESNs)

See above.

LFP pre-processing

We downsampled the LFP data from microwires that contained neurons in the hippocampus to 1,000 Hz and applied a fourth-order Butterworth bandstop filter with a centre frequency of 50 Hz (± 1 Hz) and its harmonics up to 300 Hz, to remove line noise.

LFP Artefact Rejection

For each microwire, we computed the bandpass-filtered signal between 40 Hz and 200 Hz using a first-order Butterworth filter. We identified any data points exceeding five standard deviations from the mean of this signal as artefacts and excluded the one-second intervals preceding and following them.

Identification of Episode Specific Microwires (ESWs)

We considered neural activity from the onset of the associated image to the patient's response in encoding trials, and from the cue onset to the response onset in retrieval trials. To account for edge artefacts, we extended these trial definitions by 100ms on each side. We then performed a wavelet analysis using wavelets from 40 Hz to 200 Hz in steps of 5 Hz and a width of 7 cycles, on the linenoise-removed broadband signal. After removing all artefacts (see #Artefact Rejection), we computed the mean power over all frequencies.

Trials that consisted of 50% or more artefacts during encoding or retrieval were excluded, and if fewer than nine trials remained, the microwire was not considered for further analysis. We z-scored the remaining HFA power values independently for encoding and retrieval, and afterwards excluded later forgotten trials. Finally, we defined the element-wise product of the encoding and retrieval standardized HFA power as a proxy for episode-specific reinstatement. To calculate a threshold for this episode-specific firing reinstatement we permuted the order of the encoding and retrieval episodes and recomputed the reinstatement value. We repeated this step 1,000 times and took the 99th percentile as a threshold against which we compared the empirical reinstatement value. If the empirical reinstatement exceeded the threshold and its standardized power at encoding and retrieval was at least 1.645 (≙ *p*right-tailed < 0.05), we considered this microwire an Episode Specific Microwire (ESW). This procedure allows for thresholding but does not correct for multiple comparisons on the level of a microwire. To determine whether there was a significant number of microwires that showed an episode-specific power reinstatement, we randomly drew one of the previously calculated permutations for each microwire and determined whether it would be classified as a ESW under the same criteria as before. In each of the 1,000 permutations, we summed up the number of shuffled ESW which we then used to create a null distribution against which we compared the empirically determined number of ESW. To generate Figure XX, we repeated the time-frequency analysis in the range of 3 Hz and 200 Hz in 50 logarithmically spaced steps for all microwires that exhibited a HFP reinstatement in at least one episode. For each ESW we calculated the mean HFP during reinstated and non-reinstated episodes and then averaged the respective power spectra across all ESW. To determine the statistical significance of the results, we used a cluster-based permutation test (Maris & Oostenveld, 2007).

Identification of putative Concept Specific Microwires (CSWs)

We have adapted the method created by Mormann and colleagues (Mormann et al., 2011; Mormann et al., 2008) for detecting Concept Neurons to identify microwires whose HFP (xx) was reliably increased following the presentation of a specific image. For each microwire, we divided the local field potential of the 1000ms interval post-stimulus into 19 100ms overlapping bins, with the 500ms preceding stimulus onset as the baseline period. To prevent edge artefacts, we extended the testing and baseline intervals by 100ms on either side. We performed a time-frequency analysis using wavelets in the range of 40 Hz to 200 Hz (stepsize: 5 Hz) and a width of 7 cycles, allowing us to estimate the time-resolved power. We then averaged the power over all frequencies and within each time bin. If more than one of any of the six repetitions of an image contained over 50% artefacts that time bin was discarded for all repetitions. We then compared the mean HFA power in the remaining 19 bins across all six presentations of an image with the mean HFA power of all baseline periods in the session using a Mann-Whitney U test. We corrected for multiple comparisons using the Simes’ procedure (Rødland, 2006). To test whether our dataset has a significant number of CSWs for each microwire we shuffled the trial order and recomputed the CSW detection pipeline. We repeated this step 1,000 times to generate a distribution of how many CSW to expect under the null hypothesis.

Correlation between HFP and spiking activity

After pre-processing the LFP of the microwire on which a neuron was recorded (see #LFP pre-processing) we segmented the data into separate later remembered episodes. During memory encoding the time of interest started at the onset of the associate image(s) and ended when the patient gave their response. In contrast, during memory retrieval, the time of interest started at the cue onset and ended when the patient gave their response. We added 100ms on each side to account for edge artefacts. Then, we performed a wavelet analysis between 40 Hz and 200 Hz in steps of 5 Hz and a width of 7 cycles, and averaged the power across frequencies. We then normalized the HFP across time, using a z-transformation, and concatenated this standardized power values across all episodes. To compute the instantaneous firing rate of the corresponding neuron, we convolved the firing times with a Gaussian kernel (kernel parameters: mu = 0, standard deviation = 50ms, length = 300ms, normalized peak to 1). We then z-scored this instantaneous firing rate and concatenated all episodes. Subsequently, we performed a linear correlation between the concatenated standardized HFP and the concatenated standardized instantaneous firing activity, separately for encoding and retrieval. To assess the statistical significance of the correlation we shuffled the data circularly and recomputed the correlation with this shuffled data. We repeated this step N = 10,000 times and compared the empirical correlation coefficient with the resulting null distribution of shuffled correlation coefficients. We performed this analysis twice: once for neural activity during reinstated episodes and once for all other episodes.

Results

We conducted two different experiments in which patients implanted with stereotactic Behnke-Fried depth electrodes completed a memory association task (see xx) In experiment 1 we recorded from 1011 microwires and 585 neurons in the hippocampus (16 participants, 7 female; average age = 36.13 years, from 26-53 years) and in experiment 2 we recorded from 344 microwires and 216 neurons in the hippocampus (14 participants, 7 female; average age = 33.86 years, from 19-58 years). During the encoding phase of experiment 1 patients were instructed to mentally form a vivid story containing an animal cue and two associated images (two faces, two places, or one of each). Experiment 2 only had one associate image and either cue or associate could be a face, a place or an animal. After the encoding phase a short distractor task commenced during which patients had to determine whether a series of 15 numbers were odd or even. During the retrieval phase, the cue image was presented and the patient was asked to retrieve the associated image(s). Each episode was learned and retrieved once and the experiment was completed at the participants' own speed.

Reinstatement of high frequency power

To investigate high frequency power reinstatement, we calculated the average power within a range of 40 Hz to 200 Hz in steps of 5 Hz for every microwire. During encoding we considered neural activity from the time point the associated image was presented until the patient gave their response. During retrieval the time of interest stretched from the cue onset to the response. We z-scored the power values independently for encoding and retrieval and subsequently excluded episodes that were later forgotten. We defined the element-wise product of the standardized encoding and retrieval power values as a measure of episode-specific reinstatement. Using a trial-shuffle procedure we re-computed these reinstatement values 1,000 times. If any empirical reinstatement value exceeded the 99th percentile of these permuted values and if the standardized power at encoding and retrieval during that episode exceeded a value of at least 1.645 we considered this microwire an Episode Specific Microwire (ESW). To estimate how many ESW we can expect by chance we then randomly drew one of the previously calculated permutations for each microwire and applied the same thresholding technique to these shuffled reinstatement values. This allowed us to create a distribution of ESW under the null hypothesis against which we could compare the number of empirically identified ESW. Using this approach, we found a significant number of ESW in experiment 1 (*n* = 144 out of 1010 microwires, *p* = 0.0310; permutation test; see Figure xx for an example). However, there was no significant number of ESW when limiting the analyses to later forgotten episodes (*p* = 0.305; permutation test). We subsequently contrasted the power spectra of reinstated episodes with non-reinstated episodes from 3 Hz to 200 Hz using 50 log-spaced frequency points. A cluster-based permutation test revealed that during reinstated trials, the power was significantly increased from 9.9 Hz to 200 Hz (*p* < 0.001) at encoding and from 15.3 Hz to 200 Hz (*p* < 0.001) at retrieval (see Figure 1).

HFP reinstatement is not content dependent

The second experiment included a visual tuning task, during which the same images that were used in the preceding memory task were presented repeatedly without an episodic memory component. This approach has been traditionally used to detect neurons responding to specific concepts or categories (Florian, Rodrigo xx) and allowed us to exclude all episodes that contained an image which reliably evoked a HFP increase during a visual tuning task. We defined Concept Specific Microwires (CSW) as any microwire with a significant increase of HFP in any of 19 overlapping 100ms time bins following the image presentation across all six repetitions in comparison to a 500ms pre-stimulus baseline period using a Mann-Whitney U test (see Methods). We carried out the analysis twice, once with the typically used cut-off threshold of *p* = 0.0005 and again with a more liberal cut-off threshold of *p* = 0.05. Note that no corrections were made for testing multiple images for tunings, thus making a threshold of *p* = 0.05 very liberal. No CSWs were detected at *p* = 0.0005; however, when the threshold was lowered to *p* = 0.05, we found a significant number of CSWs (86 out of 344 microwires, *p* = 0.005, permutation test). Because no CSWs were detected at a cut-off of *p* = 0.0005, no episodes were excluded in the ESW analysis. In experiment 2 we replicated our prior results and found a significant number of ESWs (*n* = 52 out of 344 microwires, *p* = 0.003). We then repeated the ESW analysis, this time excluding episodes with significant CSW activity at a threshold of *p* = 0.05. Despite this threshold change, we identified a significant number of ESWs (*n* = 50 out of 344 microwires, *p* = 0.001). Of note, although the more liberal CSW threshold led to the identification of fewer ESWs the resultant p-value is lower. This is because the reduced threshold is also applied when determining the number of permuted ESWs (i.e., ESWs expected by under the null hypothesis).

In summary, we discovered a memory code in the form of a HFP reinstatement between encoding and retrieval of individual episodes across two independent experiments. Although we were unable to detect any CSW activity using the traditionally used threshold, we detected a significant number of CSWs with a more liberal threshold. Importantly, our findings could not be accounted for by a content-specific code (i.e., CSWs).

HFP correlates with ESN and single neuron firing

Next, we examined the correlation between HFP and single neuron firing in our sample. We first determined the instantaneous firing rate of each ESN during reinstated episodes. In a separate analysis we calculated the instantaneous firing rate during non-reinstated episodes. We segmented the LFP data into later remembered episodes and performed a wavelet analysis from 40-200 Hz. For each episode we averaged the power in that frequency range. We then z-scored the instantaneous firing rate and the HFP estimate across time. Finally, we concatenated each episode separately for encoding and retrieval. We performed a linear correlation between the standardized HFP and the standardized instantaneous firing rate and assessed the statistical significance by comparing it with the correlation values that we obtained through circular shuffling. In experiment 1, HFP and ESN firing during reinstated episodes correlated with r = 0.xx during encoding (R² = 0.xx, p = 0.xx; permutation test) and r = 0.xx during retrieval (R² = 0.xx, p = 0.xx; permutation test). Firing during non-reinstated episodes significantly correlated with HFP during encoding (r = 0.xx, R² = 0.xx, p = 0.xx; permutation test) and during retrieval (r = 0.xx, R² = 0.xx, p = 0.xx; permutation test). A similar relationship was found in experiment 2, where HFP and firing during reinstated episodes correlated with r = 0.140 at encoding (R² = 0.xx, p = 0.xx; permutation test) and r = 0.135 during retrieval (R² = 0.xx, p = 0.xx; permutation test). Firing during non-reinstated episodes correlated with HFP with r = 0.xx during encoding (R² = 0.xx, p = 0.xx; permutation test) and r = 0.xx during retrieval (R² = 0.xx, p = 0.xx; permutation test).

**Chart

Description automatically generatedFigure XX. Example ESW.**

(A) The bar plots show the z-scored HFP on the y-axis for 23 episodes on the x-axis colour coded for encoding (blue) and retrieval (orange). The transparent bar encompassing the standardized HFP represent their element wise product, which is used as a measurement for episodic memory reinstatement. The dotted line represents the threshold which is calculated based on a permutation test.

(B) The time resolved HFP (y-axis) during memory encoding with the time in seconds (x-axis) starting from the associate image onset for reinstated episodes (purple) and non-reinstated episodes (green). The shaded area represents the SEM.

(C) Same as (B), but during retrieval and starting at the cue onset.

Chart, histogram

Description automatically generated

**Figure XX. Number of reinstated episodes and number of ESW expected under the null hypothesis.**

(A) Pie chart showing the number of episodes each neuron reinstated during experiment 1 (zero episodes: 598 microwires; one episode: 345 ESWs; two episodes: 60 ESWs; three episodes: 7 ESWs; four episodes: 1 ESWs).

(B) Same as (A), but for experiment 2 (zero episodes: 224 microwires; one episode: 97 ESWs; two episodes: 15 ESWs; three episodes: 3 ESWs).

(C) Distribution of the number of ESWs expected by chance and the number of empirically found ESW (red line) in experiment 1. (D) Same as (C) but for experiment 2.

Chart, histogram

Description automatically generated

**Figure XX. Power spectra for reinstated episodes (purple) and non-reinstated episodes (green) during (A) encoding and (B) retrieval.** The x-axis displays the frequency, ranging from 3 Hz to 200 Hz in 50 logarithmically spaced increments. The y-axis displays the power on a logarithmic scale to enhance visibility. The shaded regions show the SEM. The grey rectangles specify frequencies at which the power during reinstated episodes significantly exceed the power of non-reinstated episodes (9.9 Hz to 200 Hz at encoding; 15.3 Hz to 200 Hz at retrieval, based on a cluster permutation test; Maris and Oostenveld (2007)).

Discussion

Episodic memories refer to distinctive events that occurred at a specific time and space. These memories are composed of multiple components. In Chapter 1 we identified how the human hippocampus processes these episodic memories. These neurons (called Episode Specific Neurons; ESNs) increase their firing rate during encoding and retrieval of specific episodic memories. In the present chapter we extended these findings from single neurons to the population level by investigating the local field potential (LFP) as a proxy of multi-unit activity. We analysed two independent datasets that were collected using microelectrodes located in the human hippocampus while patients performed a memory association task. Power in the high frequency band (40-200 Hz) on a significant number of microwires was reinstated from encoding to retrieval of specific episodes. These findings cannot be explained by a content code (i.e., HFP induced by the presence of particular concepts). Applying the traditional criterion used to in Concept Neuron detection to detect a content code seems to be too conservative to detect significant increases in the HFP. However, when lowering this threshold, we found a significant number of microwires that show a consistent HFP increase when presenting specific concepts (CSW) despite the relatively small assembly size of Concept Neurons (R. Q. Quiroga, 2012). Importantly, the same threshold was also lowered for the group-level permutation test, which we used to determine the number of CSW expected under the null hypothesis. Concept Neuron activity might be reflected in the HFP due to the spatial clustering of Concept Neurons within the vicinity of a microwire (but see xx; neighbouring neurons often code something different). Alternatively, multiple Concept Neurons coding the same concept might be active within a short delay, leading to a higher deflection in the LFP. The development of new electrodes that enable the recording of multiple neurons that code the same concept at more precisely known locations (such as (Durand et al., 2022; Dutta et al., 2019; Jun et al., 2017; Paulk et al., 2022) might offer an answer to this question.

Our analyses revealed that the power differences between reinstated and non-reinstated episodes exceeded the frequency range of 40-200 Hz that we used to differentiate the two. Reinstated episodes were characterized by an increased power from 10 Hz (during encoding) and 15 Hz (during retrieval), implying that the distinction between reinstated and non-reinstated episodes may not be limited to 40-200 Hz, but could be attributed to either an offset or a spectral tilt of the 1/f power spectrum. Future studies will need to carefully disentangle the individual contributions of oscillatory changes, a power offset, and a spectral tilt between reinstated and non-reinstated trials.

The range of high frequency activity (40-200 Hz) overlaps with the so-called ripple band (80-120 Hz). Excitatory input from CA3 induces ripple activity in the CA1, which is characterized by highly synchronized neural firing (Buzsaki, 2015). Ripple activity has been linked to memory consolidation and replay of previous experiences (Jadhav et al., 2012; Roux et al., 2017; Vaz et al., 2019). For example, research by Vaz and colleagues (Vaz et al., 2019), has implicated coupled ripple activity to coordinate the information flow between the MTL and the temporal cortex (also see (Ngo et al., 2020)). Considering this, future studies should investigate the extent to which the here reported HFP memory reinstatement effect is driven by activity in the ripple range. Here we report a significant correlation between spiking of individual neurons and the mean power in the high frequency range from 40 Hz to 200 Hz. However, this correlation is weak, explaining only ~1-2% of variance. **Of note, we correlated the firing rate of only a single neuron with the HFP of the microwire on which it was recorded (lots of redundancy with 2 sentences prior).** Since the firing rate reflects just a single neuron, the HFP includes activity from disproportionally more neurons with different firing rates. We therefore expect a higher correlation when analysing more neurons. Moreover, it is conceivable that different microwires from the same bundle may be better suited to pick up transmembrane currents from nearby neurons. Additionally, there is substantial variance in the literature which frequency range constitutes the high frequency band (e.g., xxx). Future studies should aim to identify which frequencies are most indicative of single neuron firing taking into considerations differences between neuron types (excitatory or inhibitory cells). Furthermore, recent research has suggested that synchronous firing is more influential in increasing HFP compared to firing alone (xx). Unfortunately, due to the limited number of single neurons that can be recorded using currently available microwires we cannot resolve this question. Recording more neurons using newer electrodes may enable us to disentangle the roles of synchrony and firing in relation to HFP in the future. A larger brain coverage would also allow the investigation of what role the recorded brain area plays in moderating the relationship between neural firing and HFP (paper where different brain layers produce different findings xx).

To conclude the present chapter, consistent across two independent datasets we identified a significant number of microwires which show a HFP reinstatement during encoding and retrieval of specific memories (ESW). This HFP activity showed a low, but significant correlation with neural firing of ESNs and single neurons during encoding and retrieval. Although we did not find reliable HFP increases to specific concepts using the traditionally used threshold, we identified a significant number of concept coding microwires using a more liberal threshold (CSW). Importantly, the HFP reinstatement for specific memories could not be attributed to this content code. Taken together the present work extends findings from the level of the single neuron and provides a potential link to surface EEG recordings (Buzsaki et al., 2012).

Third “Chapter” (I won’t do chapters)

HFA reinstatement using XC

Maybe another reinstatement approach? Binning with multiple bin sizes?

Phase Slope Index (information flow from cortex to hippocampus at encoding and v.v. at retrieval)

Phase Opposition Sum of spikes in low and high theta

Maybe I have time to look into 1/f in reinstated trials

**Title: Absence of memory success induced theta oscillation increase and theta spike-field coupling in the human hippocampus during episodic memory processing**

**Abstract**

Theta oscillations play a central role in memory processing. Recent findings point towards there being not one dominant theta frequency in the human hippocampus, but rather two: a slow theta (2-5 Hz) and a fast theta (5-9 Hz) oscillation.

Recent work has suggested that successful memory processing is reflected in a narrowband theta increase as well as a ‘tilt’ in the aperiodic power spectrum, where lower frequencies are diminished and higher frequencies increased. Furthermore, according to an influential theory memory encoding and retrieval occurs in opposite theta phases so newly encoded memories do not cause catastrophic interference with older memories.

We investigated these hypotheses in two independent samples of intracranial microwire recordings. Contrary to previously reported findings, our results provide inconclusive evidence regarding narrowband slow and fast theta power and an aperiodic tilt. Our research did not reveal consistent evidence that neurons that increase their firing rate during encoding and retrieval of specific episodes (Episode Specific Neurons; ESNs) or other neurons fire at a distinct theta phase during encoding and retrieval. Likewise, we found no significant theta phase difference between neurons firing at encoding and retrieval.

**Introduction**

In the preceding chapters, we found evidence of an episode specific neurophysiological marker at both the single-neuron level and in the high frequency power of the microwire local field potential (LFP). Next, we will explore another prominent frequency in the hippocampus, the theta oscillation, and investigate how it relates to single-neuron spiking.

Research in the role of theta oscillations on learning on memory go back to the late 70s (Berry & Thompson, 1978; Winson, 1978). Winson (Winson, 1978) showed that lesioning the medium septum caused a reduced hippocampal theta rhythm along with an impaired spatial memory. In line with this, higher theta power in rabbits was associated with augmented learning (Berry & Thompson, 1978).

Since then, evidence regarding the role of theta oscillations in episodic memories has been contradictory. While most studies employing surface EEG report increases in theta power, most iEEG studies report a memory induced theta power decrease (Herweg et al., 2020). Herweg and colleagues (Herweg et al., 2020) suggested that this might be because studies frequently contrast later remembered with later forgotten memories and therefore conflate domain-general cognitive processes, such as attention and perception, with memory-specific processes. Because domain-general cognitive processes are assumed to lead to a spectral tilt (i.e., less low frequency power and more high frequency power), a narrow band theta power increase induced by memory processing might be obscured. To ameliorate this shortcoming researchers should not contrast successful memory with unsuccessful memory but instead should compare strength of memory (e.g., retrieval confidence, amount of detail in contextual retrieval, retrieved spatial distance to encoded location in a navigational task; (Herweg et al., 2020).

Another reason how surface EEG might show a theta power increase, although the LFP shows a decrease is if theta over larger areas synchronizes but decreases in amplitude. The decrease is truthfully reflected in the LFP, but activity on the scalp is integrated over larger areas and thus more synchronous theta could lead to higher scalp theta power (Herweg et al., 2020). Taken together these considerations imply theta activity as an integral part of memory processing and suggest that conflicting evidence arises due to different recording methods (EEG/iEEG), memory contrasts (success vs success or vs failure) and frequency ranges (broadband vs narrowband).

An increased narrowband theta activity is in line with the prediction from a computational model and theoretical considerations that theta synchronization in the hippocampus is necessary for memory processing (Hanslmayr et al., 2016; Parish et al., 2018). More recent findings in humans demonstrated that behavioural response times in memory tasks are modulated by theta oscillations (Ter Wal et al., 2021) and that theta binds together the multiple elements within an episode (Clouter et al., 2017; Griffiths et al., 2021; Roux et al., 2022).

A central requirement of the hippocampus is the ability to encode new information without interfering with related previous experiences. Hasselmo and colleagues developed a computational model that solves this conundrum by moving encoding and retrieval processes to opposing phases in the theta rhythm (Hasselmo et al., 2002). Empirical support for this 180° shift between memory encoding and retrieval has been recently found by Kerrén and collegues (Kerrén et al., 2018; Kerrén et al., 2022).

The relation between single neuron firing and ongoing theta oscillation contains more information than the neural firing alone (Huxter et al., 2003; Jacobs et al., 2007). Place cells in the hippocampus are neurons that code for specific spatial locations. As rodents move towards a location, a place cell fires at increasingly earlier phases of the ongoing theta oscillation. One can therefore decode the position of the rodent in relation to a place by combining the theta phase and the neural firing (O'Keefe & Recce, 1993). In humans, a stronger spike-theta coupling (Rutishauser et al., 2010) as well as neurons locking to faster theta oscillations (Roux et al., 2022) predicts successful memory.

Importantly, recent findings suggest that there are two distinct theta rhythms governing the human hippocampus: a slow (2-5 Hz) and a fast (5-9 Hz) oscillation (xx).

We therefore hypothesized that (i) later remembered episodes show a shift in the aperiodic power spectrum and an accompanying increase in oscillatory fast and slow theta power in comparison to later forgotten episodes. (ii) We also expected this change in aperiodic and periodic activity to manifest when comparing episodes in which ESNs reinstate their firing rate (as described in Chapter 1) and episodes which are not reinstated. (iii) We hypothesized that neurons, particularly ESNs, fire at distinct slow and fast theta phases during the encoding and retrieval of episodic memories, and that there is a substantial phase offset between encoding and retrieval.

Materials and Methods

Procedure of memory experiment 1 and experiment 2

See above.

Participants

See above.

Ethical approval

See above.

Behavioural analysis

See Above.

Co-Registering

See Above.

Recording System and Electrodes

See above.

Statistical analysis

All statistical analyses were conducted using MATLAB R2020a on a computer running Windows 10 Enterprise. The significance threshold for all statistical tests was set at 0.05. Unless specified otherwise, all permutation tests were implemented with *N* = 1,000 random draws.

Identification of Episode Specific Neurons (ESNs)

See above.

Periodic and aperiodic theta analysis

To investigate periodic (i.e., oscillatory) and aperiodic activity (i.e., 1/f activity), we first downsampled the LFP in every microwire to 1000 Hz and bandpassed the signal using a fourth order Butterworth filter at 50 Hz ±1 Hz and harmonics up to 300 Hz. An episode was labelled as reinstated if any neuron on the respective microwire contained a single neuron that showed a significant firing increase during encoding and retrieval (i.e., an ESN; see Chapter 1). We defined the time of interest as the period two seconds prior to the response at memory encoding and retrieval. In experiment 1, an episode was considered correctly remembered if the patient correctly chose two out of two associate images and labelled as forgotten if the patient indicated they do not remember any associates or if they chose no correct associate.

For each episode, we extracted the periodic and aperiodic part of the signal using the FOOOF implementation (Donoghue et al., 2020) in Fieldtrip (xx) in a frequency range from 1 Hz to 200 Hz. We analysed two contrasts of the periodic and aperiodic activity: (i) reinstated episodes against non-reinstated episodes in microwires with ESNs, and (ii) correctly remembered episodes against forgotten episodes (excluding reinstated episodes).

For the periodic analysis, we averaged activity within the slow (2-5 Hz) and fast (5-9 Hz) theta bands and then conducted paired-sample t-tests to compare oscillatory activity between contrasts and one-sample t-tests to test for significant oscillatory activity. For the aperiodic analysis we performed paired-sample t-tests between contrasts, with the offset and tilt as dependent variables.

Theta components and pre-processing

As a first step, we downsampled the microwire signal to 100 Hz. Because we do not know the relative position of the recorded neurons to the microwires within a bundle of electrodes by extension we do not know if the microwire on which the neuron was recorded best represents the neural input into the neuron. For this reason, we took into consideration all eight microwires and generated two theta components using generalized eigendecomposition (Cohen, 2017).

The generalization of the eigendecomposition extends the eigendecomposition to a case with two square matrices. For an eigenvalue decomposition with a singular square matrix, the eigenvector with the highest eigenvalue accounts for the maximal variance in the underlying square matrix and is pairwise orthogonal to the other eigenvectors.

In contrast, the eigenvector with the highest eigenvalue in a generalized eigendecomposition can be understood as the filter that maximizes the difference between the two input matrices. The eigenvectors in a GED are independent, but not orthogonal. In practice when applied to two covariance matrices where one matrix represents the broadband activity and the other matrix is generated using a narrowband signal the first eigenvector yields a spatial weighting that maximizes the narrowband activity and minimizes the broadband activity. This eigenvector can be applied to the narrowband filtered multichannel data to generate a narrowband component (Cohen, 2017).

Based on previous literature (Goyal et al., 2020; Kota et al., 2020) we computed a slower theta component in the frequency range of 2 Hz to 5 Hz and a second, faster component in the range of 5 Hz and 9 Hz. To generate these components, we first applied a first order Butterworth filter to bandpass the broadband signal in all eight microwire channels between 2 Hz and 5 Hz (slow theta component) or 5 Hz and 9 Hz (fast theta component). We then demeaned the signal and computed a covariance matrix using this narrowband signal, which we divided by the number of samples. Next, we computed a second covariance matrix using the entire broadband signal. We computed the generalized eigendecomposition of these two covariance matrices and used the eigenvector with the highest eigenvalue as a spatial filter for the narrowband filtered signal to generate a narrowband component. We then applied the Hilbert transform to the narrowband component to get the analytic signal.

Spike-field coupling to slow and fast theta

We considered the spikes of neurons up to two seconds preceding the patient’s response during the encoding and retrieval of later remembered episodes. Each neuron had to contain at least 11 spikes within the time of interest to be included for further analysis. We confined all spike-field analyses to spikes and LFPs that were recorded on the same Behnke-Fried electrode.

We first wanted to estimate phase preference during encoding and retrieval independently. To do this we identified the complex value at the time of each spike. We subsequently normalized each complex value and averaged across spikes. For each neuron with spikes within the time of interest we computed the preferred phase by computing the angle of this average complex number. To estimate phase preference across neurons we performed a Rayleigh test.

We next investigated whether there was a significant difference in the phase of the narrowband signal between spikes during encoding and retrieval for (i) Episode Specific Neurons in trials that were later reinstated (rESN), (ii) for Episode Specific neurons in trials that were later not reinstated (nESN) and (iii) all other neurons (SU). To this end, we computed the cosine similarity between the complex value of each spike at encoding with the complex value of each spike at retrieval. We then averaged these similarity values across spikes for each eligible neuron. We determined the statistical significance of these difference scores using a one sample test for a mean angle of 0°, which we implemented using the function *circ\_mtest* from the Circular Statistics Toolbox v1.21.0.0).

However, if only few neurons are sensitive to the ongoing theta phase an encoding-retrieval phase offset in this small number might be overshadowed by other neurons whose activity is not theta modulated. To address this, we repeated the above phase difference analysis using only neurons whose spikes showed a significant coupling to the theta phase at encoding and retrieval, as evidenced by a Rayleigh test. We proceeded with this analysis only if there were at least 11 eligible neurons.

Results

We studied recordings from two different experiments (experiment 1: 585 neurons and 1011 microwires in the hippocampus, 16 participants, 7 female; average age = 36.13 years, from 26-53 years; experiment 2: 216 neurons and 339 microwires in the hippocampus, 14 participants, 7 female; average age = 33.86 years, from 19-58 years). Patients were implanted with stereotactic Behnke-Fried depth electrodes while completing a memory association task (see xx). During the encoding phase of experiment 1 patients were instructed to mentally create a vivid story consisting of an animal cue and two associate images (two faces, two places, or a face and a place). There was only one associate image in experiment 2 and cue and associate could be either a face, a place, or an animal. Following a short distractor task where patients had to indicate whether a series of 15 numbers were odd or even the retrieval phase begun. During the retrieval phase the cue image was presented and the patient had to recall the associate image(s). Each episode was learned and retrieved only once, and the experiment was self-paced.

Periodic and aperiodic theta activity during correctly remembered and forgotten episodes

The power spectrum can be separated into periodic and aperiodic components. The periodic components reflect true oscillations, while the aperiodic component is also referred to as 1/f and is assumed to reflect general excitability (xx). We separated periodic and aperiodic components in the microwire LFP using the FOOOF (Donoghue et al., 2020) implementation available in FieldTrip (xx) over a range of 1 Hz to 200 Hz and contrasted activity of later remembered with later forgotten episodes during encoding and retrieval. We found no significant differences in the aperiodic offset during encoding in experiment 1 (all *p* > 0.54) or experiment 2 (all *p* > 0.55). However, during retrieval there was a significantly larger offset and steepness in the aperiodic signal for later forgotten episodes in both experiment 1 (offset: *toffset* (341) = 3.13, *meanremembered* = 2.23 (*s.e.* = 0.047), *meanforgotten* = 2.25 (*s.e.* = 0.050), *poffset* = 0.002; steepness: *ttilt*(341) = 3.36, *meanremembered* = 1.83 (*s.e.* = 0.020), *meanforgotten* = 1.84 (*s.e.* = 0.021), *ptilt* < 0.001) and experiment 2 (offset. *Toffset* (114) = 3.00, *meanremembered* = 2.04 (*s.e.* = 0.084), *meanforgotten* = 2.08 (*s.e.* = 0.088), *poffset* = 0.0034; steepness: *ttilt*(114) = 3.37, *meanremembered* = 1.59 (0.038), *meanforgotten* = 1.61 (*s.e.* = 0.039), *ptilt*= 0.001). We next compared the periodic theta activity between remembered and forgotten episodes. In experiment 1, there was no difference in oscillatory slow or fast theta activity between the types of episodes during either encoding or retrieval (all p > 0.059). However, in experiment 2 a difference in periodic fast theta activity during encoding emerged (*t*(114) = 2.6813, *p* = 0.0084; all other *p* > 0.065) where later forgotten episodes showed an increase in periodic power (*meanremembered* = 14.7522 (*s.e.* = 2.082); *meanforgotten* = 18.4177 (*s.e.* = 2.9803).

We found consistent evidence across experiments and experiment phase (i.e., encoding/retrieval) for periodic fast theta activity in remembered and forgotten episodes (all *p* < 0.001; see Table xx). In both experiments, forgotten trials contained significant slow theta activity during retrieval (*p* < 0.009) but not encoding (*p* > 0.07).

Remembered episodes showed slow theta activity inconsistently across experiments. There was significant periodic activity during encoding and retrieval in experiment 2 (*p* < 0.001), but not experiment 1 (*pencoding* = 0.6 and *pretrieval* = 0.025).

To conclude, we observed an increased aperiodic offset and steepness for forgotten episodes compared to remembered episodes during retrieval, but not during encoding. There was no coherent difference in periodic slow or fast theta power between forgotten and remembered episodes across experiments. We found reliable evidence for fast theta oscillations, whereas slow theta oscillations showed less clear results.

Periodic and aperiodic theta activity during reinstated and non-reinstated episodes

We next contrasted periodic and aperiodic activity of reinstated against non-reinstated episodes on microwires that contained ESNs. We found no significant difference in the offset or steepness of the aperiodic component during encoding or retrieval in Experiment 1 (all *p* > 0.3) or experiment 2 (all p > 0.5).

Next, we contrasted oscillatory activity in the slow and fast theta range between reinstated and non-reinstated episodes but found no significant differences during either encoding or retrieval in experiment 1 (all *p* > 0.16) or experiment 2 (all *p* > 0.09). We found evidence for fast theta oscillations in reinstated and non-reinstated episodes during encoding and retrieval across both experiments (p < 0.001; see Table xx). There was no reliable pattern of slow theta oscillations across experiments when contrasting reinstated and non-reinstated episodes (see Table xx).

To conclude, despite finding evidence for the existence of theta oscillations, we did not find evidence for a difference in oscillatory power between reinstated and non-reinstated trials during encoding or retrieval. Likewise, there was no difference in aperiodic offset or steepness between later reinstated trials and non-reinstated trials during encoding or retrieval.

Single neuron firing to specific theta phases during memory encoding and retrieval

We next investigated whether single neuron firing would preferably occur within a specific theta phase during encoding and retrieval of episodic memories and whether there was a neuron specific phase offset between firing during the encoding and retrieval phases.

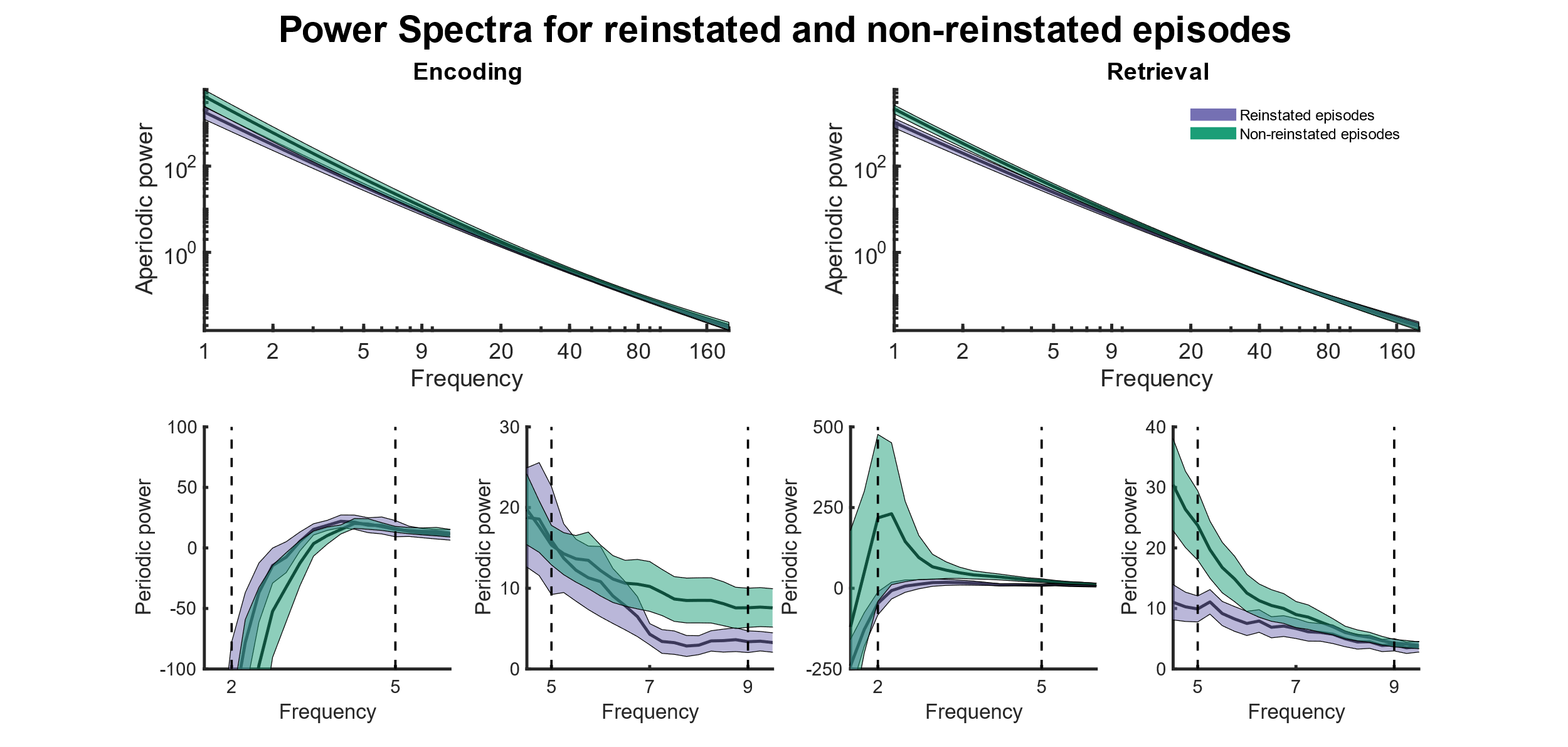
Based on previous literature no single theta frequency dominates the human hippocampus. Instead, there is a slower theta oscillation (2-5 Hz) and a faster theta oscillation (5-9 Hz) (Goyal et al., 2020; Kota et al., 2020).

We do not know which microwire best represents the dendritic input into a single neuron, so we computed theta components using a weighted average of all microwires within a microwire bundle. This was based on the generalized eigendecomposition of the narrowband theta covariance matrix and the broadband covariance matrix (see Methods). We distinguished three different categories of activity: spikes of ESN that occurred during reinstated trials (rESN), spikes of ESN during non-reinstated trials (nESN), and spikes of single units (SU). After excluding neurons with an insufficient number of spikes these analyses were based on nrESN = 36, nnESN = 116, and nSU = 380 neurons in experiment 1 and nrESN = 13, nnESN = 34, and nSU = 136 neurons in experiment 2. We first computed the preferred mean phase during encoding and retrieval for each neuron. To determine a general phase preference, we pooled this preferred phase value over all neurons within a category of neurons (rESN, nESN, SU) and used a Rayleigh test to determine statistical significant deviations from a uniform phase distribution. In experiment 1, only the SU category showed a phase preference for the slow theta component during encoding (θ = 197.5°, *p* = 0.048) and retrieval (θ = 181.9°, *p* = 0.004). After adjusting for multiple comparisons for two tests (slow and fast theta) SU only showed a slow theta phase preference during retrieval (*pencoding adj.* = 0.096; *pretrieval adj.* = 0.008; Bonferroni corrected). Neither rESN nor nESN showed any slow or fast theta phase preference during encoding or retrieval (all *p* > 0.28).

In experiment 2 the SU category showed a phase preference in the slow theta component during encoding (θ = 287.2°, *p* = 0.002) but not during retrieval (*p* = 0.633; all other *p* > 0.10). There was a statistically significant phase preference of rESN for the slow theta component during retrieval (θ = 201.3°, *p* = 0.048), however, after controlling for multiple comparisons (slow and fast theta), the effect was no longer significant (*padj.* = 0.096).

It is possible that despite an absence of phase preference during encoding and retrieval, neurons show a reliable offset between encoding and retrieval (a representative example of a 10° offset with four neurons: encoding: 0°, 90°, 180°, 270°; retrieval: 10°, 100°, 190°, 280°). To determine if there was a significant theta phase difference between neurons firing at encoding and at retrieval, we computed the mean cosine similarity of the complex value for each neuron for all spikes during encoding with all spikes during retrieval. We determined the statistical significance of the encoding-retrieval phase offset separately for each neuron type (rESN, nESN, SU) using a one-sample test with a mean angle of 0° (i.e., no phase difference between encoding and retrieval). This one-sample test is the circular equivalent of a one-sample t-test with continuous data (we used the function *circ\_mtest* from the Circular Statistics Toolbox v1.21.0.0). In experiment 1 this approach yielded no significant encoding-retrieval phase differences for any category of neurons (rESN, nESN, SU) or theta components (slow, fast) (all *p* > 0.26). Likewise, no encoding-retrieval phase differences were found in experiment 2 (all *p* > 0.4).

It is conceivable that theta activity modulates only some neurons. In this case a small proportion of theta-sensitive neurons might show a consistent phase difference between their firing at encoding and retrieval, but due to their small number this effect might be obscured. To circumvent this, we repeated the above phase difference analysis for neurons whose firing rate showed a phase coupling at encoding and retrieval using a Rayleigh test. Using this approach, we identified a significant phase offset between SU firing at encoding and retrieval in experiment 1 (θ = 14°, *puncorrected* = 0.048) that was no longer significant after correcting for multiple comparisons (*pcorrected* = 0.096; experiment 1 all other *p* > 0.39; experiment 2 all *p* > 0.435).

To conclude, we found a slow theta phase preference for SU during encoding in experiment 2 and retrieval in experiment 1. However, no neuron type (rESN, nESN, SU) showed a significant encoding-retrieval theta phase offset, which was also the case when limiting the theta phase offset analysis to neurons that showed a significant phase coupling at encoding and retrieval.

**Figure XX. Aperiodic and oscillatory fast and slow theta activity during encoding and retrieval of reinstated (purple) and non-reinstated (green) episodes. Shaded areas represented the SEM.**

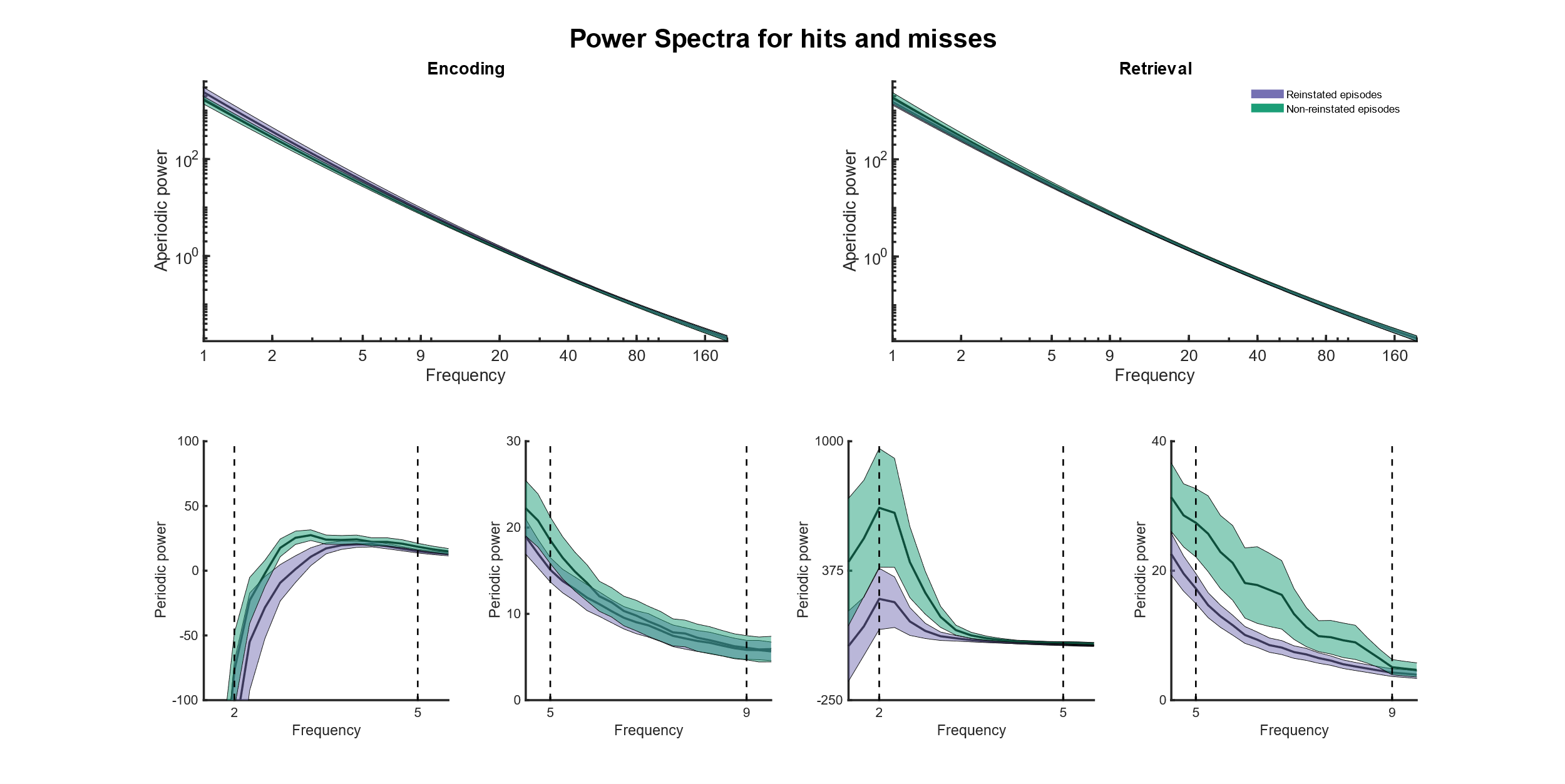
(A) Aperiodic power during encoding. Both axes are log-scaled. The x-axis shows frequencies from 1 to 200 Hz. The y-axis depicts the power at the respective frequency.

(B) Same as (A) but for retrieval

(C) Oscillatory activity in the slow theta range (2 Hz and 5 Hz) at encoding

(D) Oscillatory activity in the fast theta range (5 Hz and 9 Hz) at encoding

(E-F) Same as (C-D) but for retrieval.



**Figure XX. Aperiodic and oscillatory fast and slow theta activity during encoding and retrieval of remembered (purple) and forgotten (green) episodes. Shaded areas represented the SEM.**

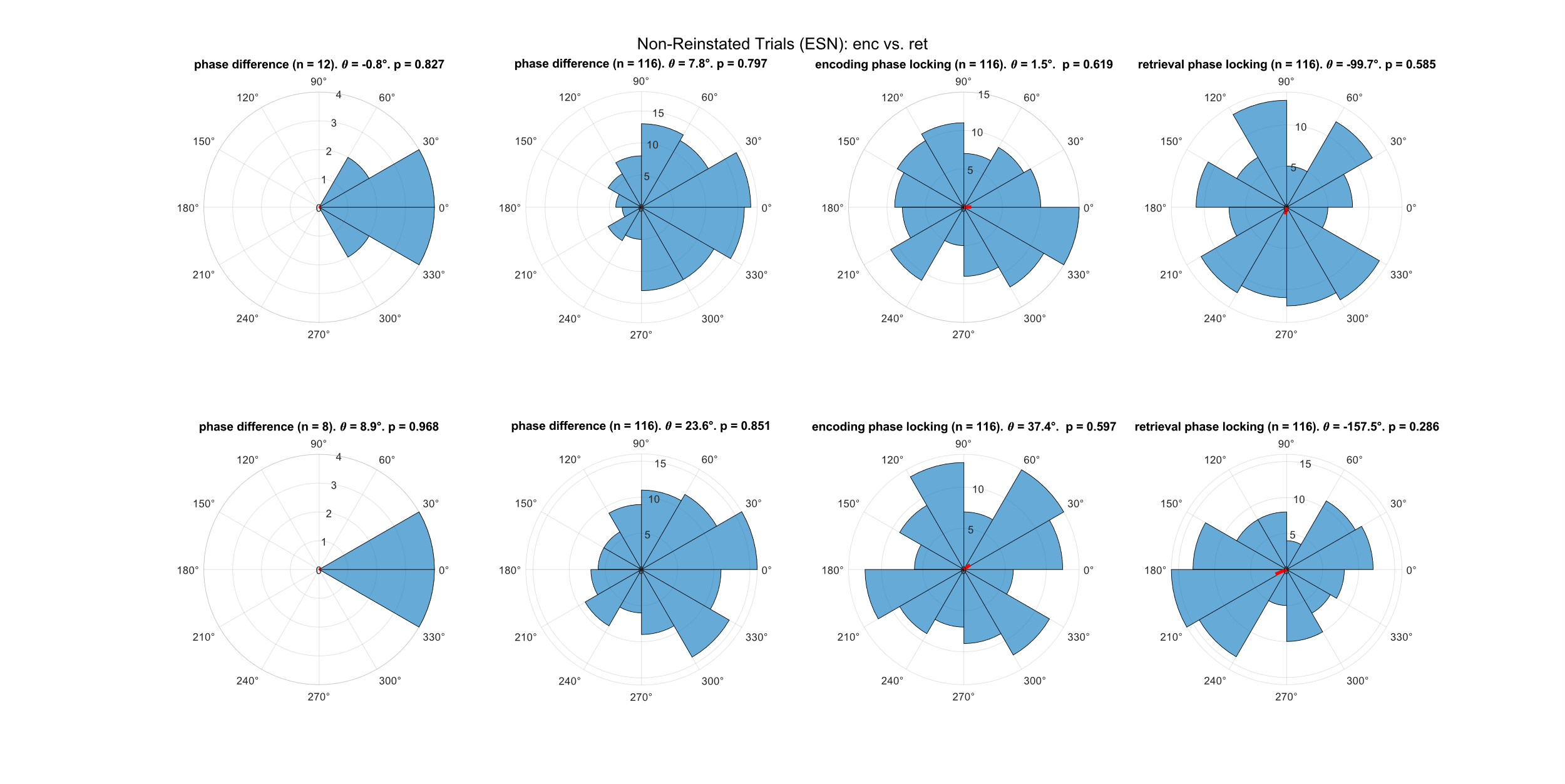
(A) Aperiodic power during encoding. Both axes are log-scaled. The x-axis shows frequencies from 1 to 200 Hz. The y-axis depicts the power at the respective frequency.

(B) Same as (A) but for retrieval

(C) Oscillatory activity in the slow theta range (2 Hz and 5 Hz) at encoding

(D) Oscillatory activity in the fast theta range (5 Hz and 9 Hz) at encoding

(E-F) Same as (C-D) but for retrieval.

**Figure XX. Polar histogram showing the phase offset between encoding and retrieval and the phase distribution during encoding and retrieval in ESNs during non-reinstated episodes.**

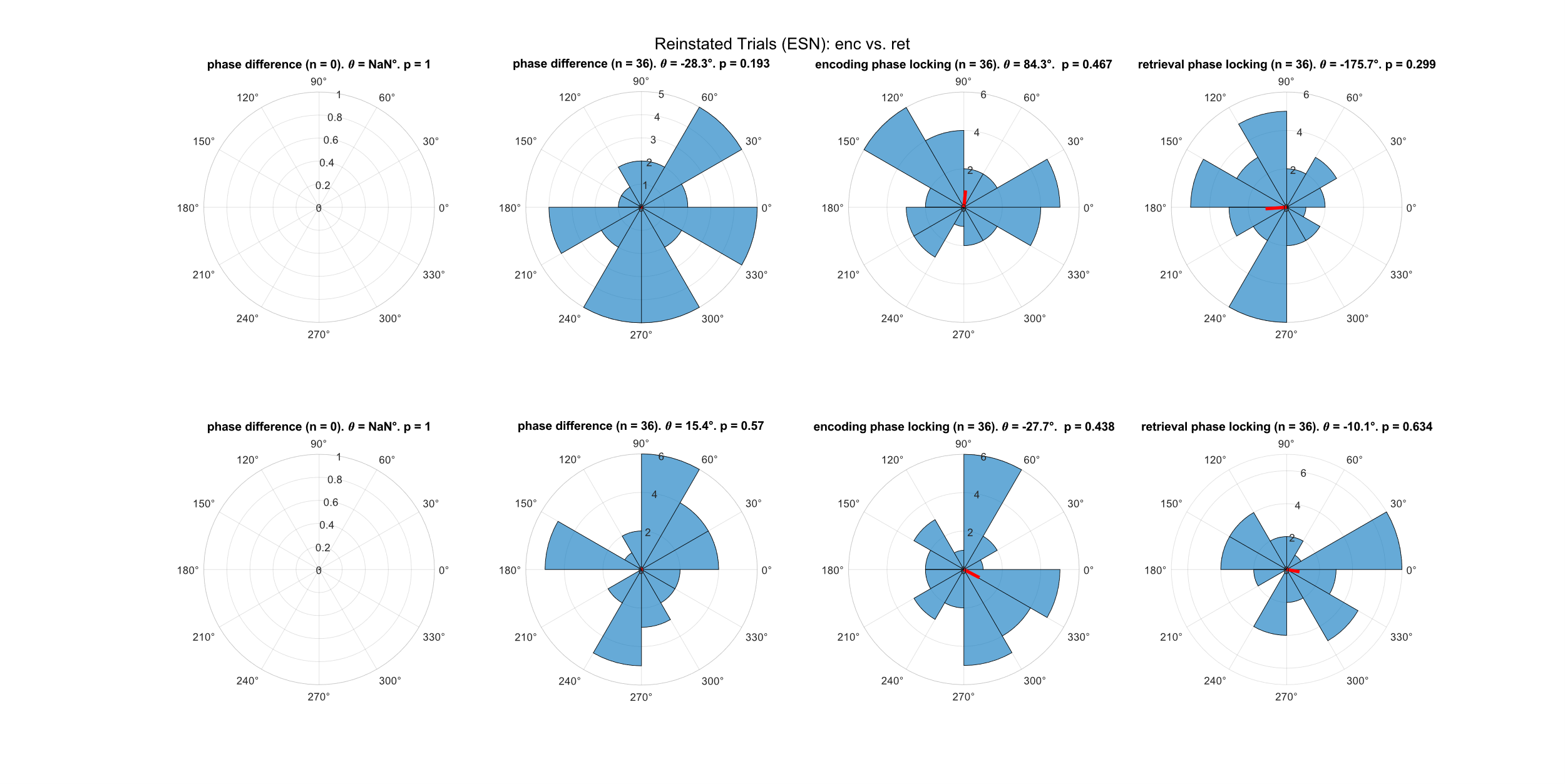
(A) Phase offset between encoding and retrieval in neurons that showed a significant theta coupling at encoding and at retrieval for slow theta (2 Hz – 5 Hz)

(B) Phase offset between encoding and retrieval across all neurons for slow theta (2 Hz – 5 Hz)

(C) Preferred phase during encoding across all neurons for slow theta (2 Hz – 5 Hz)

(D) Preferred phase during retrieval across all neurons for slow theta (2 Hz – 5 Hz)

(E-H) Same as (A-D) but for fast theta (5 Hz – 9 Hz)

 **Figure XX. Polar histogram showing the phase offset between encoding and retrieval and the phase distribution during encoding and retrieval in ESNs during reinstated episodes.**

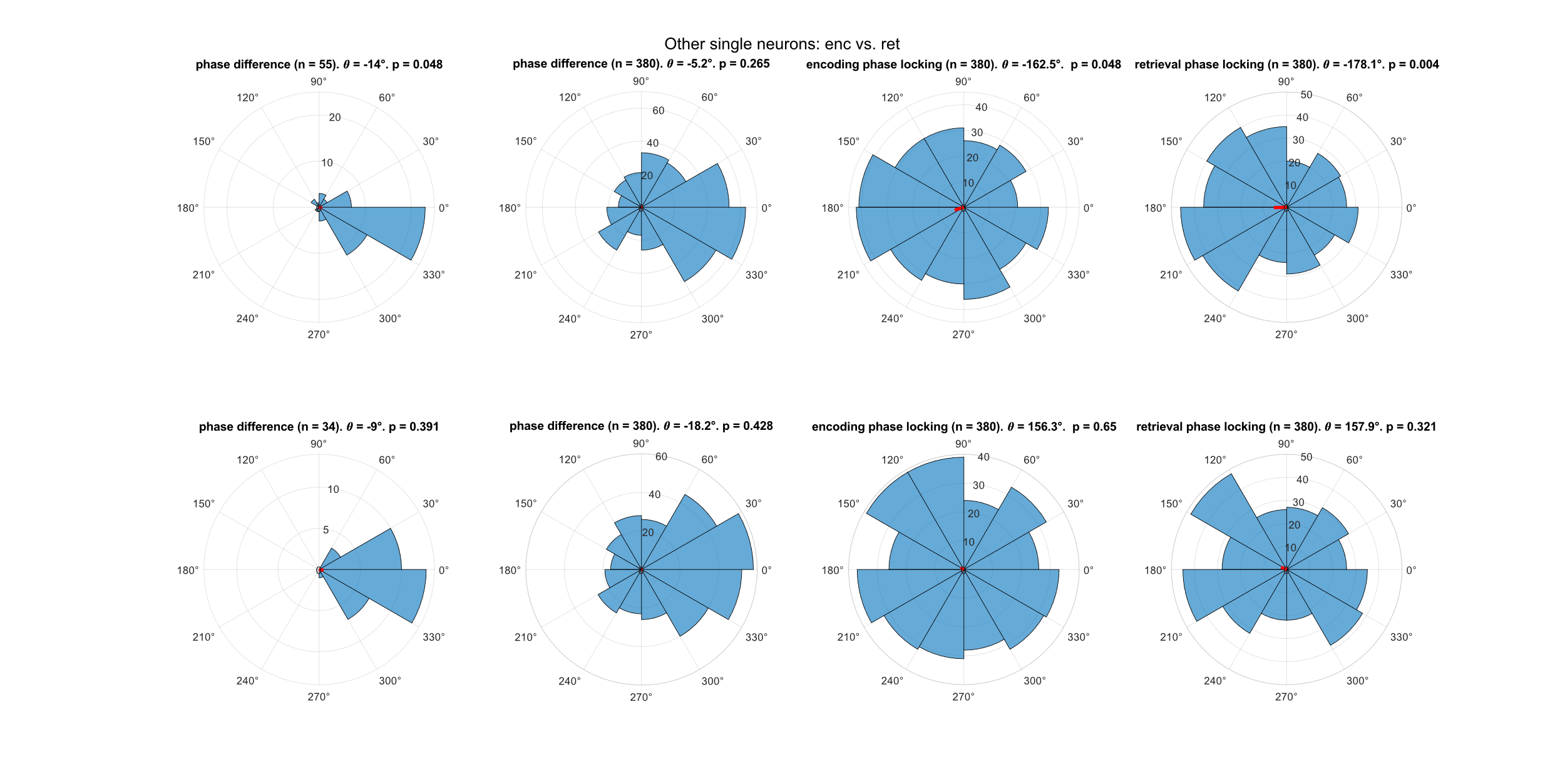
(A) Phase offset between encoding and retrieval in neurons that showed a significant theta coupling at encoding and at retrieval for slow theta (2 Hz – 5 Hz)

(B) Phase offset between encoding and retrieval across all neurons for slow theta (2 Hz – 5 Hz)

(C) Preferred phase during encoding across all neurons for slow theta (2 Hz – 5 Hz)

(D) Preferred phase during retrieval across all neurons for slow theta (2 Hz – 5 Hz)

(E-H) Same as (A-D) but for fast theta (5 Hz – 9 Hz)



**Figure XX. Polar histogram showing the phase offset between encoding and retrieval and the phase distribution during encoding and retrieval in SU.**

(A) Phase offset between encoding and retrieval in neurons that showed a significant theta coupling at encoding and at retrieval for slow theta (2 Hz – 5 Hz)

(B) Phase offset between encoding and retrieval across all neurons for slow theta (2 Hz – 5 Hz)

(C) Preferred phase during encoding across all neurons for slow theta (2 Hz – 5 Hz)

(D) Preferred phase during retrieval across all neurons for slow theta (2 Hz – 5 Hz)

(E-H) Same as (A-D) but for fast theta (5 Hz – 9 Hz)

Timeline

Description automatically generated

**Figure XX. Five second data snippet showing activity in the slow (2-5 Hz; A) and fast (5-9 Hz; B) components.** Components were generated by taking a weighted average of the narrowband signal of all microwires within a bundle. The weighted average was calculated using a generalized eigendecomposition of the broadband and narrowband covariance matrices.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Contrast | Remembered vs. forgotten episodes | | | |
| Phase | Encoding (exp 1) | Encoding (exp 2) | Retrieval (exp 1) | Retrieval (exp 2) |
| Slow theta | Remembered episodes | p = 0.603 | p < 0.001  t(114) = 6.79 | p = 0.025 | p < 0.001  t(114) = 9.13 |
| Forgotten episodes | p = 0.076 | p = 0.113 | p = 0.009  t(341) = 2.61 | p < 0.001  t(114) = 5.38 |
| Fast theta | Remembered episodes | p < 0.001  t(365) = 7.41 | p < 0.001  t(114) = 7.09 | p = < 0.001  t(365) = 8.19 | p < 0.001  t(114) = 6.61 |
| Forgotten episodes | p < 0.001  t(341) = 6.05 | p < 0.001  t(114) = 6.18 | p < 0.001  t(341) = 3.76 | p < 0.001  t(114) = 6.51 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Contrast | Reinstated vs. non-reinstated episodes | | | |
| Phase | Encoding (exp 1) | Encoding (exp 2) | Retrieval  (exp 1) | Retrieval  (exp 2) |
| Slow theta | Reinstated episodes | p = 0.318 | p = 0.003  t(32) = 3.16 | p = 0.495 | p < 0.001  t(32) = 3.81 |
| Non-reinstated episodes | p = 0.196 | p < 0.001  t(32) = 5.00 | p = 0.1814 | p < 0.001  t(32) = 3.48 |
| Fast theta | Reinstated episodes | p < 0.001  t(122) = 3.42 | p < 0.001  t(32) = 3.75 | p < 0.001  t(122) = 4.65 | p = 0.007  t(32) = 2.86 |
| Non-reinstated episodes | p < 0.001  t(122) = 3.82 | p < 0.001  t(32) = 3.92 | p < 0.001  t(122) = 4.52 | p = 0.002  t(32) = 3.48 |

**Table xx. Overview of evidence for periodic fast and slow theta activity during encoding and retrieval of (later) remembered and (later) forgotten episodes in experiment 1 and experiment 2.**

**Table xx. Overview of evidence for periodic fast and slow theta activity during encoding and retrieval of (later) reinstated or (later) non-reinstated episodes in experiment 1 and experiment 2.**

Discussion

Episodic memories consist of various multimodal elements and are embedded in a distinct temporal and spatial context (xx). The neurophysiological markers of episodic memory processing are still subject to debate, but a considerable body of literature exists that emphasizes the importance of theta oscillations for memory processing (xx). We analysed the activity of single neurons relative to the ongoing theta activity in two independent intracranially recorded datasets that were collected using microelectrodes located in the human hippocampus while patients performed a memory association task.

In a recent review Herweg and colleagues (Herweg et al., 2020) suggested that memory processing is reflected in a steeper aperiodic component and an increase in periodic theta activity. Furthermore, studies have revealed that there is not one dominant theta frequency in the human hippocampus, but rather two distinct oscillations – a slow (2-5 Hz) and a fast (5-9 Hz) theta oscillation (Goyal et al., 2020; Kota et al., 2020). We first compared the aperiodic and periodic slow and fast theta components between remembered and forgotten episodes. In a second analysis we repeated the analysis but contrasted episodes during which the neural firing rate of ESNs is reinstated with episodes without neural firing reinstatement. In line with the hypothesis proposed by Herweg and colleagues (Herweg et al., 2020) we found a higher offset and 1/f tilt during retrieval of forgotten episodes. However, this aperiodic difference was absent during memory encoding, and we found no aperiodic differences between reinstated and non-reinstated episodes. We did not find any consistent differences in oscillatory slow and fast theta power for remembered vs. forgotten episodes or reinstated vs. non-reinstated episodes. We found periodic theta activity in both contrasts and during encoding and retrieval, although this evidence was more reliable in the fast theta band.

To conclude, evidence regarding the offset and steepness of the aperiodic component was inconclusive and we found no evidence of periodic theta power being involved in memory processing.

There were no significant periodic or aperiodic differences between two categories of successful memory events (i.e., between reinstated and non-reinstated episodes). One possible reason may be that each successfully encoded memory is represented by an assembly of ESNs. As we recorded only from a small subgroup of the ones close to the microwires, the non-reinstated episodes would only differ insofar as we would not record their respective ESNs, because the LFP reflects a larger area than the area in which spikes are recorded.

However, there is a deeper problem with the argument presented by Herweg and colleagues (Herweg et al., 2020). They recommend contrasting the strength of two successful memories (e.g., how many contextual details are remembered when retrieving an episode). The idea behind that is that in both cases domain-general processes, reflected in the steepness of the aperiodic component, would be present and any differences would be driven by the memory strength. The problem with this is that it implicitly assumes that processes like task engagement, effort, perception and attention are binary. However, a more vividly remembered episode might have a shallower aperiodic component because the patient has paid more attention during the episode and not because of memory processing.

It should be noted that methods to separate periodic and aperiodic activity are far from perfect. Especially the large negative deflection in periodic activity e.g., at around 2 Hz in Figure xx C casts doubt on the validity of the aperiodic power estimation. Thus, oscillatory activity at the faster theta range may not reflect true periodicity but instead a poor 1/f fit. One might then argue that the lack of a consistent theta phase preference and no encoding-retrieval phase offset might be due to the absence of substantial periodic theta power in microwires. It is possible that macrowires instead integrate over larger areas and show more robust periodic theta activity (unless a bipolar reference is used; see Herweg et al. (2020)). However, previous studies have shown spike-field-coupling in the theta range using microwires (Jacobs et al., 2007; L. Reddy et al., 2021; Roux et al., 2022; Rutishauser et al., 2010) and spikes can couple to the phase of aperiodic components (Bush & Burgess, 2020).

One influential theoretical model proposed that encoding and retrieval of memories occur in opposite phases of the theta oscillation thereby avoiding that encoding new information causes catastrophic interference of older memories (Hasselmo et al., 2002).

We investigated how the firing activity of different previously identified neuron types relates to the phase of the ongoing theta oscillations during memory encoding and retrieval. We distinguished between spikes from ESNs during reinstated (rESN), non-reinstated episodes (nESN) and spikes from other single neurons (SU).

Although we found some rudimentary evidence that SU show a slow theta (2-5 Hz) phase preference during encoding and retrieval, this finding was not consistent across the two experiments. Apart from that we did not detect any significant encoding or retrieval theta phase preference for neural firing across experiments. We also found no significant encoding-retrieval phase offset across all neurons, nor when limiting our analysis to neurons that showed significant theta phase coupling during encoding and retrieval.

These unexpected results could be due to various reasons. Many of our recorded neurons may not have been involved in active memory processing and thus did not show any modulation induced by memory encoding and retrieval. However, this does not explain our null findings for rESN, which are, by definition, coding for that specific episode. In this case, our results may be attributed to an insufficient number of eligible neurons or the two seconds preceding the patient’s response may be a suboptimal time window for investigating spike-field coupling. Moreover, we did not differentiate between interneurons and pyramidal neurons, which are known to fire at different theta phases thus introducing more variance (Csicsvari et al., 1999).

Most neurons seem to maintain a preferred theta phase between encoding and retrieval. It is tempting to suggest that there is no theta phase preference during encoding and retrieval and that across the population of physiologically differently excitable neurons the entire theta cycle is covered leading to a uniform phase histogram at encoding and retrieval.

However, we employed a frequentist approach when analyzing our data; thus, while we did not find compelling evidence to reject the null hypothesis (i.e., no theta phase difference between spikes at encoding and retrieval), this should not be interpreted as evidence for the null hypothesis (Dienes, 2014). To further investigate this, future studies should use a Bayesian framework and use a larger sample size.

To conclude the present chapter, in line with our hypothesis we find that forgotten when compared to remembered episodes have a higher aperiodic offset and a steeper gradient. Contrary to our hypotheses, we found no such pattern during memory encoding and no periodic theta increase for correctly remembered episodes. Likewise, we did not find evidence of neural firing in specific phases during encoding and retrieval, or a phase difference between encoding and retrieval in two independent datasets.

General Discussion

Summarize the key findings here

CN -> ESN; on the symbiotic role of CN and ESN

Outlook for new studies (miwi stimulation, how to test CN-ESN, multiple retrievals, long term recordings, sleep recordings)

LFP findings

1/f increased for ESN?

Connection to cortical activity?

In the first chapter, I presented evidence of single neurons in the human hippocampus that reinstate their (temporal) firing rate during the retrieval of specific episodes. These neurons, referred to as Episode Specific Neurons (ESNs), are distinct from neurons that are tuned to specific concepts (Concept Neurons) or reoccurring time points (Leila Reddy et al., 2021; Umbach et al., 2020); Time Cells). Preliminary evidence indicates that these ESNs do not exist in the parahippocampus, although our coverage in that area is sparser than in the hippocampus. Additionally, initial evidence suggests that ESNs are likely to be excitatory pyramidal neurons. In chapter 2, we extended these findings to the high frequency band in the local field potential. Although no consensus has been reached in the literature yet, it is generally agreed upon that an increase in high frequency power reflects an increase in local neural firing (xx). In line with this, we found a significant, albeit low, correlation between single neuron firing and high frequency power. In parallel to our earlier findings, we demonstrated that power in the high frequency band (40-200 Hz) was reinstated for particular episodes in a significant number of microwires. This finding was limited to later remembered episodes and did not emerge for later forgotten episodes. Although we did find stimulus dependent high frequency power (HFP) modulations akin to the firing rate increases in Concept Neurons, these HFP changes were not responsible for the HFP memory reinstatements in ESWs. Unexpectedly, the relative power increases in reinstated episodes extended past our frequency range of interest (until ~10 Hz during memory encoding and ~15 Hz during memory retrieval). Future studies should differentiate whether this finding is driven by a power offset, an aperiodic 1/f shift, or oscillatory components.

Ample research points towards a central role of theta oscillations in episodic memory processing. Recent work has shown that hippocampal theta oscillations in humans is divided between a slow (2-5 Hz) and fast (5-9 Hz) theta oscillation.However, the exact role of theta remains elusive. Herweg and colleagues (Herweg et al., 2020) proposed that successful memory is reflected in a power shift towards higher frequencies and a circumscribed narrow-band periodic theta increase. In the third chapter we tested this hypothesis in two intracranial datasets but did not find consistent evidence to support it. Based on influential theoretical work by Hasselmo and colleagues (Hasselmo et al., 2002), we expected single neurons and ESNs to lock onto different phases of theta. Contrary to our hypothesis, we were unable to identify a consistent phase preference during encoding or retrieval of episodic memories across the two independent datasets. We also did not find evidence for a theta phase offset between encoding and retrieval. Indeed, many neurons might not be involved in processing a given memory, which provides a possible reason for our findings. However, this does not apply to ESNs which, by definition, code for a specific memory as reflected in their increased firing rates for that memory. The absence of a theta phase effect in this case may be attributed to the low number of ESNs leading to insufficient power to detect an effect.Although a complete absence of theta phase preference goes against previous findings, neurons reportedly lock to a large range of theta phases (Jacobs et al., 2007; L. Reddy et al., 2021; Roux et al., 2022; Rutishauser et al., 2010).

To conclude, we found a single neuron basis of memory processing and extended these findings to activity in a greater population of neurons reflected in the local field potential. While many exciting open questions remain, we hope to have laid a foundation for future work. In the following text we sketch some of these questions.

ESNs are maybe Index Neurons

Although our research, that culminated in compelling evidence for ESNs, was inspired by what Teyler and DiScenna (Teyler & DiScenna, 1986) called Index Neurons, we did not call them such. This is because there are features ascribed to Index Neurons that we cannot test using the two available datasets. Upon presentation of a partial input present at memory encoding the Index Neuron assembly in CA3 is pattern completed. In both of our experiments, we always use the same memory cue for encoding and retrieval. Nevertheless, we predict that using varying memory cues from the same memory should reinstate the same ESNs. Having access to multiple neurons that are allocated to the same episode would create the opportunity to ask more nuanced questions. If a cue does not initiate memory retrieval, do some neurons reinstate their firing activity, but the activation is not sufficient to pattern complete the entire assembly? Is there a relationship between strength of assembly reinstatement and detail of episodic retrieval? Is there a specific reactivation order based on the memory cue, which would indicate that within an assembly, individual neurons are responsible for specific features within the episode. It is conceivable that depending on the hippocampal subfield these answers differ. For example, reactivation in the DG might be all-or-nothing (xx) while the auto associative structure of the CA3 (xx) allows for more graded reinstatement. As we lack sufficient coverage to record multiple neurons of one assembly that codes an episode, we cannot currently investigate these questions. Conversely, pattern separation should allow the distinction of highly similar, but different episodes by assigning them to different Index Neuron ensembles. We are unable to verify this using the current experiments because the images used in each episode do not overlap.

Another important question is the stability of the ESN code over time and multiple retrievals. We expect ESNs to generally reinstate their firing pattern on all subsequent retrievals. However, it is possible that some ESNs drop out of the assembly that is allocated to a given episode, which would lead to some variance. Patients in our studies retrieved every episode only once, so we cannot investigate this question. We are currently running an experiment where each episode is retrieved multiple times but will not have a complete dataset in the foreseeable future.

An interesting, related endeavour is exploring the stability of ESNs over time. Are memory traces, as evidenced by ESN firing and HFP increases, systematically reactivated during the periods of (extended) consolidation? In this context, sleep is of particular interest as numerous studies suggest it has a role in memory consolidation by reactivating previous experiences (Born et al., 2006; Kolibius et al., 2020; Schreiner et al., 2021). Do ESNs reliably reinstate a memory days and weeks after memory encoding? Note, that it is a separate question whether the hippocampus in general stays involved in older memories (Nadel & Moscovitch, 1997) or not (McClelland et al., 1995; Squire & Alvarez, 1995). This is because it is conceivable that the original memory trace is transformed during the consolidation period between encoding and retrieval, meaning the initially allocated neurons have been replaced by other neurons or pruned. It is easy in this case to erroneously infer that the hippocampus becomes redundant in retrieving a distant episodic memory when in reality the hippocampal memory trace persists in an altered form (i.e., consisting of fewer or other neurons). In light of this, being able to record more single neurons or even multiple ESNs that reinstate the same episode would be especially insightful. In absence of this possibility our findings that episode reinstatement concurs with HFP increases could be used to investigate the stability of a memory code and the continuous involvement of the hippocampus in episodic memories. As a proxy of much broader neural firing HFP is likely robust to a drop out of some of the initially allocated neurons.

Information flow between hippocampus and neocortex

A central part of the Indexing Theory is that ongoing cortical activation is bound by neurons in the hippocampus which project back and reactivate the initial cortical pattern during successful retrieval (Teyler & DiScenna, 1986; Teyler & Rudy, 2007). There are several hurdles in showing this empirically. One is a low spatial coverage of implanted electrodes, which, on top of that, is different for each patient as not all patients have electrodes in the same part of the hippocampus or neocortex (referred to as the cortex from this point onwards). Apart from that, the number of ESNs per patient is low. These concerns should not deter the curious reader, but merely caution to the difficulty of the task.

A persisting problem has been discerning periods of memory reinstatement from background activity. One approach to this problem might be combining high frequency activity with concurrent ESN firing, which could serve as a more accurate indicator of memory reinstatement. A second approach is to correlate the instantaneous firing rate of ESNs or HFP with the output of a classifier (e.g., a linear discriminant analysis), which represents a retrieved memory in the cortex. This is preferable over computing the classifier evidence at each individual spike which would assume that each spike leads to memory reinstatement. Especially in neurons that spike frequently each spike might only reflect baseline firing. A larger increase in instantaneous firing rate would also be easier to discern from baseline firing using a thresholding procedure and could be used as a marker for memory reinstatement. A third way to detect memory reinstatement would be to apply a time-based causality measure (e.g., Granger causality, Granger (1969); Phase Slope Index, Nolte et al. (2008)) on shorter data segments during the retrieval phase, when information is thought to flow from the hippocampus to the cortex (Linde-Domingo et al., 2019). Segments in which activity in one area predicts the activity in the other are likely timepoints at which memory retrieval occurs. A fourth way would be to identify memory reinstatement in the cortex through a classifier and reverse engineer the hippocampal activity pattern that induced it (e.g., looking at the neural activity one second prior).

Finally, a fifth way would be applying a classifier to the hippocampal and cortical recordings separately and cross-correlate the two outputs or apply a Granger causality test. In this case one would not focus on the transfer of the signal as raw data, but rather the transfer of evidence for content that represents that memory. To complicate things further one may wish to use the instantaneous firing rate and/or data within the high frequency band instead of the unfiltered raw micro-/macrowire recordings as inputs to these analyses. To conclude, only future studies using other experimental designs and/or more advanced hardware will be able to ascertain whether ESNs are indeed Index Neurons. Until then, we must be satisfied to see a reinstatement of neural firing as an indicator of memory processing.

On the origin of Concept Neurons

Recent times have seen an explosion in neuron types. We find cells that code for spatial locations, such as place cells (xx) or grid cells (xx). More recently head direction cells and anker cells have been identified. In humans, neurons that code for specific concepts, so called Concept Neurons have been found consistently. Recent additions include novelty cells and familiarity cells (or neurons?) as well border and event cells (Zheng et al., 2022). In this work we introduced Episode Specific Neurons and added to this veritable embarras de richesse. We do not believe that each of these neurons represent a physiologically distinct neuron type that earns its label through a separate coding mechanism. We would like to therefore muse on the open question how CN initially develop their tuning. One possibility is that over repeated reconsolidation CNs evolve from ESNs. Imagine you meet your best friend in a coffee shop. This coffee shop episode will initially be represented by an assembly of ESNs. A few days later you meet with the same friend in a park and you remember the last time you met in the coffee shop. This reactivates the ESNs coding for the coffee shop episode. Engram literature suggests that recently active and more excitable neurons are preferentially bound to a new episode (Josselyn & Frankland, 2018, Josselyn & Tonegawa xx). This makes it likely that some of the ESNs that coded the coffee episode now also code the park episode. The common element between those two episodes is your best friend. It is conceivable that over many such episodes a proportion of the ESNs that initially coded the coffee shop episode would become "semanticized", i.e., develop a tuning for your best friend. A Concept Neuron is born. In this way ESNs can be likened to variables in a computer program to which arbitrary information is bound. In the case of episodic memory, this arbitrary information would be the complete set of features that make up an episode.

More advanced electrodes

The yield of neurons using the currently available electrodes is about a dozen per microwire bundle. In comparison, electrodes developed more recently yield hundreds of well localized neurons. Virtually all analyses described in this thesis would benefit from more neurons. We have mentioned this throughout the manuscript when appropriate. Not in all cases can this difference in neuron yield be made up by recording more participants or sessions. For instance, recording hundreds of neurons in one patient would enable the analysis of between-cell interactions, such as the detection of assemblies of Episode Specific Neurons. Something that is not feasible when recording few neurons. Beyond this, between area interactions could be investigated (Durand et al., 2022). One concrete example would be a reinstatement of neocortical neurons that represent a memory and their interaction with hippocampal ESNs. During encoding one would assume that the neocortical neurons drive ESNs, while during retrieval the cortex lags behind the hippocampus (Linde-Domingo et al., 2019).

Microwire stimulation

As I am writing this Neuralynx (xx) is seeking CE and FDA approval for microwire stimulation in patients (personal communication). If successful, microwire stimulation could provide causal evidence for an ESN based memory code. If ESNs are allocated based on excitability, as predicted by experiments in rodents, stimulating the neurons in the vicinity of a microwire should increase the probability that they are allocated to an episode. In other words, through electric stimulation hippocampal neurons may be galvanized - at the push of a button - into coding for a particular episode. Apart from shedding light on the mechanism by which ESNs are allocated this would also greatly increase the yield of ESNs per patient. Using this method, one could test the hypothesis that CN develop from ESNs by stimulating on a microwire during multiple episodes that share a common element (e.g., Jennifer Aniston in Pisa, Jennifer Aniston in Paris, …). If the stimulation causes neurons to be co-allocated to these episodes it is conceivable that some of the “tagged” neurons exhibit neural firing akin to Concept Neurons tuned to Jennifer Aniston (as she is the common element in these episodes). Likewise reactivating the previously assigned neurons through electrical stimulation should increase the probability the memory is retrieved or even induce memory retrieval in the absence of a retrieval cue. Conversely, depending on the effect of the stimulation protocol, memory retrieval may be prohibited.

Translational applications

In our everyday lives, we constantly generate and retrieve episodic memories - a marvellous ability we usually don’t think about. This makes it even more painful and disruptive when memory processing becomes dysfunctional. Alzheimer's disease is a progressive degenerative disorder that causes atrophy of neurons resulting in one of the hallmark symptoms of Alzheimer's - memory loss (xx). It is the most common form of dementia was the 7th leading cause of death in the USA in 2020 (Murphy SL, Kochanek KD, Xu JQ, Arias E. Mortality in the United States, 2020. NCHS Data Brief, no 427. Hyattsville, MD: National Center for Health Statistics. 2021. 10.15620/cdc:112079). Worldwide around 55 million people have dementia, a number which is expected to rise to 78 million by 2030 (WHO: https://www.who.int/news-room/fact-sheets/detail/dementia). I am hopeful that our research can contribute to alleviate some of the burden that comes with Alzheimer's although a cure requires a more targeted understanding of the disease. However, it might prove fruitful to electrically stimulate parts of the hippocampus during the early phases of the disease. This could help with the recruitment of ESNs to new memories or reinstate existing memories and thereby temporarily increase their quality of life.

Another disorder characterized by dysfunctional memories is the Post Traumatic Stress Disorder (PTSD). Its symptoms include vivid intrusive memories or flashbacks that can be triggered cues reminiscent of the traumatising event. Re-experiencing the traumatic memory can cause intense physical and strong emotional reactions (ICD-11). Although PTSD patients often suffer from deficits in declarative memory (Samuelson, 2011), a core issue is the pathological remembering of a disturbing event. Studies in rodents suggest that the size of the hippocampal engram does not increase with the intensity of the memory (xx). This raises the question whether it may be possible to interact with the hippocampal memory trace that represents a traumatic experience. Is it possible to downregulate the engram on demand, or even ablate it? The latter raises an important ethical concern - does erasing an entire experience also abolish the consequences of the experience or does it simply get rid of the conscious perception leaving the visceral reaction intact? Of course, the idea of simply "switching off" a memory as a treatment simplifies the underlying syndrome of PTSD. Nonetheless, the idea is that through basic research such as the work presented here we gain a better understanding of how memories are processed in humans and that this insight helps translational applications further down the road.

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