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Behavioral and brain processes supporting
the formation of episodic memories

Hannah Bernhard

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Hannah Bernhard

Supervisor:
Prof. dr. Peter de Weerd

Co-supervisors:
Dr. Joel Reithler
Dr. Rob Rouhl

Assessment Committee
Prof. dr. Alexander Sack, Chair
Dr. Linda Geerligs, Radboud University Nijmegen
Dr. Christian Herff
Prof. dr. Tobias Staudigl, Ludwig-Maximilians-University Munich

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General Introduction

Preamble

Consider the event of visiting the Strand bookstore on a Friday morning in December. During your visit, a piano concerto by Beethoven sounds through the speakers, you smell the scent of freshly printed paper, and take in the view of rows upon rows of books. When you've perused through the ground floor and the high shelves of fiction books, you make your way upstairs to the rare books room with glass cases exhibiting special editions. Upon leaving the bookstore, you enter the busy streets of New York. Even after many years, you may be able to remember this event very well: the rainy winter weather that day, the thick scarf you were wearing, the friend that you visited the store with and the conversation you had, and the books you ended up buying. Hearing the Beethoven piece playing on the radio may remind you of the many colorful spines in the shelves. This capability for mental time travel and revisiting past experiences is referred to as episodic memory: memory for specific (autobiographical) events (Tulving, 1972), including information about the spatial and temporal context that this event occurred in.

More generally, memory can be described as the ability to store and recall information. We form and retrieve memories throughout our life, but different forms of memory can be distinguished: declarative and non-declarative memory. Declarative memory, which describes the forms of memory that can be verbalized and involves conscious access to learned information, such as facts or episodes (Milner et al., 1998). Declarative memories include episodic memories, but also semantic memories, which are factual and do not require a spatiotemporal context (i.e., the knowledge that Berlin is the capital of Germany). Non-declarative memory, on the other hand, relates to changes in skilled behavior, and is improved through repeated experience and practice (i.e., learning to ride a bike). This form of memory does not require conscious access to former episodes of learning. The described visit to the Strand bookstore is an example of a declarative-episodic memory: an event, that, although experienced only once, may persist for a long time in memory, and that can be recalled and described consciously. Such episodic memories are crucial for our sense of self, as they form the narrative of who we are, and enable us to put current events into the perspective of past experience (Kandel, 2007). At the same time, episodic memories may contribute to the selection of suitable behaviors in a given situation (Clewett et al., 2019).

The work summarized in this thesis centers around behavioral and brain mechanisms underlying the encoding and consolidation of one-shot episodic memories, that is, memories based on events that are only experienced once. In the introduction I provide a summary of the current state of research on the

(human) episodic memory system and the processes involved in consolidating an experienced event into a recallable memory. To this end, the introduction is split up into two parts. **Part I** focuses on the key components of the episodic memory system and their contribution to memory formation during encoding and immediately following the experience of an event. **Part II** focuses on the long-term period following this event, and how brain dynamics during sleep may lead to remembering or forgetting.

1.1. Part I: Episodic memory encoding and immediate post-encoding processes

1.1.1. The memory engram

The lifecycle of a memory roughly consists of three stages: encoding, consolidation and retrieval. During encoding, information from the environment is sampled and processed. For instance, when looking at the cover of a book during the visit of the Strand bookstore, the outlines, shapes, colors and words are processed through activation of retinal receptors. This information is then sent along the optic nerve to the thalamus and eventually visual cortex where it is integrated over several processing layers involving other brain systems (Gluck et al., 2016). Encoding thus describes the process of transforming information from the external world and representing it in the brain through activation of neuronal populations. Consolidation, on the other hand, involves storage of information over time, which relies on brain plasticity (i.e., changes in brain structure). Because multiple systems across the brain support memory storage, these plasticity changes may occur in a distributed fashion (Hebb, 1949). Plasticity is theorized to occur through Hebbian learning: when activation of one cell consistently leads to firing of another cell, metabolic changes will occur that makes them more likely to mutually activate in the future. Cells are thus associated, and when one of these cells activates, it is likely that this triggers activity in its associated cell (Hebb, 1949). Because of the plasticity changes required for memory consolidation, the physical representation of a memory in the brain is often also called memory trace. The third stage of memory cycle, retrieval, describes the process of remembering the previously learned information, where the memory trace is reactivated and thus pulled back into conscious focus. However, the cycle does not end on retrieval. After retrieving a memory, it must be reconsolidated and may be combined with newly encoded information.

The memory engram is a concept describing the physical representation of a memory in the brain (Semon, 1921). Conceptually, an engram is the neural representation of a memory and its constituent parts, such as its spatiotemporal context, the sensory stimuli that were perceived, and the emotional associations made in this context. Accordingly, engrams involve neurons or groups of neurons across different brain structures that were activated during the encoding of a memory. Think back to the example of the bookshop visit. The auditory, olfactory, visual, and tactile information is processed by the sensory

systems and linked together by the common spatiotemporal context. These sub-representations together make up the engram of the bookstore visit. But how does this work in practice? To investigate how the engram may be implemented in the brain, we first need to understand how contextual information from the external world, such as spatial location, is represented in the brain.

1.1.2. Spatiotemporal codes: Insights from rodent research

With the decline of scientific streams like behaviorism, which argued that cognitive function could only be characterized based on observable behavior, and the advance of neurophysiological recording methods, direct investigation of the neural mechanisms underlying cognitive function became possible. Studies to localize brain regions crucial for memory and spatial cognition were historically linked to lesion studies (Milner et al., 1998; Scoville & Milner, 1957), which tested cognitive functions after resection of brain structures. In humans, these resections were undertaken due to illness or accidents, whereas rodents were lesioned for experimental purposes. Together, these studies pointed to the medial temporal lobe (MTL) as a broad region underlying episodic and spatial memory.

In 1971, O'Keefe and Dostrovsky published a seminal paper which described hippocampal place cells for the first time. Place cells are pyra-midal neurons in the hippocampus with a preference for a certain place in an environment (the so called 'place field'), at which these neurons increase their spiking activity (O'Keefe & Dostrovsky, 1971; fig. 1a). Place cells differ in their place fields, so that across neurons of the whole hippocampus, the entire environment is represented in a cognitive map (O'Keefe & Nadel, 1978; Tolman, 1948). Upon change into a different environment or alteration of the environment itself, place cells will change their place fields, a process called 'remapping' (Bostock et al., 1991; Wilson & McNaughton, 1993). In this way, different environments can be represented by a limited number of cells. Along the axis of the hippocampus, cells differ in their granularity of representing space, where cells in the anterior hippocampus show lower spatial resolution (i.e., larger place fields) than those in the posterior hippocampus (Jung et al., 1994; Kjelstrup et al., 2008).

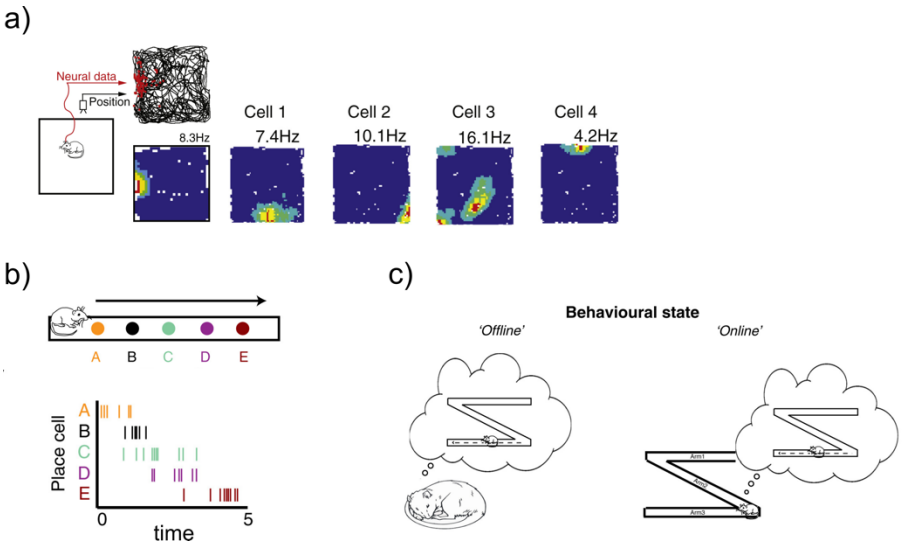

Figure 1. Encoding of spatial information in rat hippocampus. a) Classic recording setup, in which a rat explores an enclosure. Position information of the animal is measured along with neural activity in hippocampus. When combining location and neural data, 'place cells' can be found, which preferentially fire when the animal is situated in a certain position in space. Preferred firing location is shown in red, corresponding to the position in space where each cell fires most rapidly. b) The population of place cells forms a cognitive map of the environment. When an animal moves along a trajectory (top), cells fire sequentially (bottom), according to their preferred location along the trajectory. c) After an animal has explored an environment, such as a trajectory, place cell firing also occurs offline, such as during sleep (left; Wilson & McNaughton, 1994). This repetition of firing sequences is called replay. Replay can also occur online (right), such as when the animal is back on the trajectory and needs to plan future movement at a turning point. Figure adapted from (Ólafsdóttir et al., 2018).

Cognitive maps form over time, where repeated exposure to an environment stabilizes place fields (Kentros et al., 2004; McNamara et al., 2014; Rowland et al., 2011; Thompson & Best, 1990). Mechanistically, this stabilization is thought to be achieved by ways of offline reactivation of cell populations. During sleep (Wilson & McNaughton, 1994), or awake rest (Foster & Wilson, 2006) after exploring an environment (fig. 1c), place cells with adjacent place fields are seen to reactive more frequently together. In experimental setups requiring a rodent to learn a route along a trajectory (fig. 1b) or in a maze, place cells whose fields form the sequence along that route reactivate during offline periods (Lee & Wilson, 2002; Skaggs & McNaughton, 1996). This sequential reactivation of cell populations is also called 'replay'.

Through replay of activation sequences, contextual information becomes consolidated (Lee & Wilson, 2002). Co-activation of cell populations will

increase synaptic plasticity between these cells and increase the likelihood of co-firing in the future if one of them is activated. In this way, spatial learning occurs, so that when the rat re-enters the environment days later, the spatial map will be reactivated and can guide behavior, for instance by finding a food reward quicker or avoiding a painful stimulus. This reactivation of previously learned information based on cues encountered during encoding (such as the location, or any other internal or external cues) is called 'context reinstatement'.

In addition to place cells, there are also other cells in the hippocampus and surrounding structures that represent other features of space, such as grid cells (Hafting et al., 2005), and those representing the animal's body position in reference to its context, such as head direction (Taube et al., 1990) cells. Altogether, these cells are thought to track contextual factors, enabling the brain to tag individual memories with a spatiotemporal index.

The place cell research described above is based on electrophysio-logical unit recordings in the hippocampus, where high-frequency activity of single or multiple neurons is recorded from extra-cellular space. One can also record the local field potential (LFP), which is generated by synaptic currents in pyramidal neurons and reflects activity in the local network (Niedermeyer & Lopes da Silva, 2005). Replay observed in cell populations is accompanied by sharp wave ripples in the LFP, which is a transient (~100 ms) physiological pattern oscillating at high frequency (between 150 and 250 Hz in rodents, Ylinen et al., 1995; ripples in humans have been reported at lower frequency). Ripples have been linked to memory consolidation and retrieval (Carr et al., 2011), and are described in more detail in part II of the introduction.

1.1.3. Coding spatial information in the human hippocampus

Although it is a cortical structure, the hippocampus is located medially in the temporal lobe (fig. 2a and b). Up until the end of the 20th century, research of episodic and spatial memory in humans relied on behavioral testing and lesion studies with medical patients (Milner et al., 1998). Here, cognitive testing was carried out in case studies of patients who either suffered brain degradation due to medical conditions or had brain tissue resected to treat neurological conditions such as medically resistant epilepsy. The most prominent case study is that of patient H.M., who underwent bilateral resections of mediotemporal brain tissue, including the head and tail of the bilateral hippocampus, uncus, hippocampal gyrus, and amygdala, to treat severe epileptic seizures (Scoville & Milner, 1957). Although the procedure reduced the occurrence of seizures, it also triggered severe autobiographical memory loss. Whereas H.M. could recall events from his early life and recognized people he knew well from before the

surgery, he could not hold on to new information or experiences (Milner et al., 1998; Scoville & Milner, 1957). He was able to form new skills, suggesting that skill learning and episodic memory relied on different brain structures. Similar episodic memory problems were observed in other patients with bilateral resections of the medial temporal lobe, as well as those who had unilateral resections but tissue degradation in the contralateral hippocampi. Memory loss scaled with the extent of the hippocampal resections (Milner et al., 1998).

Altogether, these case studies pointed to the structures in the medial temporal lobe as crucial for episodic memory, and more specifically the formation of it. The fact that H.M. could recall early memories from before the surgery supported the notion of a distributed memory storage in the brain, where sensory information and other components of a memory event are stored in cortical areas and linked together by the spatiotemporal context of the hippocampus. Frequently repeated reactivation of this engram, as is likely with memories formed early in life, may have led to cortical changes in synaptic plasticity, and, in consequence, memory traces persisting despite the removal of the hippocampus. H.M.'s and other cases informed the theory that the hippocampus is relevant for the encoding and initial retrieval of memories, but that memories become independent from the hippocampus over time and may thus still persist after hippocampal damage or resection (Dudai, 2004; McGaugh, 2000; Squire & Alvarez, 1995).

A deeper understanding of the neural correlates of episodic memory in humans was only possible with the technological advance at the end of the 20th century, when higher computational capacities allowed for automatized data processing and non-invasive neuroimaging methods were developed that could record data in deeper brain structures, such as magnetic resonance imaging (MRI). MRI measures the magnetic signal from hydrogen atoms, which behaves differently depending on its surroundings, allowing one to distinguish between cerebral spinal fluid, white and gray matter in the brain. Functional MRI (fMRI) infers neural activity through the oxygen consumed in the brain while performing cognitive operations. Hemoglobin in capillary red blood cells has different magnetic properties depending on its state of oxygenation (Ogawa et al., 1990). Accordingly, the involvement of brain regions in cognitive processes is approximated through measuring their blood-oxygenation level dependent (BOLD) response.

Additionally, mapping of functional zones through direct brain recordings from the cortical surface (electrocorticography; ECoG) or depth structures (stereotactic electroence-phalography; sEEG) started to be undertaken more frequently to inform resection surgery. In addition to their clinical purpose,

these data were also used for fundamental scientific purposes, like testing whether knowledge of brain mapping and mechanisms gained in non-human mammals also applied to the human brain (Crone et al., 1998).

In this section, we will focus on studies investigating whether signatures reflecting memory encoding mechanisms recorded in rodents could also be found in humans. However, even with technological advances in human neuroimaging, identifying homologous memory processes in humans is not straightforward, for two reasons: First, memory signals such as place cells or replay activity across populations of cells are temporally and spatially highly specific signals, which are difficult to capture with most recording methods available in humans. Second, human memories are likely different from rodents, given the higher cognitive capacity, vastly different subjective experience, and factors such as language, which may render memories more multi-faceted. On the other hand, these differences make it possible to study how verbalized episodic, and not just spatial, memory is processed in the human brain.

The study of spatial cognition in humans predominantly employs virtual reality paradigms, in which participants incidentally learn the map of a town or arena by performing tasks such as delivering objects to specific locations or searching for certain landmarks (fig. 2c). These setups allow for complex tasks within lab settings that require the participant to remain still and close to or inside recording equipment. In this context, evidence was found for grid cells (Doeller et al., 2010; Jacobs et al., 2010) and place cells (Ekstrom et al., 2003; Miller et al., 2013), supporting the idea that basic spatial mechanisms observed in rodent hippocampus may also apply to the human brain. Human place-responsive cells were not only found in the hippocampus, but also in the amygdala, entorhinal cortex and other MTL structures (Ekstrom et al., 2003; Miller et al., 2013). Preliminary evidence also supports the idea of different scales of spatial representation in the human hippocampus (Brunec, Bellana, et al., 2018), where pattern activity in the posterior hippocampus during the exploration of a spatial environment shows more frequent transitions compared to the anterior hippocampus, akin to the anterior-to-posterior gradient of place cell selectivity in the rat hippocampus (Jung et al., 1994; Kjelstrup et al., 2008).

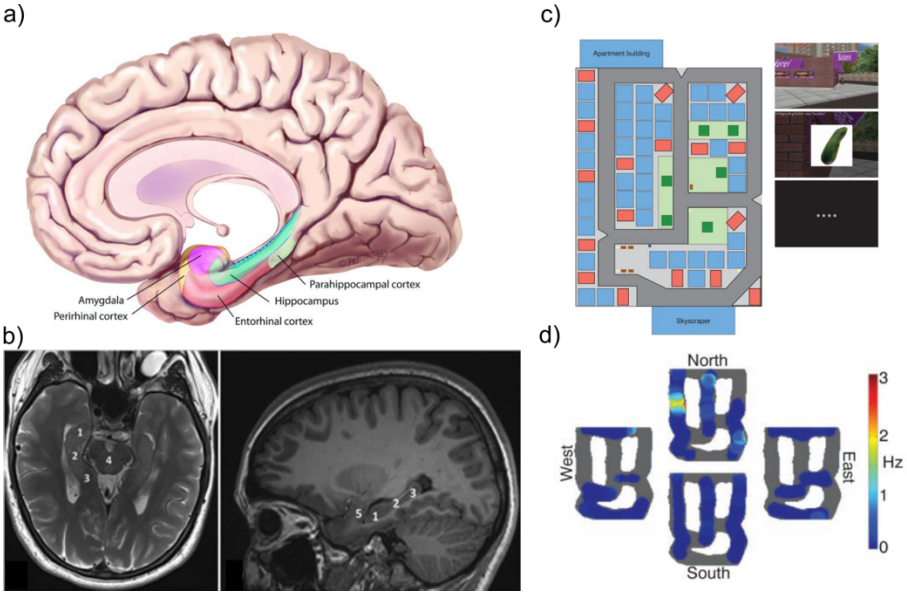

Figure 2. The human medial temporal lobe and hippocampus. a) Schematic overview of MTL structures in the human brain. b) Anatomy of hippocampal formation in transverse (left) and sagittal (right) view, in T2-weighted and 3D MPRAGE contrasts respectively. Numbered labels correspond to 1 = hippocampal head, 2 = hippocampal body, 3 = hippocampal tail, 4 = mesencephalon, 5 = amygdala. c) Virtual maze setup to test human hippocampal place cells with top-down map-view (left) and participant's view (right). Participants navigate through a virtual town and learn locations (e.g. the bakery). They are then asked to deliver objects (e.g. a zucchini) to certain locations. d) Place field of a hippocampal cell when participant was navigating through the town. This cell had its preferred location in the left arm of the maze, but only fired when participant was navigating north. Figure adapted from Dekeyzer et al., 2017; Miller et al., 2013; Raslau et al., 2015.

Evidence has also been found for context reinstatement, by analyzing similarity of item- or location-specific signals between memory encoding and retrieval, and linking this to memory performance (Gelbard-Sagiv et al., 2008; Jang et al., 2017; Manning et al., 2011; Pacheco Estefan et al., 2019; Staresina et al., 2016; Yaffe et al., 2014; Zhang et al., 2015). Although context reinstatement in humans has been studied within spatial tasks (Miller et al., 2013; Pacheco Estefan et al., 2019; Zhang et al., 2015), other setups include verbal memory tasks, in which participants study lists of individual or pairs of words and freely recall them after a temporal delay (Jang et al., 2017; Manning et al., 2011; Staresina et al., 2016; Yaffe et al., 2014). Pattern reinstatement at the moment of retrieval has been found in the neocortex (Jang et al., 2017; Manning et al., 2011; Yaffe et al., 2014; Zhang et al., 2015), and the hippocampus (Manning et al., 2011; Zhang et al.,

2015). Hippocampo-cortical reinstatement seems to be temporally coordinated, such that pattern reinstatement is first observed in the hippocampus, followed by neocortical regions (Pacheco Estefan et al., 2019). This supports the notion of the engram, where a memory trace is stored across separate brain structures. The observations detailed above support the idea that the memory of spatiotemporal context is stored in the hippocampus, while sensory and other stimulus-specific information is stored in the neocortex.

1.1.4. Connecting the dots: from spatial cognition to episodic memory

Coming back to our example of a visit to the Strand bookstore, the previous sections summarized insights into how the spatial location of a memory event (i.e. the Strand, or a specific location within the bookstore) may be processed by the brain, and contribute to later recall by reinstating the spatiotemporal context of the event. Supposedly, this spatiotemporal context reactivates cortical memory traces related to the specific content of the event. In fact, the hippocampus is activated during associative processes, that is, when multiple features of an environment must be bound together. Experimentally, this can be tested by repeatedly showing participants pairs of pictures from different categories, such as faces and houses. Associative memory is tested after a time delay by showing one picture of a pair and asking participants to recall the other (i.e., a picture of a house is presented and participants have to recall the face that was shown with this house before). Studies using this type of paradigm have shown that the hippocampus is activated during associative processing, and that hippocampal activity during encoding predicts subsequent associative memory performance (Jackson & Schacter, 2004; Kirwan & Stark, 2004; Kota et al., 2020).

For neuronal populations to be bound into a common memory trace and for synaptic changes to take place, underlying neural populations have to mutually activate within a short time window of about 100ms (Levy & Steward, 1983). This seems at odds with the way human experience unfolds, containing disparate elements that are perceived serially over an extended period of time (Tulving, 1985). That is, we perceive the features of the bookstore one after the other: maybe we notice a high shelf first, followed by colorful book spines, and then the corresponding book covers. During the experience of visiting the bookshop, representations of these features may not be active simultaneously in the brain, so how are they bound into a coherent trace? The hippocampus would have to bridge across temporal and spatial gaps to enable associative binding processes of item features that are encountered sequenially. Spatial or temporal distance between to be associated item features can be experimentally manipulated: For instance, instead of showing an image of a pink shirt (no

spatiotemporal distance), a monochrome shirt within a pink frame can be shown (spatial distance). To also increase temporal distance, the frame could follow the image of the monochrome shirt at a delay. Using this design, it was shown that hippocampal activation in subsequently successfully recalled trials scaled with increased spatiotemporal distance between features (Staresina & Davachi, 2009). This suggests that the hippocampus indeed bridges across temporal and spatial gaps to associate item information.

The example of serially processing input from the environment also raises the question how sampling of sensory information ties into the memory trace. Historically, memory and vision have largely been researched separately, but recent studies have investigated the interplay of the visual and memory systems, as well as the contribution of eye movement behavior to memory (Broers et al., 2022; Damiano & Walther, 2019; Fehlmann et al., 2020; Kafkas & Montaldi, 2011; Kragel et al., 2021; Liu et al., 2017; Mikhailova et al., 2021; Nikolaev et al., 2023; Olsen et al., 2016; Popov & Staudigl, 2022). In our example of the visit to the Strand bookstore, we perceive the environment largely through visual cues, although other sensory modalities also come into play. For visual stimuli to reach the visual cortex for further processing, they must first be sampled. Visual information from the environment is processed during gaze fixations. Given the limited size of the fovea, only a small proportion of the environment can be processed during gaze fixations. Accordingly, moving the gaze across the visual field by means of saccades or smooth pursuits is necessary to sample information from various locations (Broers et al., 2022; Nikolaev et al., 2023; Pertzov et al., 2009). It has been shown that a higher number of fixations (interspersed by saccades) during the viewing of static face or scene images predicts better memory recall (Broers et al., 2022; Damiano & Walther, 2019; Fehlmann et al., 2020; Kafkas & Montaldi, 2011; Kragel et al., 2021; Liu et al., 2017; Mikhailova et al., 2021; Nikolaev et al., 2023; Olsen et al., 2016; Popov & Staudigl, 2022). More specifically, a combination of wider sampling locations within an image of a scene, combined with dense sampling of specific elements, leads to better scene recollection (Broers et al., 2022), illustrating that memory relies on the wide-spread sampling of visual elements within a scene. Interestingly, eye movements also contribute to retrieval: gaze patterns are reinstated during mental imagery when recollecting scenes (Bone et al., 2019), or during memory recall, and suppression of gaze behavior generally leads to worse memory performance (Liu et al., 2020). This suggests that eye movements may not only be relevant for sampling the visual space, but that their movement patterns are bound into the memory trace and can act as a cue for memory reinstatement. Memory encoding of dynamic stimuli, in which elements and

their position change over time, is likely also shaped by eye movements, though this has not been investigated thus far.

With few exceptions of virtual reality paradigms, human studies investigating associative mechanisms in episodic memory rely on discrete, static stimuli, such as words or images of isolated faces, objects, or scenes. These stimuli are simplified for the sake of experimental precision, providing the advantage of adjustment of stimulus timing and exposure duration, and clear reference for time-locked analysis of neural activity. Our initial example of experiencing a special visit to the Strand bookstore and encoding it into memory is a much more complex scenario, consisting of a continuous stream of stimuli from several modalities (e.g. vision, sound) and containing a temporal progression (e.g. browsing books on the ground floor, exploring the rare books room). This complexity is not well reflected in experimental designs using static image stimuli. Dynamic stimuli, such as movie clips or feature-length movies, provide the opportunity to take a step further in approximating naturalistic (i.e., real-life experience) conditions for episodic memory formation.

1.1.5. Studying event memory with naturalistic stimuli

Throughout this introduction I have used the experience of visiting the Strand bookstore as an example for a memory event. But what really is a memory event? Previous research has shown that the brain segments the continuous stream of subjective experience into chunks of information (Baldassano et al., 2017; Geerligs et al., 2021, 2022; Kurby & Zacks, 2008; Radvansky & Zacks, 2017). These segments stem from the formation of 'event models' (Richmond & Zacks, 2017): conceptual models of the current situation which guide behavior suitable for the current circumstances (Clewett et al., 2019).

If events are units of real-life experience, a pressing question is how the brain determines the boundaries of these units of experience. Event boundaries seem to be related to detected changes in spatial, contextual, or internal cues (Brunec et al., 2020; Brunec, Moscovitch, et al., 2018; DuBrow & Davachi, 2013; Pettijohn & Radvansky, 2018a, 2018b). These changes may deem different behaviors as more appropriate to the given situation, thereby requiring updating of the event model. Previous research has shown that event boundaries also shape memory: for example, memory performance is better for items encoded within one context (i.e., in the same room) than when separated by a boundary (i.e. separated by a doorway; Horner et al., 2016).

In searching for the neural correlates of event boundaries, studies have used narratives as stimulus material, either by showing participants movies (Baldassano et al., 2017; Ben-Yakov et al., 2013; Ben-Yakov & Dudai, 2011; Ben-

Yakov & Henson, 2018) or written stories (Ezzyat & Davachi, 2011). Using short movie clips, it has been shown that activity in the hippocampus peaks at the end of clips containing an event but not those that contain scrambled footage. Moreover, the amplitude of this hippocampal response was higher for clips that were later remembered compared to those that were later forgotten (Ben-Yakov et al., 2013; Ben-Yakov & Dudai, 2011). This increase of hippocampal activity has also been found at event boundaries within feature-length movie clips. Here, hippocampal activity increased at time points which independent observers had annotated as noticeable shifts in the narrative (Baldassano et al., 2017; Ben-Yakov & Henson, 2018), and the hippocampal response at those time points was predictive of subsequent memory recall (Baldassano et al., 2017). Behavioral interruption of this process, by showing new and unrelated stimulus material immediately after an event boundary, affects memory recall for the preceding event and attenuates the amplitude of the hippocampal response to the first event's offset (Ben-Yakov et al., 2013). This observation suggests that processes immediately following an event's offset are relevant for the consolidation of the event into memory.

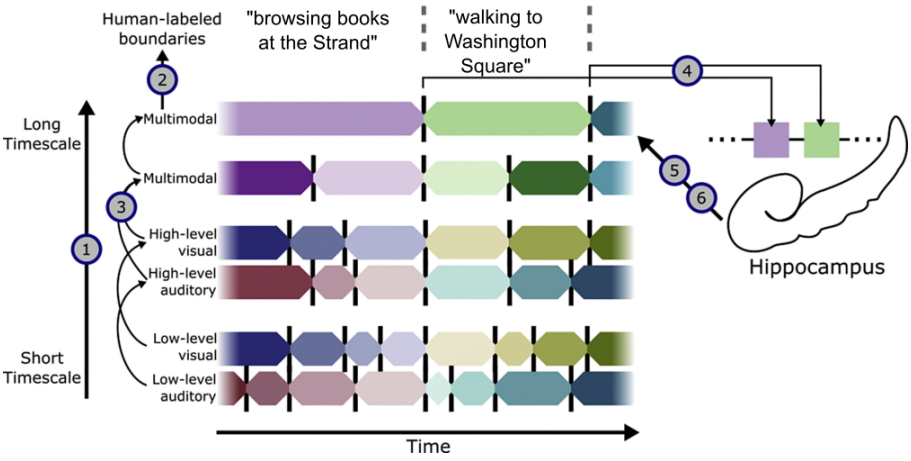

Figure 3. Model of event segmentation and memory in the human hippocampus. The brain represents events at different timescales, with lower-order sensory regions operating on smaller perceptual units of experience, which make up a larger memory event (i.e. "browsing books at the Strand"). At event boundaries, the hippocampus binds perceptual and other information processes in these lower-order areas into a memory trace. See text for descriptions of numbered labels. Figure adapted from Baldassano et al., 2017.

Using episodic events, however, is just one way of chunking experience into units. Recent theories and studies of event perception argue that the brain integrates information at different timescales (Baldassano et al., 2017; Chen et al., 2016; Geerligs et al., 2022; Hasson et al., 2015), whereby lower-order events in sensory regions may be described at the level of visual or auditory features. Figure 3 depicts this conceptual hierarchy (adapted from Baldassano et al., 2017). Moving up the cortical hierarchy, the time-scales of these representations may increase (1) and approximate the memory event of the Strand visit described throughout this introduction (2; note that the term 'event' throughout this thesis refers to the longer-scale idea of an episodic event). In this way, perceptual input from the environment may be processed at short time-scales in sensory cortex and be integrated into a model of the situation in higher order areas (3). Event boundaries may then trigger associative processes in the hippocampus to bind the information encoded throughout the event into a long-term memory trace (4). This long-term memory trace may be reinstated during later recall (5) or may contribute to new event models in similar situations (6). Evidence from fMRI studies using computational modeling of data support this notion, showing that transitions between activity patterns occur more frequently in lower-order sensory regions (i.e., visual cortex), whereas longer lasting stable activity can be observed in higher-order regions (i.e., angular gyrus). Transitions in these higher-order cortical regions overlapped with independently annotated event boundaries and increases in hippocampal activity (Baldassano et al., 2017). Altogether, these observations suggest that hippocampal processing at event boundaries may bind the elements of the just-encoded event, thereby supporting memory consolidation.

Given the amount of information and elements that events consist of, it is likely that the building of an event model relies on working memory processes. Working memory describes the capacity for transient maintenance and manipulation of information (Miller et al., 2018). As an event unfolds, representtations of processed information need to be maintained, while also allowing for integration of new incoming information. This notion is corroborated by the observation that brain structures such as the medial prefrontal cortex (Ezzyat & Davachi, 2011; Hasson et al., 2008) and the basal ganglia (Ben-Yakov & Dudai, 2011) show increased activation during the processing of dynamic stimuli containing narratives. These structures have otherwise been associated with working memory manipulation and selection of incoming information. Upon the occurrence of an event boundary, this stored information may then be bound together by the hippocampus, effectively marking the transfer of working memory information to long-term memory (Ezzyat & Davachi, 2011; Kurby & Zacks, 2008).

1.2. Part II: Memory consolidation during sleep

After introducing the key components of the episodic memory system and contribution of the neocortex and hippocampus to memory formation and retrieval, Part II will focus on the consolidation period following this event. Long-term memories of any kind (episodic, semantic, non-declarative) rely on structural changes in the brain, for instance through the growth of new synapses or the strengthening of existing synapses. Long-term potentiation, which is the molecular process that is thought to underlie synaptic growth (Bliss & Lømo, 1973), requires repeated stimulation of the neuronal populations involved (Josselyn et al., 2015). Repeated stimulation can occur either by repetition of stimuli (e.g. having an animal run repeatedly through a circuit or a human repetitively studying word lists), or through spontaneous reactivation in 'offline' states (fig. 1). On the systems level, this recurrent reactivation of hippocampal traces is thought to engage neurons in the neocortex to strengthen the engram's connections. Although reactivation can also occur during rest, we will focus here on memory consolidation during sleep.

During sleep, the brain cycles through a number of stages (fig. 4a). These stages are marked by differences in electrophysiological patterns in the brain, which can be measured using intracranial or scalp EEG, as well as the muscle tonus (measured via electromyography on the chin) and movement of the eyes (measured via sensors around the eyes). The combination of these three measures is called polysomnography, which is used as a basis to categorize segments of sleep into the substages: from wake, sleep stages 1 to 4 to rapid eye movement (REM) sleep (Iber et al., 2007; Rechtschaffen & Kales, 1968).

1.2.1. Nested oscillations during sleep

The different sleep stages are marked by recurring patterns of neuronal population activity (i.e., oscillations) that can be measured in electrophysiology either inside the brain, or on the scalp via electroencephalography (EEG). Figure 4b depicts these hallmark oscillations. Particularly slow oscillations (SOs), sleep spindles and ripples have been associated with memory consolidation.

SOs are high amplitude oscillations which originate in the neocortex and reflect synchronized network population activity (Klinzing et al., 2019), where the down-state reflects membrane hyperpolarization and the up-state membrane depolarization. Accordingly, neuronal firing is suppressed during the

down-state and more likely during the up-state (fig. 4c, top). SOs occur during non-REM (NREM) sleep, particularly during deeper sleep stages.

Sleep spindles are transient patterns of activity at 8 to 16 Hz, which last for 0.5 to 2 s. They are mostly associated with NREM sleep stages 2 and 3. Although they originate in the thalamus (Contreras et al., 1997; Steriade, 2005; Steriade et al., 1993), they can also be observed in the neocortex (Andrillon et al., 2011; Mak-McCully et al., 2017; Nir et al., 2011) and the hippocampus (Staresina et al., 2015). Studies into the relevance of sleep spindles in human memory consolidation (Gais et al., 2002; Petzka et al., 2022; Schabus et al., 2004) primarily rely on cortical spindles that can be measured in scalp EEG. Here, spindles have been shown to predominantly occur in those regions that were activated during a learning session on the previous day and that this overlap in cortical spindle topography between learning session and sleep predicts memory consolidation (Petzka et al., 2022). Ripples are transient oscillatory patterns at 80 to 140 Hz, whose occurrence in the hippocampus has been linked to memory reactivation (Zhang et al., 2018). Although ripples have been frequently reported in the hippocampus, they can also be observed in the neocortex, where they may reflect neuronal ensemble reactivation (Vaz et al., 2019).

Although SOs, spindles and ripples originate in different structures in the brain, they can occur in a nested fashion (Gonzalez et al., 2018; Staresina et al., 2015). That is, they do not only co-occur, but aligned with each other's excitatory phases. Figure 4c depicts this nesting, where spindles are more likely to occur at the SO up-state (Klinzing et al., 2016, 2019; Staresina et al., 2015). Ripples, in turn, tend to be nested in the troughs of spindles (Staresina et al., 2015). Figure 4d illustrates the underlying interplay between neocortex, thalamus, and hippocampus, where certain states (e.g. cortical SO down-states) suppress, and others (SO up-states) trigger generation of other oscillations. Overall, there seems to be a top-down and bottom-up dialogue between these structures regulating the oscillatory signals. It is thought that the nesting of SOs, spindles and ripples underlies memory consolidation. Evidence for this has been found in studies in rodents: although external induction of thalamic spindles generally triggered hippocampal ripples, it had to be timed to the SO up-state to trigger cortical ripples and to improve memory performance (Latchoumane et al., 2017).

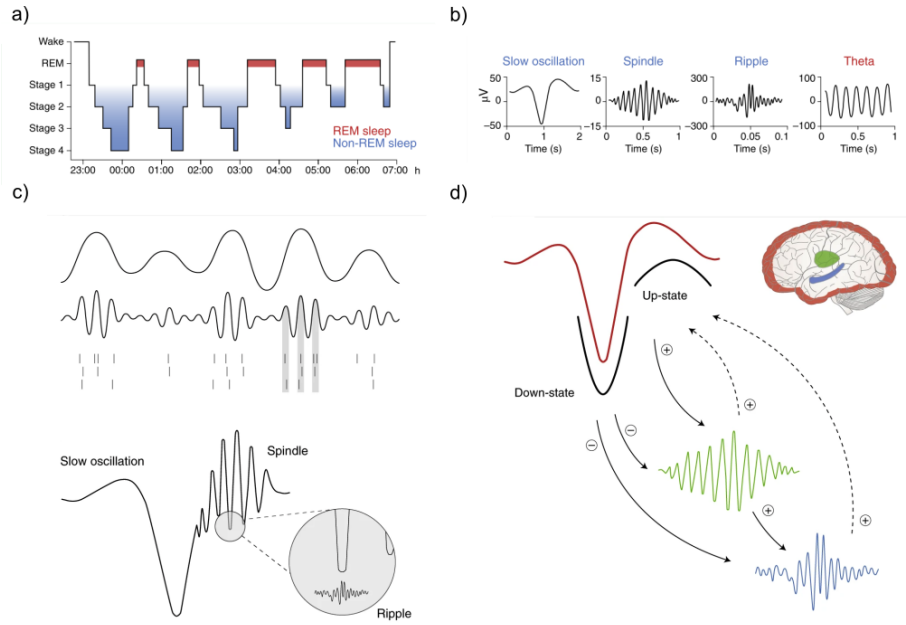

Figure 4. Sleep stages and oscillations in the brain. a) Schematic human polysomnogram of one night of sleep, scored into stages. Note the progression through the different NREM sleep stages, followed by a period of rapid eye movement REM sleep. b) Hallmark oscillatory patterns during sleep: SOs, spindles and ripples which occur during NREM sleep and theta oscillations, which occur during REM sleep. c) Nested oscillations regulating systems consolidation. Top depicts an example of a neuron population engaging in nested slow (top) and fast (middle) oscillation. Note that the single spikes of a cell within this population (bottom) align with the peaks of the faster oscillation. At this depolarized phase, neurons are more likely to fire, increasing the likelihood of mutual firing with other neurons in the population, promoting plasticity within the population. Bottom: Triple-nested SO, spindle and ripple. The spindle occurs at the up-state of the SO, with ripples nested into the troughs of the spindle. d) Overview of main brain regions involved (inset) and corresponding nested sleep oscillations: SOs in the neocortex (red), spindles in the thalamus (green), and ripples in the hippocampus (blue). It is thought that oscillations in these regions are both regulated in top-down and bottom-up fashion (arrows). Spindles are thought to synchronize ripples to their troughs, thereby coordinating when the hippocampus reactivates. Figure adapted from Klinzing et al., 2019.

To briefly summarize, it seems that triple nesting of SOs, spindles and ripples promotes memory consolidation during sleep. However, reactivation of hippocampo-cortical neuronal assemblies must be specific to the populations involved in the engram. Although to date it is unknown how reactivation is coordinated across brain systems in a targeted manner, it is possible that the thalamus times network reactivation through spindle activity (Klinzing et al., 2019).

1.2.3. Systems memory reactivation may be coordinated by the thalamus

The thalamus is a mid-brain structure situated in the diencephalon, consisting of several nuclei with distinct connections to cortical and subcortical structures (Cappe et al., 2009; Jankowski et al., 2013; Zhang et al., 2010). Originally, the thalamus has been considered as a relay station in the brain responsible for transmitting incoming sensory information to the primary cortices for further stimulus processing (Gluck et al., 2016; McCormick & Bal, 1994), and suppression of incoming input during sleep. However, the rich connections of the thalamus in cortical and subcortical networks also make it a viable structure to coordinate activity across brain systems in a targeted manner, as required for memory reactivation (Klinzing et al., 2019).

A newer theory argues that because of its rich connections, the thalamus could work as a connector hub between distinct cortical networks (Kawabata et al., 2021). It has been shown that targeted suppression and activation in thalamic nuclei enhances processing within, and interplay between, specific cortical networks to adapt to changing task demands when mice are awake (Schmitt et al., 2017). Similar patterns can be observed during spindle generation in rodent NREM sleep (Chen et al., 2016; Halassa et al., 2014). This preliminary evidence supports the notion that the thalamus could function as a switchboard to activate distinct cortical circuits implicated in memory traces. Selective activation of neuronal populations could be achieved through activation of different neuronal subtypes in the thalamus. These thalamic cell types differ in their projections to and feedback from cortical regions, with core cells being spatially selective and targeting single cortical areas, whereas matrix cells project diffusely (Piantoni et al., 2016).

Although its neuroanatomical features make the thalamus a good candidate for selective network activation, this does not explain how spindles may induce plasticity for memory consolidation. Early studies in the feline brain have shown that spindles change responsiveness in cortical neurons (Timofeev et al., 2002). Newer evidence from rodent models indicates that spindles also induce metabolic processes conducive to long-term potentiation (Niethard et al., 2018; Seibt et al., 2017), which could lead to changes in brain plasticity after a learning session. In humans, it has been shown that cortical spindles induce increased firing in pyramidal and inter-neurons, and that spindle coherence between cortical sites increased mutual firing of neurons (Dickey et al., 2021). Although still preliminary, this suggests that spindles may provide neuronal populations in the hippocampus and neocortical locations implicated in a memory trace with time windows of excitability during which reactivation can take place.

1.3. Conclusion

In brief, episodic memory is the cognitive capacity for encoding, consolidating and recalling (autobiographical) events. Across species, the hippocampus has been consistently linked to memory operations, where it is thought to encode the spatiotemporal context of learned events. Events consist of elements from multiple modalities that unfold over time. Associative processes to bind the event's elements also relies on the hippocampus, and it is thought that this binding occurs at contextual boundaries. Returning to the example of our memory event, hippocampal processes upon leaving the bookstore may ensure association of all the constituent elements (which books we saw, the music that was playing, the visit of the rare book room etc.) into a memory trace. For this trace to be stored for later memory recall, the neuronal populations implicated in it must undergo plasticity changes. This consolidation process is thought to predominantly occur during sleep, through an organized concert of oscillatory patterns.

Throughout the introduction I have summarized existing research into encoding, consolidation and retrieval processes that may underlie episodic memory for events. Nonetheless, several open questions remain. Firstly, the study of episodic memory with dynamic stimuli is still relatively new. Accordingly, to date only few studies on event processing, the role of the hippocampus, and the importance of event boundaries exist. Even fewer of those have been replicated. For instance, it is unclear how unrelated input that co-occurs with event boundaries affects binding of event information. Furthermore, the relevance of sampling visual information from the environment for episodic memory has not been systematically investigated for dynamic stimuli. Lastly, although it has been suggested that thalamic spindles may coordinate reactivation in memory systems during sleep, the extent to which spindles systematically co-occur in human thalamocortical loops has not been investigated. This thesis aims to address these open questions.

1.4. Outline of this thesis

Episodic memory processes during and immediately after the encoding of events rely on associative binding of the information experienced during the event (i.e. as sampled through eye movements). The hippocampus is thought to support this binding process. Only recently, studies have adopted dynamic stimuli, as opposed to static pictures or word lists, to investigate event processing in setups with higher similarity to day-to-day life experience. The first three chapters of this thesis address gaps in understanding event processing and episodic memory formation in dynamic stimuli. Because of the relevance of sleep for memory consolidation, we tested memory recall after 24 hours across all studies. To this end, we used short movie clips containing a progression of events. Across several chapters, we studied event boundary processes by behaviorally interfering with the offset of the movie clips (chapter 2), the role of eye movements during the encoding of these clips (chapter 3), and peri- and post-event processes in the hippocampus and other brain regions using fMRI data (chapter 4).

Episodic memory of events seems to hinge on processing at event boundaries, where unrelated input retroactively interferes with memory of the preceding event (Ben-Yakov et al., 2013). In **Chapter 2**, we aimed to replicate this retroactive memory interference and further characterize the observation. EEG activity after event boundaries is characterized by reactivation of patterns found during encoding (Silva et al., 2019; Sols et al., 2017; Wu et al., 2023). It is conceivable that information input at event boundaries perturbs this reactivation. Yet, EEG pattern reinstatement occurs within 1.5 s after an event boundary. We hypothesized that input should only retroactively interfere with the preceding event when it is presented immediately after its boundary, but not after a delay. In addition, we studied putative differences in retroactive memory interference for recall after 20 minutes compared to 24 hours.

The role of eye movement for the encoding of static images has been established in several studies, where a higher number of fixations, interspersed with saccades, predicts better recollection memory after short retention delays (cf. Broers et al., 2022). However, it is not clear how eye movements shape memories after longer retention delays, and for dynamic stimuli, which contain changes in the depicted elements and in their position (e.g. an actor may move across the screen). Particularly in this context, as compared to static images, smooth pursuit eye movements may play an important role. Smooth pursuits are slow eye movements used to track moving stimuli. Humans rarely engage in

smooth pursuits during static stimuli, but they may be relevant to process information in dynamic setups. Accordingly, we hypothesized that not only fixations, but also smooth pursuits during encoding may be relevant for later memory performance. In **Chapter 3**, we investigated eye movements, include smooth pursuits, during the viewing of short movie clips.

Previous research has shown that hippocampal BOLD activity increases after event boundaries (Baldassano et al., 2017; Barnett et al., 2022; Ben-Yakov & Henson, 2018) and at the offset of short movie clips (Ben-Yakov et al., 2013; Ben-Yakov & Dudai, 2011), and that this signal increase scales with subsequent memory performance, particularly after short retention delays (Baldassano et al., 2017; Ben-Yakov & Dudai, 2011). This suggests that the hippocampus may be engaged in associative binding processes at the offset of events, as suggested by the model introduced in figure 3. However, it has not been explored thus far if the whole hippocampus behaves the same way at event boundaries, or if processes may differ along its longitudinal axis. Using spatial tasks, differences in resolution of spatial representation of the environment have been shown in rodents (Kjelstrup et al., 2008; Komorowski et al., 2013) and humans (Brunec, Bellana, et al., 2018). In **Chapter 4**, we investigated event boundary processes in the head, body and tail of the hippocampus by analyzing 7T BOLD responses to the offset of short eventful and uneventful movie clips, and sorting trials based on memory performance after 24 hours. We furthermore aimed to extend the results of activity changes outside the hippocampus reported by Ben-Yakov et al. (2011) using fMRI at a higher field strength.

Episodic memories are consolidated during offline states. Nested oscillation patterns during sleep are thought to reflect network and local population excitability, during which memories can be reactivated (Klinzing et al., 2019). The systematic and coordinated reactivation of memory traces in hippocampo-cortical networks during sleep is hypothesized to be timed by thalamic spindles. Due to difficulties in recording signals in the human thalamus, few studies have reported co-occurrence of spindles in the thalamus and cortex (Bastuji et al., 2020; Mak-McCully et al., 2017; Schreiner et al., 2022). Yet, for the thalamus to play a role in coordinated activity in cortical loops, mutual spindle activity across thalamus and cortex would need to be systematic. This open question was addressed in **Chapter 5**, by studying thalamocortical spindle co-occurrence patterns using recordings from deep-brain stimulation implants in the thalamus combined with scalp EEG. We devised a statistical analysis to assess whether mutual spindle activity across thalamocortical sites differed from patterns that would be expected by chance. Additionally, we investigated

putative differences in spindle co-occurrence patterns and topographies based on spindle frequencies.

Chapter 6 summarizes the observations made in the different empirical studies and discusses them in context of each other and the wider episodic memory literature.

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Chapter 2:

Stimulus presentation following event boundaries immediately, but not after a delay, interferes with event memory

Chapter 3:

Fixation behavior during encoding of dynamic stimuli predicts memory recall

Chapter 4

Probing activity during memory encoding of narrative episodes using multi-echo fMRI at 7T

Chapter 5:

Spatiotemporal patterns of sleep spindle activity in human anterior thalamus and cortex

Corresponding manuscript:
Bernhard, H., Schaper, F. L., Janssen, M. L., Gommer, E. D., Jansma, B. M., Van Kranen-Mastenbroek, V., Rouhl, R., de Weerd, P., Reithler, J., Roberts, M.J. & DBS study group (2022). Spatiotemporal patterns of sleep spindle activity in human anterior thalamus and cortex. *NeuroImage*, *263*, 119625.

5.1. Abstract

Sleep spindles (8 - 16Hz) are transient electrophysiological events during non-rapid eye movement sleep. While sleep spindles are routinely observed in the cortex using scalp electroencephalography (EEG), recordings of their thalamic counterparts have not been widely studied in humans. Based on a few existing studies, it has been hypothesized that spindles occur as largely local phenomena. We investigated intra-thalamic and thalamocortical spindle co-occurrence, which may underlie thalamocortical communication. We obtained scalp EEG and thalamic recordings from 7 patients that received bilateral deep brain stimulation (DBS) electrodes to the anterior thalamus for the treatment of drug resistant focal epilepsy. Spindles were categorized into subtypes based on their main frequency (i.e., slow (10±2Hz) or fast (14±2Hz)) and their level of thalamic involvement (spanning one channel, or spreading uni- or bilaterally within the thalamus). For the first time, we contrasted observed spindle patterns with permuted data to estimate random spindle co-occurrence. We found that multichannel spindle patterns were systematically coordinated at the thalamic and thalamocortical level. Importantly, distinct topographical patterns of thalamocortical spindle overlap were associated with slow and fast subtypes of spindles. These observations provide further evidence for coordinated spindle activity in thalamocortical networks.

Highlights:

- Sleep spindles were measured in human anterior thalamus and on the scalp
- Both fast and slow spindles occurred in the anterior thalamus
- 25% of spindles spanned multiple channels in thalamus and cortex
- A novel statistical approach confirmed that spindle co-occurrences were not random
- Cortical spindle patterns depended on thalamic involvement and spindle frequency

Keywords: thalamus, sleep spindle, thalamocortical coupling, human, intracranial EEG

5.2. Introduction

Sleep spindles are transient (0.3 – 2s) electrophysiological patterns (8 – 16Hz) and a characteristic of non-rapid eye movement (NREM) sleep in humans and other mammals (Fernandez & Lüthi, 2020). Recent research suggests that spindles may be involved in coordinating information transfer in hippocampal and neocortical networks to support memory consolidation and learning (Andrillon & Kouider, 2020; Klinzing et al., 2019; Latchoumane et al., 2017; Ngo et al., 2020; Staresina et al., 2015). Spindles across the brain can occur in a number of ways. They could be locally restricted to specific (cortical) sites or they could spread from thalamus to cortex in a set manner. Both options would preclude spindles from flexibly coordinating information transfer. Alternatively, spindles might follow function-specific spatiotemporal patterns. To the best of our knowledge, the extent to which spindles systematically co-occur within thalamic and across thalamocortical networks is unknown. Here, we investigated in humans whether sleep spindle activity in anterior thalamus and cortex follows coordinated spatiotemporal patterns.

To date, only few studies on human thalamic spindles exist. The reasons for this are twofold. Most human spindle research has relied on scalp electroencephalography (EEG), whose limited spatial resolution does not permit inferences about thalamic spindles. Thalamic signals can only be recorded using invasive procedures, but invasive thalamic recordings are generally performed during awake brain surgery or under anesthesia (Schaper et al., 2019). Deep brain recordings acquired via externalized deep brain stimulation (DBS) leads (Schreiner, Kaufmann, et al., 2021; Szalárdy et al., 2021), provide a safe (Feldmann et al., 2021) opportunity to study thalamic spindles in clinical populations of patients with epilepsy. Several studies have performed recordings from the posterior thalamus and cortex. For example, Mak-McCully et al. showed that thalamic spindles can precede spindles in neocortex (Mak-McCully et al., 2017). Bastuji et al. reported a tendency of localized thalamic spindles to also have more localized cortical counterpart, while diffuse thalamic spindles were correspondingly more diffusely represented in the cortex (Bastuji et al., 2020). Based on these data, the authors have inferred coordinated spindle activity across networks. However, their analysis did not account for random temporal overlap of spindle activity. In addition, these studies did not differentiate between so called slow (8 – 12Hz) and fast (12 – 16Hz) spindles. This differentiation seems relevant, because different types of spindles might have different functions. Fast spindles have been linked to memory processes

(Ngo et al., 2013). The functional role as well as the physiology of slow spindles is understudied, as the majority of studies filtered in the spindle frequency range of 11 to 16Hz (Bastuji et al., 2020; Mak-McCully et al., 2017). Furthermore, due to clinical constraints, these studies focused on the unilateral posterior thalamus. Consequently, the spindle behavior within the anterior thalamus and between anterior thalamus and cortex remains unknown. A characterization of spindles in the anterior thalamus is pertinent, because of its relation to memory function in animals (Aggleton et al., 2010; Frost et al., 2021) and humans (Sweeney-Reed et al., 2014, 2017, 2021).

Here, we addressed these issues and took a statistical approach to measure patterns of slow and fast spindle (co-)occurrence in the anterior thalamus and cortex by controlling for random spindle co-occurrence using permutation testing. This approach allowed us to compare temporal spindle coupling observed in the data with distributions of random spindle co-occurrence, thereby informing whether spindles in thalamocortical loops occur in systematically coordinated patterns. We recorded data in 7 individuals with epilepsy who underwent implantation of DBS quadripolar electrodes targeting the anterior nucleus of the thalamus (ANT). The setup of bilateral thalamic depth electrodes allowed us to assess co-occurrences of thalamic spindles within and across hemispheres. Simultaneous 19-channel scalp EEG recordings made it possible to study thalamocortical spindle co-occurrence globally across the cortex. We categorized spindles based on their main frequency as slow (10±2Hz) or fast (14±2Hz) spindles, as well as their level of thalamic involvement (spindles restricted to one channel, or spanning more than one channel unilaterally or bilaterally). We found that both slow and fast thalamic and thalamocortical spindles were more likely to co-occur across channels than expected by chance. Additionally, distinct topographical patterns of spindle overlap were associated with the different spindle subtypes. These observations provide support that slow and fast spindle activity are systematically coordinated in thalamocortical circuits.

5.3. Materials & Methods

Study design

The participants included in this study were admitted to Maastricht University Medical Centre for the implantation of DBS as treatment for drug-resistant focal epilepsy. In- and exclusion criteria followed the FDA and CE mark of the SANTE trial and are described elsewhere (Fisher et al., 2010; Salanova et al., 2015; Schaper et al., 2020). Patients were monitored with EEG and intracranial registration of their DBS electrodes for 22 hours after DBS implantation, including one night of sleep. The study procedure (DBSSEPI study (Dutch Trial Register NL4440 (NTR4562))) was approved by the medical ethical committee of Maastricht University Medical Centre, the Netherlands (ID: METC 14-4-126), and was conducted in accordance with the declaration of Helsinki.

Participants

Seven individuals with drug-resistant focal epilepsy were included in this study (2 female; mean age 35 years; range 18 – 59 years). Six other individuals, who were part of the DBSSEPI study (Schaper et al., 2019, 2020) were excluded due to missing scalp electrodes and/or seizures during the night of recording.

DBS surgery

As part of standard clinical practice, all participants had a pre-operative 3-T MRI (Philips, Eindhoven, The Netherlands) or 1.5-T MRI (Philips, Eindhoven, The Netherlands) in case of an implanted vagal nerve stimulator for stereotactic planning of the electrode trajectory. The sequences used were a 3D T1 with gadolinium, axial T2 and a T1 inversion recovery.

DBS electrode implantation was performed by directly targeting the ANT on pre-operative MRI. The most distal contacts were placed at the termination point of the mammillothalamic tract (MTT) in the ANT, also called the ANT-MTT junction (Lehtimäki et al., 2019). DBS surgery was performed under general anesthesia with remifentanil and propofol using a Leksell stereotactic frame, under guidance of intraoperative microelectrode recording. Details of the DBS surgery and lead placement are described elsewhere (Schaper et al., 2019, 2020).

P	Sex	Age	Treatment (medication, dosage)	MRI abnormalities	EEG electrodes
1	m	26	levetiracetam 2d1000mg, oxcarbazepine 2d450mg, lacosamide 1d50mg, clobazam 3d5mg	Normal	19 channel
2	m	23	phenytoin 2d150mg, lamotrigine 2d250mg, perampanel 1d10mg	Normal	19 channel
3	m	59	carbamazepine 200mg-400mg, clobazam 1d5mg, lacosamide 2d200mg	generalized atrophy	19 channel
4	f	19	carbamazepine 2d200mg, lamotrigine 250mg-200mg, perampanel 1d2mg, topiramate 2d75m	Normal	19 channel
5	m	51	lacosamide 2d150m	Normal	22 channel (incl. EOG)
6	f	49	phenytoin 125mg-112,5mg, carbamazepine 2d600mg	frontobasal scars	22 channel (incl. EOG)
7	m	18	brivaracetam 2d100mg, carbamazepine 2d200mg, clobazam 2d10mg, lamotrigine 2d200mg	Normal	22 channel (incl. EOG)

Table 1. Individual clinical and MRI data and EEG setup.

DBS lead localization

DBS leads were localized in a common space (Montreal Neurological Institute (MNI) 1522009b template (Fonov et al., 2011)) using Lead-DBS software (https://www.lead-dbs.org) with default settings (Horn et al., 2019), in line with previous studies (Horn et al., 2017). In short, pre-operative T1 / T2 MRI sequences and post-operative MRI / CT images (suppl. table 1) were linearly co-registered using SPM (Friston et al., 1994), version 12. Co-registration was

further refined using the *brainshift correction* option (Schönecker et al., 2009) and images were normalized to MNI space using the Advanced Normalization Tool (http://stnava.github.io/ANTs/; Avants et al., 2008). DBS electrode trajectories and contacts were automatically pre-localized and manually refined using Lead-DBS. DBS lead trajectories were visualized in MNI space in relation to a thalamic mask, derived from the Thomas atlas (Su et al., 2019) and can be seen in figure 1.

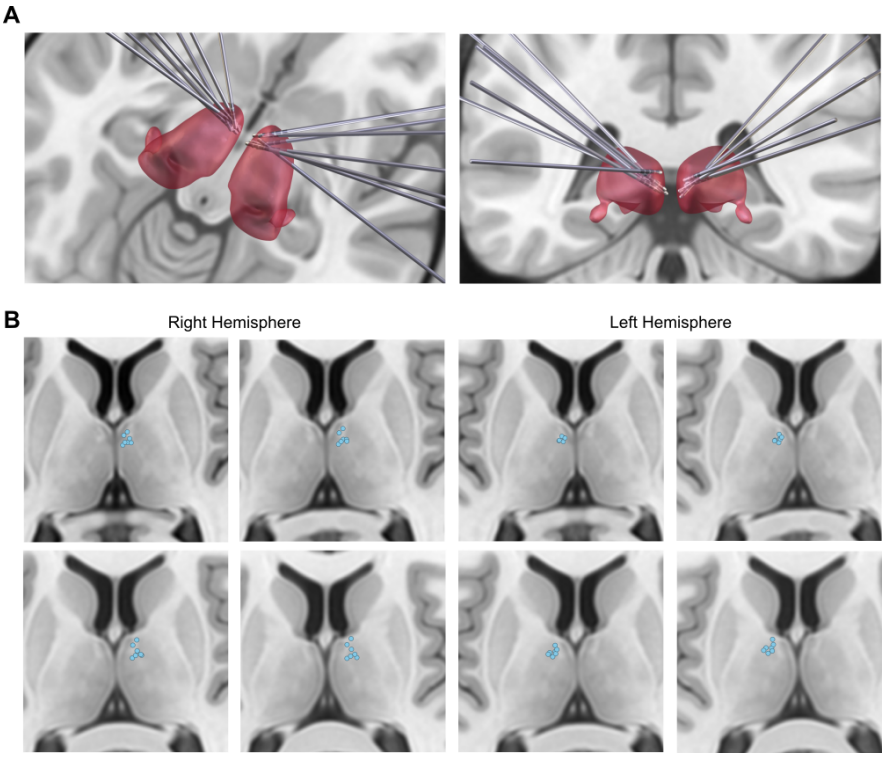

Figure 1. DBS lead locations and anterior thalamic recording sites in a common atlas space. A) Bilateral trajectories of implanted DBS leads for all participants (n=7) in transversal (left) and coronal (right) view. Note that each lead contained four electrode contacts. B) Contact positions (blue dots) of all participants projected onto a thalamic mask derived from the Thomas atlas (Su et al., 2019). MNI coordinates of each contact can be found in supplementary table 1.

Data acquisition of electrophysiological recordings

Thalamic local field potentials (LFPs) were acquired using quadripolar externalized DBS leads (model 3389, Medtronic, Dublin, Ireland). Scalp EEG was measured using a 19-channel setup, with Ag/AgCl cup electrodes placed

according to the standard 10-20 system. Data were recorded in Brain RT version 3.3 at a sampling rate of 2048 Hz, using Braintronics BrainBox-1166-64.

Sleep scoring

Sleep scoring was performed in Brain RT version 3.3. For three out of seven participants, eye movement activity was measured with electro-oculogram (EOG) electrodes placed on the outer corners of the eyes, recording horizontal (HEOG) and vertical (VEOG) eye movements, respectively. For sleep scoring, a bipolar montage was applied to HEOG and VEOG channels, respectively. Four patients did not have EOG recordings. In this case, the frontal scalp EEG channels (Fp1 and Fp2, as well as F7 and F8, respectively) were re-referenced to bipolar derivations to show maximum eye movement activity for the polysomnographic scoring (i.e., Fp1 to F7, and Fp2 to F8), enabling the identification of wake and phasic REM periods. Channels F3, C3, O1, were re-referenced to the right mastoid, channels C4 and O2 to the left mastoid. Using a notch-filter at 50 Hz and a window size of 30s, a sleep scoring expert (H.B.) manually scored the data into sleep stages according to the American Academy of Sleep Medicine (AASM) criteria (Berry et al., 2015).

Data Preprocessing

The Fieldtrip toolbox (Oostenveld, Fries, Maris, & Schoffelen, 2011; http://fieldtriptoolbox.org), Sleeptrip toolbox and MATLAB R2018a and 2021b (Mathworks Inc., Sherbom, MA) were used for data analysis. Hypnograms and continuous data were exported from Brain RT to Matlab. A high pass filter at 0.4Hz (Butterworth, two-pass filter, 5^{th} order) was applied to the continuous data, before down-sampling to 512 Hz. Electrodes were checked manually and, in case of continuous high noise levels, excluded. This was only the case for one electrode (A2) in one participant.

Scalp EEG activity was then re-referenced to the average of the mastoid electrodes (A1, A2), or to the average of all scalp electrodes for the patient missing A2. For the thalamic electrodes, bipolar derivations were computed between neighboring electrodes, resulting in 6 thalamic channels per participant (3 per hemisphere). We chose bipolar derivations to obtain a more localized signal. Where a certain thalamic nucleus is named in the supplementary material of this paper, one or both of the electrodes in the bipolar derivation were localized within this nucleus. However, given the length (1.5mm), diameter (1.27 mm) and spacing (0.55 mm) of electrode contacts, bipolar derivations may not be located in one nucleus but span several.

Both thalamic LFP and scalp EEG data were subjected to a low pass filter at 100Hz (Butterworth, two-pass, 5th order), and a line noise filter at 50Hz to reduce electrical noise. Subsequently, the continuous data were transformed into 30s epochs and linked with the sleep hypnogram. Epochs with manually identified artifacts (e.g., visibly noisy signal) or arousals were excluded, as well as any epochs not pertaining to NREM sleep (N2 and N3, respectively).

Spindle detection

Automatized spindle detection was performed in Sleeptrip, based on a pipeline used in previous literature (Weber et al., 2020). Spindles were detected in NREM sleep. Slow and fast spindles were detected separately because of the 1/f drop-off in power with frequency and the observation that slower spindles had a higher amplitude (the trend for which is visible in fig. 4C, although it doesn't reach statistical significance). Because we used an amplitude-threshold based algorithm to automatically detect spindles, considering the spindles as stemming from one frequency band (8-16Hz) would have biased our analysis towards slow spindles and only included the highest amplitude fast spindles. We thus filtered the data separately for slow and fast spindles and carried on analysing them separately throughout the paper.

To summarize, data from NREM segments were subjected to a FIR filter with a peak-frequency of 10±2Hz for slow and 14±2Hz for fast spindles, respectively. Next, the absolute value of the Hilbert transform of the filtered signal was taken to detect the spindles. Segments in which the envelope surpassed the threshold of the mean ±1.5 SD were marked as putative spindles. If the threshold crossings fell into the duration limits of 0.3 to 2s, and a second threshold of 2.25 SD was passed at least once, these segments were considered as spindles. Spindles were not merged if they closely followed each other. Spindle detection was implemented separately for each channel and participant.

Spindle detection control analysis

To verify that detected time windows included true spindles and not false positives, e.g. due to broadband frequency changes, we implemented a control analysis based on Ngo et al. (2020). This control analysis was done separately for slow and fast spindles. For each channel, time-frequency representations (TFRs) of each spindle were created using wavelets (5 cycles; 5 to 15Hz for slow spindles; 9 to 19Hz for fast spindles; in 0.5Hz steps, time window of ± 0.75s in 2ms steps). Per event, frequencies were averaged between - 0.5 to + 0.5s. Then, the *findpeaks* function was used on the resulting spectrogram. A putative spindle was considered a true spindle when the peak surpassed 0.2 prominence, and if

the peak was between 8 to 12Hz (for slow spindles) or 12 to 16Hz (for fast spindles). All false positives were excluded from further analyses. For the number of detected spindle events and those that passed the control analysis, see table 2.

Spindle characteristics

To quantify spindle parameters in the anterior thalamus, we computed several descriptive measures per channel and per participant for slow and fast spindles separately, namely: Spindle duration (difference of spindle offset and onset); spindle density (number of spindles per minute) and spindle amplitude (maximum peak to trough potential difference). Putative differences of spindle characteristics between fast and slow spindles were compared using paired t-tests per channel, setting the two-tailed significance level at $\alpha = 0.05$. In a separate step, we examined whether channels in certain nuclei differed in spindle characteristics (supplementary material and supplementary fig. 2), but caution against their overinterpretation due to several factors: Our definition of the exact nucleus can only be viewed as an approximation, the number of electrode contacts differ between thalamic nuclei, and the age range of the sample may lead to variability within a given nucleus.

To visualize the presence of slow and fast spindles in the thalamic LFP, we selected time-windows of ±1s around the midpoint of algorithmically detected slow and fast spindles. Next, the TFR of each spindle was created using wavelets (5 cycles; 6 to 18Hz, time window of ± 0.75s in 1ms steps), and TFRs were averaged separately for time-windows marked as slow or fast by the algorithm. To visualize the spatial layout of spindles in the scalp EEG, density values per scalp electrode were averaged across participants, separately for the algorithmically detected slow and fast spindles.

Thalamic and thalamocortical spindle co-occurrence

To evaluate whether spindles occurred in isolation or co-occurred across channels, we created continuous spindle time-courses across all channels. That is, a channel x time point matrix spanning the total duration of artifact-free NREM sleep was marked with absence or presence of spindles (0 or 1, respectively). The continuous spindle time-courses of a participant's channels were divided into separate spindle events as follows: First, each time point was summed across channels. The resulting vector contained information on how many channels showed spindle activity at a given time point. Time points with 0 values meant no spindle activity occurring in any of the channels, and served to

define boundaries between individual spindles. Time points with values > 0 were categorised as a (co-)occurring spindle.

We refer to the different combinations of thalamic and thalamocortical channels participating in a spindle event as spindle scenarios. Figure 2B schematizes these spindle scenarios at the thalamic (T spindles, 2B.I), the thalamocortical (TC spindles, 2B.II) and the cortical (T_0C spindles, 2B.II) level. At the thalamic level, spindles were further categorized into clusters spanning 1 to 6 channels (T_1 to T_6 spindles, according to the number of thalamic channels involved in each spindle event). Spindle events occurring in >1 thalamic channel were further categorized as unilateral or bilateral, depending on whether they occurred in one hemisphere (unilateral; fig. 2B.I & II, second column) or in both (bilateral; fig. 2B.I & II third column). Because we recorded from 3 channels in each thalamic hemisphere, spindle clusters T_4 or greater were bilateral by definition, while T_2 and T_3 could be either unilateral or bilateral clusters.

Given the difference in spindle numbers per scenario and participant, rates of each scenario are reported as the percentages rather than as raw counts. The analysis was carried out separately for thalamic and thalamocortical spindles. For thalamic spindles, we did not consider scalp EEG activity, and report the spindle events per thalamic channel as a percentage of all thalamic spindle events. For thalamocortical spindles, the information about spindle occurrence of all scalp EEG channels were collapsed into one cortical channel (fig. 2B.II). That is, whenever a spindle occurred in one scalp EEG electrode, this was marked in the common scalp channel. Thalamocortical spindle events are reported as a percentage of all spindle events occurring across thalamus and cortex. We refer to thalamocortical spindles as T_xC, where X correspond to the number of thalamus channels involved. In addition, we recorded how many scalp EEG spindle events did not have a thalamic counterpart as a percentage of the total number of detected spindles across all channels in a participant (T_0C; fig. 2B.II, fourth column). All analyses were computed separately for slow and fast spindles.

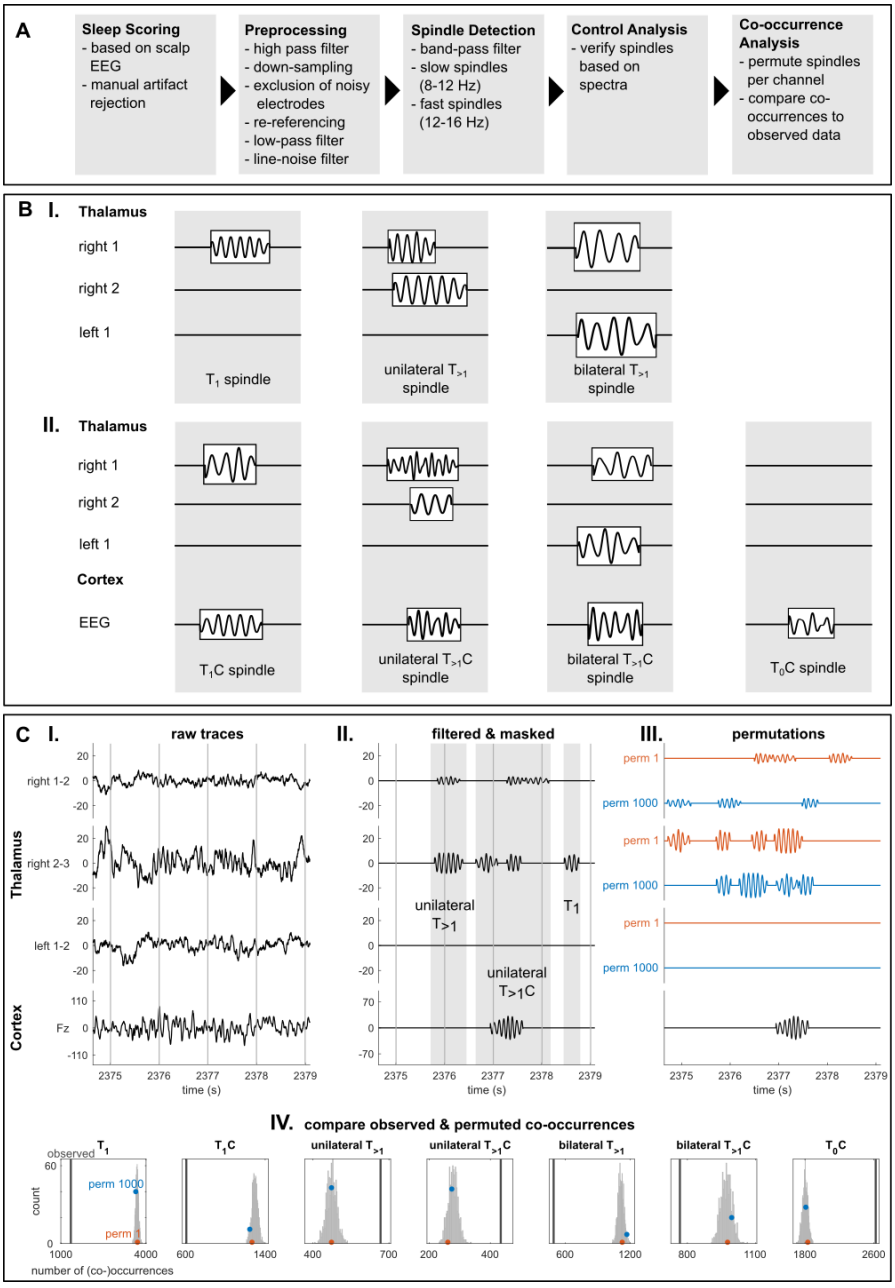

Figure 2. Overview of (pre-)processing steps to measure spindle (co-)occurrence in thalamic and scalp signals. A) Sequence of pre-processing steps and spindle detection. B) Schematized overview of spindle scenarios, i.e., possible channel combinations involved in a thalamic (B.I) or thalamocortical (B.II) spindle event. Three thalamic (2 right, 1 left) channels and the cortex time-course (all scalp EEG activity collapsed into one channel) are schematized. Subscripts describe the

number of thalamic channels participating in a given spindle, so that a T_1 spindle describes a spindle occurring in one thalamic channel. For more data examples for the spindle scenarios, see supplementary figure 1. C) Analysis steps are visualized on a data segment from three thalamic (2 right, 1 left) channels and one cortical channel (Fz) of one participant. Shown are an example raw trace (C.I) and filtered signal (C.II), in which only the detected spindles are shown and other data are masked. The grayscale columns mark (co-)occurring spindle activity and categorization of the shown spindles according to the spindle scenarios detailed in B). C.III depicts exemplar permutations of this data segment. Note that NREM time points were re-shuffled in each thalamic channel keeping the number and duration of spindles constant. The observed and permuted (co-)occurrences were then compared, as visualized in C.IV. Per spindle scenario, the number of co-occurrences were counted in the observed (black line) and permuted data (permutation 1 and 1000 are indicated in the histogram with the y-values at the lowest and highest bin-counts, respectively). To evaluate the likelihood of the observed value stemming from the permuted distribution, we assessed the proportion of permuted values that were more extreme than the observed value. In the analysis of thalamic and thalamocortical spindle clusters, we report the difference between the observed value and the mean of the permuted distribution. Note that the observed value and permuted distributions are shown as untransformed data for visualization purposes, but that we report percentages to enable comparison across subjects.

Thalamocortical spindle co-occurrence maps

To evaluate putative topographical differences in thalamocortical spindle co-occurrence for the thalamic spindle scenarios (T_1C, unilateral or bilateral $T_{>1}C$), we focused on the continuous spindle time-courses across individual scalp EEG channels. To measure the co-occurrence of thalamic spindles with cortical spindles, we considered each thalamus channel as seed and each cortical channel as target channels. Only time points were included during which the seed and at least one cortical channel showed spindle activity. The spindle activity overlap was then calculated as the proportion of time points during which the target channel showed spindle activity at the same time as the seed channel out of the total number of T_1C, unilateral or bilateral $T_{>1}C$ spindle time points of a seed channel. That is, in order to have 100% overlap with T_1C spindles, the target channel would need to show spindle activity at all the time points that the seed channel was engaged in a spindle with no other thalamic channel showing simultaneous spindle activity. Consequently, a 50% overlap with T_1C spindles would signify that only half of the target's spindle activity temporally coincided with the seed's spindle activity, when this particular thalamic seed channel showed a spindle that was not shared by any other thalamic channels. This analysis was carried out for each of the six thalamic channels, and separately for slow and fast spindles.

Paired time-lag analysis of thalamocortical overlap

Thalamocortical overlap maps were calculated at a 0 time-lag, and thereby might not capture time-delayed relationships between seed and target channels. To

evaluate whether there were any consistent non-zero time-lag relationships between seed and target channels across participants, histograms for the time lag between the onset of seed and target spindles were computed, sorted into bins of 0.05s between ±0.75s and averaged across seed, target channels and participants. The resulting histograms contain the average distribution of lags between thalamus and cortex, for which we tested the difference from 0 by means of a one-sample t-test.

Within-participant statistical analyses

Spindle co-occurrence across channels may happen by chance according to the number and duration of spindles in a given channel pair. To test whether and to what extent observations of thalamic or thalamocortical spindle co-occurrence were due to chance, we tested within-participant statistical significance by permuting the data 1000 times. Figure 2C.III and IV visualizes this procedure. The time points of artifact-free NREM sleep of each thalamic channel were shuffled, keeping number and duration of spindles constant. Spindle co-occurrences in the permuted data were quantified as in the observed data, by using 0-columns in the channel x time matrix as bounds between spindle events (as shown in 2C.II). When permuting the data, $T_{>1}$ spindles may be broken up into many T_1 spindles, thus permuted data could contain a higher total number of spindle events than the original data. In order to keep permuted and non-permuted data comparable, we assessed the counts of spindle clusters as percentages of the sum of spindle events in a given participant and permutation. For the analysis of slow and of fast thalamic spindle clusters, we report the observed spindle events as the percentage of all respective spindle events across all thalamic channels. We repeated this analysis for slow and fast thalamocortical spindles separately, where we report the observed spindle events as the percentage of all respective spindle events across thalamic and cortical channels.

For statistical testing, the permuted and non-permuted data were z-scored based on the mean and standard deviation of the permuted data. The z-scored percentage of spindles in the non-permuted data were tested two-sided against the z-scored permuted distribution of percentage of spindles, with the p-value calculated as the proportion of permuted values that were more extreme than the observed value. For a visualization of the comparison between observed and permuted data, consider figure 2C.IV, which depicts exemplar permuted distributions and observed values for each of the thalamic and thalamocortical spindle scenarios for one participant. Lastly, the difference percentage of observed and mean permuted spindle clusters was computed. Positive values thus indicate cases in which specific spindle scenarios occurred more frequently

in the observed than permuted data (e.g. 2C.IV, unilateral $T_{>1}C$ spindles), whereas negative values reflect spindle scenarios that occurred less frequently in the observed than permuted data (e.g., 2C.IV, T_1 spindles). Consequently, scenarios that are more likely in the observed than permuted data suggest systematic temporal co-occurrence of spindle activity.

For mapping thalamocortical spindles, temporal spindle overlap was quantified based on the continuous spindle time-courses. Hence, the permuted data were not separated into spindle events, but quantified as the overlap of spindle time points of the target electrode with the seed electrode. The overlap thus describes the percentage of time a channel pair engages in spindle activity simultaneously. Only channels with p-value < 0.025 (indicating an observed value more extreme on either side of the permuted distribution) were considered in further analyses.

Group level statistical analyses

Between-participant effects were tested by running repeated measures analysis of variance (RMANOVA) on the difference in spindle percentage between observed and permuted values. Where sphericity was violated, Greenhouse-Geisser corrected degrees of freedom are reported. For significant main and interaction effects, post-hoc pairwise comparisons were computed and adjusted for multiple comparisons using Bonferroni correction, and confidence intervals (CIs) at 95% are reported. The significance level was set at $\alpha = 0.05$.

For analysis of thalamic spindle clusters, a RMANOVA was computed with factors *cluster size* (1…6 channels involved) and *spindle type* (slow vs. fast). For testing thalamic uni- vs. bilateral involvement, the RMANOVA factors were *cluster size* (2 vs. 3 channels involved), *hemispheric involvement* (unilateral vs. bilateral) and *spindle type* (slow vs. fast). To assess the thalamocortical spindle behavior, the following RMANOVA factors were defined: *thalamic involvement* (T_1, unilateral or bilateral $T_{>1}$), *cortical involvement* (T vs. TC spindles) and *spindle type* (slow vs. fast). EEG spindle events that did not have a thalamic counterpart were compared between slow and fast spindles, using paired samples t-tests.

To investigate thalamocortical maps across participants, we computed the median difference of observed from permuted thalamocortical overlap across thalamic seed channels, hemispheres and participants, but separately per *spindle type* (slow vs. fast spindles), and *thalamic involvement* (T_1C, unilateral or bilateral $T_{>1}C$). We only included datapoints that were significantly different from permuted data at the within-participant level.

To qualitatively compare the topographies of different spindle types and thalamic classes, we computed the center of gravity (CoG) per category. CoG quantifies the mean location of thalamocortical overlap, as the average x- and y-position of all electrodes weighted by their values of thalamocortical overlap. The x- and y-position are both relative to the central EEG electrode (Cz) and indicate lateralization and placement on the anterior/posterior axis, respectively. Given that thalamocortical maps were averaged across hemispheres, positive, zero and negative lateralization values describe ipsilateral, midline and contralateral locations, respectively. Positive, zero and negative y-values describe anterior, central and posterior locations, respectively. To test the robustness of estimating the CoG based on the average maps, we used a bootstrapping approach, in which we resampled the population of statistically significantly overlapping scalp EEG channels (n = 42), with replacement 1000 times. For each iteration, a new CoG was computed per condition (spindle type and thalamic involvement). Additionally, the difference between slow and fast spindles for each thalamic class was calculated for x and y coordinates, respectively.

Statistical testing was carried out to evaluate whether the CoG was significantly lateralized and/or shifted along the anterior/posterior axis for each of the thalamic classes and spindle types. For this, we tested the difference of each of the 6 bootstrapped distributions from 0, both for the x- and y-coordinates, by calculating the proportion of cases < 0 in each distribution. Next, we evaluated whether the difference of lateralization and/or shift along the anterior/posterior axis differed between slow and fast spindles, by calculating the proportion of cases < 0 in the distribution of the difference.

Data availability

The data sets analyzed in the current study are not publicly available due to privacy concerns as outlined in the consent form.

5.4. Results

To investigate thalamocortical spindle co-occurrence we analyzed data recorded from 7 participants during one night of sleep. On average, participants slept for 8.15 ± 1.55 hours (489 ± 93 minutes), of which 5.98 ± 1.46 hours (359 ± 87.33 min) were spent in NREM sleep (stages N2 and N3; see table 2 for participant-specific details of minutes spent in each sleep stage). We algorithmically detected slow and fast spindles in a total of 42 bipolar thalamic channels (6 per participant) and 133 scalp EEG electrodes (19 per participant). Note that because electrode placement into the specific subnuclei varied inter-individually, we assessed spindles in the anterior thalamus as a whole rather than its subnuclei. Accordingly, we generally refer to the anterior thalamus as the broad region. In contrast, we refer to the specific subnucleus as the anterior nucleus of the thalamus or ANT.

Table 2 reports the number of putative spindle events detected in the thalamic LFP and scalp EEG per participant, and the number of spindle events after the spindle control analysis. Only spindle events that passed the control analysis were included in all further analyses.

Participant	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Sleep stages (minutes)							
Wake	121.5	14.5	124	41	118	27.5	141.5
N1	118	25.5	164	35.5	38.5	54.5	221.5
N2	324.5	93.5	223.5	208	157	177.5	258
Artifact-free	315	79.5	181	200.5	141.5	155.5	248.5
N3	101.5	418.5	110	117.5	97.5	195.5	30.5
Artifact-free	101	376.5	91.5	115	90	183.5	30
REM	38.5	37	0	47.5	31	72.5	24.5
Spindle events (number)							
Thalamus							
slow	23555	29316	18991	20339	17431	26645	18622
corrected	12656	8399	12588	12640	7587	18399	12243
fast	22779	28696	17148	18872	14220	20642	16697
corrected	12475	9937	2836	6130	2283	5271	5402
Cortex							
slow	81181	99071	63906	69564	46902	108052	64270
corrected	50359	17247	14969	27419	7908	83608	43461
fast	102576	103823	63155	76032	47722	79243	59343
corrected	72181	32085	19625	26515	9744	12521	21622

Table 2. Overview of participants' sleep architecture and detected spindle events before and after control analysis (corrected). Sleep staging data are reported in minutes, for spindle events the total number of events are reported (pooled across thalamic or scalp EEG electrodes, respectively).

Slow spindles occur more frequently in anterior thalamus than fast spindles

We first investigated whether slow and fast spindles were present in the anterior thalamus and scalp EEG. Both slow and fast spindles were present in all participants. Figure 3 A shows an example of a slow followed by a fast spindle in one participant (for another example, see supplementary fig. 1B). The average TFRs of all slow and fast spindles in the same channel are shown in figure 3B. Note that the TFRs were computed across the slow and fast spindle band. Consequently, if the distinction into slow and fast spindles were arbitrary, this should be seen by an overlap in the TFR. Figure 3C depicts the average slow and fast spindle distributions in the scalp EEG across all participants.

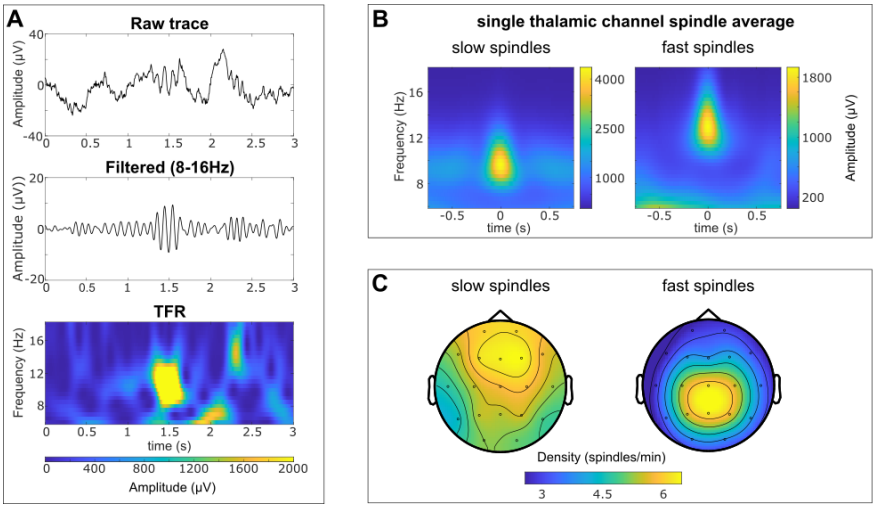

Figure 3. Slow and fast spindles in anterior thalamus and scalp EEG. A) Data of a slow (at 1.5s) and fast (at 2.25s) thalamic spindle, here represented in the raw trace (top panel), filtered trace (middle panel) and time-frequency representation (TFR; bottom panel). Data are shown for right thalamic channel 2-3 of participant 3. B) Averaged TFRs centered on time windows containing slow (left) and fast (right) spindles in one thalamic channel. The represented channel is the same as in A. C) Density of slow (left) and fast (right) spindles in scalp EEG averaged across all subjects.

Next, we assessed whether anterior thalamic spindles differed in their density, duration or amplitude. Figure 4 shows the spindle density (A), duration (B) and amplitude (C) for slow and fast spindles. Both data pooled across thalamic channel per participant (top row) are shown, as well as the histograms of the differences (slow – fast spindles) at the channel level (bottom row). On average, spindle density (fig. 4A) was higher ($t(41) = 5.52$; $p < 0.001$) for slow (6.34 ± 3.22 spindles/min) than fast spindles (3.01 ± 1.46 spindles/min). Spindle duration (fig. 4B; $t(41) = 1.45$; $p = 0.15$) and spindle amplitude (fig. 4C; $t(41) = 1.97$; $p = 0.06$) did not differ between slow and fast spindles.

We evaluated the same descriptive spindle measures when categorizing the data according to their corresponding estimated thalamic nuclei (suppl. material and suppl. fig. 1) and did not find any differences between thalamic nuclei.

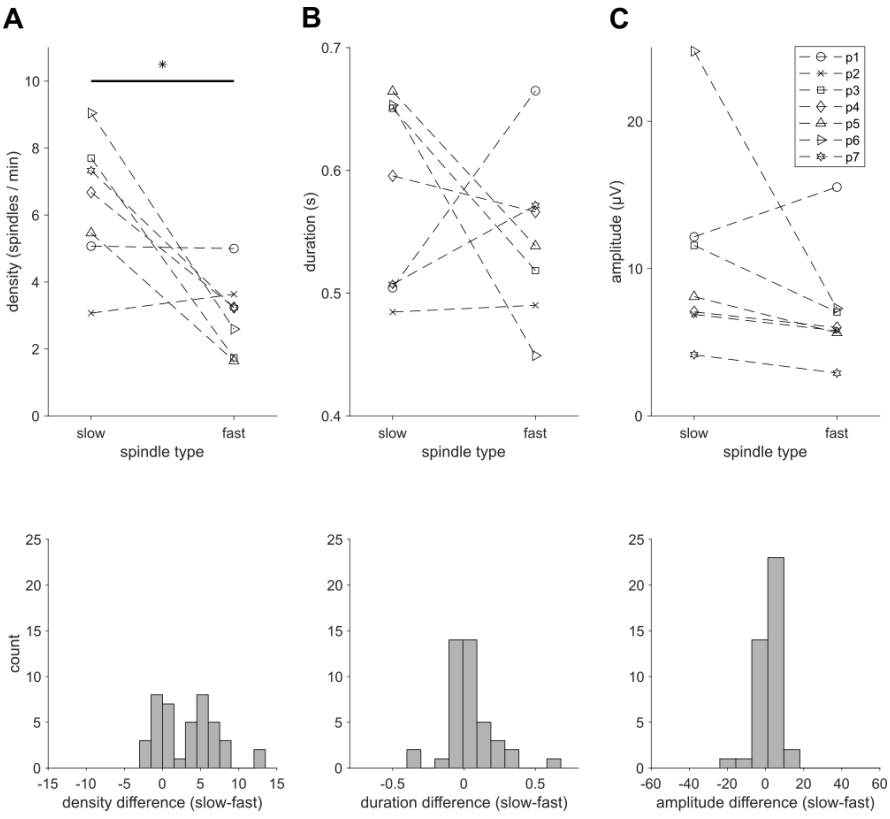

Figure 4. Spindle characteristics in anterior thalamus. Electrodes pooled per participant are shown (p1 to 7, legend) for slow and fast spindles (top row) and histograms of the differences (slow-fast) per electrode (bottom row). A) Average spindle density (number of spindles per minute) was on

average higher for slow than for fast spindles; B) Spindle duration and C) spindle amplitude (peak to trough difference) did not differ between slow and fast spindles. Asterisk marks paired t-test difference significant at $p < 0.05$ (uncorrected).

Characterization of spindles in the anterior thalamus

In a next step, we investigated whether slow and fast spindles co-occurred across channels in the thalamus (without considering the cortical EEG channels). We refer to the number of thalamic channels involved in a spindle as a channel cluster. Figure 5A depicts the channel cluster size of spindle events as a proportion of all thalamic spindles, separately for slow (upper panel) and fast (lower panel) spindles. To control for random spindle co-occurrence, we next ran permutations of the spindle time courses, with number and duration of spindles at each channel kept constant. These permuted data allowed us to estimate how often spindles occurred in isolation or co-occurred by chance. We compared these observed distributions of spindle clusters with the permuted distributions. Figure 5B shows the population difference between observed and permuted data for all 6 channel clusters for slow and fast spindles, respectively. Since the spindle cluster sizes are expressed in percentages, the likelihoods of T_1 and $T_{>1}$ clusters are interdependent and should be interpreted accordingly. T_1 spindles, which were the most dominant type in the observed data (cf. fig. 5A), were less likely to occur in the observed compared to the permuted data. In contrast, spindle co-occurrences ($T_{>1}$) occurred more often than expected by chance ($F_{1.29, 7.71} = 119.41$; $p < 0.001$ in RMANOVA; mean differences of observed – chance values between spindle cluster of T_1 spindles vs. $T_{2...6}$ channels range from -26.65 to -35.13%; $p \leq 0.001$). This suggests that thalamic spindles co-occur more often than would be assumed based on permuted distributions. Slow and fast spindles did not co-occur differently in the anterior thalamus ($F_{1,6} = 0.18$; $p = 0.69$).

It is noteworthy that some spindle events spanned more than three channels, pointing to the bilateral involvement of the thalamus in these spindle events (since our setup included three channels in each thalamic hemisphere). We subdivided the observed T_2 and T_3 clusters to investigate whether spindle clusters of two or three channels involved one or both hemispheres of the thalamus. Figure 5C depicts the difference in proportion of unilateral and bilateral T_2 and T_3 spindles between observed and permuted data, for slow and fast spindles, respectively.

T2 and T3 spindles showed distinct unilateral and bilateral involvement ($F_{1,6} = 128.17$; $p < 0.001$ in RMANOVA). We observed that unilateral T_2 spindles occurred more often than by chance (15.58%; 95% CI, 11.99 to 19.18), while

bilateral T_2 spindles occurred less often than by chance (-5.68%; 95% CI, -7.46 to -3.89). This can also be seen in figure 5C when looking at the T_2 data. This difference between unilateral and bilateral spindles was absent for T_3 spindles: in this situation, both unilateral (3.36%; 95% CI, 1.95 to 4.77) and bilateral spindles (4.81%; 95% CI, 3.32 to 6.31) occurred equally more often than by chance (cf. fig. 5C, both unilateral and bilateral T_3 bars are positive). We also observed a difference in the amount of slow and fast spindles spanning 2 or 3 channels in the thalamus ($F_{1,6}$ = 7.88; p = 0.03 in RMANOVA). There were more fast T_2 spindles (6.66%; 95% CI, 4.21 to 9.12) than slow T_2 spindles (3.24%; 95% CI, 0.14 to 6.34), but equally many slow (4.44%; 95% CI, 3.70 to 5.18) and fast T_3 spindles (3.74%; 95% CI, 2.56 to 4.93).

To conclude, these analyses indicated that compared to the permuted data, T_2 spindles tended to be unilateral, whereas T_3 spindles were equally likely unilateral or bilateral. Overall, it seems that thalamic spindle events did not only co-occur across channels, but were temporally coordinated within and across thalamic hemispheres.

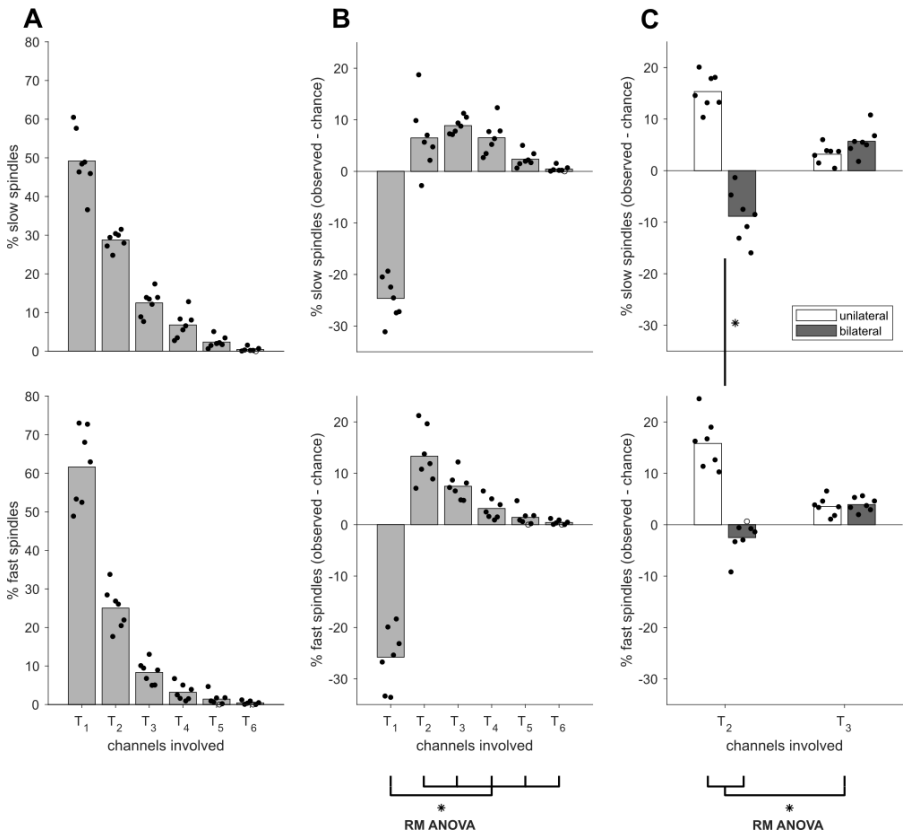

Figure 5. Observed thalamic spindles and the number of thalamic channels they spanned (channel clusters T_1...T_6) for slow (top) and fast (bottom) spindles. A) Observed percentages for each channel cluster out of the total number of slow or fast thalamic spindles, respectively. B) Difference of observed percentages from permuted data for thalamic spindle co-occurrences. Positive values demarcate when observed data are more likely to occur than by chance, negative values less likely than by chance. C) T_2 and T_3 spindles further subdivided into unilateral and bilateral slow and fast thalamic spindles. The difference of observed and permuted data is shown. Bars depict the group average, dots the individual participant data. Individual datapoints are systematically offset so that horizontal order corresponds to participants 1 to 7. Filled in dots are significantly different from permuted data at the within-participant level at $p < 0.05$ (two-sided), open dots depict data points failing to meet significance at the within-participant level. The asterisks mark differences significant at $p < 0.05$ in the RMANOVA (corrected for multiple comparisons), brackets stand for differences between means, forking out brackets for differences between individual bars.

Multi- (but not single-) channel thalamic spindles show above-chance cortical counterparts

Next, we investigated whether observed thalamic spindle activity was systematically related to cortical spindle activity. For this analysis, spindles were subdivided according to the extent of thalamic involvement into T_1, unilateral $T_{>1}$ or bilateral $T_{>1}$ spindles. We then categorized whether these spindles occurred solely in the thalamus (T spindles) or also had a cortical counterpart (TC spindles). We compared the occurrences of each spindle scenario to the total number of recorded spindles, rather than the total number of thalamic spindles, which was the case in the analysis up until now. Figure 6 shows the observed spindle percentages (A), and the difference between observed and permuted data (B), both for slow and fast spindles in the top and bottom row, respectively.

T and TC spindles were more or less likely than in the permuted data, depending on whether they occurred in one channel or were unilateral or bilateral ($F_{1.04,6.24} = 24.74$; $p = 0.002$ in RMANOVA). While most observed slow and fast spindles involving the thalamus were T_1 or T_1C spindles (fig. 6A, most leftward bars), these types of spindles were less likely than expected by chance (cf. 6B, most leftward bars have negative values). Unilateral $T_{>1}$ (3.87%; 95% CI, 1.70 to 6.05) and $T_{>1}C$ (5.12%; 95% CI, 3.73 to 6.51) spindles were more likely to occur than expected by chance. In contrast, bilateral $T_{>1}$ spindles occurred less often than predicted (-2.0%; 95% CI, -2.80 to -1.20), whereas bilateral $T_{>1}C$ spindles occurred more often than predicted (2.96%; 95% CI, 1.30 to 4.67). These observations did not differ between slow and fast spindles ($F_{1.03,6.18} = 0.62$; $p = 0.46$ in RMANOVA).

Except for bilateral $T_{>1}$ spindles without a cortical counterpart, spindles spanning more than one thalamic channel were more likely to occur than

expected by chance. These multichannel spindles were also more likely to co-occur with cortical spindles. This can be seen in figure 6B, when looking at the unilateral and bilateral spindles (middle left and middle right bars). These results suggest that spindles at the thalamic (except for bilateral $T_{>1}$ spindles) and thalamocortical level tend to co-occur across multiple channels systematically, indicating coordinated spindle activity.

We separately confirmed the absence of a systematic temporal offset between thalamic and cortical spindles (supplementary material & suppl. fig. 3). A temporal shift would bias analyses of spatiotemporal spindle overlap toward channel pairs with a smaller temporal offset, but this was not the case.

Not all cortical spindles have an anterior thalamic counterpart

The data in the present study were recorded from a subsection of the thalamus and may have only captured a subsample of spindles occurring in the thalamus. Because cortical spindles are thought to depend on thalamic spindles and, more specifically, the projections of the different thalamic nuclei to distinct cortical networks, our data may also contain cortical spindles that did not have a thalamic counterpart (T_0C spindles). To test this, we counted the occurrences of cortical-only spindles out of all recorded spindles, and compared them to permuted data. The most rightward bar in each panel of figure 6 shows the percentage of these T_0C spindles. Indeed, in the observed data (fig. 6A), around half of observed cortical EEG spindles did not have a thalamic counterpart (slow: 41.46 ± 4.12%; fast: 53.0 ± 9.04%). This observation was higher than expected by chance (slow: 14.38 ± 4.60%; fast: 8.88 ± 3.74%), but did not differ between slow and fast spindles ($t(6) = 1.87$; $p = 0.11$). It may be that these T_0C spindles had counterparts in thalamic subparts not sampled in our setup, such as more posterior nuclei. The centro-posterior cortical topography of fast T_0C spindles (suppl. fig. 3) could suggest that these may be spindles that co-occur with posterior thalamic spindles, which themselves do not have a (strong) anterior thalamic counterpart that could be recorded in our setup. However, due to our sparse sampling of the thalamus we can only truly speak to spindles that positively occur at the electrode locations and not their absence. Consequently, we caution against over-interpretation of this observation.

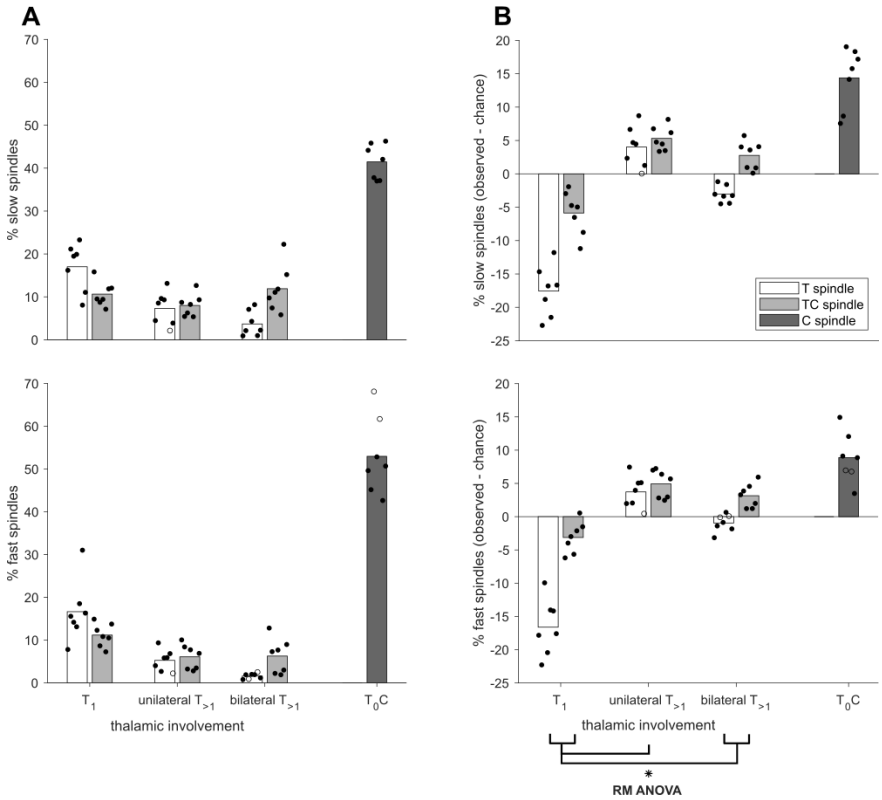

Figure 6. Observed spindles in thalamus and cortex, subdivided according to the level of involvement in the thalamus (T_1 spindles or $T_{>1}$ spindles, unilateral and bilateral), and whether they were accompanied by a cortical spindle (TC spindle, light grey) or not (T spindle, white). Cortical spindles without thalamic counterparts are also shown (T_0C spindles, dark grey). A) Observation of each scenario as a percentage of the total number of slow (top) and fast (bottom) spindles. B) Difference of observed from permuted percentages for slow (top) and fast (bottom) spindles. Bars depict the group average, dots the individual participant data. Individual datapoints are systematically offset so that horizontal order corresponds to participants 1 to 7. Filled in dots are significantly different from permuted data at the within-participant level at $p < 0.05$ (two-sided), open dots depict data points failing to meet significance at the within-participant level. The asterisks mark differences significant at $p < 0.05$ in the RMANOVA (corrected for multiple comparisons), brackets stand for differences between means, forking out brackets for differences between individual bars. Note that there was no difference between slow and fast spindles.

Patterns of thalamocortical spindle overlap depend on level of thalamic involvement and spindle subtypes

Lastly, we investigated cortical topographies of spindles dependent on the type of thalamic spindle and thalamic channel involvement. If coordinated thalamocortical spindle activity were to support targeted engagement of

thalamocortical networks, different cortical spindle topographies should be observed based on different thalamic spindle properties. To compare differences in topography for thalamocortical spindles, depending on the spindle type (slow vs. fast) and the level of thalamic involvement (T_1C, unilateral $T_{>1}C$ or bilateral $T_{>1}C$ spindles), we computed the thalamocortical overlap between all thalamic and cortical channel pairs across participants. The overlap quantifies the percentage of data points during which two channels (here, a seed channel in the thalamus and a target cortical channel) show simultaneous spindle activity. Since we verified that thalamocortical spindle onset occurred predominantly simultaneously (suppl. fig. 4), this analysis was carried out at 0-lag.

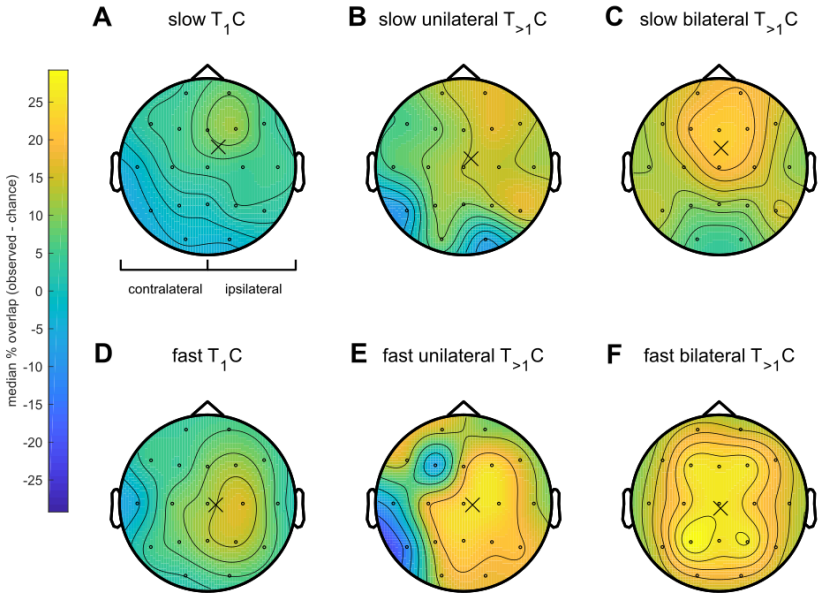

Figure 7. Thalamocortical spindle topographies. The difference of observed and permuted temporal overlap is shown as the group median. Accordingly, positive values describe a higher than by chance overlap, negative values a lower than by chance overlap. Data are averaged across thalamic channels, participants and hemispheres, such that values on the left within each topoplot describe the intensity of overlap contralateral and values on the right ipsilateral to the thalamus seed. The Center of Gravity is marked with a cross in each topoplot. Data are subdivided into spindle type and thalamic involvement: A) slow T_1C spindles; B) slow unilateral $T_{>1}C$ spindles; C) slow bilateral $T_{>1}C$ spindles; D) fast T_1C spindles; E) fast unilateral $T_{>1}C$ spindles; F) fast bilateral $T_{>1}C$ spindles.

Figure 7 shows the median group difference of observed from permuted data for each category. The median group data were calculated based on the individual data that differed significantly from chance levels. The resulting topographies do not depict overlap in the left- and right hemisphere, but rather contra- or ipsilateral to the thalamic source, respectively, because data were averaged across participants, thalamus channels and hemispheres. Visual inspection suggests that thalamocortical overlap for slow spindles had a frontal topography (fig. 7 A-C), and fast spindles had a more central topography (fig. 7 D-F). Furthermore, the topographies seem to progress from lateralized to more widespread as more thalamic channels were involved in a thalamocortical spindle, which can be seen when comparing T_1C (fig. 7 A & D) to unilateral (fig. 7 B&E) and bilateral (fig. 7 C&F) $T_{>1}C$ spindles, respectively.

To summarize these different topographies, we computed the Center of Gravity (CoG), which provides the spatial average weighted by the value of overlap at each cortical position. The CoG is marked with a cross in each panel of figure 7. Note, that the CoG does not necessarily exactly match the location suggested by the color scheme, given the different ways of calculating CoG and interpolating the colored surface.

For statistical analysis, we split the CoG into its x- and y-position allowing for quantifying the thalamocortical overlap's lateralization and shift along the anterior/posterior (a/p) axis relative to a central scalp EEG topography (Cz). We used bootstrapping to statistically evaluate the differences in CoG between conditions. Figure 8 shows the bootstrapped CoG data for lateralization (fig. 8 A-D) and a/p-shift (fig. 8 E-H), split into the different conditions of thalamic involvement and spindle type (slow spindles in blue, fast spindles in orange).

Lateralization of spindles (fig. 8 A-C) was significantly different from 0 for slow T_1C (0.055 ± 0.025; p = 0.001), slow unilateral $T_{>1}C$ (0.046 ± 0.02; p = 0.004), and fast T_1C (0.045 ± 0.012; p = 0) spindles, indicating that the average thalamocortical overlap for these conditions tended to be focused ipsilateral to the thalamic spindles. Slow bilateral $T_{>1}C$ (0.008 ± 0.013; p = 0.224), as well as fast unilateral $T_{>1}C$ (0.055 ± 0.034; p = 0.095) and fast bilateral $T_{>1}C$ (0.01 ± 0.013; p = 0.19) spindles were not significantly lateralized. A/p-shift was significantly different from 0 for slow T_1C (0.137 ± 0.029; p = 0), slow unilateral $T_{>1}C$ (0.075 ± 0.024; p = 0), and slow bilateral $T_{>1}C$ (0.108 ± 0.022; p = 0) spindles. Thus, the average CoG of thalamocortical spindle overlap of slow spindles was shifted anteriorly while fast T_1C (0.018 ± 0.015; p = 0.102), fast unilateral $T_{>1}C$ (0.018 ± 0.028; p = 0.198) and fast bilateral $T_{>1}C$ spindles (0.004 ± 0.03; p = 0.471) were not shifted along the a/p axis.

When comparing slow and fast spindles, T_1C slow spindles were significantly more anterior than T_1C fast spindles (0.12 ± 0.033; p = 0) but equally lateralized (0.009 ± 0.028; p = 0.355). Slow and fast unilateral $T_{>1}C$ spindles did not differ in terms of lateralization (-0.009 ± 0.039; p = 0.585) or shift on the anterior/posterior axis (0.056 ± 0.037; p = 0.076). Slow bilateral $T_{>1}C$ spindles were significantly more anterior than fast bilateral $T_{>1}C$ spindles (0.106 ± 0.039; p = 0.008), but did not differ in their lateralization (-0.002 ± 0.018; p = 0.556).

These results point towards differences in thalamocortical overlap depending on the spindle type (slow vs. fast) and level of thalamic involvement. As such, we observed that slow spindles tended to co-occur with cortical slow spindles in frontal scalp EEG sites. Co-occurrence of thalamic and cortical fast spindles was distributed more widely across the cortex, which was reflected by a more centralized average of thalamocortical overlap. Additionally, the level of involvement of the thalamus in spindles was related to the cortical lateralization of thalamocortical overlap: During T_1C spindles and slow unilateral $T_{>1}C$ spindles, cortical electrodes ipsilateral to the thalamus channel(s) tended to engage in spindles together, whereas bilateral $T_{>1}C$ spindles showed a higher overlap with bilateral cortical electrodes.

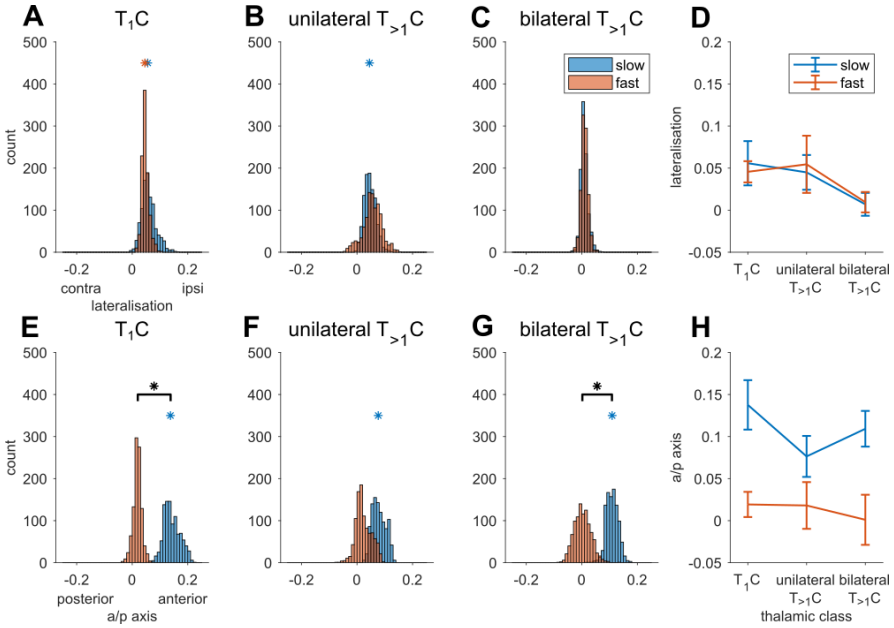

Figure 8. Center of Gravity of thalamocortical overlap, split into its x- and y-position, which describe lateralization and shift along the anterior/posterior (a/p) axis relative to electrode position Cz, respectively. Top row depicts the lateralization for A) T_1C spindles; B) unilateral $T_{>1}C$ spindles; C) bilateral $T_{>1}C$ spindles; D) median values and standard deviations of the distributions in A-C. Bottom row depicts a/p shift for E) T_1C spindles; F) unilateral $T_{>1}C$ spindles; G) bilateral $T_{>1}C$ spindles; H) median values and standard deviations of the distributions in E-G. Colored asterisks mark the distributions that differ from 0 at $p < 0.05$. Black asterisks and brackets mark distributions that differ from each other at $p < 0.05$.

5.5. Discussion

In the current study, we investigated the spatiotemporal coordination of spindles in the human anterior thalamus and cortex. Both slow and fast spindles were detected in the anterior thalamus, with a higher density of slow spindles. We categorized spindles based on their main frequency (slow vs. fast spindles) and level of thalamic involvement (spindles spanning one channel vs. multiple channels unilaterally vs. bilaterally) and compared observed patterns of spindle co-occurrence with permuted data. Figure 9 graphically summarizes predominant scenarios of thalamic and thalamocortical spindle (co-)occurrence we found in the permuted (A) and observed (B) data. We found that simultaneous multichannel spindle activity within thalamic and thalamocortical circuits occurred more often than expected by chance (fig. 9B). This points to spindle activity as a coordinated phenomenon across brain sites. Interestingly, multichannel spindles did not only co-occur unilaterally (fig. 9B.4&7), but also bilaterally within the thalamus (fig. 5; fig. 9B.5&6). When contrasting the different spindle subtypes based on their main frequency and the level of thalamic involvement, we observed similar rates of co-occurrence, but differences in the spatial pattern of cortical overlap (fig. 7). Overall, these results point to coordinated and differential patterns of sleep spindle co-occurrence in thalamocortical circuits.

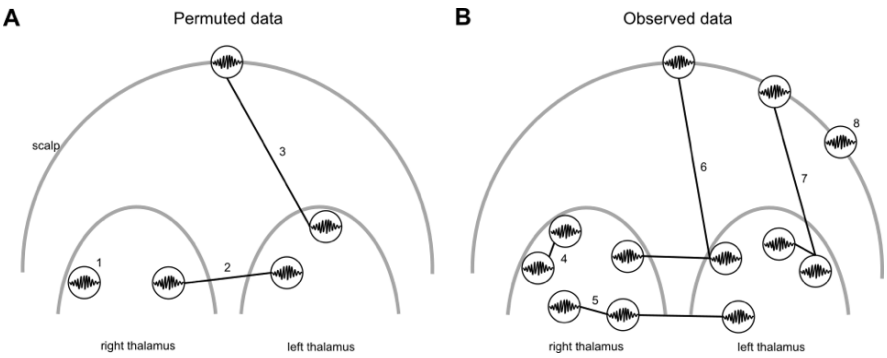

Figure 9. Graphical summary of thalamic and thalamocortical spindle (co-)occurrence, depicting spindle scenarios (numbered; cf. fig. 2 B) that appeared more often in the A) permuted or B) observed data. Bilateral thalamus and scalp are depicted schematically. Individual channels are indicated by circles, simultaneous spindle activity is indicated by connecting lines. A) In the permuted data, locally restricted spindles predominated. Thalamic spindles were predominantly T_1 spindles (1) or bilateral T_2 spindles (2), and thalamocortical spindles mostly T_1C spindles (3). B)

In the observed data, the most common spindle scenarios included two or more channels, indicating coordinated spindle activity. At the thalamic level, unilateral $T_{>1}$ spindles (4) predominated, as well as bilateral $T_{>2}$ spindles (5; here illustrated by a T_3 spindle). Thalamocortical spindles were predominantly bilateral $T_{>1}C$ (6) or unilateral $T_{>1}C$ spindles (7). There were also more cortical spindles without a thalamic counterpart (T_0C spindles; 8) than expected by chance.

There are alternative scenarios to coordinated and differentiated spindle activity across brain areas: For instance, spindles could be restricted to specific sites, which would preclude them from timing communication between brain areas during NREM sleep. Previous iEEG studies reported spindles as predominantly local in the cortex (Andrillon et al., 2011; Frauscher et al., 2015; Peter-Derex et al., 2012) and with low synchrony between cortical spindles and those in deeper brain structures like insula and hippocampus (Frauscher et al., 2015). These reports gave rise to the idea of sleep spindles as a local phenomenon. Although we observed many occurrences of spindles restricted to one channel, we also saw that spindles often spanned multiple channels, both at the thalamic (~50% of spindles) and the thalamocortical level (~35% of all spindles engaged at least one thalamic and one cortical channel). Most of these multi-channel spindle scenarios occurred more often than predicted by chance, suggesting that spindle activity in thalamocortical networks is indeed coordinated rather than always being locally restricted. While we cannot exclude the possibility that some multichannel spindles in our data reflect volume conduction from a single source, volume conduction clearly cannot explain many of the patterns we observed, among which bilateral spindles picked up by electrodes in opposite hemispheres (cf. suppl. fig. 1C), or spindle onset and duration differences between neighbouring channels (cf. fig. 1C.I and II). Moreover, the proportion of multi-channel thalamic and thalamocortical spindles observed here, may be an underestimation as the exact thalamic recording sites differed between individuals, and consequently only sample a subsection of the thalamus. Events categorized here as T_1, T_1C or T_0C spindles may have engaged thalamic regions not sampled in our setup. Our findings thus indicate that many spindles are not local, and do not co-occur merely by chance, but are coordinated over wide areas of the thalamocortical network.

Although pathological brains differ from healthy ones, treatment with benzodiazepines and anticonvulsant drugs may influence the sleep architecture (for a comprehensive review see Jain & Glauser, 2014) and spindles (Leong et al., 2022; Plante et al., 2015) and the wide age range in our sample may bias spindle-related characteristics (Muehlroth & Werkle-Bergner, 2020), clinical populations are presently the only opportunity to directly measure time-

resolved recordings of the human thalamus. Previous studies have reported thalamocortical spindles in medial/anterior thalamus and cortex (Schreiner, Kaufmann, et al., 2021; Szalárdy et al., 2021) as well as posterior thalamus and cortex (Bastuji et al., 2020; Mak-McCully et al., 2017), albeit only one of these studies investigated thalamic and thalamocortical spindle co-occurrence in a more systematic fashion (Bastuji et al., 2020). In that study, around half of all thalamic spindles occurred in one channel and the remaining half spread across two or more channels. These reports were limited to the posterior thalamus due to clinical demands, were not contrasted with chance spindle co-occurrence and only investigated fast spindles. Our data now add information on the anterior thalamus.

In contrast to previous literature, which focused on fast spindles, we systematically investigated thalamic and thalamocortical spindle co-occurrence separately for slow and fast spindles. In our data, both spindle types were coordinated in a largely similar fashion, although some differences were also found. Slow and fast spindles were more likely to span multiple channels in the observed than permuted data, both unilaterally and bilaterally, and co-occurred between thalamus and neocortex. Our time lag analysis revealed simultaneous activation of thalamic and cortical spindles. The lack of temporal sequence in our data does not provide information on causal relations of spindle activity across regions. However, our findings provide evidence that both slow and fast spindles are present in the (anterior) thalamus, and that both engage in a coordinated fashion with the neocortex. It is thus not likely that slow spindles are specific to cortico-cortical interactions, as suggested previously (Mölle et al., 2011). The slow spindle frequency range overlaps with the alpha band, which makes discriminating spindles from alpha bursts challenging, particularly when only scalp EEG is available. However, their overall similarity to fast spindles (e.g. similar duration, amplitude, involvement of other thalamic/cortical electrodes) suggests that slow spindles are de facto spindles, while differences also exist. In addition to differences in density, slow and fast thalamocortical spindles did show different topographical patterns of thalamocortical overlap: slow thalamic spindles showed higher overlap with frontal scalp EEG electrodes, whereas fast thalamic spindles showed higher overlap with centro-posterior scalp EEG electrodes. These topographies of overlap are reminiscent of sites of slow and fast spindle activity observed in previous (i)EEG studies (Andrillon et al., 2011; Mölle et al., 2011; Peter-Derex et al., 2012). Note, however, that the different patterns we uncovered cannot be merely explained by the higher density of slow spindles in frontal EEG channels (cf. fig. 3C). We used permutation testing to control for the bias of higher spindle density/duration on spurious spindle

overlap. Thus, although the frequency-based spindle dichotomy has been criticized as overly simplified (Gonzalez et al., 2021), our results suggest that thalamic spindles engage different cortical networks, depending on their main frequency.

The topographical patterns of thalamocortical spindle overlap were not only related to the main frequency of a given spindle, but also to the level of involvement of the thalamus. Andrillon and colleagues (2011) reported that spindles occasionally span multiple channels across the cortex. In this scenario, spindles travel along the cortical posterior-to-anterior axis (Andrillon et al., 2011; Muller et al., 2016). In the present study, we observed that multi-channel thalamic spindles were related to an increase in the number of cortical channels showing high thalamocortical spindle overlap. Consequently, the different cortical spindle scenarios (localized vs. global cortical events) reported in previous (i)EEG studies could be related to differential involvement of the thalamus in these spindles. Accordingly, multichannel cortical spindles could arise as a consequence of multichannel thalamic spindles. That is, when a spindle travels through the thalamus, this may be reflected as a cortical traveling spindle mediated by thalamocortical projections with the engaged thalamic sites. In fact, the patterns of thalamocortical spindle co-occurrence in our data (cf. fig. 7) suggest that this might be the case. For instance, the thalamocortical overlap of fast spindle events differed between spindles occurring in one thalamic channel and those spanning bilateral thalamic channels: In the former case, the thalamocortical overlap was spatially restricted to centro-posterior cortical sites ipsilateral to the thalamic site (cf. fig. 7 & 8), whereas in the latter case, the overlap was more widespread across the cortex bilaterally. Interestingly, cross-hemispheric co-occurrence of cortical spindles is preserved in callosotomized patients, suggesting that cortical spindle spread may depend on thalamocortical instead of cortico-cortico connections (Bernardi et al., 2021). The results of the present study add to the currently limited literature on interhemispheric spindle co-occurrence by showing that cortical spread of spindle activity is related to the level of involvement of the thalamus. More broadly, the involvement of different thalamic sites in a spindle may engage different cortical networks.

Spindles may facilitate network communication during memory consolidation

Memory consolidation requires reactivation in hippocampo-cortical circuits to trigger structural changes (Josselyn et al., 2015). This reactivation must be specific to the neuronal populations involved in encoding the memory, and thus requires coordinated activity across (sub)cortical networks. Hippocampal

ripples (80 – 140 Hz), which have been linked to memory reactivation (Zhang, Fell, & Axmacher, 2018), co-occur with spindles in the hippocampus and cortex (Ngo et al., 2020; Staresina et al., 2015), the anterior thalamus (Szalárdy et al., 2021) and in the anterior thalamus and hippocampus (Sarasso et al., 2014). Spindles are hypothesized to align windows of excitability in hippocampo-cortical circuits, and thereby coordinate the information transfer necessary for long term memory consolidation (Klinzing et al., 2019).

The thalamus is richly connected with cortical and subcortical structures (Cappe, Morel, Barone, & Rouiller, 2009; Jankowski et al., 2013; Zhang, Snyder, Shimony, Fox, & Raichle, 2010) and has been proposed as a connector hub between distinct cortical networks (Kawabata et al., 2021). Recent research hints at an active role of the thalamus in memory consolidation during sleep (Klinzing et al., 2019). According to this view, thalamus functions as a switchboard to enhance processing within and between specific cortical networks as required by task demands (Nakajima & Halassa, 2017; Schmitt et al., 2017). Those same mechanisms seem to be engaged during spindle generation in rodent NREM sleep (Chen et al., 2016; Halassa et al., 2014). Regarding memory reactivation, spindles in the thalamus (within or across thalamic nuclei) could selectively engage (sub)cortical sites through its rich projections. This could lead to carefully timed communication between the hippocampus and the cortical network(s) implicated in a specific memory and thereby enable reactivation of memory traces crucial for memory consolidation.

Previous studies have reported the importance of temporally (Latchoumane et al., 2017; Schreiner, Petzka, et al., 2021) and spatially (Petzka et al., 2022) organized cortical spindles during memory consolidation processes. Although our study did not include a memory task, here we add to this literature by showing that thalamocortical spindle activity is temporally coordinated and spatially specific. Further research using memory tasks needs to be conducted to directly study how the thalamocortical processes reported here may support memory consolidation.

5.6. Conclusion

The present study investigated (co-)occurrence of sleep spindles in the anterior thalamus and neocortex. Up to half of the recorded spindles co-occurred across multiple channels, both at the thalamic and thalamocortical level. Although spindles were more likely to engage the thalamus unilaterally, we also observed simultaneous bilateral thalamic spindles. Slow and fast spindles were observed in the anterior thalamus. Both slow and fast, as well as single channel and multichannel thalamic spindles were associated with distinct topographical patterns of spindle co-occurrence in the cortex. As such, slow thalamocortical spindles generally co-occurred with more frontal scalp EEG channels, while fast thalamocortical spindles showed a wider cortical overlap, averaging highest at central scalp EEG sites. Single channel and unilateral thalamic spindles had more lateralized cortical co-occurrence patterns than bilateral thalamic spindles. These diverse patterns of thalamocortical spindle co-occurrence could be involved in selective engagement of thalamocortical networks during memory consolidation and other cognitive processes.

6.7. References

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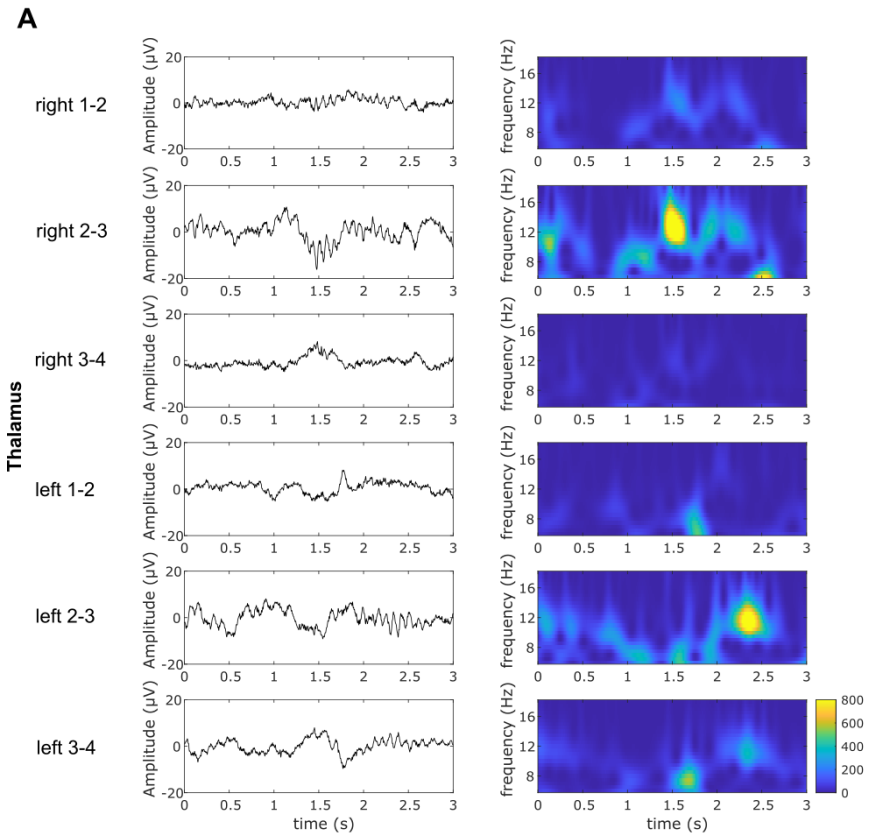
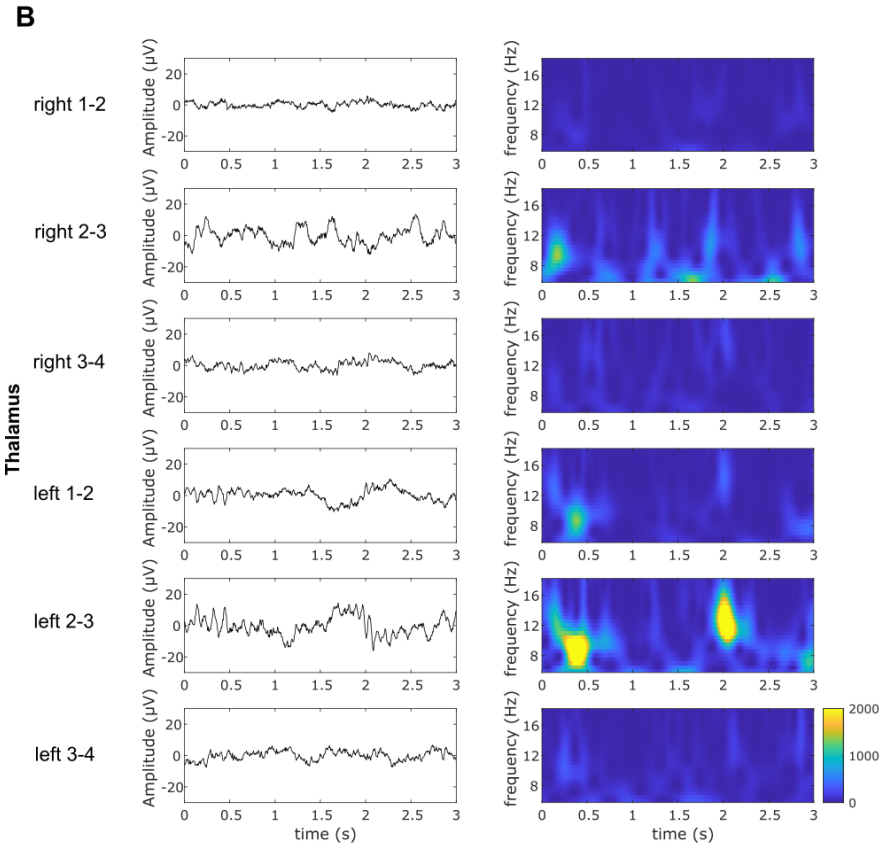
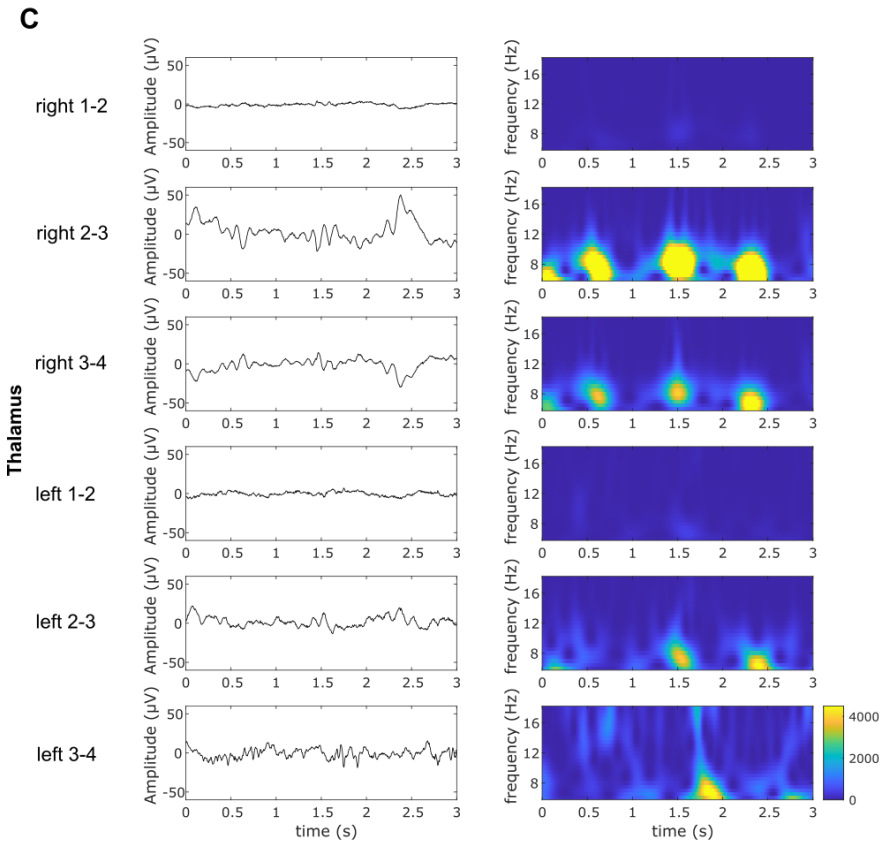
6.8. Supplementary material




B
Thalamus
right 1-2
right 2-3
right 3-4
left 1-2
left 2-3
left 3-4
Amplitude (μV)
frequency (Hz)
time (s)
20
0
-20
16
12
8
0
0.5
1
1.5
2
2.5
3
2000
1000
0



Supplementary figure 1. Representative data of thalamic spindles, shown in raw trace (left column) and time-frequency representations (TFRs; right column) from three different participants. A) A fast spindle in right thalamus (channel 2-3) at 1.5s is accompanied by a slow spindle in left thalamus (channel 3-4). This spindle is, in consequence, bilateral. At 2.3s, a unilateral spindle in the left thalamus occurs (channels 2-3 and 3-4). B) A slow bilateral spindle (channels right 2-3, left 1-2 and left 2-3) occurs at the beginning of the epoch. Though the peak of these spindles occurs at different time points, the tails of the spindles overlap, qualifying them as co-occurring spindles in our analysis. At 2s, we see a local fast spindle in channel left 2-3. C) A unilateral spindle in the right thalamus (channels 2-3, 3-4) at 0.5s, followed by bilateral spindles at 1.5s and 2.3s in the right (channels 2-3 and 3-4) and left (left 2-3, and partially left 3-4) thalamus.

P	ANT	VA	RT	Lam.med.	MD
1		7.51, -6.25, 7.88 9.02, -4.90, 9.03 -7.92, -6.28, 8.96 -9.51, -5.73, 10.40	6.00, -7.61, 6.73 -6.34, -6.83, 7.52	4.49, -8.96, 5.58 -4.75, -7.38, 6.08	
2		7.40, -8.25, 10.65 -6.88, -8.53, 7.51 -8.82, -8.13, 8.77 -10.75, -7.72, 10.02		4.24, -9.40, 7.11 5.82, -8.82, 8.88 -4.95, -8.94, 6.26	2.65, -9.97, 5.33
3		6.95, -7.92, 8.96 8.72, -7.43, 10.52 -7.77, -7.02, 8.45 -9.28, -6.85, 10.22	[1] -4.74, -7.37, 4.90 -6.25, -7.20, 6.67	5.19, -8.41, 7.39 [1]	3.42, -8.90, 5.82
4	4.35, -5.01, 11.70 5.87, -3.67, 12.03 -5.51, -6.84, 9.23 -6.73, -5.44, 9.98	[2]	7.39, -2.33, 12.36 8.90, -0.98, 12.68 -7.95, -4.03, 10.73[2] -9.17, -2.63, 11.48		
5		7.15, -7.65, 9.18 9.08, -7.80, 9.99 11.00, -7.96, 10.79 -8.50, -7.46, 8.69 -8.50, -7.46, 8.69 -11.76, -5.60, 10.14	5.23, -7.50, 8.37 -6.87, -8.39, 7.97		
6	4.50, -5.26, 8.32 5.97, -4.22, 9.70	-7.33, -5.52, 8.35 -8.86, -4.53, 9.48 [3]	7.44, -3.18, 11.08[3] -5.80, -6.52, 7.23		3.04, -6.29, 6,95 -4.27, -7.51, 6.11
7		7.29, -8.30, 7.73 8.78, -7.53, 9.10 10.27, -6.75, 10.46 -8.43, -7.82, 8.13 -10.39, -7.25, 8.92 -12.35, -6.67, 9.71	[4] [5]	5.80, -9.08, 6.37[4] -6.47, -8.40, 7.34[5]	

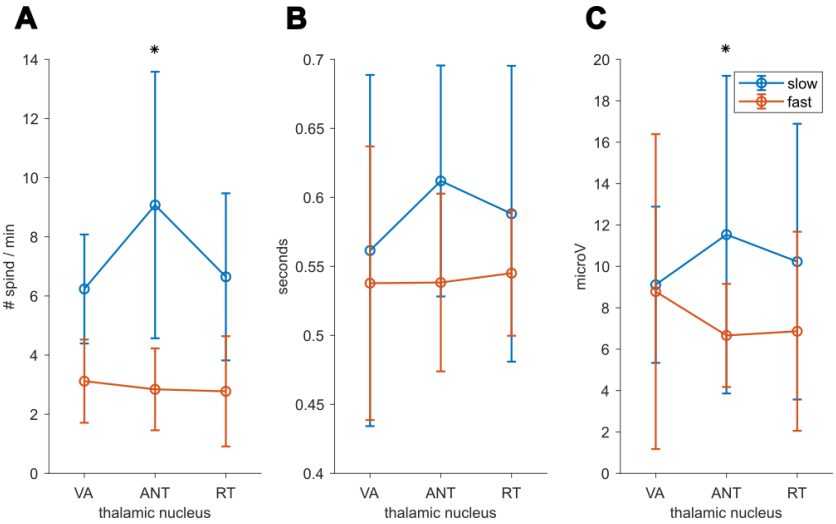
Supplementary Table 1. Coordinates in MNI-space (with respective nuclei guided by the Mai atlas) of the locations of electrode centers for all participants. Abbreviations: ANT: anterior nucleus of the thalamus; VA: ventral anterior nucleus; RT: Reticular nucleus; Lam.Med.: Lamina medialis; MD: mediodorsal nucleus. Electrode locations at borders between nuclei are indicated by superscripts.

Spindle characteristics per nucleus

We evaluated descriptive spindle measures when categorizing the data into their corresponding thalamic nuclei, selecting the three most common nuclei in our data. Note, that there was considerable uncertainty associated with the anatomical labeling. Three separate generalized linear mixed-effects models were constructed with density, duration, or amplitude as dependent variable, spindle type (slow vs. fast), thalamic nucleus (VA, ANT, RT) and their interaction as fixed effects, and participant as random intercept. The two-tailed significance level was set at $\alpha = 0.05$. Mixed-effects models were computed using the Matlab function *fitglme*.

In the generalized linear model (GLM) analysis, there was a significant main effect of spindle type ($F_{1,60} = 60.45$; $p < 0.001$) on spindle density, but no main effect of nucleus ($F_{2,60} = 1.48$; $p = 0.24$) and no significant interaction of spindle type and nucleus ($F_{2,60} = 2.29$; $p = 0.11$) on spindle density. Post-hoc comparisons revealed that slow spindles (6.90 ± 2.88 sp/min) had significantly higher density ($t(60) = 7.78$; $p < 0.001$) than fast spindles (2.94 ± 1.52 sp/min). The intercept term was significant ($F_{1,60} = 243.01$; $p < 0.001$), pointing to inter-individual differences in spindle density.

When modelling spindle duration with nucleus, spindle type and their interaction as mixed effects, and participant as random intercept, there was no significant effect of spindle type ($F_{1,60} = 4.01$, $p = 0.05$), nucleus ($F_{2,60} = 0.49$, $p = 0.62$), or their interaction ($F_{2,60} = 0.35$; $p = 0.71$), but a significant intercept ($F_{1,60} = 1033.5$, $p < 0.001$). When modeling spindle amplitude, there was a significant main effect of spindle type ($F_{1,60} = 6.80$; $p = 0.01$), but no significant main effect of nucleus ($F_{2,60} = 0.40$, $p = 0.67$) and no interaction effect of spindle type and nucleus ($F_{2,60} = 1.63$; $p = 0.20$). Post-hoc comparisons revealed that slow spindles (9.96 ± 5.53 μV) had significantly higher amplitude ($t(60) = 2.61$; $p = 0.01$) than fast spindles (7.67 ± 5.85 μV) The intercept was significant ($F_{1,60} = 36.47$, $p < 0.001$), suggesting inter-individual differences in both spindle duration and amplitude.



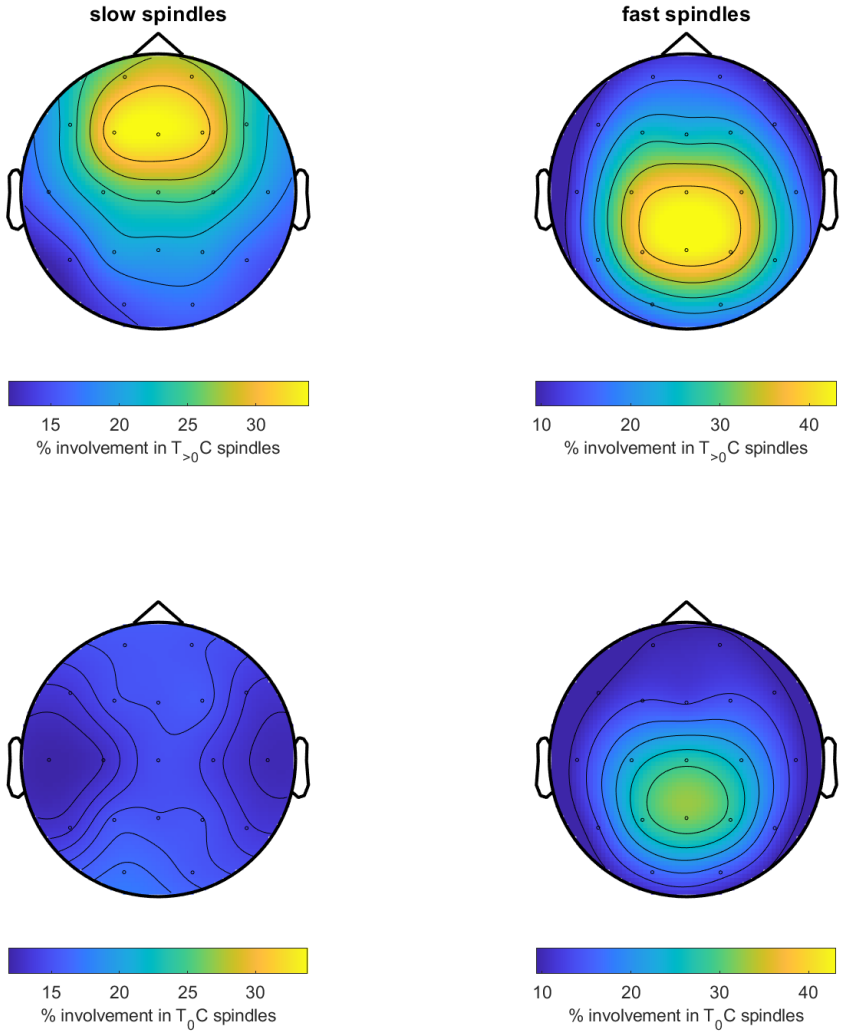
Supplementary figure 2. Average spindle characteristics per putative thalamic nucleus (see methods for limitations when estimating thalamic electrode locations). Values are shown for A) density; B) duration and C) amplitude. Asterisks mark significant results of spindle type at $p < 0.05$ in generalized linear mixed model. VA: ventral anterior nucleus of the thalamus; ANT: anterior nucleus of the thalamus; RT: Reticular nucleus of the thalamus.

Cortical spindles with and without thalamic counterparts

Since we reported that not all cortical spindles had a thalamic counterpart (figure 6), we further explored how these spindles were distributed across the cortical electrodes. To this end, we calculated the percentage of time each cortical electrode was involved in a T_0C spindle. Supplementary figure 3 contrasts to which extent scalp EEG electrodes are involved in thalamocortical ($T_{>0}C$ spindles) or cortical spindles without a recorded thalamic counterpart (T_0C).

Note, however, that our setup comprised a sparse sampling of anterior thalamic sites, which is why spindles that we call T_0C likely comprise both cortical spindles that truly have no (anterior) thalamic counterpart, and spindles that in fact do have an (anterior) thalamic counterpart that we did not record. Given these considerations, a comparison of true T_0C and $T_{>0}C$ properties is beyond the data at our disposal. For exploratory purposes, however, it is interesting that the electrodes with and without an anterior thalamic counterpart have a similar topography for the fast spindles, whereas the topology appears different for slow spindles. This could hint at fast spindles being linked to more posterior nuclei of the thalamus, which are not sampled in our setup. Possibly, some of these spindles also spread to the anterior thalamus,

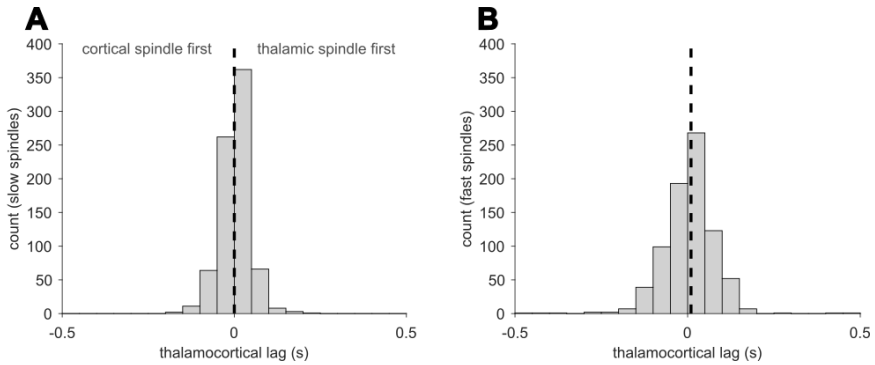
which are then captured in the $T_{>0}C$ spindle group. However, we strongly caution against over-interpreting these results, given the limitations of the data.



Supplementary figure 3. Cortical involvement in spindles with (top row; $T_{>0}C$ spindles) and without (bottom row; T_0C spindles) a thalamic counterpart recorded in our setup. The mean involvement of each electrode (not corrected for chance-level occurrence) in slow/fast $T_{>0}C$ or T_0C spindles is shown, respectively (calculated as time points of spindle activity per electrode as a percentage of the total time points of slow/fast $T_{>0}C$ or T_0C activity). Mean data across subjects are shown.

Simultaneous spindle onset in anterior thalamus and cortex

The previous analyses have shown that spindles tend to co-occur systematically. In order to understand this co-occurrence better, we investigated potential shifts in onset of thalamic and cortical spindles. Thalamic spindles might precede or follow cortical spindle activity. On the one hand, this would allow inferences on a causal relation of spindles between the two regions, but on the other hand this temporal shift would bias analyses of spatiotemporal spindle overlap toward channel pairs with a smaller temporal offset. The temporal shift was investigated by means of lag analysis. The lag was calculated as the time difference between spindle onsets in cortical relative to thalamic channels. Consequently, spindles with an earlier onset in the thalamus are indicated by a positive lag, and spindles with an earlier onset in cortical channels by a negative lag. Supplementary figure 3 depicts the median thalamocortical lag across participants and channels, for slow (A) and fast spindles (B), respectively. The distributions of thalamocortical lag for both slow (0.001 s ± 0.043) and fast (0.004 s ± 0.075) spindles were centered around 0. A one-sample t-test showed that both the slow ($t(778) = 0.35$; $p = 0.72$) and the fast ($t(797) = 1.45$; $p = 0.15$) spindles did not differ significantly from 0. This implies that spindle onset in thalamus and cortex was, on average, simultaneous.



Supplementary figure 4. Histograms of thalamocortical lag of spindle onset across all thalamocortical channel pairs and participants. A positive lag indicates spindle onset in thalamus first, a negative lag in cortex first. A) slow spindles; B) fast spindles. Dashed line marks the median lag of cortical relative to thalamic spindle onset.

Chapter 6:

General Discussion

This thesis investigated behavioral and brain processing during the encoding of naturalistic memories, as well as network processes during sleep. During the encoding of episodic memories, elements experienced during an event must be associated for event retention and retrieval. This process is thought to be supported by the hippocampus, which shows an increase in activity upon detecting contextual boundaries (Baldassano et al., 2017; Ben-Yakov & Dudai, 2011). Human episodic memory research predominantly has relied on static stimuli, which do not reflect the complexity of day-to-day experience. More recently, studies have started using dynamic stimuli, such as movies, to recreate naturalistic experience conditions for the study of episodic memory and event boundary processes (Baldassano et al., 2017; Ben-Yakov & Dudai, 2011). The main goals of this thesis were to **1)** probe the temporal specificity of event boundary processes and their susceptibility to interfering information, **2)** explore how sampling of elements in an event through eye movements affects its later memory, and **3)** investigate which brain regions support boundary processing and whether differences can be observed within hippocampal subregions. Furthermore, based on theories of thalamic contribution to organization of consolidation-related signals (Klinzing et al., 2019), we aimed to **4)** investigate if activity patterns during sleep were coordinated within thalamus and with the cortex. I will summarize our observations from the empirical chapters of this thesis and discuss them in the wider context of episodic memory function, mirroring the two-part structure of the introduction. **Part I** focuses on encoding and immediate post-encoding processes and their relevance for episodic memory. **Chapters 2 to 4** are part of this and will be discussed in context of each other, given that the same stimulus material was employed for the three empirical studies. **Part II** is centered around offline processes and systems consolidation during sleep, and will include a discussion and contextualization of **Chapter 5**.

6.1. Part I: The importance of immediate post-encoding processes for episodic memory

In **Chapters 2 to 4** of this thesis, we investigated behavioral and brain processes related to processing of event boundaries in dynamic stimuli. We used short movie clips containing a narrative to recreate naturalistic experience. In **Chapter 2**, we investigated the extent to which processing at event boundaries is susceptible to incoming information. It has been reported that showing two movie clips in immediate succession impaired memory for the first clip and attenuated the hippocampal response to the first clip's offset (Ben-Yakov et al., 2013). This suggests that brain processes at event boundaries are crucial for later memory performance. In this chapter, we probed the temporal specificity of this previously reported retroactive memory interference effect. Previous EEG research has shown that patterns of activation observed during encoding are reinstated within 1.5s after event boundaries, and that this is predictive of memory performance (Silva et al., 2019; Sols et al., 2017; Wu et al., 2023), suggesting that associative binding processes are limited to a short time window after event boundaries. Based on this observation, we hypothesized that following a movie clip with a second clip after a 2s time delay should not interfere with memory of the first clip. We showed participants movie clips in isolation, or followed by a second clip, where we manipulated the time delay between first and second clip (0 versus 2 seconds). Memory recall of the clips was tested after 20 minutes or 24 hours in free and cued recall. We replicated the previously reported retroactive interference effect for cued recall after 20 minutes and 24 hours but only when movies were shown in immediate succession. Introducing the 2s time delay between clips negated the interference effect of the second clip. Based on these observations, we conclude that the brain is susceptible to incoming information within a 2s time window after detecting event boundaries, and that this disrupts processes which may be crucial for memory consolidation.

The processing at event boundaries is thought to reflect binding of item information, such as the different elements encountered during an event (Baldassano et al., 2017). Visual information is sampled during fixations, which are interspersed with saccadic eye movements during the viewing of static images. Previous research has shown that a high number of fixations predicts memory performance. More specifically, memory for scene images seems to rely on a combination of distributed and densely grouped fixations to process specific elements (Broers et al., 2022). However, research on the link between

eye movements and memory performance has focused on static images, which do not resemble memory encoding in real-life scenarios. The aim of **Chapter 3** was to test whether fixation behavior is also relevant for memory encoding in dynamic stimuli. Dynamic stimuli, unlike static images, require smooth pursuits to track moving objects. An additional goal was to test whether smooth pursuits play a role in memory encoding. We recorded gaze behavior while participants viewed short movie clips, to probe fixation and smooth pursuit behavior. We reported that a higher number of fixations during the viewing of a movie clip increased the likelihood of its successful memory recall, both after 20 minutes and after 24 hours. However, the contribution of smooth pursuits to memory performance was less clear, as they predicted memory performance only in some experimental conditions and statistical models. We conclude that fixation behavior is also predictive of memory performance during the viewing of dynamic stimuli, but that further research is needed to understand the role of smooth pursuits for memory performance.

In **Chapter 4**, we investigated the brain processes triggered by event boundaries in 7T fMRI, using eventful and uneventful short movie clips. The study aimed to extend the observations by Ben-Yakov et al. (2011) on hippocampal BOLD increases after eventful movie clip offsets, using a higher field strength. The higher field strength, and multi-echo imaging, allowed us to test whether these hippocampal processes differed between hippocampal subregions along the longitudinal axis of the hippocampus. Based on observations of an anterior-to-posterior gradient of coarse-to-fine resolution in processing spatial environments (Brunec et al., 2018; Kjelstrup et al., 2008; Komorowski et al., 2013), we hypothesized that the head and tail of the hippocampus would show different signals related to the offset of movie clips. Indeed, we found that the body and tail of the hippocampus showed larger signal increases at the offset of eventful movies compared to the head of the hippocampus. At the whole brain level, we observed offset-related processes in the basal ganglia and cerebellum, replicating the results by Ben-Yakov et al. (2011). However, in this analysis, no hippocampal cluster was evident. Instead, we report activity close, but not in, the anterior hippocampus. We did not observe a subsequent memory effect in this cluster outside of the hippocampus. Neither was there a difference in the offset response in the hippocampal ROI analysis between subsequently remembered and forgotten clips. Although the lack of a clear hippocampal cluster at whole brain level complicates interpretation of the results, we provide evidence that there may be a longitudinal gradient in the hippocampus for event boundary processing. This observation provides further evidence for distinct hippocampo-cortical

networks along the hippocampal longitudinal axis (Adnan et al., 2016; Barnett et al., 2021; Ritchey et al., 2015). Our results also suggest that the hippocampus is active during event boundaries, putatively for binding of item information, but that this is not predictive of memory performance at a 24 hour retention delay.

When considering the results of the three empirical chapters in light of each other, what can they tell us about memory encoding of naturalistic stimuli? The eye movement data from **Chapter 3** were recorded in the context of **Chapter 2**, in which we interfered retroactively with memory. We analyzed the contribution of fixations to memory performance per movie presentation condition and found that even in the presence of a strong behavioral manipulation (i.e. when two movies were shown in immediate succession), a higher number of fixations predicted a higher chance of remembering a clip. Because of the behavioral manipulation, however, memory recall was less likely when the clip was immediately followed by a second clip, even if many fixations were made during the clip. Although these results do not allow inferences about the involved brain regions during this process, they are in line with the model of event segmentation (Baldassano et al., 2017). According to this model, lower-order events must be integrated across time to form higher-order events. Our observations from **Chapters 2 and 3** suggest that encoding of (visual) elements in an event occurs through fixations, where more fixations lead to better information sampling of the scene. Presentation of irrelevant information at an event boundary may then affect associative binding of this information.

Based on the observation that interfering with processes at event boundaries affects event memory, one would expect that hippocampal signals at event boundaries predict subsequent memory performance. This was the case in previous studies (Ben-Yakov et al., 2011; 2013), but it contrasts with the lack of a subsequent memory effect despite an existing offset response we report in **Chapter 4**. In **Chapter 2**, we report that unrelated input immediately following an event boundary retroactively impairs memory performance for this event. Because we did not measure fMRI in this chapter, it is unknown whether this interference was linked to an attenuated hippocampal offset response. A possible explanation that reconciles the observations from these two chapters is that immediately following a clip with a second one affects working memory processes. It has been reported that visual stimuli followed by longer inter-trial-intervals allow stimuli to be processed in working memory for a longer period of time and lead to better memory performance in subsequent testing (Souza & Oberauer, 2017). This effect is also present when active rehearsal is suppressed (Oberauer, 2022). Displaying two clips in immediate succession may limit the time window for working memory processes related to the first stimulus and

lead to impaired long-term memory, whereas a time window of 2 seconds between stimuli may allow sufficient processing to take place. In this way, the retroactive memory interference effect could be explained by interruption of working memory processes rather than boundary-related item binding of information in the hippocampus. In **Chapter 4**, we report stronger negative BOLD responses during the viewing of subsequently remembered stimuli in the right caudate nucleus, whose activity has been associated to working memory processing (Marvel et al., 2019; Provost et al., 2010). An alternative explanation is that hippocampal offset processes are necessary but not sufficient for intact memory performance. In this view, event boundaries would trigger associative processes to bind information encountered during the event. Whether this event is consolidated sufficiently for successful recall after 24 hours or not may hinge on other processes. However, interruption of this process, by displaying information at the event boundary, would impair subsequent memory because binding of information cannot occur in the first place.

As outlined in **Chapter 1**, offline periods after learning are crucial for episodic memory consolidation. Plasticity changes to store recently encoded memories are particularly seen during sleep, when reactivation of activity patterns triggers molecular processes to promote synaptic growth (Niethard et al., 2018; Seibt et al., 2017). However, the majority of studies that have contributed to our understanding of episodic memory and associative hippocampal processes have tested memory only within short time intervals of encoding (within 20 minutes to 1 hour, or on the same day). This seems counter-intuitive given the importance of offline periods, including sleep, after encoding for memory consolidation. Sleep may be particularly relevant for weakly encoded memories, as shown in a recent study which reported that items that were less well remembered in a memory test immediately after learning were preferentially reactivated during subsequent rest periods. The extent to which they were reactivated predicted memory performance after sleep but not awake rest (Schapiro et al., 2018).

The bias towards short memory retention intervals in episodic memory studies likely biases our knowledge on these memory processes. For projects in this thesis, we thus opted to test memory after 24 hours in **Chapter 4** and to directly contrast memory after 24 hours with memory after 20 minutes in separate samples in **Chapters 2 and 3**. We did not report differences in the effect of behavioral memory interference or in the role of eye movements on cued recall between short and long retention delays. However, some differences were found when testing free recall. Overall free recall performance after 24 hours was reduced compared to after 20 minutes. We also found that fixation behavior

only contributed to free memory recall after 24 hours of retention delay. This suggests that denser stimulus sampling during encoding leads to better memory after 24 hours, a finding that conflicts with the previously reported preference of reactivation for weakly encoded memories. In **Chapter 4**, we tested memory after 24 hours and failed to observe the subsequent memory effect of hippocampal offset responses previously reported for retention delays of 20 minutes (Ben-Yakov et al., 2011; 2013). This lack of a subsequent memory effect puts into question whether hippocampal boundary-related processes are sufficient to explain whether a memory trace persists or not. Furthermore, it illustrates that results obtained at shorter retention delays may not extend to longer retention periods. Future research into behavioral and brain mechanisms for the encoding of naturalistic stimuli should include longer retention delays to understand their contribution to the long-term retention of event memories.

6.1.1. The use of naturalistic stimuli for memory research: Considerations and limitations

Naturalistic stimuli were used for the experiments carried out in **Chapters 2 to 4**. In addition to their higher resemblance to natural processing in day-to-day life, these types of stimuli have several advantages over static stimuli: first, they include unfolding events which are consistent and lack the arbitrariness of random word lists or unrelated paired associates. These stimuli are also more engaging, and, lastly, they allow dissociation of processes linked to the onset versus offset of stimuli, which cannot be achieved with short presentation of static stimuli. Here, we carefully selected naturalistic stimuli to fulfill several criteria that we established as necessary for our purposes: 1) the movie clips needed to contain a narrative that was not evident from the first frame of the movie, so cued recall could be tested based on this first frame; 2) the narratives needed to differ, so as to make movies identifiable based on participants' written descriptions; 3) they needed to be short to allow for a high number of trials within a given time frame; and 4) the movies had to be silent so that they were language-independent for use in several study populations. Silent movies are also easier to present in the MRI environment, where loud noise may otherwise affect understanding of the narrative. In **Chapter 4**, we selected an additional set of movies lacking a narrative, to compare brain activations between eventful and uneventful movies. Throughout the empirical work in this thesis, several difficulties with these stimuli became evident, which I will discuss here.

Although opting to select clips without sound made the stimuli more appropriate for our testing setup, it simplifies stimulus material and only makes it naturalistic to a certain extent. This choice is likely to have particularly affected the eye movement behavior, which we reported on in **Chapter 3**. Choosing stimuli with sound, and potentially including dialogue, puts demands on other brain systems. Although eye movements are still likely relevant for sampling visual elements in audiovisual stimuli and to understand the narrative, their contribution to memory performance might be slightly lower than in exclusively visual stimuli.

It is possible that the choice of visual clips contributed to differences in neuroimaging findings between our work and the experiments by Ben-Yakov et al. (2011), in which audiovisual clips with dialogue were used. According to our results in **Chapter 4**, the body and tail of the hippocampus responded to the offset of movie clips when they contained narratives, but responses did not differ for subsequently remembered and forgotten stimuli. Possibly, the involvement

of the hippocampus in event boundary processing for later memory retention is different for multimodal dynamic stimuli compared to unimodal stimuli. To process audiovisual stimuli, multimodal item information must be bound. It could be that this process is more reliant on the hippocampus than exclusively visual events. However, this seems unlikely given the many reports of hippocampal associative binding in setups using static visual stimuli described in **Chapter 1**.

Naturalistic stimuli include motion of their constituent elements or of the camera, which may trigger smooth pursuit eye movements. Because the influence of eye movement behavior on episodic memory has only been investigated using static stimuli, the role of smooth pursuits for stimulus encoding and memory has not, to our knowledge, been previously investigated. In **Chapter 3**, we investigated smooth pursuits, but we did not find clear evidence for their relationship to subsequent memory performance. Although we found a moderate effect of smooth pursuits on memory performance in certain conditions, the majority of our analyses did not suggest that smooth pursuits played a meaningful role in encoding of information for later memory performance. This lack of clear contribution to subsequent memory contrasts with the robust finding that a higher number of fixations predicts subsequent memory, even in presence of a behavioral manipulation. Smooth pursuits seem to be stimulus-driven (Agtzidis et al., 2020; Dar et al., 2021; Dorr et al., 2010; Goettker et al., 2020; Ross & Kowler, 2013), in that they are triggered by slow movement of objects or a panning camera. Although subjects can suppress smooth pursuits, it is hard to voluntarily engage in them in dark settings or with static stimuli (Sharpe & Wong, 2005). Because they depend on the stimulus to be encoded, smooth pursuits seem to contribute less to stimulus sampling and memory encoding in comparison to fixations. It is possible that clips differed in terms of movement and required smooth pursuits, thereby confounding our analysis. To further explore the role of smooth pursuits in sampling elements within an event for its later memory, one could quantify movement of actors, objects or the camera in the stimulus material. Including this movement as a predictor in the analysis would be necessary to assess whether deviation from stimulus-triggered smooth pursuits contribute to subsequent memory performance. Ideally, this should be tested in a simple design without behavioral interference manipulation, to maximize the trial count for different element/camera movement scenarios.

In conclusion, although the use of dynamic stimuli is necessary to understand episodic memory processes in more naturalistic settings, these stimuli are complex and might introduce confounds. Accordingly, additional consideration is recommended when opting to use naturalistic stimuli.

6.2. Part II: Memory consolidation during sleep – a role for thalamic spindles?

During offline states, such as sleep, reactivation in neuronal populations leads to consolidation of memories. At the systems level, oscillations reflect network and local population excitability, and nesting of oscillation patterns supports reactivation in hippocampo-cortical networks (Klinzing et al., 2019). Thalamic spindles are theorized to be involved in the timing and coordination of these network oscillations, but only a few studies have recorded spindles in the human thalamus and characterized their activation patterns during sleep (Bastuji et al., 2020; Mak-McCully et al., 2017; Schreiner et al., 2022). Moreover, in studies recording intracranially at the cortical level, spindles have been reported to occur in isolation relative to the rest of the recorded cortex (Andrillon et al., 2011; Frauscher et al., 2015; Peter-Derex et al., 2012). For spindles to be involved in reactivation processes across brain systems, they would need to occur in tandem, rather than be locally restricted at all times.

In **Chapter 5**, we investigated whether spindle activity in the thalamus and cortex is coordinated, by analyzing intracranial and scalp EEG data from the anterior thalamus in patients who underwent implantation of deep brain stimulation electrodes for epilepsy treatment. Although previous studies have reported mutual spindles in the thalamus and cortex (Bastuji et al., 2020; Mak-McCully et al., 2017; Schreiner et al., 2022), these studies did not systematically investigate whether observed spindle co-occurrences differed from what would be expected by chance. In this study, we used a novel statistical approach to quantify random spindle co-occurrences, and compared these with observed co-occurrences to infer whether mutual spindle activity was coordinated. We found that spindles mutually occurred more often than expected by chance, both at the thalamic and thalamocortical level. Moreover, we observed distinct topographical patterns of spindle co-occurrence in the cortex depending on the thalamic channels involved in a given spindle. Altogether, our observations suggest that the extent of cortical spindle activity is related to the spread of a spindle within the thalamus.

Although this study did not test memory, our observations provide evidence for coordinated processes in thalamocortical loops that could underlie the selective engagement of cortical structures during memory consolidation. Reactivation of memory traces needs to be spatially and temporally specific whereby dispersed neuronal populations involved in a memory must reactivate

simultaneously for neuronal plasticity changes to take hold (Josselyn et al., 2015). How may this be achieved at the systems level?

During NREM sleep, sleep oscillations, namely SOs, ripples and spindles, occur in a nested fashion (Gonzalez et al., 2018; Staresina et al., 2015), where the phase of SOs in the neocortex reflect hyperpolarized and depolarized states. The depolarized state marks a time window of neuronal excitability during which information processing could occur (Klinzing et al., 2019), however, SOs are too wide-spread and slow to signal selective reactivation in specific neuronal populations. Hippocampal ripples create the high-frequency stimulation conducive to long term potentiation, which triggers the molecular processes thought to underlie synaptic plasticity changes (Sadowski et al., 2016). The timing signal to coordinate hippocampal with neocortical processes during memory consolidation could come from the thalamus in the form of spindles. The thalamus shares connections with many cortical areas and distinct systems through its numerous nuclei (Cappe et al., 2009; Jankowski et al., 2013; Zhang et al., 2010). Moreover, core and matrix cells, which differ in their projections and spatial selectivity to output structures, may achieve a balance of wide-spread and targeted connections to subcortical and cortical areas (Piantoni et al., 2016). Because of these neuroanatomical properties, the thalamus seems like a viable structure to selectively engage neuronal populations across brain systems, as required during memory consolidation (Klinzing et al., 2019).

In this speculative view of the thalamus as a coordinator for systems consolidation, spindles may provide brain systems with time signals during which communication and information transfer between hippocampus and neocortex can take place. Spindles are transient and spatially specific signals. At the cortical level, a higher density of spindle activity in areas engaged during a spatial associative memory task has been observed during post-training sleep and linked to better learning performance (Petzka et al., 2022). Spindles trigger metabolic processes underlying plasticity changes in rodent models (Niethard et al., 2018; Seibt et al., 2017), and have been shown to increase mutual firing of neurons across cortical sites in humans (Dickey et al., 2021). In this way, spindle activity at different thalamic sites could engage different cortical networks. In **Chapter 5**, we provided evidence that mutual spindle activity in human thalamocortical systems is not random but coordinated. Moreover, we showed that involvement of cortical sites in spindles is related to spindle spread within the thalamus. This chapter indirectly supports the emerging theory on the coordinating role of the thalamus during systems consolidation. Future studies should include memory paradigms to elucidate the role of thalamocortical spindle coordination for memory consolidation.

6.3. Conclusion

Episodic memory encoding relies on associative processing of elements encountered during an event, a process which is thought to occur upon detection of contextual boundaries (Baldassano et al., 2017; Ben-Yakov & Dudai, 2011). Only recently there has been a shift from static images to dynamic stimuli recreating naturalistic conditions to study episodic and event memory. In this thesis we addressed several open questions on behavioral and brain processes supporting the formation of event memories. Binding of an event's constituent information is thought to occur at event boundaries, a process which we found to be susceptible to incoming information only within a time window of 2s after an event boundary. This interference affected memory performance both after 20 minutes and 24 hours. We furthermore found that visual information sampled throughout an episode affects later memory recall, where more fixations predict better memory performance both after 20 minutes and 24 hours. Activity in the body and tail of the hippocampus seem to be involved in boundary-related processing, but this activity may not be sufficient to explain whether an event will be remembered or forgotten after retention delays of 24 hours. As to mechanisms of consolidation during sleep, it is thought that the thalamus coordinates network reactivation for memory replay (Klinzing et al., 2019). Although we did not test memory, we found evidence for coordinated oscillatory patterns between thalamus and cortex during sleep. These observations provide a stepping-stone for future research into the involvement of thalamic processes for hippocampo-cortical coordination during systems memory consolidation.

6.4. References

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Appendix

Appendix A: Impact Paragraph

When you think back to your last birthday, you may remember the people you spent the day with, the cake you ate, the surprise party that was thrown for you, or the way you tried to move throughout the day trying to forget about the fact that you were growing older. Whichever way you celebrated or didn't celebrate your birthday, chances are that you remember the events of this day and can tell them to another person if asked.

The human capacity of episodic memory makes it possible for us to mentally time travel back and revisit past experiences (Tulving, 2002). Episodic memory describes the memory for autobiographical events. A big part of what defines us as people is our past as seen through the lens of memory. Unlike other forms of learning, episodic memory is formed after single experiences of situations and does not require repetition of information (think of studying for a vocabulary test, for example, or learning to drive a bike). Although we don't consciously rehearse the information of what happened during our birthday, while we sleep, our brains reactivate information to manifest experience into plastic changes in the brain.

This thesis investigated how behavioral and neural processes during the experience of events, and the period immediately following those events, may lead to remembering or forgetting. To this end, we showed participants short movie clips in several conditions. In **Chapter 2**, we show that watching two clips in immediate succession decreases memory performance. This is in contrast to intact memory performance for clips that are shown in isoluation, or followed by a short time window before showing the next clip. Together, these results suggest that the period right after the end of an event is relevant for memory processing in the brain. In **Chapter 3**, we show that viewing behavior (i.e., where we look) during events predicts better memory performance. In **Chapter 4**, we describe the brain regions involved in encoding these clips. In the second part of the thesis and **Chapter 5**, we analyzed data from deep brain structures in the sleeping brain in patients with epilepsy. These data are rare due to their invasive nature, but they allowed us investigate how different brain networks may coordinate their activity. Although we did not test memory in this study, the results of this study are important for memory research, because storage of memory information relies on the interaction of different brain networks during sleep.

The main purpose of this thesis was fundamental research into human episodic memory processing. Fundamental research often paves the way for

more applied research settings, which tend to have a higher direct impact on society. Nonetheless, several ways in which the work of this thesis impacts the scientific field and society can be named.

Chapter 1 outlines difficulties of recording data from deep structures in the human brain, particularly time-resolved data which cannot yet be captured with non-invasive neuroimaging techniques. The in-depth study of these brain regions would hardly be possible without multidisciplinary collaborations. This PhD project was made possible by the effort of two faculties, including several departments. **Chapter 5** illustrates how the collaboration between medical doctors and neuroscientists leads to production of knowledge that would otherwise not be gained. The outcome of this work has been published in two papers (Bernhard et al., 2022; Jacobs et al., 2022).

Chapter 2 to **4** used the same stimulus material to study behavioral and brain processes related to encoding and early consolidation stages of episodic memory, using dynamic stimuli such as movie clips. **Chapter 2** illustrates that irrelevant information presented right after a movie clip makes it more likely that this clip will be forgotten later on. This observation may be particularly relevant for the way information is presented on social media platforms nowadays. On apps like TikTok or Instagram, it is not uncommon to have short video clips quickly follow each other. The results of our study highlight how the speed at which information is presented can be problematic for memory retention of content.

The scientific field in general, and cognitive neuroscience in particular, suffers from a replication crisis, where results are not stable across studies. This may stem from the complexity of the human brain and human behavior that we study, but also small study populations (Button et al., 2013), variability in hypothesis definition, analysis workflows (Botvinik-Nezer et al., 2020), and a bias for publishing hypothesis-confirming results. Two studies in this thesis (**Chapter 2** and **Chapter 4**) were conducted with the goal of extending previously published results to new experimental conditions (**Chapter 2**) and advanced neuroimaging techniques (**Chapter 4**). In **Chapter 4**, we did not exactly replicate the results of previous studies, putting into question whether observations of the involvement of the hippocampus in event boundary processing are robust across studies. Overall, this thesis thus contributes to replication of neuroscientific research and to probing robustness of results across analysis techniques and experimental conditions.

Lastly, I want to outline my contributions as a scientist to society outside of the specific work of this thesis. At a broader level, I believe science is a dialogue, and should be handled as such. Without proper communication, the scientific

endeavor is nothing more than the indulgence of curiosity. Unfortunately, limited funding resources and a too-strong focus on citation metrics lead to competition and, in consequence, to strategic decisions of not sharing data or code. But the scientific dialogue is not limited to sharing knowledge with other scientists, it describes a broad spectrum of actions that can be undertaken for dissemination of knowledge. Throughout my PhD, I have undertaken several steps to share the outcomes of my studies. Some were more conventional, like discussions on academic twitter, presentations at conferences of for undergraduate students. But I also believe that it is necessary to make science more approachable for the general public, especially to counteract developments of scientific distrust and to empower women and marginalized groups in STEM fields. To do so, I have shared insights into life as a graduate student on my otherwise social media accounts, assembled book recommendations about women in STEM on my book-related Instagram account, and am currently working on a novel set in an academic context.

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Appendix B: General Summary

Episodic memory describes the human capacity for mental time travel and revising past experiences. Life is experienced in a continuous manner, yet when we remember past experience, we often think back to specific events: Our last vacation, the afternoon at that special bookstore in New York we have been wanting to visit. This chunking of continuous experience into events is thought to occur based on contextual boundaries or shifts in internal goals, for instance leaving the bookstore and stepping onto the busy streets of New York. These boundaries trigger hippocampal responses, which are relevant for later memory recall and are thought to reflect associative binding of information encountered during the event. Most research on episodic memory processing and event boundaries relies on static image stimuli, which do not reflect the complexity of everyday life experience. Only in recent years has there been a shift to study episodic memory in dynamic stimuli recreating naturalistic conditions. In this thesis we addressed open questions on behavioral and brain processes underlying event memory formation (Part I) and systems consolidation (Part II). The main aims of this thesis were to 1) probe the temporal specificity of event boundary processes and their susceptibility to interfering information, 2) explore how sampling of elements in an event through eye movements affects its later memory, and 3) investigate which brain regions support boundary processing and whether differences can be observed within hippocampal subregions. Systems memory consolidation during sleep relies on reactivation across several brain structures. Recent theories suggest that the thalamus and sleep spindles may play a role in coordinating these processes. We aimed to 4) study systematic mutual activity of sleep spindles in thalamocortical circuits.

Part I

The aim of Part I was to study behavioral and brain processes underlying event and event-boundary processing for memory retention. To this end, we used short movie clips containing a narrative to recreate naturalistic experience.

In **Chapter 2**, we aimed to investigate to which extent processing at event boundaries is susceptible to incoming information. Previous research has shown that presentation of two movie clips in immediate succession attenuates the hippocampal boundary-related response and impairs memory for the first clip. The temporal specificity of this retroactive memory interference effect had been hitherto unknown. Hypothesizing that the window of susceptibility to external information might be related to the 1.5 s post-event time window during which

reactivation of event-related activity patterns can be observed, we compared memory for clips that were shown in isolation, or followed by a second clip at 0s or 2s. Memory recall of the clips was tested after 20 minutes or 24 hours in free and cued recall. In cued recall, we replicate the observation that presenting new information at an event boundary retroactively interferes with memory of the previous clip. This retroactive memory interference effect is still present when memory is tested after 24 hours. A 2s time delay between clips did not affect memory performance for the first clip, suggesting that the brain is susceptible to incoming information within 2s after detecting an event boundary. The brain processes occurring in this time window seem to be highly relevant for memory consolidation, seeing as their disruption decreased memory performance.

In **Chapter 3**, we aimed to study how gaze behavior during an event affects its later memory. Brain processes at event boundaries are thought to reflect associative binding of information encountered during an event. Visual information is sampled through eye movements, where input is processed during fixations. Previous research has shown that a high number of fixations, interspersed with saccades, predicts memory performance. Research on the link of eye movements and memory performance has hitherto focused on static images. Static stimuli render smooth pursuit movements, which are used to track moving objects, obsolete. In **Chapter 3**, we investigated to which extent fixation and smooth pursuit behavior influence event memory. We report that a higher number of fixations during the viewing of a movie clip increased the likelihood of its successful memory recall, both after 20 minutes and after 24 hours. However, the contribution of smooth pursuits to memory performance was less clear. Smooth pursuits predicted memory performance only in some experimental conditions and statistical models. We thus conclude that fixation behavior is also predictive of memory performance during the viewing of dynamic stimuli, but that further research is needed to understand the role of smooth pursuits for memory performance.

In **Chapter 4**, we aimed to investigate the neural correlates of memory encoding and boundary processing in 7T fMRI. In addition to the eventful movie clips used in the previous chapters, we also presented uneventful clips to participants, which did not contain a narrative. Previous studies reported hippocampal BOLD responses related to the offsets of eventful movie clips, whose amplitude scaled with subsequent memory performance. The goal of this chapter was to extend these findings to a higher field strength, and probe whether offset processes differ along the hippocampal anterior-to-posterior axis. Here, we found that the body and tail, but not the head, of the hippocampus showed signal increases at movie offsets, and that the response was stronger for

eventful compared to uneventful movies. However, BOLD responses to movie clip offset did not differ between movies that were remembered 24 hours later and those that were forgotten. When extending the analysis to the whole brain level, we report offset responses in the basal ganglia and cerebellum. This analysis, however, did not reveal offset responses in the hippocampus. Instead, we found offset-related processes in a cluster near the head of the hippocampus. None of the reported regions showed offset-response modulations related to subsequent memory. We provide evidence that there may be a longitudinal gradient in the hippocampus for event boundary processing. Our results also suggest that the hippocampus is active during event boundaries, putatively for binding of item information, but that the lack of a clear subsequent memory effect suggests that this is not predictive of later memory performance.

Part II

Consolidation of memories relies on reactivation of neuronal populations during offline states, such as sleep. Oscillations during sleep reflect neuronal and network excitability, and their nesting is thought to support reactivation across systems. The thalamus has been proposed to coordinate reactivation during systems consolidation, yet only few studies have recorded spindles in the human thalamus and characterized their activation patterns during sleep. In studies recording intracranially at the cortical level, spindles have been reported to occur in isolation, although they seem to co-occur between thalamus and cortex. **Chapter 5** aimed to systematically analyze to which extent spindle activity in the thalamus and cortex is coordinated. Data were collected in intracranial electrodes from the anterior thalamus and scalp EEG in patients who underwent implantation of deep brain stimulation electrodes for epilepsy treatment. By comparing observed patterns of mutual spindle activity to randomized spindle co-occurrences, we quantified whether mutual spindle activity was systematically coordinated. We report that spindles mutually occurred more often than expected by chance, both at the thalamic and thalamocortical level. Moreover, distinct topographical patterns of spindle co-occurrence in the cortex could be observed, depending on the thalamic channels involved in a given spindle. Based on these observations, we conclude that cortical spindle activity is related to the spread of a spindle within the thalamus. Although memory was not tested, our observations in this chapter support notions on the coordinating role of the thalamus during systems consolidation through systematic spindle coordination in thalamocortical loops.

Conclusion

In this thesis, we report behavioral and brain processes underlying event memory formation. In Part I, we highlight the importance of using dynamic stimuli and longer retention intervals to study event memory in naturalistic conditions. Because our results partially conflict with existing literature, more research is needed to elucidate the role of the hippocampus for event memory processing. In Part II, we provide evidence for a basic assumption of thalamocortical coordination during sleep, but further research needs to be carried out to pinpoint whether this mechanism underlies systems consolidation.

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Curriculum Vitae

Hannah Bernhard was born in Berlin, Germany, on December 3rd 1993. After obtaining her high school diploma at Heinrich-von-Kleist Gymnasium, Berlin, in 2012, she studied a bachelor's degree in psychology at Dresden University of Technology. During her bachelor's, she worked as a student assistant at the Cochlear Implant Centre at the University Hospital Carl-Gustav-Carus under the supervision of Dr. Anja Hahne, where she got her first taste of neuroscientific research. After graduating with a Bachelor of Science in 2016 (*cum laude*), she was accepted to the Research Master in Cognitive and Clinical Neuroscience at Maastricht University. During her master's, Hannah completed a scientific internship and wrote her thesis on neural dynamics during mind wandering under the supervision of Dr. Chie Nakatani and Prof. Dr. Cees van Leeuwen (Katholieke Universiteit Leuven) and Prof. Dr. Peter de Weerd (Maastricht University). She graduated with a Master of Science (*cum laude*) in 2018 and started her PhD trajectory at Maastricht University in the same year. She now works as a research associate in the emotion cognition lab with Dr. Deborah Talmi at University of Cambridge.