PATTERN SEPARATION IN THE HUMAN HIPPOCAMPUS:

NOVEL INSIGHTS FROM

NATURAL LESION MODELS AND SLEEP-DEPENDENT MEMORY CONSOLIDATION

Dissertation
zur Erlangung des Doktorgrades
der Philosophischen Fakultät
der Christian-Albrechts-Universität
zu Kiel

vorgelegt von

Annika Katrin Hanert

Kiel, 05.03.2019

Erstgutachterin: Prof. Dr. Anya Pedersen

Zweitgutachter: Prof. Dr. Christian Kaernbach

Tag der mündlichen Prüfung: 18.06.2019

Durch den Prodekan für Studium und Lehre Prof. Dr. Ulrich Müller zum Druck genehmigt: 12.07.2019

Abstract

Within an ever-changing world, the formation of stable and enduring memories is essential in defining our self and identity. High demands on cognitive functions in daily life, therefore, call for an efficient memory system that reduces interference between memories and enables generalizations across similar events. By means of pattern separation, similar memories are stored as distinct and non-overlapping representations, whereas during pattern completion, previously stored memories are reactivated by partial environmental cues. These two complementary functions are critically reliant on the hippocampus. The combination of a unique cytological and network architecture forms the neural substrate of hippocampal mnemonic functions. Regarding pattern separation and completion, evidence from computational models, studies in rodents, as well as human data support the idea that those processes are mediated by the hippocampal dentate gyrus and CA3 regions. However, studies in humans lack information regarding mechanistic aspects of causality regarding the anatomical structures of the hippocampus and pattern separation and completion. The aim of this thesis was to elucidate the role of the human hippocampus and its subfield-specific contributions to pattern separation. We examined natural lesion models, by means of selective CA1 lesions during a transient global amnesia, and in a rare form of limbic encephalitis, where neurodegeneration preferentially shows in the dentate gyrus and CA3. The results showed that pattern separation measured by a mnemonic similarity task was best predicted by the volume of the DG, whereas recognition memory was stronger associated with the volume of CA1. We also found a strong deficit in pattern separation performance associated with selective CA1 lesions. We then examined pattern separation performance after post-encoding sleep in healthy humans to clarify the neurobiological processes of memory consolidation. We demonstrated the relevance of hippocampal information processing during sleep, in the stabilization of separated information. This might also suggest a link between pattern separation and completion processes during sleep-dependent memory consolidation.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ANOVA	*Analyses Of Variance*
CA	*Cornu Ammonis*
DG	*Dentate Gyrus*
EC	*Entorhinal Cortex*
EEG	*Electroencephalogram*
FLAIR	*Fluid Attenuated Inversion Recovery*
fMRI	*Functional Magnetic Resonance Imaging*
GC	*Granule Cell Layer*
ISI	*Inter-Stimulus Interval*
LGI1	*Anti-Leucine- Rich Glioma-Inactivated 1*
LTD	*Long-Term Depression*
LTP	*Long-Term Potentiation*
MST	*Mnemonic Similarity Task*
MWT	*Mehrfachwahl-Wortschatz Intelligenztest*
NMDA	*N-Methyl-D-Aspartat*
PatSep	*Pattern Separation*
RAVLT	*Rey Auditory Verbal Learning Test*
REM	*Rapid Eye Movement*
RM	*Recognition Memory*
RWT	*Regensburg Word Fluency Test*
SO	*Slow Oscillation*
SWS	*Slow Wave Sleep*
TGA	*Transient Global Amnesia*
TMT	*Trail Making Test*

1 Introduction

One of the most important challenges in an ever-changing environment is the correct identification of differences and similarities in the outside world. It can be difficult, for example, to distinguish between memories of the last summer holidays if they were all spent at the same place with the same company. In contrast, one would not want to miss vibrant remembrance of a wonderful day at the beach when seeing a picture of sand, deckchairs, and palm trees.

In the face of the high encoding demands of everyday life, an essential feature of memory formation consists in the coordination of discrimination between similar events with simultaneous generalization across similar impressions. These operations are highly relevant in the utilization of all memory capacity in the context of forming stable long-term memories (Marr, 1971). The discrimination between two similar events requires the separate storage of overlapping memories. Additionally, long-term memories of past events should be easily retrieved by means of environmental cues (Rolls, 2016). These opposite but complementing processes are supported by the hippocampus: The hippocampal process of pattern separation allows a distinct and non-overlapping storage of similar mnemonic information to reduce interference of overlapping memories during retrieval to maximize memory capacity. Pattern completion, on the other hand, by means of the extraction of generalities, enables retrieving previously encoded memories in the presence of partial environmental cues (Marr, 1971; McClelland, McNaughton, & O'Reilly, 1995; Norman & O'Reilly, 2003; Rolls, 2016; Figure 1-1).

Beside the role in physiological memory formation, the hippocampus is also implicated in the stabilization of long-term memories in the process of system consolidation. Here, memory representations are redistributed from the hippocampal short-term store to neocortical long-term

stores (Frankland & Bontempi, 2005; McClelland et al., 1995). This stabilization due to redistribution to long-term stores is most effective during sleep (Marr, 1971; McClelland et al., 1995).

Figure 1-1. Similar beach scenes for an illustrative example of pattern separation and completion. The discrimination of similar events (e.g., the last two summer holidays) is facilitated by means of pattern separation. The recall of previously encountered events by environmental cues (e.g., remembering the last summer holidays from a picture of palm trees) is supported by pattern completion. Wilfredor, Wikimedia Commons, licensed by CreativeCommons-Lizenz by-sa-2.0-de, URL: http://creativecommons.org/licenses/by-sa/2.0/de/legalcode

Memory impairment is commonly accompanied by hippocampal disturbances due to neurological disorders or healthy aging (Bartsch & Wulff, 2015; Small, Schobel, Buxton, Witter, & Barnes, 2011). However, there are no suitable lesion models to show the contribution of the human hippocampus to pattern separation and completion processes. Also, mechanisms that stabilize pattern separation and completion are largely unknown. The studies presented in this thesis aimed at showing the mechanistic contribution of the human hippocampus to pattern separation processing and demonstrating the neurobiological processes within the hippocampus during consolidation by the example of sleep. Those aims were achieved by the investigation

of natural hippocampal lesions models in memory impaired patients with selective hippocampal damage, and by the examination of the effect of sleep on pattern separation.

1.1 The role of the hippocampus in memory

The famous patient HM became amnesic because of a surgical removal of most of both hippocampi due to epilepsy treatment (Scoville & Milner, 1957). HM was impaired in forming new memories and retrieving those of current personal experiences. However, remote memories, technical skills as well as general intelligence were preserved (Scoville & Milner, 1957). Those studies provided the first evidence that different entities of memory are dependent on different brain structures. The characterization of the types of memories that are reliant on the hippocampal formation was promoted by the examination of memory deficits in a range of amnesic syndromes (Zola-Morgan, Squire, & Amaral, 1986). Those studies have shown that the medial temporal lobe was critical for the recollection of personal past experiences and the retrieval of previously learned facts with regard to general knowledge (Squire, Knowlton, & Musen, 1993; Squire & Zola, 1996; Squire & Zola-Morgan, 1991; Tulving, 1991). Patient HM's preserved abilities lead to the presumption that long-term memory can be divided into different components that operate on different brain systems (Squire et al., 1993). Personal past experiences and the integration of those events into a spatiotemporal context form episodic memories (Tulving, 1991). On the other hand, factual and general knowledge are subsumed under semantic memories (Tulving, 1972). Those two types of memories can be consciously recollected; they are referred to as explicit memories and assigned to the declarative memory system (Cohen & Squire, 1980; Graf & Schacter, 1985). Non-declarative forms of memory are subsumed under various implicit processes including procedural memory, skill learning, priming, and conditioning (Milner, Squire, & Kandel, 1998; Squire, 1986; Squire & Wixted, 2011; Squire & Zola, 1996). In contrast to declarative memory, non-declarative memories are independent from the

medial temporal lobe and mediated by various brain systems. Procedural and skill learning mostly rely on corticostriatal areas, whereas priming is associated with the neocortex depending on the mode of sensory input (Squire et al., 1993). Similarly, regarding conditioning, the neural substrate is dependent on the task at hand. For instance, fear conditioning is dependent on emotional responses that involve the amygdala, whereas conditioned muscular responses activate cerebellar areas (Squire & Zola-Morgan, 1991). Regarding the analysis of pattern separation in the human hippocampus and its neural substrates on the subfield level, the studies conducted in this thesis will concentrate on hippocampal processing in declarative memory.

1.2 Formation and consolidation of hippocampus-dependent memories

The hippocampus is involved in all stages of memory formation that include encoding, consolidation, and retrieval (Frankland & Bontempi, 2005; Squire, 1992). For accurate encoding and retrieval of past events the hippocampus performs the processes of pattern separation and completion (O'Reilly & McClelland, 1994). By means of pattern separation, similar and ambiguous information are rapidly encoded and the overlap of activity pattern is reduced (Marr, 1971; McClelland et al., 1995). Pattern separation reduces interference by orthogonalization (i.e. decorrelation) of similar inputs into distinct, non-overlapping representations and small differences are amplified into large differences (Knierim & Neunuebel, 2016). This process thus allows the hippocampus to separately store sequentially processed, overlapping input and minimizes the loss of previously stored information due to catastrophic interference (McClelland et al., 1995). In addition, for later retrieval, during pattern completion the hippocampus can reinstate a previously stored pattern by means of incomplete or degraded cues (Norman & O'Reilly, 2003). This process enables the extraction of generalities from new and previously encoded representations (Yassa & Stark, 2011; Figure 1-2).

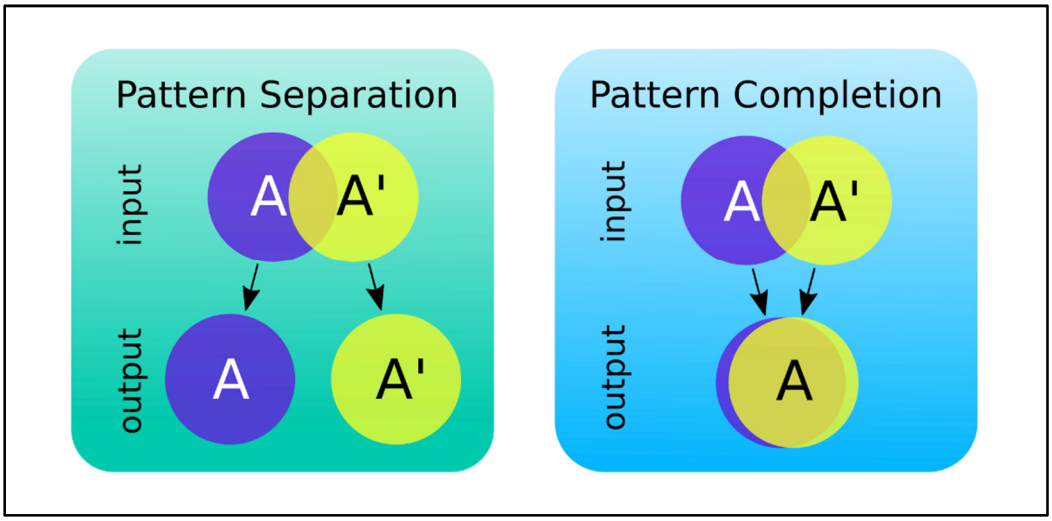

Figure 1-2. Concept of pattern separation and completion modified after Yassa and Stark (2011). Pattern separation makes similar input pattern (i.e., A and A') more distinct and reduces the overlap between representations. In contrast, pattern completion increases the overlap between two similar input patterns.

After an initial experience, neural patterns of perceptual information are encoded in the primary and associative cortices – in this connection, the hippocampus collects and integrates information of multiple features from those different areas into one memory trace (Frankland & Bontempi, 2005; Morris et al., 2003). At this stage, the memory representation is rather labile and susceptible to interference (Müller & Pilzecker, 1900). To establish long-term memories after encoding, the representations must be set to more stable and permanent traces within the process of consolidation (Dudai, 2004; Müller & Pilzecker, 1900). Memory consolidation takes place on two levels (Frankland & Bontempi, 2005):

System consolidation is considered as a temporal change of a memory's dependence on the hippocampus to more distributed neocortical areas (McClelland et al., 1995). That the role of the hippocampus in memory is time-limited has been proposed due to the finding that hippocampal damage often causes malfunction of recent but not remote memories (Scoville & Milner, 1957). In this context, the standard two-stage model of memory consolidation suggests two complementary learning systems: the hippocampus rapidly encodes new information for

temporary storage, whereas the neocortex serves as a slow-learning long-term store (Marr, 1971; McClelland et al., 1995). The gradual redistribution from the hippocampus to neocortex is reliant on repeated reactivation of the memory trace (Dudai, 2004) and has been proposed to be most effective during quiet wakefulness and sleep, where encoding demands are absent and the interference level is low (Marr, 1971; O'Reilly & McClelland, 1994).

Complementing the system level, memory consolidation also takes place at the synaptic level (Dudai, 2004). The formation of long-term memories is dependent on a reorganization of synaptic connectivity (i.e., strengthening or weakening) as the basis for synaptic plasticity (see Redondo and Morris (2011) for a review). This rather fast cellular process is thought to support the slower mechanisms of system consolidation (Dudai et al., 2015). Encoding induces synaptic plasticity via long-term potentiation (LTP; Bliss & Lomo, 1973) or long-term depression (LTD; Dudek & Bear, 1992). Generally, LTP is thought to increase the strength of the presynaptic transmission as well as the activation of the postsynaptic cells (Bliss & Collingridge, 1993; Hebb, 1949), whereas LTD decreases the synaptic transmission (Dudek & Bear, 1992; Manahan-Vaughan, 1997). The modification of synapses critically underlies the temporal order of the spiking of the pre- and postsynaptic synapse (Abbott & Nelson, 2000). The rule of this so-called spike-timing dependent plasticity proves that synaptic connectivity is influenced by LTP, when the postsynaptic neuron is activated shortly after the presynaptic neuron. However, the synaptic transmission is depressed via LTD when the postsynaptic neuron is activated before the presynaptic neuron (Bi & Poo, 1998; Markram, Lübke, Frotscher, & Sakmann, 1997).

1.3 Anatomical organization of the hippocampal formation and connectivity within the tri-synaptic circuit

The critical role of the hippocampus in processing new mnemonic information and forming stable and permanent episodic memories relies on its complex circuit structure and connectivity.

The combination of a unique anatomy with cytoarchitectonically distinct subfields and a special neural organization of intrinsic connections support the spectrum of hippocampal cognitive and mnemonic functions (Amaral & Lavenex, 2007).

Originally, the neuroanatomist Rafael Lorente de Nó defined the hippocampus solely as the cornu ammonis (CA) region (Lorente de Nó, 1934). In recent literature, the term 'hippocampus' varies across broader definitions that include all areas of the hippocampal formation, meaning the five further regions of the medial temporal lobe: dentate gyrus (DG), entorhinal cortex (EC), subiculum, presubiculum, and parasubiculum (Insausti & Amaral, 2004). It became standard to at least relate to the adjacent regions of the CA area - DG and subiculum - when speaking of the 'hippocampus' as is the case in this thesis.

The hippocampal formation processes multidimensional input from the limbic system and subcortical areas with backprojections to cortical areas (Amaral & Lavenex, 2007; Amaral & Witter, 1989; Lavenex & Amaral, 2000). The so-called hippocampal tri-synaptic circuit is characterized by unidirectional excitations from EC to DG over CA3 to CA1 (Amaral & Witter, 1989). Information processing starts with projections from the EC layer II neurons to the molecular layer of the DG and area CA3 via the perforant path (Steward & Scoville, 1976; Witter & Amaral, 1991). CA1 receives projections from EC layer III neurons via the perforant path at temporal levels and the alvear path at more septal levels (Deller, Adelmann, Nitsch, & Frotscher, 1996). Within the unidirectional circuit structure, CA3 neurons also get projections from DG granule cells that forward information via mossy fibers (Blackstad, Brink, Hem, & Jeune, 1970; Rosene & Van Hoesen, 1977). From CA3, information are processed via Schaffer collaterals to CA1 (Rosene & Van Hoesen, 1977; Swanson, Wyss, & Cowan, 1978). With its recurrent collateral input, CA3 forms an autoassociative network, where axons fire back to dendrites of neurons within CA3 creating a recursive feedback loop (Amaral, Ishizuka, & Claiborne, 1990; Amaral & Witter, 1989; Witter, 2007). CA1 pyramidal cells mainly forward

information to the subiculum (Amaral, Dolorfo, & Alvarez-Royo, 1991; Rosene & Van Hoesen, 1977). The main output of the hippocampus and backprojection to the EC originates in CA1 and the subiculum, which are linked to deep layers (i.e. layer V/VI) of the EC (Steward & Scoville, 1976; Swanson & Cowan, 1977; Tamamaki & Nojyo, 1995; Witter, 1993; Figure 1-4).

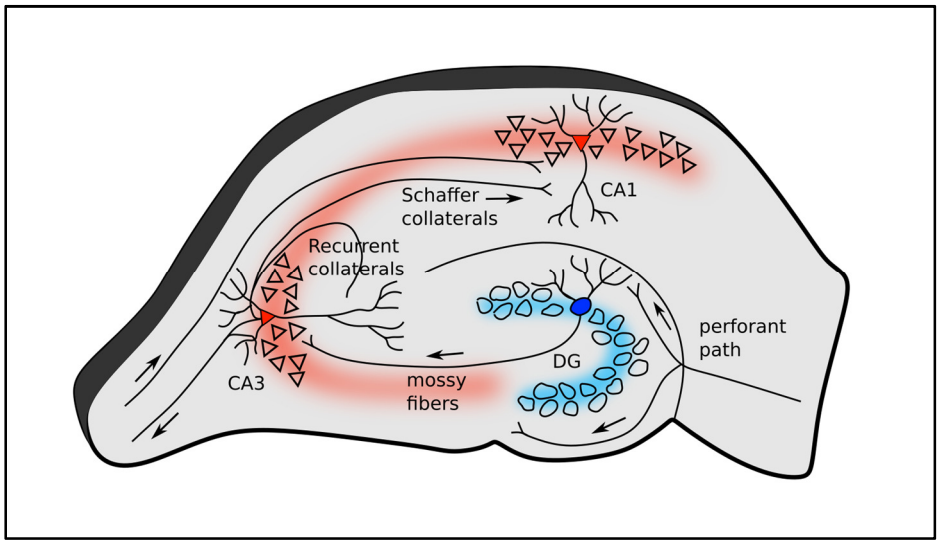

Figure 1-3. Schematic view of the hippocampus with intrinsic subfield connections presented by the example of the rat brain modified after Yassa and Stark (2011). Perforant path input from the EC (not shown) projects to the DG and CA3. Mossy fibers project from the DG to the area CA3. Backprojections from CA3 on its own neurons are formed by recurrent collaterals. Schaffer collaterals serve as input from CA3 to the CA1 region.

The reported information about the hippocampal architecture and especially its connectivity are mostly derived from experimental studies in rodents and non-human primates. However, memory research demonstrated high similarities in the structure and function across mammalian hippocampi (Clark & Squire, 2013). The described anatomy has thus a great significance for research in humans and provides a basis for discussions and interpretations regarding the involvement of hippocampal structure and function in human cognition and behavior.

1.4 Neural substrates of pattern separation and completion in the hippocampus

Hippocampal memory regarding precise encoding and correct retrieval of past events is facilitated by pattern separation and completion computations (O'Reilly & McClelland, 1994). Those essential functions are a significant subordinate of the unique molecular organization and connectivity of different hippocampal subfields. In this context, computational models assume that the DG and CA3 are especially implicated in pattern separation and completion computations (Marr, 1971; McClelland et al., 1995; Rolls, 2016). Pattern separation reduces similarity between input and output patterns so that similar representations are transformed to dissimilar, non-overlapping representations (McClelland et al., 1995; Yassa & Stark, 2011; Figure 1-4). The DG is thought to accomplish pattern separation by providing distinct neural codes within the feedforward pathway from EC to the DG and to CA3 (O'Reilly & McClelland, 1994). First, similar input patterns from the EC are spread over a five times larger cell population of the DG granule cells (200,000 EC neurons vs. 1,000,000 DG granule cells in the rat) (Amaral et al., 1990) so that the patterns are decorrelated and less overlapping on the level of the DG (O'Reilly & McClelland, 1994; Rolls, 2007). Second, the firing activity of dentate granule cells is very sparse (i.e. relatively few neurons are active, but still provide powerful connections) leading to a small number of mossy fiber connections between DG and CA3 (Kesner & Rolls, 2015). Together with a higher number of DG granule cells compared with CA3 pyramidal cells (160,000 in the rat) (Amaral et al., 1990) an even sparser activity in CA3 is produced (Kesner & Rolls, 2015; Rolls, 2007).

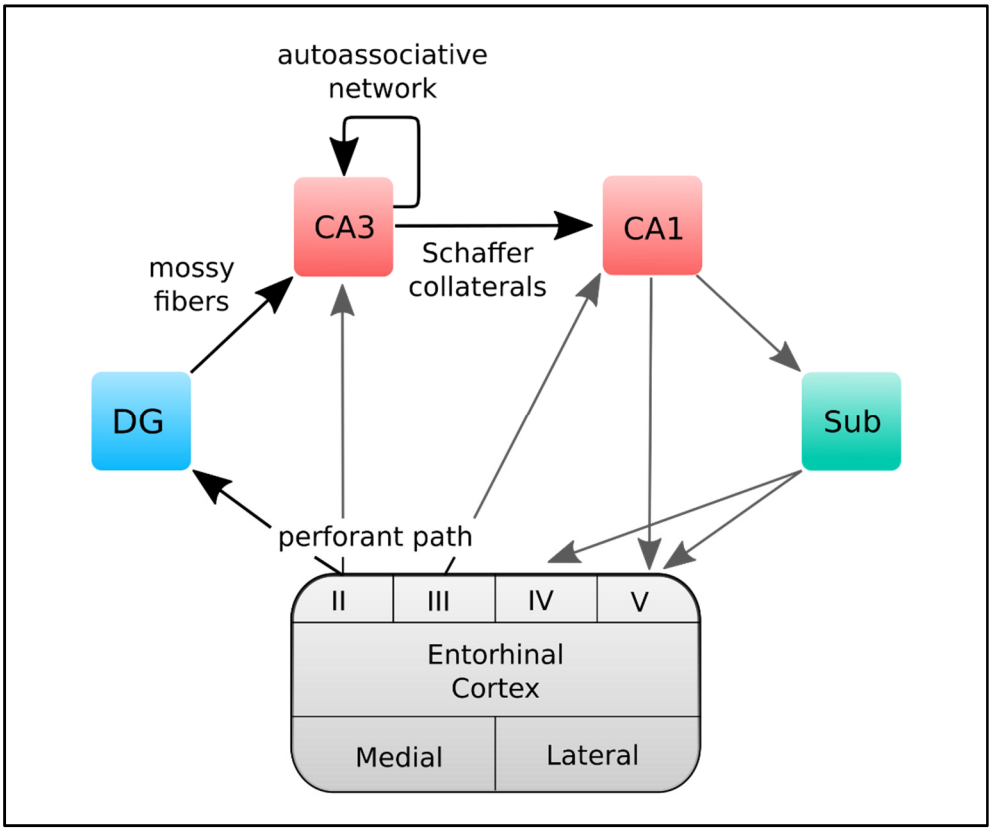

Figure 1-4. Schematic representation of the hippocampal tri-synaptic circuit and connections to the EC (modified after Clark and Squire (2013)). Black arrows highlight the projections that hallmark the feed-forward circuit structure that is especially involved in hippocampal mnemonic functions (i.e. pattern separation and completion). EC layer II projects to both DG and CA3 via the perforant path. DG projects to CA3 via mossy fiber projections. CA3, in turn, forms an autoassociative network via backprojections on itself. CA1 receives projections from EC layer III via the perforant path as well as from CA3 via the Schaffer collaterals. CA1 as the main output region of the hippocampus projects the hippocampal readout to the subiculum, that is like CA1 connected to deeper layers of the EC (see Yassa and Stark (2011) for a review). Sub = Subiculum.

During pattern completion, a previously encoded cortical activity pattern is reinstated by reactivation of partial or noisy versions of the pattern by a sensory cue (O'Reilly & McClelland, 1994; Rolls, 2016; Figure 1-5). Here, most theories suggest that pattern completion is dependent on the area CA3 and its intrinsic connections generated by recurrent collaterals that function as an auto-associative network (McClelland et al., 1995; Treves & Rolls, 1992). The auto-associative memory within CA3 is complemented by two excitatory afferents: the mossy fiber input of dentate granule cells initiates storage of information, whereas the perforant path input from

the EC promotes retrieval of preexisting neural ensembles (Treves & Rolls, 1992). The relative strength and plasticity within the synaptic connections regulate the function of CA3. During storage, the orthogonalized input from the DG via strong mossy fiber projections outperform the recurrent collateral activations within the CA3 network (Treves & Rolls, 1992). Thus, new firing patterns are stored within recurrent collateral connections in CA3 that represents novel input (Rolls, 2013; Treves & Rolls, 1992, 1994). The perforant path input from the EC provides the cues for retrieval. Importantly, due to the recurrent collateral system and the large number of perforant path inputs, even a partial cue, that does not have to be very powerful, suffice for retrieval within CA3 (Treves & Rolls, 1992).

1.5 Evidence from rodent studies: electrophysiological recordings during exploration of different and similar environments

The assumptions made by computational models have repeatedly been confirmed by studies in rodents. To provoke pattern separation and completion in the rodent hippocampus, different behavioral setups are commonly used. For instance, the animals explore gradually morphed environments from circular to rectangular shapes (J. K. Leutgeb, Leutgeb, Moser, & Moser, 2007; Muller & Kubie, 1987) or are exposed to one environment where local and distal cues are rotated (Lee, Yoganarasimha, Rao, & Knierim, 2004; Neunuebel & Knierim, 2014). The simultaneous recording of neural activity in different subfields of the hippocampus using single cell recordings or immediate early gene techniques rendered proofs of a functional specialization of hippocampal subfields (Lee et al., 2004; S. Leutgeb, Leutgeb, Treves, Moser, & Moser, 2004; Vazdarjanova & Guzowski, 2004). The comparison of CA3 and CA1 ensemble firing of freely moving rats in two different environments showed high changes in the firing of neurons in CA3 (i.e. pattern separation) and a larger overlap of firing ensembles in CA1 (S. Leutgeb et al., 2004). A similar study, but using an installation with low environmental change, demonstrated homogenous firing responses in CA3 compared with CA1 (Lee et al., 2004). Those two

studies together suggest that CA3 exhibits different tuning functions considering input similarity (Guzowski, Knierim, & Moser, 2004; Yassa & Stark, 2011). In this context, using immediate-early gene-based imaging of the rodent brain confirmed that CA3 and CA1 showed a high overlap of firing ensembles when the environmental change was small. Indeed, an even higher overlap was observed in CA3 (i.e. pattern completion). By contrast, exposure to two completely different enclosures providing a large change of the environmental input caused a higher rate of non-overlapping firing patterns in both CA3 and CA1. The overlap of neural ensembles was even lower in CA3 compared with CA1 (i.e., pattern separation in CA3; Vazdarjanova & Guzowski, 2004). Those results indicate different transfer functions of CA3 and CA1: Hippocampal CA3 neurons perform pattern completion when the environmental change is small, and, conversely, exhibit pattern separation when the environmental change is large. Conversely, the input-output function of CA1 can be thought of as a linear transformation (Guzowski et al., 2004; Figure 1-5). Regarding the function of the hippocampal DG, mice with dysfunctional granule cells due to cell-specific NMDA receptor knock-out, were unable to distinguish similar contexts in a standard fear conditioning paradigm (McHugh et al., 2007). Using the same apparatus including local and global cues as I. Lee et al. (2004), Neunuebel and Knierim (2014) provided evidence that the DG implements pattern separation on its EC inputs, whereas CA3 performs pattern completion on the inputs received from the DG and EC. The difference of neural pattern separation in the DG and CA3 has vividly been illustrated by means of spike recordings in freely moving rats. In gradually morphed environments from circular to squared borders (or vice versa), only a slight deformation of the enclosure was enough to strongly separate the dentate granule cells' firing pattern. However, a major distortion of the environment was needed to show pattern separation in CA3 (J. K. Leutgeb et al., 2007; Figure 1-5).

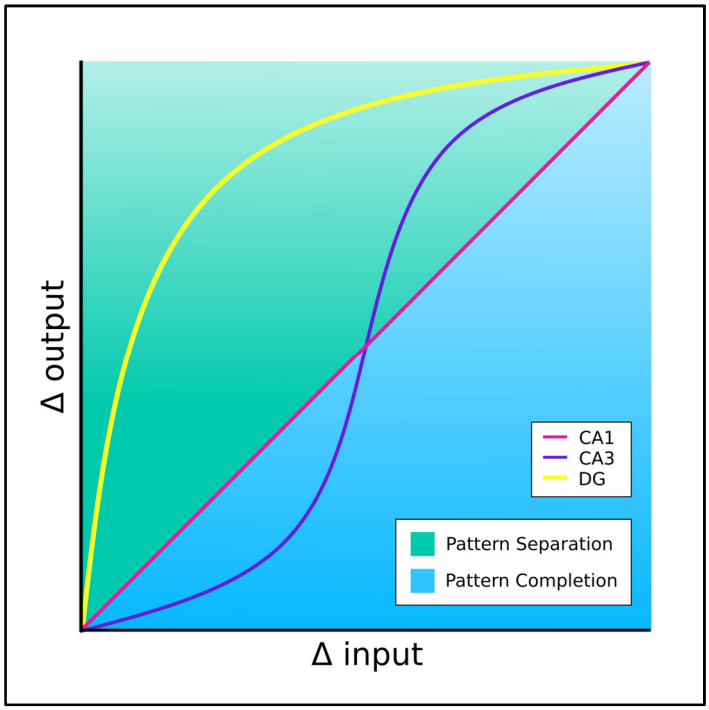

Figure 1-5. Expected input/output transfer function of the DG, CA3, and CA1 based on rodent studies as summarized in Guzowski et al. (2004). The DG shows a non-linear input-output transformation as it is more sensible regarding the performance of pattern separation when input changes are small. CA3 is represented by a sigmoidal curve with performing pattern completion when environmental changes are small, and pattern separation when input changes are large. CA1 is described by a linear transfer function. Figure modified after Yassa and Stark (2011).

1.6 Evidence from human studies: behavioral pattern separation and fMRI

Inspired by animal literature, behavioral pattern separation in humans is commonly measured by means of specific match-to-sample tasks that include similar stimuli (Bakker, Kirwan, Miller, & Stark, 2008; Berron et al., 2016; Holden, Hoebel, Loftis, & Gilbert, 2012; Kirwan & Stark, 2007; Stark & Stark, 2017; Stark, Yassa, Lacy, & Stark, 2013). Generally, those memory tests comprise an encoding phase of items of one specific category (i.e., faces, objects, or scenes) and a retrieval phase, where the learned items have to be distinguished from items that are similar, commonly called ‘lure items’ (see Berron et al. (2016); Kirwan and Stark (2007);

Stark et al. (2013) for examples). Importantly, those similar lures tax hippocampal pattern separation so that correctly identifying lures as similar implies successful pattern separation abilities, whereas confusing similar lures with their corresponding targets indicates a bias towards pattern completion (Lacy, Yassa, Stark, Muftuler, & Stark, 2011; Stark et al., 2013; Yassa et al., 2010).

The first study that provided evidence for a functional specialization of hippocampal subfields regarding pattern separation in humans used the Mnemonic Similarity Task (MST; Kirwan & Stark, 2007; Stark et al., 2013) during fMRI. Here, Bakker et al. (2008) measured lure-related novelty signals in the medial temporal lobe by making use of repetition-suppression effects. In short, if the activity in response to a lure stimulus was more similar to the response to a novel stimulus, the region was supposed to be engaged in pattern separation. If the activity response to a lure stimulus was more similar to the response to an old stimulus, the region was suggested to perform pattern completion. It has been found that the CA3/DG region responded akin to pattern separation, whereas the hippocampal CA1 region, subiculum, and entorhinal cortex, as well as the parahippocampal cortex showed activity that was related to pattern completion. Complementing those findings, Lacy et al. (2011) examined gradual levels of similarity of lures to the corresponding target. The results were comparable to the findings in rodents, where the testing environments were gradually morphed (J. K. Leutgeb et al., 2007). The CA3/DG region was sensitive to small changes, so that activity levels were high for even very similar lures. In contrast, in the hippocampal CA1 activity was mapped in a linear fashion: Low activity levels responding to highly similar items and higher activity levels responding to highly dissimilar items (Lacy et al., 2011). Another study that used scenes instead of objects as the behavioral paradigm as well as a higher resolution due to 7 Tesla fMRI, demonstrated that CA1 showed a decreased activation regarding old items in comparison to new items, whereas only the DG

showed an increased activation regarding lures (Berron et al., 2016). Importantly, the high resolution allowed separating the DG region from adjacent CA3 to make individual statements regarding both regions. However, there was no lure-specific activity in the CA3 region. This study was the first to provide evidence for pattern separation in the DG separated from CA3 in humans.

1.7 Physiology of sleep and sleep-dependent memory consolidation

Sleep is a natural and innate state characterized by reduced reactivity to the external world and loss of consciousness. The sleep-wake cycle is regulated by homeostatic mechanisms that cause a rebound of sleep after loss or deprivation (Borbély & Achermann, 1999; Borbély, Daan, Wirz-Justice, & Deboer, 2016). While the relatively passive state of sleep has long been questioned regarding its function, and the exact mechanisms are still not fully understood, it is widely acknowledged that sleep is critically involved in the adaptation to environmental demands by regulation of metabolic processes, energetic utilization, and immune functions (Rasch & Born, 2013). With regard to the sleeping brain, specific field potential oscillations hallmark different sleep stages that alternately occur in 90 minutes cycles (Diekelmann & Born, 2010). Typically, the first half of nocturnal sleep (early sleep) is predominated by slow wave sleep (SWS), whereas rapid eye movement (REM) sleep prevails in the second half (late sleep; Diekelmann & Born, 2010; Stickgold, 2005). SWS is represented by high amplitude and low frequency slow oscillations (peak frequency at ~ 0.75 Hz), whereas REM sleep is accompanied by ponto-geniculo-occipital (PGO) waves and wakelike hippocampal theta activity (4-8 Hz). As the term indicates, REM sleep is reflected by phasic rapid eye movements and muscle atonia (Rasch & Born, 2013). Concerning the memory function of sleep, SWS and REM sleep have been found to be differentially involved: It is widely acknowledged that SWS supports the formation and

consolidation of hippocampus-dependent declarative memories, whereas REM sleep specifically processes non-declarative memory content (Maquet, 2001). For instance, after SWS verbal (Drosopoulos, Wagner, & Born, 2005), emotional (Groch, Zinke, Wilhelm, & Born, 2015), and spatial memories (Plihal & Born, 1999) were stabilized, while REM-rich periods of sleep have been found to facilitate procedural memory and the effect of priming (Plihal & Born, 1997, 1999).

Declarative memory consolidation is critically reliant on the rapid encoding of new information in the hippocampus and transformation to neocortical long-term stores (Frankland & Bontempi, 2005; McClelland et al., 1995). One of the first direct evidences that neuronal ensemble activity is gradually transferred across brain regions has been provided by studies in rats (Buzsáki, 1996, 1998). It is assumed that the core mechanism for this transfer is based on repeated reactivation of newly encoded memories providing an active system consolidation process (Frankland & Bontempi, 2005; Marr, 1971; McClelland et al., 1995). Those reactivations occurring during slow wave sleep (SWS) result in reorganization and stabilization of representations in the long-term store (Frankland & Bontempi, 2005; Rasch & Born, 2013).

During SWS, the neocortical slow oscillation (SO) at a frequency of ~ 0.75 Hz (Steriade, Contreras, Curró Dossi, & Nuñez, 1993; Steriade, Nuñez, & Amzica, 1993), serves as a frame for the synchronization of simultaneously present neural activity in the thalamus and hippocampus (Mölle & Born, 2011; Mölle, Yeshenko, Marshall, Sara, & Born, 2006; Steriade, 2006, 2006). First, the part of oscillatory dynamics that are essentially involved in the active system consolidation process are the thalamo-cortical spindles (~ 10-15 Hz oscillations) that emerge in the form of two types. Fast spindles (~ 13-15 Hz) are distributed over central and parietal regions, whereas slow spindles (~ 10-12 Hz) concentrate on frontal areas (De Gennaro & Ferrara, 2003). Second, hippocampal sharp-wave ripples play a crucial role in memory transfer from hippo-

campus to neocortex (Buzsáki, 1996). Sharp-waves are generated in the hippocampal CA3 region and are overlaid by ripples generated in CA1 (~200 Hz high-frequency bursts in rodents (Buzsáki, Horváth, Urioste, Hetke, & Wise, 1992) and 80-100 Hz oscillations in human hippocampal recordings (Clemens et al., 2007, 2011)). Those two events jointly form sharp-wave ripples (Buzsáki, 1986; Csicsvari, Hirase, Czurkó, Mamiya, & Buzsáki, 1999; Girardeau & Zugaro, 2011). In particular, sharp-waves accompany the repeated reactivation of previously encoded memory traces during sleep (Sirota & Buzsáki, 2005; Wilson & McNaughton, 1994). The neocortical SO cause a temporal grouping of spindles and sharp-wave ripples into hyperpolarizing down-states and depolarizing up-states (Haider, Duque, Hasenstaub, & McCormick, 2006; Shu, Hasenstaub, & McCormick, 2003; Steriade, 2006; Steriade, Timofeev, & Grenier, 2001): During the hyperpolarizing down-states of the SO the generation of thalamo-cortical spindles and hippocampal sharp-wave ripples is inhibited, whereas the depolarizing up-state releases a new increase (rebound effect) of spindle and sharp-wave ripple activity (Clemens et al., 2007; Isomura et al., 2006; Mölle & Born, 2011; Peyrache, Battaglia, & Destexhe, 2011). The synchronization further allows for the formation of spindle-ripple events that mediate the hippocampal to neocortical transfer (Clemens et al., 2011; Siapas & Wilson, 1998; Sirota & Buzsáki, 2005; Sirota, Cicsvari, Buhl, & Buzsáki, 2003; Staresina et al., 2015). Here, ripples are temporally nested into spindle troughs (Clemens et al., 2011; Siapas & Wilson, 1998; Figure 1-6). During this process, the SO orchestrates the linkage of hippocampal, thalamic, and neocortical regions under a top-down control allowing communication (Buzsáki, 1996). This so-called hippocampal-neocortical dialogue forms the basis for system consolidation of hippocampus-dependent memories (Frankland & Bontempi, 2005). On the synaptic level, it is assumed that due to homeostasis within synaptic connections, the synaptic strength after encoding during wakefulness is downscaled in subsequent periods of sleep (Tononi & Cirelli, 2003, 2006). Higher demands on encoding mechanisms during wakefulness result in higher amplitudes of

SO during SWS (Huber, Ghilardi, Massimini, & Tononi, 2004; Mölle, Marshall, Gais, & Born, 2004). Downscaling of the synaptic strength leads to sparing of energy and space by means of deterioration of weakly encoded memories and enhancement of strongly encoded memories, and, importantly, to a facilitation of new encoding after sleep (Dash, Douglas, Vyazovskiy, Cirelli, & Tononi, 2009; Tononi & Cirelli, 2006; Vyazovskiy, Cirelli, Pfister-Genskow, Faraguna, & Tononi, 2008). Whether those processes of consolidation leading to a redistribution of memory representations or a facilitation of encoding after sleep also apply to hippocampal pattern separation is not clear.

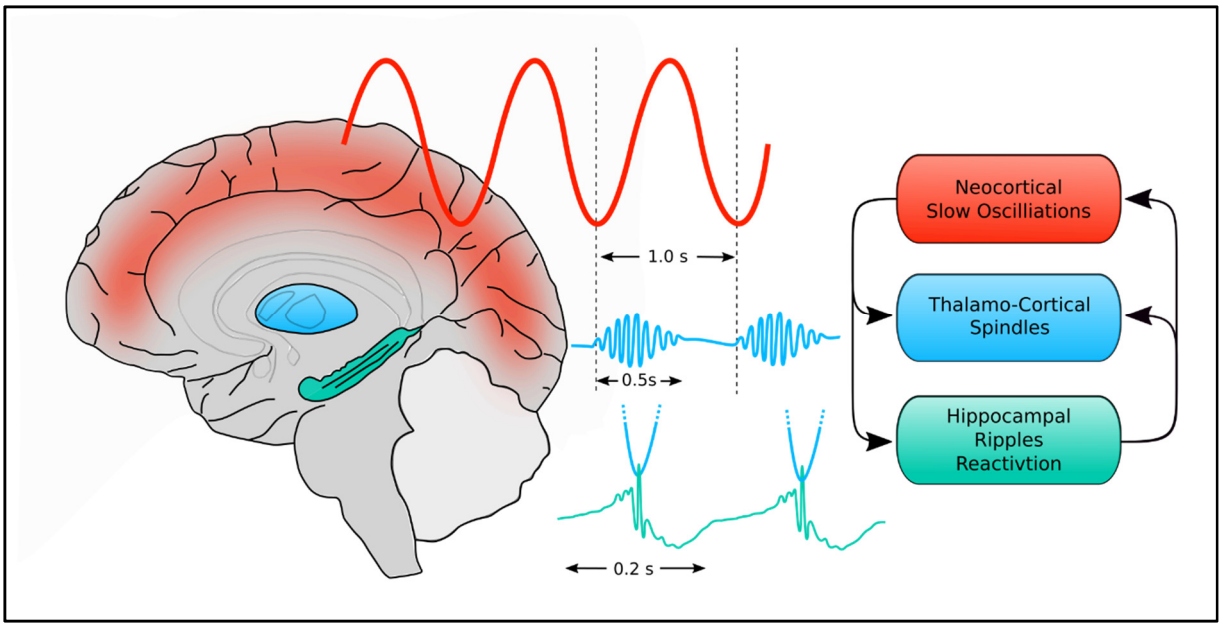

Figure 1-6. Schematic model of the hippocampal-neocortical dialogue. During SWS neocortical SO temporally couple thalamo-cortical spindles and hippocampal sharp-wave ripples. This synchronization coordinates the formation of spindle-ripple events that mediate the hippocampal to neocortical transfer. Repeated reactivation of newly encoded memories is accompanied by sharp-wave ripples. The dialogue during SWS thus forms the basis for system consolidation of hippocampus-dependent memories. Figure inspired by Born and Wilhelm (2012).

1.8 Aims and hypotheses

Theories based on the computational models of pattern separation processing and rodent studies that measured the behavioral outcome on the basis of hippocampal place cell remapping both

conclude that the DG/CA3 network is critically involved in pattern separation (Lee et al., 2004; J. K. Leutgeb et al., 2007; Treves & Rolls, 1994). Studies in humans support this finding by means of fMRI investigations that measured the activity of hippocampal areas during behavioral paradigms that tax pattern separation (Bakker et al., 2008; Berron et al., 2016; Lacy et al., 2011). Although regional neural activity of the hippocampus can be displayed by means of fMRI (see Berron et al. (2016) for an example), those studies lack information regarding mechanistic aspects of causality about the subfield-specific computational processes and the causal role of hippocampal structure and its function. Therefore, we examined two hippocampal lesion models, where specific hippocampal subfields are impaired due to neurological diseases. In Study I, the transient global amnesia (TGA) served as a model for a selective disruption of hippocampal CA1 neurons. TGA is characterized by a cognitive deficit limited to a clear anterograde amnesia in the acute phase that is resolved within 24 hours (Bartsch et al., 2010; Hodges & Warlow, 1990). Typically, focal lesions restricted to area CA1 accompany the disturbance (Bartsch, Alfke, Deuschl, & Jansen, 2007; Bartsch et al., 2006). It was hypothesized that a selective impairment of CA1 during TGA causes a deficit in pattern separation abilities. To further reveal the causal role of hippocampal subfield contributions to pattern separation, an extremely rare patient cohort positive for LGI1 antibodies, who develop limbic encephalitis with persisting memory deficits (Bettcher et al., 2014; Butler et al., 2014) and structural damage to the hippocampal system (Irani et al., 2011, 2013; Malter et al., 2014), was examined in a second study. The aim of Study II was to predict pattern separation performance from DG and CA3 that are predominantly affected by neuroinflammatory changes due to LGI1 encephalitis (Finke et al., 2017; Miller et al., 2017). The hypothesis included that inflammatory lesions within the DG and CA3 subfields correlate to hippocampal pattern separation.

Surprisingly, few attention has been paid to the neurobiological role of the hippocampus and its subfield-specific computations during consolidation. Therefore, Study III concentrates on

this gap in research: Pattern separation abilities were tested before and after a night of sleep compared to a delay period covering wakefulness. It was hypothesized that the effect of sleep-dependent consolidation would be reflected in stabilized pattern separation after post-encoding sleep. Participants and patients in all three studies, were tested using a mnemonic similarity task (MST) that has been validated as assessing behavioral pattern separation and taxing the associated hippocampal function of neural pattern separation (Bakker et al., 2008; Lacy et al., 2011; Stark et al., 2013).

In summary, the three studies conducted within this thesis were expected to clarify the relation of the hippocampus to pattern separation in describing structure-function relationships in memory as well as consolidation.

2 Study I

Transient hippocampal CA1 lesions in humans impair pattern separation performance

A. Hanert, A. Pedersen, and T. Bartsch

Published in *Hippocampus, January 2019, doi: 10.1002/hipo.23073, [Epub ahead of print]*

2.1 Abstract

Day-to-day life involves the perception of events that resemble one another. For the sufficient encoding and retrieval of similar information, the hippocampus provides two essential computational processes. Pattern separation refers to the differentiation of overlapping memory representations, whereas pattern completion reactivates memories based on noisy or degraded input. Evidence from human and rodent studies suggest that pattern separation specifically relies on neuronal ensemble activity in hippocampal subnetworks in the dentate gyrus and CA3. Although a role for CA1 in pattern separation has been shown in animal models, its contribution in the human hippocampus remains elusive. In order to elucidate the contribution of CA1 neurons to pattern separation, we examined 14 patients with an acute transient global amnesia (TGA), a rare self-limiting dysfunction of the hippocampal system showing specific lesions to CA1. Patients' pattern separation performance was tested during the acute amnestic phase and follow-up using an established mnemonic similarity test. Patients in the acute phase showed a profound deficit in pattern separation ($p < .05$) as well as recognition memory ($p < .001$) that recovered during follow-up. Specifically, patients tested in a later stage of the amnesia were less impaired in pattern separation than in recognition memory. Considering the time dependency of lesion-associated hippocampal deficits in early and late acute stages of the TGA, we

showed that the pattern separation function recovered significantly earlier than recognition memory. Our results provide causal evidence that hippocampal CA1 neurons are critical to pattern separation performance in humans.

Keywords: CA1, hippocampus, pattern separation, recognition memory, transient global amnesia

2.2 Introduction

In daily life, we are constantly exposed to episodes that resemble one another in temporal, spatial and contextual features. The formation of episodic memories from this mnemonic information requires the detection of novelty and similarity. The hippocampus provides two operations that support rapid storage of this new mnemonic information separately from preexisting similar representations. The function of pattern separation is to produce non-overlapping representations of similar neuronal input in order to reduce interference and to facilitate the formation of a novel memory representation. On the contrary, pattern completion supports retrieval of memories based on incomplete or degraded cues (Knierim & Neunuebel, 2016; McClelland, McNaughton, & O'Reilly, 1995; Yassa & Stark, 2011). Recent progress in rodent physiology led to critical advances and reformulation of the mechanistic aspects of pattern separation and completion processes in the hippocampus. Here, the activation of hippocampal subnetworks has been found to be associated with distinct mnemonic functions. The hippocampal dentate gyrus (DG) is particularly involved in pattern separation whereas CA3 has been shown to be capable of performing both, pattern separation and completion computations depending on the similarity of the sensory input (Guzowski, Knierim, & Moser, 2004; I. Lee, Yoganarasimha, Rao, & Knierim, 2004; J. K. Leutgeb, Leutgeb, Moser, & Moser, 2007; Neunuebel & Knierim, 2014). The CA1 region serves as the primary output of the hippocampus that relays information from CA3/DG networks to neocortical areas (Witter & Amaral, 2004). Here, results

from a variety of rodent studies suggest a linear input-output function of CA1 neuronal activity in the discrimination of similar mnemonic information (Guzowski et al., 2004; I. Lee et al., 2004; S. Leutgeb, Leutgeb, Treves, Moser, & Moser, 2004; Vazdarjanova & Guzowski, 2004). In both rodents and humans, the role of CA1 neurons in the process of pattern separation and completion is incompletely understood. In trying to identify the contribution of CA1 to pattern separation and completion processes in humans, imaging studies showed a bias toward pattern completion in the CA1 region (Bakker, Kirwan, Miller, & Stark, 2008), whereas a later study showed that CA1 exhibits pattern separation-like activity when the change of the input increases (Lacy, Yassa, Stark, Muftuler, & Stark, 2011). Due to methodological reasons of image resolution in fMRI a fine-grained analysis of a causal relationship between hippocampal subfields and hippocampus-dependent behavior is complex. To mechanistically enlighten the contribution of individual hippocampal subfields and their operation in pattern separation and completion, specific lesion models are needed. For instance, Baker et al. (2016) provided evidence for a particular role of the DG in pattern separation in one patient with bilateral ischemic lesions in the DG. This case study provides insight into the causal contribution of hippocampal subfields to mnemonic processes and its structural foundation. To further elucidate the mechanistic contribution of CA1 to pattern separation processes, we here examined patients in the acute stage of transient global amnesia (TGA) that is a natural lesion model of hippocampal CA1 neurons (Bartsch et al., 2006, 2010). As a behavioral paradigm, we used the mnemonic similarity task (MST) that has previously been shown to validly tax hippocampal pattern separation (Hanert, Weber, Pedersen, Born, & Bartsch, 2017; Kirwan & Stark, 2007; Stark, Yassa, Lacy, & Stark, 2013). We aimed at showing that a transient hippocampal CA1 dysfunction within acute stages of a TGA impairs the operation of pattern separation highlighting the role of CA1 in the context of the formation of episodic memory.

2.3 Materials and procedure

2.3.1 Study cohort

A cohort of 14 patients (66.86 ± 2.29 years, range 53 – 80, 50% female) participated in the study. Patients presented to our neurological emergency unit during the acute phase of a TGA. Patients were diagnosed according to the criteria of a TGA (Bartsch et al., 2010; Caplan, 1985; Hodges & Warlow, 1990) that includes (a) that attacks are witnessed by an observer present for most of the attack, (b) a clear anterograde amnesia during the attack, (c) no clouding of consciousness or loss of personal identity, (d) that the cognitive impairment is limited to the amnesia, (e) no focal neurological symptoms or epileptic signs, (f) no recent history of head injuries or seizures, and (g) that the attack is resolved within 24 hr. The TGA shows a characteristic time course with an abrupt onset of the pronounced hippocampal deficit and with a gradual recovery of the hippocampal functions in the last third of the attack. Patients were studied by one neurologist who remained 24/7 on-call for this study. All patients had a standard neurological examination on admission and follow-up and underwent a structured interview to assess the time course of TGA evolution as well as clinical factors and a history of cardiovascular and neurological diseases. As a control group, 14 healthy subjects matched by age and gender (67.86 ± 2.15, 50% female) were tested. All participants gave written informed consent to the study that was approved by the Ethical Committee of the University of Kiel.

2.3.2 Materials and procedure

Patients were tested in the acute phase of the TGA (4.21 ± 0.38 hr after onset of symptoms) and in a follow-up testing procedure when fully recovered from all TGA symptoms (212.29 ± 19.28 d, at least 2 months after the acute phase). The mnemonic similarity task (MST) (Kirwan & Stark, 2007; Stark et al., 2013; https://faculty.sites.uci.edu/starklab/mnemonic-similarity-task-mst/, stand-alone version for windows v 0.8) as well as the Rey Auditory Verbal Learning Test

(RAVLT; Rey, 1941) were performed in the acute phase of the TGA, in the follow-up measurement and by the control group. TGA patients performed parallel versions of the MST (Set C in acute phase, Set D in follow-up phase) and RAVLT at follow-up. The RAVLT was used to assess the quantifiable degree and magnitude of the hippocampal deficit.

2.3.3 Mnemonic similarity task

For the assessment of behavioral pattern separation we used the MST (Kirwan & Stark, 2007; Stark et al., 2013). The encoding phase of the task consisted of 128 items displaying everyday objects that patients were supposed to identify as either indoor or outdoor object via button press on a keyboard. The following immediate test phase comprised 192 items displaying in each case one third as exact repetitions of the encoded objects (64 targets), objects similar to the encoded items (64 lures), and items that were totally new (64 foils). In this phase, participants indicated whether the objects were ‘old,’ ‘similar,’ or ‘new’ to the previously encoded targets via button press. Participants performed the whole task on a computer with items presented on the screen as color photographs on a white background for 3 s and 1 s inter-stimulus interval. Responses had to be given in the 3 s stimulus presentation for recording of data (Figure 2-1).

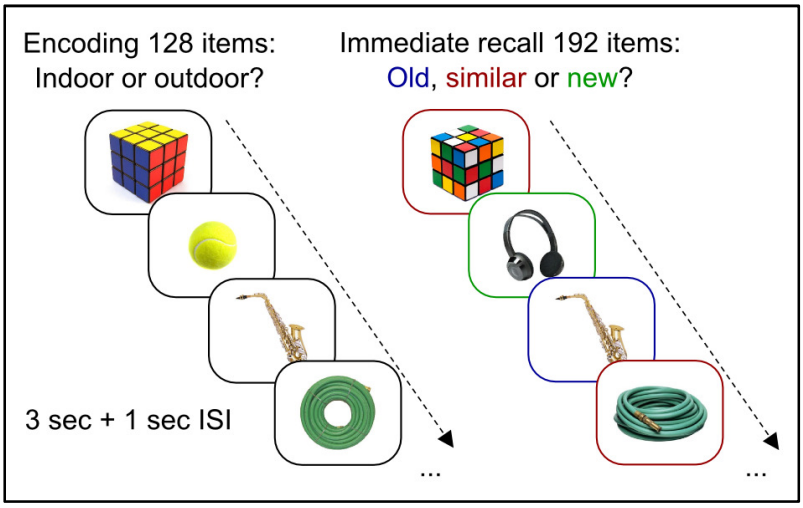

Figure 2-1. Procedure of the MST. Participants first encoded 128 items of everyday objects presented on a computer screen thereby judging whether items were indoor or outdoor objects. Thereafter participants decided whether the items were old, similar or new to the previously encoded target items in an immediate recall condition containing 192 items. The items displayed in the graph are taken from the original image data base of the MST. ISI, inter-stimulus interval.

The responses to lure items were of particular interest with the correct 'similar' response indicating successful pattern separation, whereas an 'old' response would indicate a bias toward pattern completion (Bakker et al., 2008; Lacy et al., 2011; Yassa et al., 2010). The lure objects were grouped into five degrees of similarity to a target object ranging from 1 (most similar) to 5 (least similar). Thus, pattern separation performance was also assessed as a function of lure similarity (Lacy et al., 2011; Yassa et al., 2010). In summary, the MST allows the calculation of a Pattern Separation score (PatSep score) in previous studies often termed Behavioral Pattern Separation (BPS) score or Lure Discrimination Index (LDI) (Stark & Stark, 2017; Stark et al., 2013) and a Recognition Memory (RM) score, each corrected for a response bias: (a) Pattern Separation (PatSep) score: PatSep = [p (correct 'similar' response to lures) – p (false 'similar' response to foils)], (b) RM score: RM = [p (correct 'old' response to targets) – (false 'old' response to foils)] (Stark et al., 2013; Yassa, Lacy, et al., 2011; Yassa et al., 2010).

2.3.4 Declarative memory testing

For evaluating episodic declarative memory and assessing the magnitude of the amnestic deficit as well as the severity of TGA and thus hippocampal dysfunction the RAVLT was used. Overall, the test measures immediate memory, new verbal learning, susceptibility to interference, delayed recall, and recognition. It thus enables to evaluate encoding, consolidation and retrieval of verbal memory.

2.3.5 General neuropsychological testing

Healthy controls and patients at time of the follow-up measurement completed a general neuropsychological test battery for the assessment of (a) executive functioning via Trail Making Test A and B (TMT; Reitan, 1979) (b) verbal fluency via Regensburg Word Fluency Test (RWT; Aschenbrenner, Tucha, & Lange, 2000) (c) working memory via digit span (Wechsler, 1997) and (d) an estimate of premorbid general intellectual ability via a 37-item multiple choice vocabulary test as a German equivalent of the National Adult Reading Test (Lehrl, 2005). Participants all performed within a normal range (Table 2-1). Handedness was assessed via the Edinburgh Handedness Inventory (EHI; Oldfield, 1971).

Table 2-1. Neuropsychological data of TGA patients (follow-up) and controls (mean ± SEM)

	TGA	Controls	t(df = 26)	Z	p	95% CI
Age (years)	66.86 ± 2.29	67.86 ± 2.15	-0.32	-	0.753	[-7.47, 5.47]
TMT-A	40.71 ± 3.87	41.86 ± 4.41	-	-0.23	0.839	
TMT-B	109.57 ± 19.79	85.82 ± 5.99	-	-0.09	0.946	
MWT-B	32.14 ± 0.85	29.86 ± 1.76	-	-0.40	0.701	
RWT-fore-names	27.14 ± 1.84	29.43 ± 2.47	-0.74	-	0.465	[-8.62, 4.05]
RWT-S	13.50 ± 1.29	15.71 ± 1.45	-1.14	-	0.264	[-6.20, 1,77]
Digit span total	12.86 ± 0.94	13.14 ± 0.94	-0.22	-	0.831	[-3.02, 2.45]

TMT, Trail-making test; MWT, Mehrfachwahl-Wortschatz Intelligenztest; RWT, Regensburg word fluency test, 95% confidence intervals are calculated for parametric tests.

2.3.6 Magnetic resonance imaging

Whole brain clinical MRI's of patients were acquired 24-72 h after onset of TGA symptoms when the detectability of hippocampal lesions is highest (Bartsch, Alfke, Deuschl, & Jansen, 2007) High-resolution were performed on a 3 Tesla unit (Philips Achieva) using diffusion weighted Echo Planar Imaging (DW-EPI) (TR/TE/FA = 3,234/72/90, slice thickness 2 mm, voxel size 1.67 x 2.12 x 3 mm) with subsequent maps of the apparent diffusion coefficient (ADC), as well as additional T2-weighted turbo spin echo sequences (TR/TE/FA = 4,455/100/90, slice thickness 2mm, voxel size 0.51 x 0.65 x 2 mm) transverse oblique plane parallel to the hippocampus and coronal perpendicular to the hippocampus. All images were inspected with respect to structural abnormalities in the whole brain including temporal and frontal lobe structures.

Lesions were considered a CA1 hippocampal lesion only when detectable in both DWI and T2-weighted images with hyperintense DWI and T2 lesions corresponding to identical locations within the different sectors of the cornu ammonis in the coronal plane and the rostral-occipital position within the hippocampus. MR images were visually inspected by two neuroradiologists

and one neurologist experienced in the detection of structural changes in hippocampal signals in TGA. Lesions were mapped within the different sectors of the cornu ammonis after Lorente de Nó according to the anatomical reference atlas of Duvernoy (Duvernoy, Cattin, & Risold, 2013; Lorente de Nó, 1934; Figure 2-7. a-d).

2.3.7 Statistical Analyses

For investigating differences between the TGA patients' performance in the acute phase and follow-up, repeated measures analyses of variance (ANOVAs) with condition as within subjects factor were performed.Patients' performances in both conditions (acute and follow up) were compared with the control group by means of two factorial ANOVAs with condition (acute vs. controls) or (follow-up vs. controls) as between -subjects factor. Correct responses (‘old|target’ vs. ‘similar|lure’ vs. ‘new|foil’), incorrect responses to lures (‘old’ vs. ‘new’), and performance in different degrees of lure similarity (Lure 1 to Lure 5) were compared by adding a within -subjects factor to the ANOVAs, respectively. For the analysis of differences in PatSep and RM Scores as well as the RAVLT scores between acute and follow-up paired samples t tests or Wilcoxon tests, depending on distribution, were performed. The same comparison with regard to the control group was done using independent samples t tests or Mann-Whitney-U-tests according to distribution. The Shapiro Wilk test was performed for the pretesting of normal distributions and Levene's test for assessing homogeneity of variances. Welch's t tests were performed for independent samples when variances were heterogenous. If the assumption of sphericity for repeated measures ANOVAs was violated, degrees of freedom would be reported according to Greenhouse-Geisser correction. Post-hoc pairwise comparisons were conducted to specify significant effects highlighted by the ANOVA.

As the RAVLT reflects the degree of hippocampal recovery after the onset of symptoms, the TGA acute group was split by their declarative memory performance in the RAVLT. Here,

either independent samples t tests, for comparison of the acute groups, or paired samples t test, for comparison of acute groups and their follow-up performance, were calculated. For the comparison of the acute groups regarding lure similarities, a mixed ANOVA was conducted with lure similarity as within -subjects factor (Lure 1 to Lure 5) and the testing time after onset of symptoms (early acute vs. late acute) as between-subjects factor. Moreover, for the comparison of both acute groups with the follow-up phase, repeated measures ANOVA with lure similarity (Lure 1 to Lure 5) as the first and condition (acute vs. follow-up) as the second repeated-measure factor was performed. Differences in response behavior on stimulus type level were analyzed using either two-way ANOVA for independent samples for the comparison of both acute groups, or two-way repeated measures ANOVA for dependent samples for the comparison of both acute groups with their follow-up performance.

To statistically prove increases in performance when lure similarity decreases (i.e., lures are easier to distinguish from corresponding targets), Spearman's Rho expressing the relation between PatSep scores and lure similarity was calculated for every participant separately. The significances of the slopes of the average correlations for all three conditions were tested using Wilcoxon signed ranks tests against zero. The same procedure was done for the relation between the sequential number of the RAVLT learning trials and the related number of words to show a significant increase in learning from trial to trial.

To analyze the relationship between scores of the MST and RAVLT, Pearson product-moment correlations were calculated for normal distributed variables, otherwise, Spearman's Rho was used. The equality of performance in neuropsychological tests of TGA patients and the control group was examined by independent samples t tests or Mann-Whitney-U-tests according to distribution. To demonstrate independence of test scores from number, lateralization and posi-

tion of lesions in the sagittal plane, eta coefficients and Spearman's Rho were computed. Adjustments for multiple testing were done according to Bonferroni-Holm. The significance level was set to $p < 0.05$, two-tailed for all tests. Data are specified as mean ± SEM.

2.4 Results

2.4.1 Mnemonic similarity task performance

We performed a 2 x 3 repeated measures ANOVA with condition (TGA acute vs. TGA follow-up) and stimulus type (target vs. lure vs. foil) as within -subjects factors for the analysis of correct responses in the MST. The ANOVA revealed a significant condition x stimulus type interaction ($F(2, 26) = 51.26$, $p < 0.001$, $\eta^2p = 0.80$). Post-hoc pairwise comparisons showed that patients in the acute phase of the TGA performed significantly worse than at follow-up considering correct responses to targets ($p < 0.001$) and lures ($p < 0.001$). There was no difference regarding answers to foils ($p = 0.566$). A 2 x 3 mixed ANOVA with group (TGA follow-up vs. controls) as between subjects factor and stimulus type (target vs. lure vs. foil) as within subjects factor. There was no significant interaction effect ($F(2, 52) = 1.23$, $p = 0.117$, $\eta^2_p = 0.08$). As expected, the test performance at follow-up was not different from the performance of the control group for any stimulus type (all p's > 0.310). There was a significant group x stimulus type interaction in the 2 x 3 mixed ANOVA with group (TGA acute vs. controls) as between subjects factor and stimulus type (target vs. lure vs. foil) as within subjects factors ($F(2, 52) = 39.72$, $p < 0.001$ 0.117, $\eta^2_p = 0.60$). Clearly, post-hoc between-subjects contrasts revealed that patients in the acute phase of the TGA performed significantly worse compared with the control group considering correct responses to targets ($p < 0.001$) and lures ($p < 0.001$), but no significant difference between the responses to foils ($p = 0.550$) (Figure

2-2).

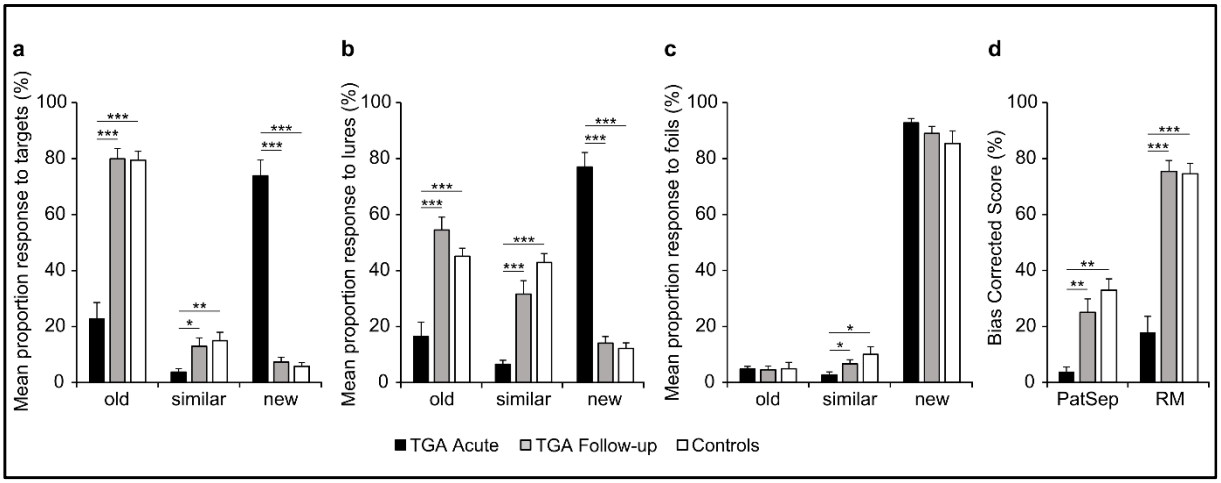

Figure 2-2. a-c) Mean (± SEM) proportion of responses to targets, lures, and foils in performing the MST for TGA acute vs. follow-up vs. controls. The values indicate percentages of responses relative to each stimulus type. Note the reduced correctness of patients in the acute phase of the TGA regarding target and lure items as well as the bias towards the 'new' response. d) Mean (± SEM) of the PatSep and RM scores for all groups. TGA patients were impaired in correctly separating and recognizing items of the MST. Adjustment for multiple testing was done using the Bonferroni-Holm correction. *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ n.s. = non-significant.

As we were interested in the answers to lures that pose the highest demands on pattern separation, we analyzed the errors responding to those items. The 2 x 2 repeated measures ANOVA with condition (TGA acute vs. follow-up) and error type (old vs. new) as within - subjects factors revealed a significant condition x error type interaction ($F(1, 13) = 121.39$, $p < 0.001$, $\eta^2_p = 0.90$). Patients in the acute phase of the TGA were more prone to incorrectly respond 'new' to lures than in the follow-up phase ($p < 0.001$). In contrast, for the follow-up phase, the 'old' response to lures was more frequent than in the acute phase of the TGA ($p < 0.001$). Comparing patients' performance at follow-up with the control group showed no interaction effect ($F(1, 26) = 1.25$, $p = 0.273$, $\eta^2_p = 0.05$). There was no difference between the committed errors of patients in the follow-up phase and the control group (both p's > 0.189). As expected, the comparison between patients in the acute phase and the control group revealed a significant interaction effect ($F(1, 26) = 72.90$, $p < 0.001$, $\eta^2_p = 0.74$). Patients in the acute

phase responded significantly more ‘new’ to lures than the control group ($p < 0.001$), whereas the control group used the ‘old’ answer more frequently ($p < 0.001$). Considering the patients' response behavior in the acute phase, they made significantly more ‘new’ responses to lures (77.00 ± 5.18) than ‘old’ responses (16.43 ± 5.17) ($p < 0.001$). However, at follow-up, the incorrect ‘old’ answer was more frequent (54.50 ± 4.59 vs. 14.07 ± 2.35) ($p < 0.001$). Likewise, control group participants used the ‘old’ answer more frequently than the ‘new’ answer (45.07 ± 2.91 vs. 12.14 ± 2.03) ($p < 0.001$) (Figure 2-2). All p-values in multiple tests for the analysis of response behavior were adjusted according to Bonferroni-Holm.

Calculating the MST scores, pairwise t-tests displayed that the scores in the acute phase were significantly lower than in the follow-up phase of the TGA (PatSep: $t(13) = -4.06$, $p = 0.004$, 95% CI [-32.36, -9.89]; RM: $t(13) = -8.77$, $p < 0.0001$, 95% CI [-71.66, -43.34]; Bonferroni-Holm adjusted). The same held true comparing scores of TGA patients in the acute phase with the control group using the Welch test and Mann-Whitney U-test (PatSep: $t(16.74) = -6.54$, $p < 0.001$, 95% CI [-38.37, -19.63]; RM: $Z = -4.18$, $p < 0.001$, Bonferroni-Holm adjusted). There were no differences between the scores in the follow-up phase of the TGA and the control group (PatSep: $t(26) = -1.23$, $p = 0.456$, 95% CI [-20.99, 5.24]; RM: $t(26) = 0.17$, $p = 0.865$, 95% CI [-10.21, 12.06]; Bonferroni-Holm adjusted) (Figure 2-2). The results indicate profound deficits in successfully separating and recognizing previously encoded stimuli during the acute phase of the TGA.

Considering different degrees of lure similarity, we found deficits in separating lures for all degrees over the course of the acute phase. For the PatSep score, a 2 x 5 repeated measures ANOVA with condition (TGA acute vs. follow-up) and similarity (Lure 1 to Lure 5) as within-subjects’ factors revealed a significant condition x similarity interaction ($F(4, 52) = 4.18$, $p < 0.01$, $\eta^2_p = 0.24$). Post-hoc pairwise tests of simple effects showed for every degree of lure similarity a higher score in the follow-up phase compared with the acute phase (all p's < 0.05,

Bonferroni-Holm adjusted). A 2 x 5 ANOVA with group (TGA acute vs. controls) as between -subjects factor and similarity (Lure 1 to Lure 5) as within -subjects factor revealed a significant interaction effect ($F(4, 104) = 5.42$, $p < 0.001$, $\eta^2p = 0.17$). We found significantly higher scores for every degree of lure similarity in the control group compared with the acute phase (all p's < .001). The interaction of a 2 x 5 ANOVA with group (TGA follow-up vs. controls) as between -subjects factor and similarity (Lure 1 to Lure 5) as within subjects factor was non-significant ($F(2.58, 67.17) = 2.04$, $p = 0.125$, $\eta^2p = 0.07$). There was no difference for any degree of lure similarity (all p's > 0.258) (Figure 2-3).

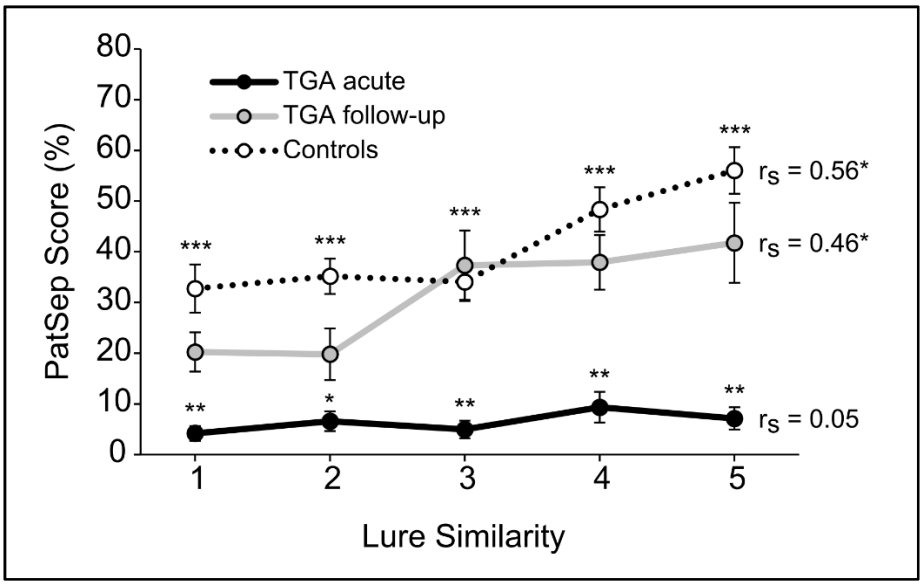

Figure 2-3. Pattern separation performance (means ± SEM) as a function of lure similarity. Asterisks above the black line indicate significance of post-hoc comparisons between acute and follow-up. Asterisks above the dotted line indicate significance of comparisons between the acute group and controls. PatSep scores are higher in the follow-up phase of the TGA compared to the acute phase for every degree of lure similarity. Note the gradual increase of performance as a function of lure similarity in the follow-up phase and in the control group. * $p < 0.05$, ** $p < 0.01$.

To further characterize the relation of correctly separating lures of different similarities during hippocampal dysfunction, we calculated correlations between lure similarity and the PatSep score for every participant separately. For three patients, it was not possible to calculate a correlational coefficient in either condition (i.e., acute vs. follow-up) as the scores of all five

bins were equal, so that the variable was constant without any variance. Thus, the following analysis was reduced to n = 11 in the acute phase and n = 12 in the follow-up phase. The average correlation was significantly different from zero only in the follow-up phase ($r_s = 0.46 \pm 0.13$; $Z = 2.62$, $p < 0.05$, adjusted for Bonferroni-Holm) and the control group ($r_s = 0.56 \pm 0.11$; $Z = 2.77$, $p < 0.05$, adjusted for Bonferroni-Holm), whereas the correlation equaled zero in the acute phase of the TGA ($r_s = 0.05 \pm 0.19$; $Z = 0.27$, $p = 0.79$, adjusted for Bonferroni-Holm). The results show, that the patients' deficits in pattern separation during the acute phase of the TGA were not limited to a specific degree of lure similarity. Indeed, as there was no graduation in performance in the acute phase, the findings show that all functions of pattern separation were impaired during the amnesia (Figure 2-3).

2.4.2 Declarative memory

Pairwise comparisons revealed profound deficits in verbal declarative learning for patients in the acute phase as measured by the RAVLT sum score, RAVLT retention score, RAVLT delayed recall, and RAVLT recognition score. At follow-up declarative memory performance of patients no longer differed from the control group's performance (Table 2-2).

Table 2-2. Mean ± SEM of the RAVLT scores and pairwise comparisons for TGA patients (acute and follow-up) and controls

	TGA acute			TGA follow-up		Controls		
RAVLT sum	26.43 ± 1.22			48.50 ± 1.76		50.50 ± 2.04		
RAVLT retention	1.29 ± 0.55			10.85 ± 0.78		11.86 ± 0.49		
RAVLT delayed	1.14 ± 0.48			10.50 ± 0.69		12.14 ± 0.69		
RAVLT recognition	2.64 ± 0.75			12.14 ± 1.00		13.43 ± 0.47		
pairwise comparisons	acute vs. follow-up			follow-up vs. controls		acute vs. controls		
	t(13)/Z	p	95% CI	t(26)/Z	95% CI	t(26)/Z	p	95% CI
RAVLT sum	-10.82	***	[-26.48, -17.67]	-0.74	[-7.53, 3.53]	-10.14	***	[-28.95, -19.19]
RAVLT retention	-9.37†	***	[-11.67, -7.26]	-1.12†	[-2.87, 0.85]	-4.59‡	***	
RAVLT delayed	-11.02	***	[-11.19, -7.52]	-1.68	[-3.65, 0.36]	-4.56‡	***	
RAVLT recognition	-3.25‡	**		-0.43‡		-4.53‡	***	

†Degrees of freedom are reduced to df=12 (paired samples) and df=25 (independent samples) because of a missing value in the retention score in the follow-up phase; ‡ Non-parametric tests (Mann-Whitney-U-test for independent and Wilcoxon test for dependent samples); 95% confidence intervals are calculated for parametric tests; ** $p < 0.01$. *** $p < 0.001$; Bonferroni-Holm adjusted for 12 comparisons.

2.4.3 Pattern separation dependent on degree of hippocampal impairment

The TGA shows a characteristic time course with an abrupt onset of the pronounced hippocampal deficit and with a gradual recovery of the hippocampal functions in the later parts of the episode. This time-dependency of the hippocampal deficit is reflected in the correlation of the acute hippocampal deficit as measured in the RAVLT sum score with the time point of testing after onset of symptoms ($r = 0.872$, $p < 0.0001$). We thus classified the TGA patients into two groups by means of their performance either above or below 3 SD from the mean of the RAVLT sum score of the control group (50.5 ± 7.61) to incorporate a measure of the time-dependent degree of hippocampal impairment. We defined the groups as *early acute* (n = 9) and *late acute* (n = 5). The latency between onset of symptoms and behavioral tests was significantly different between both groups ($t(12) = 2.92$, $p = 0.013$, 95% CI [0.47, 3.22], 3.56 ± 0.38 h vs. 5.40 ± 0.51 h). Further analysis of performance during the RAVLT learning trials revealed a significant increase in verbal declarative learning in both acute groups. We calculated the correlation between the learning trials and number of correct remembered words for every patient separately. Wilcoxon signed ranks test against zero revealed a positive slope of the average correlation in both the early ($r_s = 0.71 \pm 0.09$; $Z = 2.66$, $p < 0.05$, adjusted for Bonferroni-Holm) and the late acute group ($r_s = 0.88 \pm 0.07$ $Z = 2.02$, $p < 0.05$, adjusted for Bonferroni-Holm) (4).

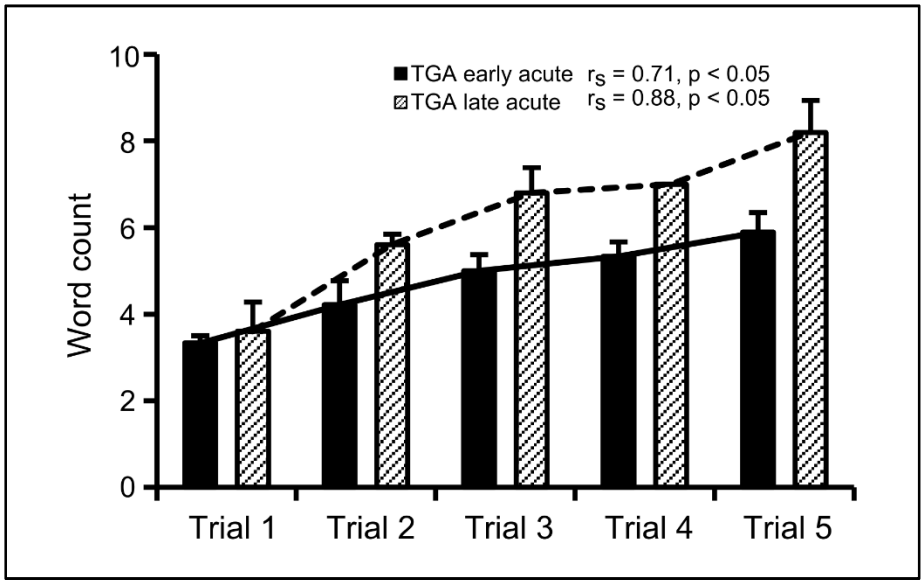

Figure 2-4. RAVLT learning trials for early and late acute TGA patients. Both patient groups show a statistically significant increase in learning from the first to the last trial.

In the following, we also analyzed the differences between both acute groups and their follow-up performance to consider individual memory performance. Comparing the early and late acute groups with regard to performance on the MST revealed neither difference in the PatSep score ($t(12) = 1.10$, $p = 0.295$, 95% CI [-3.56, 10.77]) nor the RM score ($t(12) = 1.87$, $p = 0.232$, 95% CI [-0.03, 0.44]). Regarding the differences between acute and follow-up, paired samples t tests showed that the PatSep score only differed between the early acute group and the follow-up ($t(8) = -3.36$, $p < 0.05$, 95% CI [-41.30, -7.69]). There was no difference comparing the late acute group with the follow-up condition ($t(4) = -2.37$, $p = 0.232$, 95% CI [-32.73, 2.62]). However, the RM score was significantly lower in both acute groups compared with follow-up (early acute: $t(8) = -7.81$, $p < 0.001$, 95% CI [-82.04, -44.62]), late acute: $t(4) = -4.46$, $p < 0.05$, 95% CI [-76.29, -17.71]) indicating that pattern separation shows an earlier recovery than RM performance (Figure 2-5e). All p values were adjusted for multiple tests.

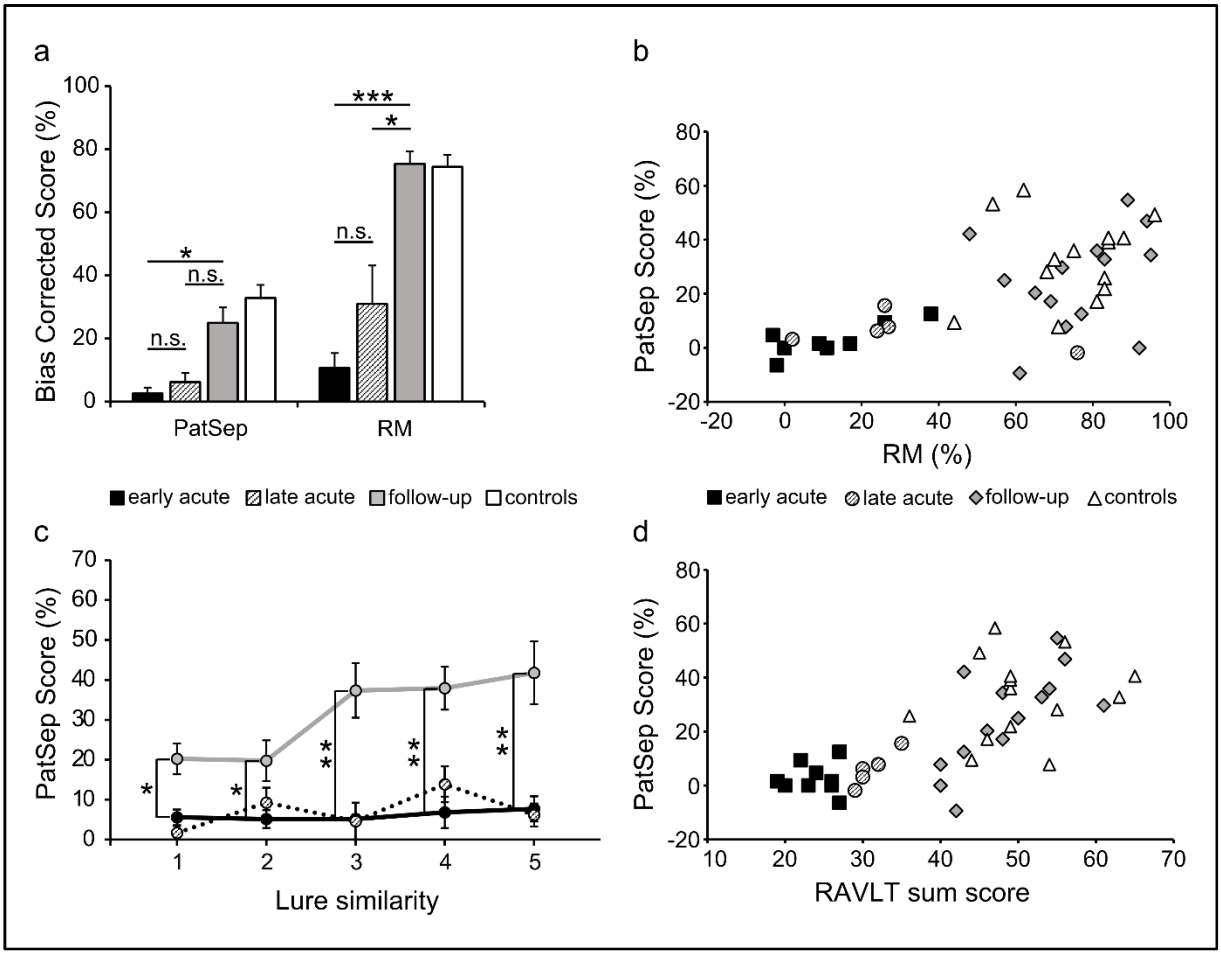

Figure 2-5. Comparison of group performance in different scores. a) The early acute group differed significantly between acute and follow-up in PatSep test performance and recognition memory performance, whereas the late acute patients only differed from follow-up regarding recognition memory. White bars for the control group are depicted for descriptive illustration but were not included in the analysis. b) PatSep in relation to the RAVLT sum score. c) The difference between early acute and follow-up remained considering lure similarities. d) PatSep in relation to recognition memory. Note that b) and d) serve as graphical illustration only of the time course of the amnesic syndrome from early acute to full recovery. Correlational analyses were not performed on the illustrated data. Comparisons between acute and follow-up were analyzed by paired samples t-tests so that mean ± SEM for follow-up depicted in a) and c) do not reflect the values used for the statistical test. Adjustment for multiple testing was done using the Bonferroni-Holm correction. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

With regard to patients' performance in the MST, we observed an increased rate of false 'new' responses to lures in the acute phase (Figure 2-5b). However, the hypothesized deficit in pattern separation should rather involve a heightened false 'old' response to lures reflecting the

ability or inability of discriminating similar from previously seen items. By contrast, the false ‘new’ response indicates an overall deficit in recognition. We thus tested whether early and late acute groups differed in responses to lures and whether the bias toward ‘new’ responses was initially driven by early acute patients who were more impaired.

A 2 x 2 factorial ANOVA with the group (early vs. late acute) as between -subjects factor and error type (old vs. new) as within subjects factor revealed a significant interaction effect ($F(1, 12) = 7.55$, $p = 0.018$, $\eta^2_p = 0.39$). In the early acute phase, patients showed a significantly higher proportion of ‘old’ responses to lures compared to ‘new’ responses ($p < 0.001$), whereas we found no difference between ‘old’ and ‘new’ responses to lures in the late acute phase of the amnesia ($p > 0.05$). Moreover, the rate of ‘old’ responses was significantly higher in the late acute group compared with the early acute group ($p < 0.05$), whereas the proportion of ‘new’ responses was higher in the early acute group ($p < 0.05$). The 2 x 2 repeated measures ANOVA for the comparison of follow-up with the corresponding performance in the acute phase revealed a significant interaction for both the late ($F(1, 4) = 38.76$, $p = 0.003$, $\eta^2_p = 0.91$) and the early acute group ($F(1, 8) = 130.21$, $p < 0.0001$, $\eta^2_p = 0.94$) with a higher proportion of ‘new’ responses in both acute groups compared with a higher rate of ‘old’ responses at follow-up (Figure 2-6). The p values are adjusted according to Bonferroni-Holm.

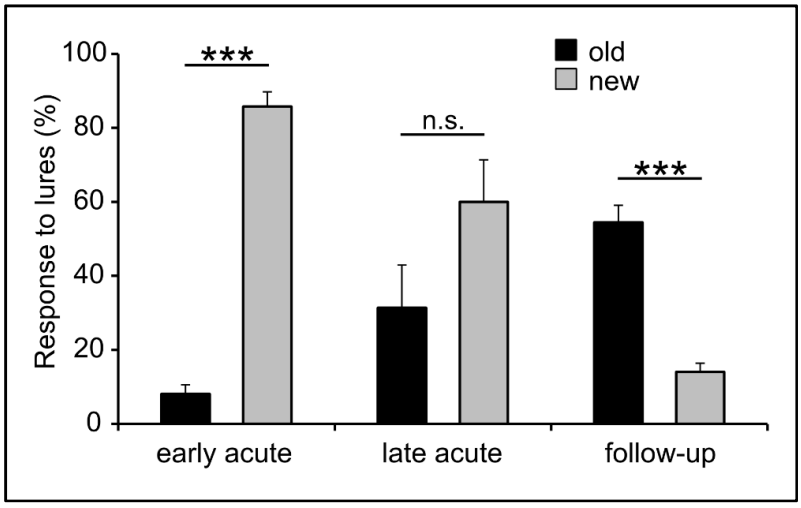

Figure 2-6. Mean (± SEM) proportion of responses to lures in performing the MST for TGA early acute vs. late acute vs. follow-up. The values indicate percentages of responses to lures. Patients in the early acute phase were more prone to respond 'new' to lures, whereas the response bias was reversed over recovery. Adjustment for multiple testing was done using the Bonferroni-Holm correction. *** $p < 0.001$ n.s. = non-significant.

Including lure similarity in the analysis, we conducted a mixed ANOVA with the group (early acute vs. late acute) as between-subjects factor and similarity (Lure 1 to Lure 5) as within-subjects factor. For the PatSep score, the ANOVA revealed no significant interaction ($F(4, 48) = 1.44$, $p = 0.236$, $\eta^2_p = 0.11$), but a significant main effect of group ($F(1, 12) = 18.48$, $p < 0.01$, $\eta^2_p = 0.61$). Further analyses of simple main effects showed that there was no difference between the early acute group and the late acute group regarding any degree of lure similarity (all p's > 0.211). In the second step, we performed within-subjects ANOVAs regarding both acute groups and the follow-up phase with lure similarity (Lure 1 to Lure 5) as the first and condition (acute vs follow-up) as the second within subjects factor. Considering the early acute group, there was a significant interaction effect ($F(4, 32) = 3.40$, $p < 0.05$, $\eta^2_p = 0.30$). The follow-up condition showed a significantly higher score for every degree of lure similarity (all p's < 0.028). For the late acute group, there was no significant interaction ($F(1.49, 5.95) = 1.96$, $p = 0.22$, $\eta^2_p = 0.33$). Post-hoc pairwise comparisons showed no differences between any degrees (all p's > 0.2, corrected for Bonferroni-Holm) (Figure 2-5c).

2.4.4 Relationship between MST and RAVLT performances

Relationships between the MST performance and RAVLT based on correlational analyses turned out significant for the RAVLT sum score and the delayed recall test. Both scores were correlated with the PatSep score in the follow-up phase (RAVLT sum $r = 0.695$, $p = 0.006$; RAVLT delayed recall: $r = 0.564$, $p = 0.036$). In the acute phase, a correlation only revealed for the late acute group with the RAVLT sum score ($r = 0.951$, $p < 0.05$) (Figure 2-5d), that was a reflection of the RAVLT sum score serving as a criterion for differentiating the acute groups.

2.4.5 General neuropsychological assessment

Patients did not differ from controls in general neuropsychological test performance at follow-up (Table 3-1). In patients with TGA, working memory and executive functions typically remain intact during the amnestic episode (Bartsch & Deuschl, 2010; Bartsch et al., 2010; Quinette et al., 2003).

2.4.6 MRI Study

In 11 of 14 patients, we detected a total of 19 hippocampal lesions in a time window of 24-72 hr after onset of TGA symptoms. Six patients showed only one lesion, two patients had two lesions, and, in three patients, we detected three lesions in total. In four patients, lesions were only detected within the left, in five patients within the right cornu ammonis, and in two patients, lesions were detected bilaterally. Eta coefficients verified that there was neither a relation between lateralization of lesions and the performance on MST nor RAVLT (all p's > 0.05). Also, the performance on the tests was independent from the number of lesions (all p's > 0.05, Spearman's rho) as well as from the position on the longitudinal axis of the hippocampus (all p's > 0.05, eta coefficient).

The detailed analysis of lesion distribution revealed that all lesions were selectively found in the area corresponding to the CA1 sector of the hippocampal cornu ammonis and randomly

distributed along the anterior-posterior axis within the hippocampus as typically seen in TGA. Performing a detailed whole brain analysis provided no evidence for diffusion restricted lesions outside the hippocampal cornu ammonis (Figure 2-7). MRI did not show any abnormalities in temporal or frontal lobe structures such as focal atrophy or mesial temporal sclerosis.

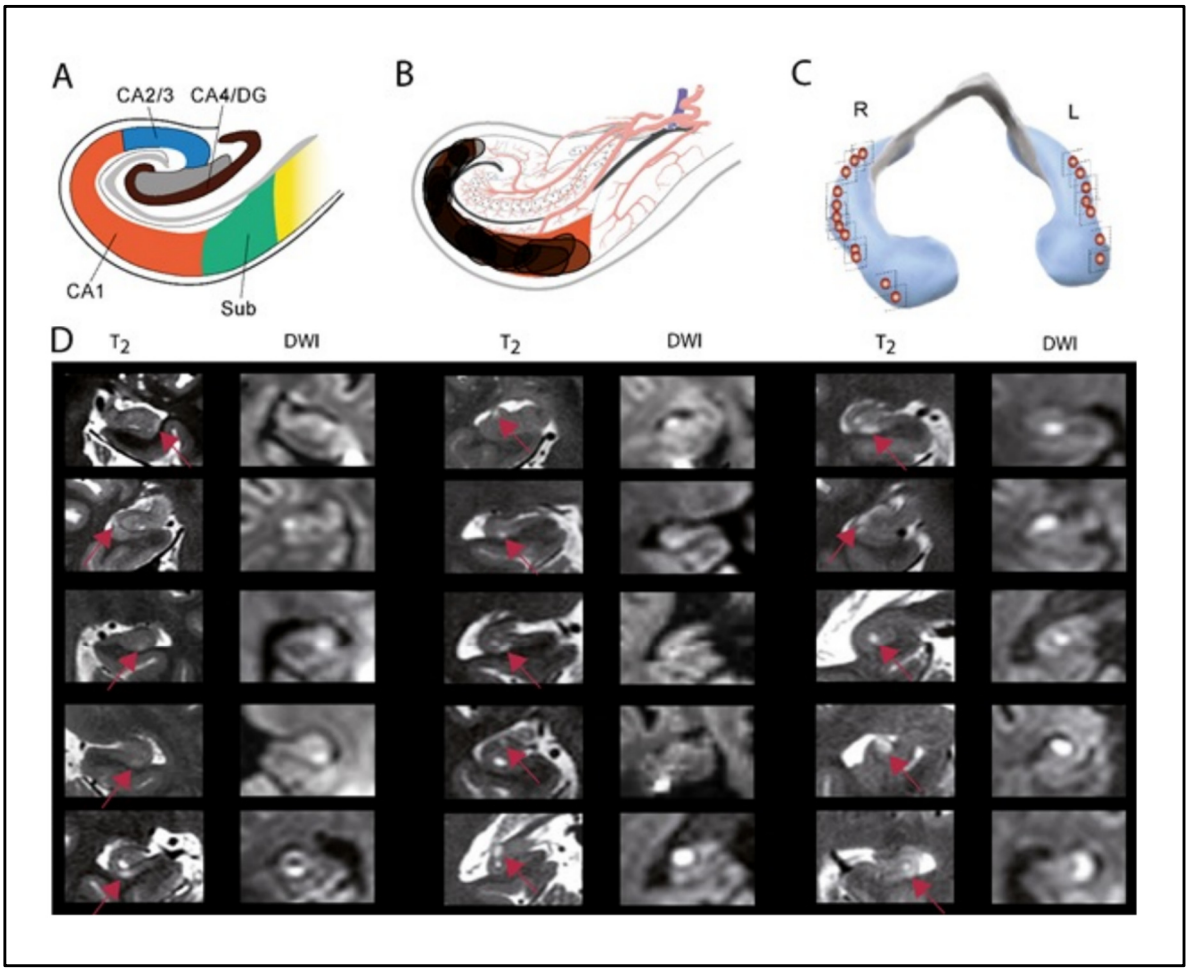

Figure 2-7. a-d) a) Anatomical template showing a representative coronary slice of the hippocampal cornu ammonis indicating sectors after Lorente de Nó. b) Synopsis of all DWI/T2 lesions transferred to an anatomical template of the cornu ammonis. c) Three-dimensional model of the hippocampus showing the anterior-posterior distribution of hippocampal CA1 lesions. Lesions were located in the lateral hippocampus and were distributed along the anterior-posterior axis of the hippocampus. d) MRI of representative lesions shows that lesions were confined to the CA1 area of the cornu ammonis.

2.5 Discussion

Our results provide causal evidence that hippocampal CA1 neurons are critical to pattern separation performance in humans. We found that patients with focal lesions restricted to the CA1 sector of the hippocampus were profoundly impaired in behavioral pattern separation performance. Considering the time dependency of lesion-associated hippocampal deficits in patients in early and late acute stages of the TGA, we also showed that the behavioral pattern separation performance recovered significantly earlier compared to RM. recognition memory. This points to a differential affection and reorganization of both functions during hippocampal disconnection in TGA.

The formation of episodic memory requires the ability to discriminate between similar experiences that depend on the hippocampus and its pattern separation function. Pattern separation produces non-overlapping representations of newly encoded mnemonic information to avoid interference (Knierim & Neunuebel, 2016; McClelland et al., 1995; Yassa & Stark, 2011). Our data show that this function was impaired in patients with acute hippocampal CA1 lesions. Patients were impaired in both recognition of previously seen items and correctly responding to stimuli that were similar to already seen targets. The function was restored at follow-up. The results complement the current picture regarding the contribution of hippocampal subfields to pattern separation: Computational models assume that the DG performs pattern separation by means of sparse coding of neural activity from the entorhinal cortex (EC) to CA3 thereby decorrelating overlapping neural assemblies (Rolls, 2016; Treves & Rolls, 1994). Interconnected pyramidal neurons of the CA3 region function as an auto-associative network so that a stored representation can be retrieved from an incoming partial cue supporting the process of pattern completion (Norman & O'Reilly, 2003; O'Reilly & McClelland, 1994). Within the tri-synaptic circuit, CA3 ensemble activity passes CA1 as the main output relay area of the hippocampus to be transferred to neocortical areas (Insausti & Amaral, 2004; Knierim & Neunuebel, 2016).

(Wilson & McNaughton, 1994) suggested that synaptic modifications especially during sleep support CA1 in transmitting signals from CA3 to neocortical areas. In this context, we recently showed that pattern separation performance in the human hippocampus was influenced by oscillatory dynamics during sleep resulting in a stabilization of the memory representation (Hanert et al., 2017).

By recording activity from DG and CA3, a variety of rodent studies showed that the hippocampal DG is indeed involved in pattern separation, whereas the area CA3 performs pattern separation or pattern completion depending on the change of sensory input thereby balancing the discriminative function in the context of the formation of a new memory representation (Guzowski et al., 2004; I. Lee et al., 2004; J. K. Leutgeb et al., 2007; Neunuebel & Knierim, 2014). In this context, importantly, exposure to different environments caused differences in the ensemble activity of CA1 and CA3 neurons using both neural ensemble recordings and imaging of immediate early gene activity (I. Lee et al., 2004; S. Leutgeb et al., 2004; Vazdarjanova & Guzowski, 2004). These experiments indicate stronger pattern separation in CA3 than in CA1 (Guzowski et al., 2004). The neural response in CA3 suggests a sigmoidal function, where large changes to the input cause pattern separation, and small changes to the input cause pattern completion. The area CA1, on the contrary, displays a linear relationship between input and output showing that CA1 indeed exhibits pattern separation in a different way (Guzowski et al., 2004). Our results in patients with transient CA1 lesions show impaired behavioral pattern separation performance strongly supporting this assumption. Considering that pattern separation in the hippocampus is mainly performed by means of the DG subnetwork providing distinct neural codes within the feedforward pathway to CA3 pyramidal cells (Neunuebel & Knierim, 2014), it is most likely that the downstream circuit of CA1 is disrupted in relaying the orthogonalized information. Accordingly, a different transfer function of similar representations in humans has been shown for CA1 compared with DG/CA3 using high-resolution fMRI studies.

Those studies first demonstrated separation-like signals only in the DG (Baker et al., 2016; Berron et al., 2016) found a discontinuous response in the DG/CA3 area compared with CA1 (Lacy et al., 2011). By varying the similarity of the presented items of the MST, it was shown indeed that CA1 was resistant to small changes with a linear response pattern, whereas DG/CA3 was sensitive to small changes (Lacy et al., 2011). Those findings suggest that CA1 may not be involved in neural computations of pattern separation processes per se, but in subsequent forwarding of the orthogonalized DG/CA3 input. In accordance with those results, we found behavioral pattern separation performance completely impaired in patients with lesions restricted to CA1.

With regard to the transient hippocampal dysfunction, patients in the late acute stage of the TGA were less impaired in pattern separation than in RM. Specifically, the late acute group differed from follow-up only in pattern separation performance, whereas the groups were comparable in RM. We assume that the differential recovery was a result of the dependence of those memory functions on distinct hippocampal subnetworks. RM may be more reliant on CA1, whereas pattern separation is also dependent on computations in upstream hippocampal regions DG/CA3 (McClelland et al., 1995; Yassa & Stark, 2011). Thus, considering the hippocampal perturbations on the CA1-neural level, our study might also reflect a functional dissociation of pattern separation and RM.

Previous studies in humans regarding disease-related structural changes to the DG, CA3 and CA1 found deficits in pattern separation. These studies examined patients with amnestic mild cognitive impairment (Yassa et al. 2010; Stark et al. 2013) as well as Alzheimer's disease (Ally et al. 2013), where CA1 neurons are also affected early in the disease course (West, Coleman, Flood, & Troncoso, 1994; West, Kawas, Martin, & Troncoso, 2000; West, Kawas, Stewart, Rudow, & Troncoso, 2004). Functional MRI studies investigating subregion-associated net-

work functions of the hippocampus suggested DG and CA3 to be associated with pattern separation performance, whereas findings regarding the contribution of CA1 activity yielded divergent outcomes (Bakker et al., 2008; Lacy et al., 2011).

To further clarify the function of CA1 in pattern separation, we compared the behavioral output of a task that taxes pattern separation of both impaired and intact output structures of the hippocampal circuit. Here, CA1 transmits its output information and from DG/CA3 to other brain regions in the context of hippocampus-dependent cognition and behavior (Knierim & Neunuebel, 2016). In our lesion model, we see the effect of an impaired hippocampal CA1 network, but we cannot make a statement regarding intrinsic CA1 computational processes as such or the contribution of further upstream CA3/DG network functions in the hippocampal trisynaptic pathway. Thus, what we can show is that CA1 has an essential and integrative relay function in the pattern separation processing involved in hippocampus-dependent memory, cognition, and behavior. Experimental and conceptual evidence of pattern separation and pattern completion has been provided mainly in rodent studies where, for example, the input/output function, the discharge and response properties of neuronal ensembles in hippocampal subnetworks as well as behavioral evidence are tightly linked to hippocampal functions (see Guzowski et al., 2004; I. Lee et al., 2004; J. K. Leutgeb et al., 2007).

It is to be noted, that it is difficult to assess pattern completion processes in our study. The bias toward responding ‘old’ to lure items of the MST has been defined as a shift from pattern separation to pattern completion (Ally, Hussey, Ko, & Molitor, 2013; Yassa et al., 2010). However, the validity of measuring behavioral pattern completion based on the MST has repeatedly been discussed (Hunsaker & Kesner, 2013; Liu, Gould, Coulson, Ward, & Howard, 2016). As the test lacks partial cues that reactivate previously encoded memory representations - as suggested by the theoretical construct of pattern completion - drawing conclusions regarding the underlying neural processes is challenging. However, there is evidence that CA1 is involved in pattern

completion processes. For instance, in an fMRI study in humans, Bakker et al. (2008) found completion-like activity in CA1. We confined our analysis as well as interpretation of behavioral data to pattern separation. The MST has already been well validated in a variety of studies with regard to assessing behavioral pattern separation performance (Lacy et al., 2011; Stark et al., 2013; Yassa, Mattfeld, Stark, & Stark, 2011; Yassa et al., 2010). However, to further clarify the role of CA1 in pattern completion the application of specific tests that explicitly assess pattern completion function in humans is suggested. Thus, behavioral paradigms shall be used in prospective studies to elaborate pattern completion related functions in lesion models restricted to hippocampal CA1 neurons.

Data from human behavioral studies as well as from experimental rodent examinations suggest that CA1 encompasses several major functions that include temporal processing of information (i.e., temporal order memory), association across time, intermediate memory, and consolidation of new memory (Kesner, Morris, & Weeden, 2012; Rolls, 1996; Rolls & Kesner, 2006). Specifically, CA1 integrates temporal and object representations also referred to as temporal pattern separation (Hunsaker, Lee, & Kesner, 2008; Rolls & Kesner, 2006). In this context, CA1 plays an important role in match/mismatch as well as novelty detection evidenced by human and rodent studies (Duncan, Ketz, Inati, & Davachi, 2012; Hasselmo, 2005; Knierim & Neunuebel, 2016; Inah Lee, Hunsaker, & Kesner, 2005; McClelland et al., 1995; Vinogradova, 2001). Furthermore, CA1 neurons play a necessary role in the ability to learn map-like representations of an environment referring to a critical function in spatial memory (Bartsch et al., 2010). This is well in accordance with our results considering the relation of spatial coding to pattern separation performance with regard to place cell remapping (Colgin et al., 2010; I. Lee et al., 2004; J. K. Leutgeb et al., 2007, 2005). In the context of pattern separation, hippocampal remapping produces distinct representations in populations of place cells in exposure to similar environments. Here, pattern separation is mainly expressed in global remapping that relates to basically

different rates and fields of firing during encoding of different environments (Colgin, Moser, & Moser, 2008). In rodents, recordings of spike activity from hippocampal place cells that measure the remapping of place fields after the rats' exploration of similar enclosures evinced the relation between remapping on a neural level and pattern separation on the part of behavior (J. K. Leutgeb et al., 2007, 2005). Summarizing those results, our data further illuminate the connection between spatial learning and pattern separation operations both involving CA1 specific computations within an unrestricted feedforward processing in the tri-synaptic hippocampal circuitry.

The tri-synaptic pathway provides the prerequisites for pattern separation and completion processes by means of its unique cytoarchitectonic structure and different subfield functions. However, the mono-synaptic path projects from EC Layer III directly to CA1 bypassing the area DG/CA3 (Witter & Amaral, 2004). With this connection, CA1 is assumed to compare direct input from CA3 and EC to perform match/mismatch calculations between previous experiences and current environmental input (Duncan et al., 2012; Hasselmo, 2005). The focal CA1 lesions in TGA patients most likely interfered with those computations and thus further affect the output generation of pattern separation and completion processes.

As we tested patients in different stages of the acute amnesia, we observed floor effects in memory performance. This effect might be the result of an encoding deficit in the acute phase of the TGA as the hippocampal CA1 is assumed to be involved in both encoding and retrieval processes in memory formation (Duncan, Tompary, & Davachi, 2014). We cannot rule out that these deficits may also involve the bias towards 'new' instead of 'old' or 'similar' responses to lures that complicated the interpretation regarding pattern separation. A quantification of encoding abilities in our study cohort is made possible by means of the RAVLT learning trials that assess learning performance of episodic memory. Even though the RAVLT performance in the acute phase was significantly lower compared to follow-up testing and the control group,

both early and late acute TGA patients showed a significant positive increase in learning from the first to the last trial. Considering this, patients were indeed able of encoding, albeit deficiently. The impaired performance in the recall conditions of both MST and RAVLT thus might be due to a failure of consolidation and retrieval. This can be seen particularly in the early acute patients' disproportionately high number of 'new' answers. The response behavior indicates a failure in recognition that might have interfered with the behavioral pattern separation outcome. It should be noted, however, that the TGA patients' results who were tested later in the course of the disease are valuable in interpreting our hypothesis. Specifically, those patients were able to encode and consolidate but were impaired in recognition memory and behavioral pattern separation performance.

The interpretation of our data critically relies on the selectivity of CA1 lesions. Indeed, beyond our own studies (Bartsch et al., 2007, 2006, 2010) a variety of other studies also observed focal hyperintense MR-lesions selectively in the area of CA1 thus providing a natural lesion model of CA1 neurons (Sedlaczek et al., 2004; Weon, Kim, Lee, & Kim, 2008). The neurons in CA1 are highly vulnerable against metabolic and vascular noxious input (Bartsch et al., 2015). Typically, lesions outside CA1 or outside the hippocampus are not detected in TGA (Bartsch & Deuschl, 2010; Bartsch et al., 2015).

In sum, using TGA as a human hippocampal lesion model with selective and focal disruption of CA1-associated neural functions, we highlight the critical role of hippocampal CA1 neurons in terms of a relay function for pattern separation performance in humans. Our analysis of the temporal course of recovery of cognitive functions also points to a possible dissociation of pattern separation and RM in the context of a differential recovery of subregion-associated network functions of the hippocampus.

2.6 References - Study I

Ally, B. A., Hussey, E. P., Ko, P. C., & Molitor, R. J. (2013). Pattern separation and pattern completion in Alzheimer's disease: evidence of rapid forgetting in amnestic mild cognitive impairment. *Hippocampus*, *23*, 1246–58. doi:10.1002/hipo.22162

Aschenbrenner, S., Tucha, O., & Lange, K. W. (2000). *Regensburger Wortflüssigkeitstest*. Göttingen: Testzentrale.

Baker, S., Vieweg, P., Gao, F., Gilboa, A., Wolbers, T., Black, S. E., & Rosenbaum, R. S. (2016). The human dentate gyrus plays a necessary role in discriminating new memories. *Current Biology*, *26*(19), 2629–2634. doi:10.1016/j.cub.2016.07.081

Bakker, A., Kirwan, C. B., Miller, M., & Stark, C. E. (2008). Pattern separation in the human hippocampal CA3 and dentate gyrus. *Science*, *319*, 1640–2. doi:10.1126/science.1152882

Bartsch, T., Alfke, K., Deuschl, G., & Jansen, O. (2007). Evolution of hippocampal CA-1 diffusion lesions in transient global amnesia. *Annals of Neurology*, *62*(5), 475–480. doi:10.1002/ana.21189

Bartsch, T., Alfke, K., Stingele, R., Rohr, A., Freitag-Wolf, S., Jansen, O., & Deuschl, G. (2006). Selective affection of hippocampal CA-1 neurons in patients with transient global amnesia without long-term sequelae. *Brain*, *129*(11), 2874–2884. doi:10.1093/brain/awl248

Bartsch, T., & Deuschl, G. (2010). Transient global amnesia: Functional anatomy and clinical implications. *Lancet Neurology*, *9*, 205–14. doi:10.1016/S1474-4422(09)70344-8

Bartsch, T., Döhring, J., Reuter, S., Finke, C., Rohr, A., Brauer, H., … Jansen, O. (2015). Selective neuronal vulnerability of human hippocampal CA1 neurons: lesion evolution, temporal course, and pattern of hippocampal damage in diffusion-weighted MR imaging. *Journal of Cerebral Blood Flow and Metabolism*, *35*, 1836–45. doi:10.1038/jcbfm.2015.137

Bartsch, T., Schönfeld, R., Müller, F. J., Alfke, K., Leplow, B., Aldenhoff, J., … Koch, J. M. (2010). Focal lesions of human hippocampal CA1 neurons in transient global amnesia impair place memory. *Science*, *328*, 1412–5. doi:10.1126/science.1188160

Berron, D., Schütze, H., Maass, A., Cardenas-Blanco, A., Kuijf, H. J., Kumaran, D., & Düzel, E. (2016). Strong evidence for pattern separation in human dentate gyrus. *Journal of Neuroscience*, *36*, 7569–79. doi:10.1523/JNEUROSCI.0518-16.2016

Caplan, L. (1985). Transient global amnesia. In P. J. Vinken, G. W. Bruyn, & H. L. Klawans (Eds.), *Handbook of Clinical Neurology* (pp. 205–218). Amsterdam: Elsevier.

Colgin, L. L., Leutgeb, S., Jezek, K., Leutgeb, J. K., Moser, E. I., McNaughton, B. L., & Moser, M. B. (2010). Attractor-map versus autoassociation based attractor dynamics in the hippocampal network. *Journal of Neurophysiology*, *104*, 35–50. doi:10.1152/jn.00202.2010

Colgin, L. L., Moser, E. I., & Moser, M. B. (2008). Understanding memory through hippocampal remapping. *Trends in Neurosciences*, *31*, 469–77. doi:10.1016/j.tins.2008.06.008

Duncan, K., Ketz, N., Inati, S. J., & Davachi, L. (2012). Evidence for area CA1 as a match/mismatch detector: a high-resolution fMRI study of the human hippocampus. *Hippocampus*, *22*, 389–98. doi:10.1002/hipo.20933

Duncan, K., Tompary, A., & Davachi, L. (2014). Associative encoding and retrieval are predicted by functional connectivity in distinct hippocampal area CA1 pathways. *Journal of Neuroscience*, *34*(34), 11188–11198. doi:10.1523/JNEUROSCI.0521-14.2014

Duvernoy, H. M., Cattin, F., & Risold, P.-Y. (2013). *The Human Hippocampus: Functional Anatomy, Vascularization and Serial Sections with MRI*. Berlin, Heidelberg: Springer. doi:10.1007/978-3-642-33603-4

Guzowski, J. F., Knierim, J. J., & Moser, E. I. (2004). Ensemble dynamics of hippocampal regions CA3 and CA1. *Neuron*, *44*, 581–4. doi:10.1016/j.neuron.2004.11.003

Hanert, A., Weber, F. D., Pedersen, A., Born, J., & Bartsch, T. (2017). Sleep in humans stabilizes pattern separation performance. *Journal of Neuroscience*, *37*(50), 12238–12246. doi:10.1523/JNEUROSCI.1189-17.2017

Hasselmo, M. E. (2005). The role of hippocampal regions CA3 and CA1 in matching entorhinal input with retrieval of associations between objects and context: theoretical comment on Lee et al. (2005). *Behavioral Neuroscience*, *119*(1), 342–345. doi:10.1037/0735-7044.119.1.342

Hodges, J. R., & Warlow, C. P. (1990). Syndromes of transient amnesia: towards a classification. A study of 153 cases. *Journal of Neurology, Neurosurgery and Psychiatry*, *53*(10), 834–843.

Hunsaker, M. R., & Kesner, R. P. (2013). The operation of pattern separation and pattern completion processes associated with different attributes or domains of memory. *Neuroscience and Biobehavioral Reviews*, *37*(1), 36–58. doi:10.1016/j.neubiorev.2012.09.014

Hunsaker, M. R., Lee, B., & Kesner, R. P. (2008). Evaluating the temporal context of episodic memory: the role of CA3 and CA1. *Behavioural Brain Research*, *188*, 310–5. doi:10.1016/j.bbr.2007.11.015

Insausti, R., & Amaral, D. G. (2004). Hippocampal formation. In G. Paxinos (Ed.), *The human nervous system* (2nd ed., pp. 871–914). Amsterdam: Elsevier. doi:10.1016/B978-012547626-3/50024-7

Kesner, R. P., Morris, A. M., & Weeden, C. S. S. (2012). Spatial, Temporal, and Associative Behavioral Functions Associated with Different Subregions of the Hippocampus. In E. A. Wasserman & T. R. Zentrall (Eds.), *The Oxford Handbook of Coparative Cognition*. doi:10.1093/oxfordhb/9780195392661.013.0018

Kirwan, C. B., & Stark, C. E. L. (2007). Overcoming interference: an fMRI investigation of pattern separation in the medial temporal lobe. *Learning & Memory*, *14*, 625–33. doi:10.1101/lm.663507

Knierim, J. J., & Neunuebel, J. P. (2016). Tracking the flow of hippocampal computation: Pattern separation, pattern completion, and attractor dynamics. *Neurobiology of Learning and Memory*, *129*, 38–49. doi:10.1016/j.nlm.2015.10.008

Lacy, J. W., Yassa, M. A., Stark, S. M., Muftuler, L. T., & Stark, C. E. L. (2011). Distinct pattern separation related transfer functions in human CA3/dentate and CA1 revealed using high-resolution fMRI and variable mnemonic similarity. *Learning & Memory*, *18*, 15–8. doi:10.1101/lm.1971111

Lee, I., Yoganarasimha, D., Rao, G., & Knierim, J. J. (2004). Comparison of population coherence of place cells in hippocampal subfields CA1 and CA3. *Nature*, *430*, 456–9. doi:10.1038/nature02739

Lee, Inah, Hunsaker, M. R., & Kesner, R. P. (2005). The role of hippocampal subregions in detecting spatial novelty. *Behavioral Neuroscience*, *119*(1), 145–153. doi:10.1037/0735-7044.119.1.145

Lehrl, S. (2005). *Mehrfachwahl-Wortschatz-Intelligenz-Test, MWT-B* (5th ed.). Balingen: Spitta Verlag.

Leutgeb, J. K., Leutgeb, S., Moser, M. B., & Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science*, *315*, 961–6. doi:10.1126/science.1135801

Leutgeb, J. K., Leutgeb, S., Treves, A., Meyer, R., Barnes, C. A., McNaughton, B. L., … Moser, E. I. (2005). Progressive transformation of hippocampal neuronal representations in "morphed" environments. *Neuron*, *48*, 345–58. doi:10.1016/j.neuron.2005.09.007

Leutgeb, S., Leutgeb, J. K., Treves, A., Moser, M. B., & Moser, E. I. (2004). Distinct ensemble codes in hippocampal areas CA3 and CA1. *Science*, *305*, 1295–8. doi:10.1126/science.1100265

Liu, K. Y., Gould, R. L., Coulson, M. C., Ward, E. V., & Howard, R. J. (2016). Tests of pattern separation and pattern completion in humans-A systematic review: Tests of Pattern Separation and Pattern Completion. *Hippocampus*, *26*(6), 705–717. doi:10.1002/hipo.22561

Lorente de Nó, R. (1934). Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. *Journal Für Psychologie Und Neurologie*, *46*, 113–177.

McClelland, J. L., McNaughton, B. L., & O'Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychological Review*, *102*, 419–57.

Nelson, H. E., & O'Connell, A. (1978). Dementia: the estimation of premorbid intelligence levels using the New Adult Reading Test. *Cortex*, *14*, 234–44.

Neunuebel, J. P., & Knierim, J. J. (2014). CA3 retrieves coherent representations from degraded input: direct evidence for CA3 pattern completion and dentate gyrus pattern separation. *Neuron*, *81*, 416–27. doi:10.1016/j.neuron.2013.11.017

Norman, K. A., & O'Reilly, R. C. (2003). Modeling hippocampal and neocortical contributions to recognition memory: a complementary-learning-systems approach. *Psychological Review*, *110*, 611–646. doi:10.1037/0033-295X.110.4.611

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, *9*, 97–113.

O'Reilly, R. C., & McClelland, J. L. (1994). Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off. *Hippocampus*, *4*(6), 661–682. doi:10.1002/hipo.450040605

Quinette, P., Guillery, B., Desgranges, B., de la Sayette, V., Viader, F., & Eustache, F. (2003). Working memory and executive functions in transient global amnesia. *Brain*, *126*(9), 1917–1934. doi:10.1093/brain/awg201

Reitan, R. M. (1979). *Trail Making Test (TMT)*. Göttingen: Hogrefe.

Rey, A. (1941). L'examen psychologique dans les cas d'encéphalopathie traumatique. *Archives de Psychologie*, *28*, 21.

Rolls, E. T. (1996). A theory of hippocampal function in memory. *Hippocampus*, *6*, 601–20.

Rolls, E. T. (2016). Pattern separation, completion, and categorisation in the hippocampus and neocortex. *Neurobiology of Learning and Memory*, *129*, 4–28. doi:10.1016/j.nlm.2015.07.008

Rolls, E. T., & Kesner, R. P. (2006). A computational theory of hippocampal function, and empirical tests of the theory. *Progress in Neurobiology*, *79*, 1–48. doi:10.1016/j.pneurobio.2006.04.005

Sedlaczek, O., Hirsch, J. G., Grips, E., Peters, C. N. A., Gass, A., Wöhrle, J., & Hennerici, M. (2004). Detection of delayed focal MR changes in the lateral hippocampus in transient global amnesia. *Neurology*, *62*(12), 2165–2170.

Stark, S. M., & Stark, C. E. L. (2017). Age-related deficits in the mnemonic similarity task for objects and scenes. *Behavioral Brain Research*, *333*, 109–117. doi:10.1016/j.bbr.2017.06.049

Stark, S. M., Yassa, M. A., Lacy, J. W., & Stark, C. E. L. (2013). A task to assess behavioral pattern separation (BPS) in humans: Data from healthy aging and mild cognitive impairment. *Neuropsychologia*, *51*, 2442–9. doi:10.1016/j.neuropsychologia.2012.12.014

Treves, A., & Rolls, E. T. (1994). Computational analysis of the role of the hippocampus in memory. *Hippocampus*, *4*, 374–91. doi:10.1002/hipo.450040319

Vazdarjanova, A., & Guzowski, J. F. (2004). Differences in hippocampal neuronal population responses to modifications of an environmental context: evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles. *Journal of Neuroscience*, *24*, 6489–96. doi:10.1523/jneurosci.0350-04.2004

Vinogradova, O. S. (2001). Hippocampus as comparator: role of the two input and two output systems of the hippocampus in selection and registration of information. *Hippocampus*, *11*(5), 578–598. doi:10.1002/hipo.1073

Wechsler, D. (1997). *WMS-III Administration and scoring manual*. San Antonio, TX: The Psychological Corporation. Harcourt Brace & Co.

Weon, Y. C., Kim, J. H., Lee, J. S., & Kim, S. Y. (2008). Optimal diffusion-weighted imaging protocol for lesion detection in transient global amnesia. *American Journal of Neuroradiology*, *29*, 1324–8. doi:10.3174/ajnr.A1105

West, M. J., Coleman, P. D., Flood, D. G., & Troncoso, J. C. (1994). Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet*, *344*, 769–72.

West, M. J., Kawas, C. H., Martin, L. J., & Troncoso, J. C. (2000). The CA1 region of the human hippocampus is a hot spot in Alzheimer's disease. *Annals of the New York Academy of Sciences*, *908*, 255–9.

West, M. J., Kawas, C. H., Stewart, W. F., Rudow, G. L., & Troncoso, J. C. (2004). Hippocampal neurons in pre-clinical Alzheimer's disease. *Neurobiology of Aging*, *25*, 1205–12. doi:10.1016/j.neurobiolaging.2003.12.005

Wilson, M. A., & McNaughton, B. L. (1994). Reactivation of hippocampal ensemble memories during sleep. *Science*, *265*, 676–9.

Witter, M. P., & Amaral, D. G. (2004). Hippocampal Formation. In George Paxinos (Ed.), *The Rat Nervous System* (3rd ed., pp. 635–704). Burlington: Academic Press. doi:10.1016/B978-012547638-6/50022-5

Yassa, M. A., Lacy, J. W., Stark, S. M., Albert, M. S., Gallagher, M., & Stark, C. E. L. (2011). Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults. *Hippocampus*, *21*, 968–79. doi:10.1002/hipo.20808

Yassa, M. A., Mattfeld, A. T., Stark, S. M., & Stark, C. E. L. (2011). Age-related memory deficits linked to circuit-specific disruptions in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 8873–8. doi:10.1073/pnas.1101567108

Yassa, M. A., & Stark, C. E. L. (2011). Pattern separation in the hippocampus. *Trends in Neurosciences*, *34*(10), 515–525. doi:10.1016/j.tins.2011.06.006

Yassa, M. A., Stark, S. M., Bakker, A., Albert, M. S., Gallagher, M., & Stark, C. E. L. (2010). High-resolution structural and functional MRI of hippocampal CA3 and dentate gyrus in patients with amnestic Mild Cognitive Impairment. *NeuroImage*, *51*, 1242–52. doi:10.1016/j.neuroimage.2010.03.040

3 Study II

Hippocampal dentate gyrus atrophy predicts pattern separation impairment in patients with LGI1 encephalitis

A. Hanert, J. Rave, O. Granert, A. Pedersen, J. Born, C. Finke, and T. Bartsch

Published in *Neuroscience, February 2019, 400:120-131,*

doi: 10.1016/j.neuroscience.2018.12.046. [Epub January 2019]

3.1 Abstract

Day-to-day life involves the perception of events that resemble one another. For the sufficient encoding and correct retrieval of similar information, the hippocampus provides two essential cognitive processes. Pattern separation refers to the differentiation of similar input information, whereas pattern completion reactivates memory representations based on noisy or degraded stimuli. It has been shown that pattern separation specifically relies on the hippocampal dentate gyrus (DG), whereas pattern completion is performed within CA3 networks. Lesions to these hippocampal networks emerging in the course of neurological disorders may thus affect both processes. In anti-leucine-rich, glioma-inactivated 1 (LGI1) encephalitis it has been shown in animal models and human imaging studies that hippocampal DG and CA3 are preferentially involved in the pathophysiology process. Thus, in order to elucidate the structure-function relationship and contribution of hippocampal subfields to pattern separation, we examined patients (n=15, age range: 36-77 years) with the rare LGI1 encephalitis showing lesions to hippocampal subfields. Patients were tested 3.53 ± 0.65 years after the acute phase of the disease. Structural sequelae were determined by hippocampal subfield volumetry for the DG, CA1, and CA2/3. Patients showed an overall memory deficit including a significant reduction in pattern

separation performance ($p = 0.016$). In volumetry, we found a global hippocampal volume reduction. The deficits in pattern separation performance were best predicted by the DG ($p = 0.029$), whereas CA1 was highly predictive of recognition memory deficits ($p < 0.001$). These results corroborate the framework of a regional specialization of hippocampal functions involved in cognitive processing.

Key words: episodic memory, hippocampus-dependent memory, hippocampal sclerosis, hippocampal subfield segmentation, limbic encephalitis

3.2 Introduction

In everyday life, we are experiencing a constant string of episodes that can be more or less similar with regard to time, objects, location and content. The formation of episodic memory, however, requires that similar experiences are transformed into unique and nonoverlapping episodes that can be differentiated into distinct memories. To prevent these memories from interference and to ensure correct retrieval of newly encoded episodes, the hippocampus provides two neural operations which differentiate similar episodes and store them as distinct neural representations (Knierim & Neunuebel, 2016; McClelland, McNaughton, & O'Reilly, 1995; Rolls, 2016). First, a pattern separation process is critical for the separation and storage of similar and overlapping memory representations. During encoding, the neural input is orthogonalized and de-correlated by associating distinct neural codes to the similar representations (Treves & Rolls, 1994). Secondly, pattern completion involves the reactivation of previously stored memories in case of noisy, incomplete or degraded input (Yassa & Stark, 2011). At retrieval, the pre-existing memory representation is reactivated as the overlapping input is used as a retrieval cue (McClelland et al., 1995; Norman & O'Reilly, 2003; Rolls, 2016). Animal models and human imaging studies suggest that the hippocampal dentate gyrus (DG) is partic-

ularly involved in pattern separation whereas CA3 is capable of performing both, pattern separation and completion computations depending on the variance of the sensory input (Bakker, Kirwan, Miller, & Stark, 2008; Berron et al., 2016; Lee, Yoganarasimha, Rao, & Knierim, 2004; J. K. Leutgeb, Leutgeb, Moser, & Moser, 2007; S. Leutgeb, Leutgeb, Treves, Moser, & Moser, 2004). Animal findings show that neuronal ensembles in CA1 are also involved in pattern separation but differ from CA3 as they show a more linear input-output function in response to environmental changes. However, studies in humans characterizing the contribution of CA1 to pattern separation and completion are still scarce (Vazdarjanova & Guzowski, 2004).

In this context, the study of specific lesion models allows further clarification of the causal relationship of individual hippocampal subfield function and their operation in hippocampus-dependent memory processing (Bartsch, Döhring, Rohr, Jansen, & Deuschl, 2011; Bartsch et al., 2010; Döhring et al., 2017). Considering this, we examined patients with an anti-leucine-rich, glioma-inactivated 1 (LGI1) encephalitis who show lesion-associated and degenerative changes in hippocampal subfields. Patients who are positive for LGI1 antibodies develop limbic encephalitis and exhibit memory impairments and hippocampus-associated epileptic seizures in the acute stage (Irani et al., 2011, 2013; Malter et al., 2014), whereas in post-acute stages, significant and disabling memory deficits persist (Bettcher et al., 2014; Butler et al., 2014). Interestingly, the LGI1 gene transcript in the mouse is mainly expressed in the pyramidal and granular layers of the DG and CA3 field of the hippocampus, where the perforant path fibers from the entorhinal cortex project onto dendrites of the DG granule cells (Bartsch & Wulff, 2015; Herranz-Pérez, Olucha-Bordonau, Morante-Redolat, & Pérez-Tur, 2010; Kalachikov et al., 2002). Thus, the features of the LGI1 pathogenesis involving both the hippocampal DG and CA3 regions offer a lesion model to study the function of DG and CA3 within memory processing in the hippocampal network. In these patients, significant atrophy in the hippocampal CA2/3 and CA4/DG regions and a chronic memory impairment has been reported in the post-

acute stage (Finke et al., 2017). Also, Miller et al. (2017) found a bilateral CA3 atrophy in patients with this rare form of limbic encephalitis.

The aim of the present study was to further elucidate the structure-function relationship and the contribution of hippocampal subfields to pattern separation in humans. We expected to find deficits in pattern separation in patients with LGI1 encephalitis as a result of hippocampal atrophy and as a consequence of limbic encephalitis (Malter et al., 2014). Against this background, inflammatory lesions particularly expressed in DG and CA3 that are characteristic of LGI1-antibody mediated encephalitis should correlate with impairments in subfield-specific computations as seen in a greater variability in hippocampal subfield volumetry (Finke et al., 2017; Miller et al., 2017). Therefore, we tested LGI1 patients on a behavioral task, i.e., the Mnemonic Similarity Task (MST), that has been shown to tax hippocampal pattern separation (Kirwan and Stark, 2007; Stark et al., 2013; Hanert et al., 2017) and correlated task performance to structural sequelae in the hippocampus using high-resolution volumetry of the hippocampus. Hippocampal volumetry for the subfields of interest was assessed using the automated segmentation method Freesurfer 6.0.0.

3.3 Experimental Procedures

3.3.1 Study cohort

Fifteen patients (mean age: 64.47 ± 3.28 years, range: 36-77, 9 male) with anti-LGI1 encephalitis participated in the study. All reported data were collected after the acute stage of the limbic encephalitis with a mean time between symptoms onset and study examination of 3.53 ± 0.65 years. Early symptoms of the limbic encephalitis before the onset of the acute phase (i.e., hippocampus-associated temporal lobe seizures, uni- or bilateral faciobrachial dystonic and other types of seizures) were reported by 10 patients (66 %). The acute phase of limbic encephalitis was accompanied by typical clinical features such as amnesia, confusion, and behavioral and

mood disturbances. Patients were moderately neurologically impaired measured by the modified Rankin Scale (mRS) score (mean: 1.53 ± 0.26, range: 0-3). Fifteen control participants (mean age: 65.13 ± 3.11, range: 40-80, 9 male) were individually matched according to sex, age and educational background including profession and years of formal education. The study was approved by the local ethics committee. All participants gave written informed consent for the procedures. The clinical and laboratory characteristics of some of these patients have been published (Finke et al., 2017). The present study provides an additional and new assessment of cognitive performance as well as a new analysis of the MRI data. The behavioral testing including neuropsychological assessment and acquisition of MRI data were no longer than 6 months apart.

3.3.2 Behavioral tests

Mnemonic similarity task

Behavioral pattern separation was assessed by means of the Mnemonic Similarity Task (MST; Kirwan & Stark, 2007; Stark et al., 2013; http://faculty.sites.uci.edu/starklab/mnemonic-similarity-task-mst/). The computer-based task presents items on the screen as color photographs of everyday objects on a white background. The encoding phase included 128 items that had to be identified as either indoor or outdoor object. The immediate test phase comprised 192 items displaying in each case one third as exact repetitions of the encoded items (64 targets), similar items (64 lures), and items that were totally new (64 foils). In this phase, participants indicated whether the objects were ‘old’, ‘similar’ or ‘new’ to the previously encoded targets. Of particular importance were the responses to lure items with the correct ‘similar’ response indicating successful pattern separation, whereas incorrect ‘old’ responses to lures suggest a bias toward pattern completion (Bakker et al., 2008; Lacy, Yassa, Stark, Muftuler, & Stark, 2011; Yassa et al., 2010). The lure objects were divided into five degrees of similarity to a target object ranging from 1 (most similar) to 5 (least similar). Therewith, behavioral pattern separation was also

assessed as a function of lure similarity (Lacy et al., 2011; Yassa et al., 2010). In both the encoding and recall phases the stimuli were presented for 3 s with 1 s inter stimulus interval. For recording of data, participants had to respond via button press within the 3-s stimulus presentation (Figure 3-1). By means of participants' responses at recall a Pattern Separation score (PatSep score) and a Recognition Memory (RM) score were computed each corrected for a response bias: i) *Pattern Separation (PatSep) score: PatSep = [p (correct similar response to lures) – p (false similar response to foils)]*, ii) *Recognition Memory (RM) score: RM = [p (correct old response to targets) – (false old response to foils)]* (Stark et al., 2013; Yassa, Lacy, et al., 2011; Yassa et al., 2010).

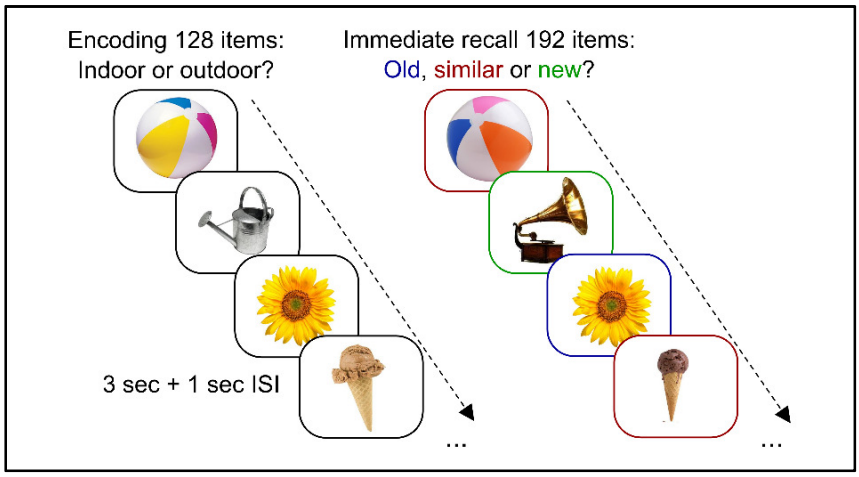

Figure 3-1. Procedure of the MST. First, participants encoded 128 items of everyday objects by judging the items as indoor or outdoor objects. Then participants were supposed to decide whether the items were old, similar or new to the previously seen targets in an immediate recall condition containing 192 items. Displayed pictures are taken from the original image data base of the MST. ISI, inter-stimulus interval.

Neuropsychological assessment

A comprehensive neuropsychological test battery was used to test episodic memory (Rey auditory verbal learning test, RAVLT; Rey, 1941), visuospatial memory (Rey-Osterrieth complex figure, ROCF), working memory (digit span forwards and backwards), executive functioning (Trailmaking Test A and B, TMT; Reitan, 1979), verbal fluency (Regensburg word fluency

test, RWT; Aschenbrenner, Tucha, & Lange, 2000), and premorbid general intelligence (Mehrfach-Wortschatz-Intelligenztest-B, MWT-B, as a German equivalent of the National Adult Reading Test; Lehrl, 2005) as described in Finke et al. (2017).

3.3.3 MRI acquisition and hippocampal subfield segmentation

Whole-brain MRI were acquired using 3 Tesla MRI Scanners (Siemens Tim Trio, Siemens, Erlangen, Germany; Philips Achieva, Philips, Best, The Netherlands). T1-weighted MRI scans were recorded using a three-dimensional magnetization prepared rapid gradient-echo sequence (3D MPRAGE, matrix size = 240x240, 176 slices, voxel size = 1x1x1 mm^3). The evaluation of clinical images was based on T2-weighted turbo spin echo sequences as well as a 3D isotropic T2-weighted fluid attenuated inversion recovery (FLAIR).

Hippocampal subfield volumetric segmentation was performed on the T1-weighted scans using the freely available software Freesurfer image analysis suite version 6.0.0 (http://surfer.nmr.mgh.harvard.edu/). The standard processing steps of Freesurfer 6.0.0 are described as follows: first, nonbrain tissues were removed using a hybrid watershed/surface deformation procedure (Ségonne et al., 2004). Then, images were automatically transformed to Talairach coordinates and subcortical white matter and deep gray matter volumetric structures containing the hippocampal formation were segmented (Fischl et al., 2004). The process was followed by intensity normalization (Sled, Zijdenbos, & Evans, 1998) and tessellation of the gray matter/white matter boundary (Ségonne, Pacheco, & Fischl, 2007). The automated hippocampal subfield segmentation was performed by means of Bayesian inference and a probabilistic atlas of the hippocampal formation (Fischl et al., 2004; Van Leemput et al., 2009). The segmentation results were visually rechecked for accuracy in all subjects. Freesurfer 6.0.0. provides results regarding the volume of the alveus, parasubiculum, presubiculum, subiculum, CA1, CA2/3, CA4, granule cell layer of the DG (GC-DG), hippocampus-amygdala-transition-

area, fimbria, molecular layer for subiculum and CA fields, hippocampal fissure and hippocampal tail. However, the analysis regarding hippocampal volumetry was hypothesis-driven and focused on the hippocampal regions of interest that are critically involved in pattern separation and completion processes (i.e. CA1, CA3 and DG) (Yassa & Stark, 2011). Given the segmented subfields by Freesurfer 6.0.0, the analyses thus included CA1, CA2/3, GC-DG, and CA4. The DG is originally formed by the granule cell, polymorphic, and molecular layers (Amaral, Scharfman, & Lavenex, 2007). Freesurfer 6.0.0 assigns the DG's polymorphic and molecular layer to the CA4 region and keeps the DG separate with the layer of the granule cells (Iglesias et al., 2015). Thus, we included CA4 to the GC-DG region in our analysis to make plausible predictions about the global DG and its contribution to hippocampal pattern separation. Throughout the analysis, we use the term 'DG' referring to the volume of the segmented GC-DG and CA4 regions.

The updated technique of the Freesurfer 6.0.0 version provides significant advantages over the earlier method used in Freesurfer 5.3 described in (Van Leemput et al., 2009). Due to the use of an atlas based on ex vivo MRI data, the precision of the segmentation of subfield boundaries was improved that also affected the accuracy of hippocampal subfield volumes. Particularly, the delineation and segmentation of volumes of CA1 and CA2/3 are much more congruent with previous histological studies (Iglesias et al., 2015). The volumes of each subfield were corrected for inter-individual head size by means of the estimated total intracranial volume (eTIV). The correction was computed according to an atlas normalization formula (Buckner et al., 2004).

3.3.4 Statistical Analyses

The Shapiro-Wilk test was used for pretesting of normal distributions and Levene's test was performed for the assessment of homogeneity of variances. Differences between the patient and control group were examined with paired samples t-tests or Wilcoxon signed–rank tests de-

pending on distribution. Accordingly, confidence intervals were calculated for either the difference of the means or medians. 2 x 5 repeated measures ANOVA with group as repeated factor (patient vs. control) and similarity (1 to 5) as within-subjects factor were performed to show differences in PatSep scores regarding lure similarities. Spearman's ρ expressing the relation between PatSep scores and lure similarity was calculated for every participant separately and the mean correlation for both groups was calculated. Significances of the average correlations were tested using Wilcoxon signed–rank tests against zero. Depending on distribution Pearson's r or Spearman's ρ was used to characterize the relationship between the PatSep score and the RM score as well as the scores from the neuropsychological test battery. To analyze differences in hippocampal volume a three-way repeated measures ANOVA with group as repeated factor (patient vs. control) and subfield (CA1, CA2/3, DG) as well as side (left vs. right) as within-subject factors was conducted. Degrees of freedom were corrected according to Greenhouse-Geisser adjustment if the assumption of sphericity was violated. ANOVA were followed by planned post-hoc pairwise comparisons to specify significant main and interaction effects. To predict behavioral outcome variables (i.e., PatSep and RM scores) by hippocampal subfield volumes, multiple linear regression analyses were performed. The independence of residuals was checked by the Durbin-Watson statistic. For testing the distribution of residuals regarding normality, the Shapiro-Wilk test was performed. Homoscedasticity of residuals was tested using the Breusch-Pagan test. As the independent variables were correlated, the established method of backward elimination was used to find best predictors of PatSep and RM scores. Adjustment for multiple testing was done using Benjamini & Hochberg's False Discovery Rate. The significance level was set to $p < 0.05$, two-tailed for all tests. Data are specified as mean ± SEM if not otherwise stated.

3.4 Results

3.4.1 Mnemonic similarity task

Paired samples t-tests showed that pattern separation performance of LGI1 patients was significantly lower (22.01 ± 4.60) than the performance of controls (35.83 ± 3.92) ($t(14) = 3.10$, $p = 0.016$, 95% CI [4.24, 23.41]). With regard to recognition memory, patients performed worse than controls (patients: 65.67 ± 6.02, controls: 79.80 ± 2.46, $t(14) = 2.56$, $p = 0.023$, 95% CI [2.31, 25.96]) (Figure 3-2). There was no significant correlation between pattern separation performance and recognition memory neither in the patient ($r = 0.354$, $p = 0.196$) nor in the control group ($r = 0.420$, $p = 0.120$). To further ensure that the pattern separation deficit was not secondary to a general impairment in recognition memory as well as to prevent that the results are biased by floor effects, we reran paired samples t-tests with the exclusion of patients ($n = 3$) whose recognition performance was below 3 standard deviations from the mean of the control group (79.80 ± 9.52). The exclusion of highly impaired patients showed that recognition memory performance was equal in both groups (patients: 75.00 ± 3.65, controls: 82.00 ± 2.45, $t(11) = 1.78$, $p = 0.102$, 95% CI [-1.65, 15.65]), whereas pattern separation performance still differed significantly (patients: 22.93 ± 5.57, controls: 34.38 ± 4.73, $t(11) = 2.21$, $p < 0.05$, 95% CI [0.02, 22.88], no alpha adjustment).

Separate comparisons regarding the response types revealed no difference between the groups regarding the ‘old’ response to lures (patients: 43.93 ± 5.21, controls: 42.93 ± 2.82, $t(14) = -0.21$, $p = 0.833$, 95% CI [-11.01, 9.01]). In contrast, patients were more prone to incorrectly respond ‘new’ to lures (patients: 26.87 ± 5.35, controls: 12.40 ± 2.06, $t(14) = -2.90$, $p = 0.017$, 95% CI[-25.15, -3.78]). However, excluding the highly memory impaired patients (≤ Q1 in recognition memory) led to equal results across groups regarding ‘new’ responses to lures (patients: 19.25 ± 4.12, controls: 12.17 ± 2.49, $t(11) = -1.98$, $p = 0.074$, 95% CI[-14.97, 0.80]). Results for paired samples t-tests for all response types are displayed in Figure 3-2 B-D.

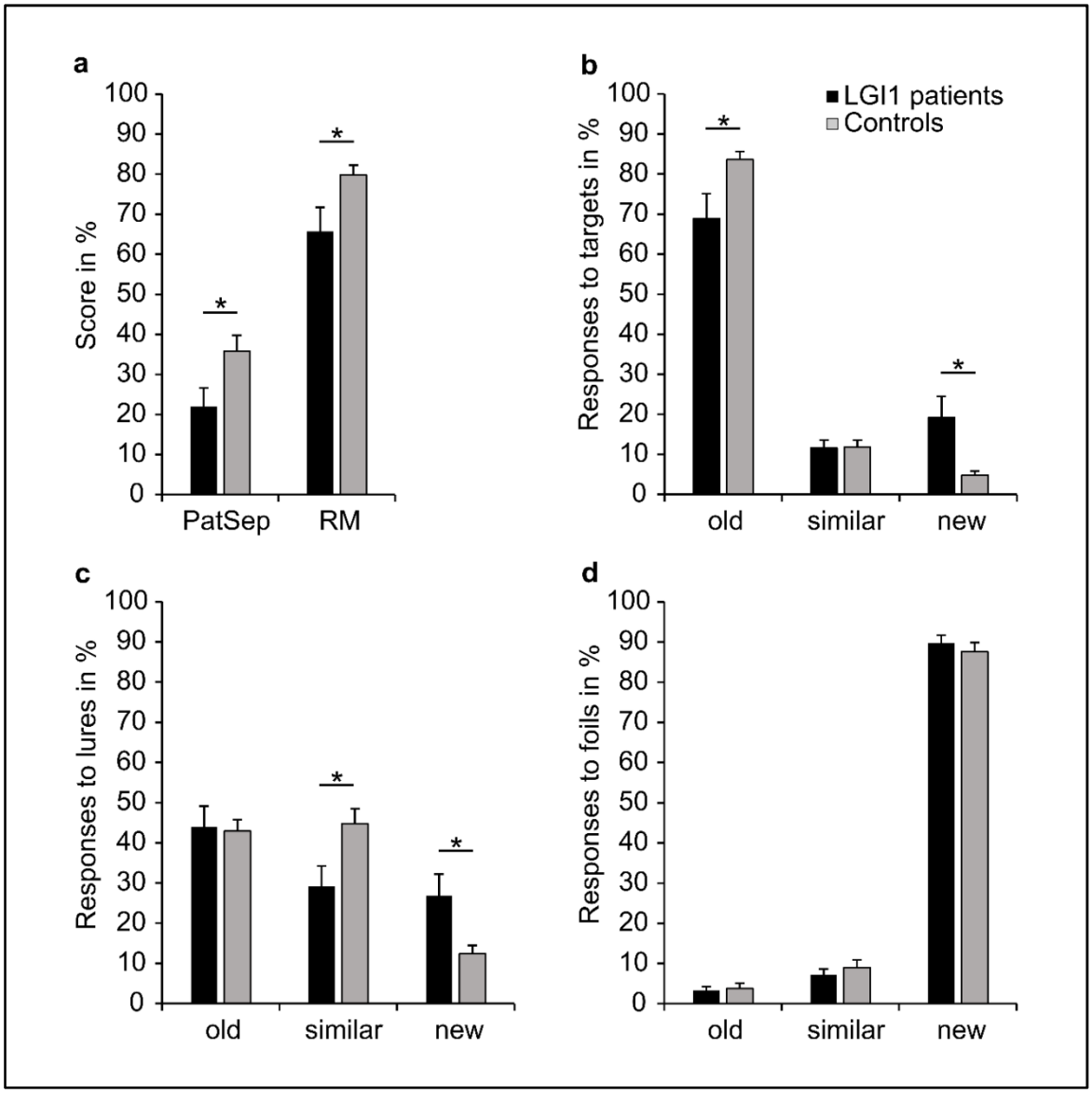

Figure 3-2. Results of the MST including the PatSep and RM scores as well as all response types. Note that the PatSep and RM scores are bias corrected scores. Values of the response types are given in percent corresponding to the item types. Adjustment for multiple testing was done using Benjamini & Hochberg's False Discovery Rate. * $p < 0.05$.

Entering the PatSep scores for 5 degrees of lure similarity in a 2 x 5 repeated measures ANOVA revealed no group x similarity interaction ($F(4, 56) = 0.647$, $p = 0.632$) but significant main effects for group ($F(1, 14) = 10.03$, $p = 0.007$) as well as similarity ($F(4, 56) = 8.65$, $p < 0.0001$). Post-hoc pairwise tests of simple effects demonstrated superior performance for the control group in every lure similarity, though not statistically significant for every degree (Lure 1: $t(14) = 1.83$, $p = 0.088$, 95% CI [-1.86, 23.81], Lure 2: $t(14) = 3.17$, $p = 0.034$, 95% CI [5.39, 27.94],

Lure 3: t(14) = 1.85, p = 0.088, 95% CI [-1.53, 20.49], Lure 4: t(14) = 2.46, p = 0.046, 95% CI [2.08, 30.22], Lure 5: t(14) = 2.66, p = 0.046, 95% CI [3.21, 30.12]) (Figure 3-3).

We further analyzed different degrees of lure similarity by means of a calculation of Spearman's rank correlation coefficients between lure similarity and the PatSep scores for every patient and control. The PatSep score was positively correlated with lure similarity for both the patient (r_s = 0.515 ± 0.14, Z = 2.84, p = 0.008, for test against 0) and control group (r_s = 0.378 ± 0.13, Z = 2.36, p = 0.018, for test against 0).

The results indicate that LGI1 patients were impaired in correctly separating lures from related targets. Notably, the same held true not only for the overall PatSep score but also for the scores related to lure similarities. The significant slope in pattern separation performance in both groups demonstrates that patients did not show a differential impairment in separating either highly similar or least similar lures as the deficit in pattern separation was equally dispersed across all degrees of similarity (Figure 3-3).

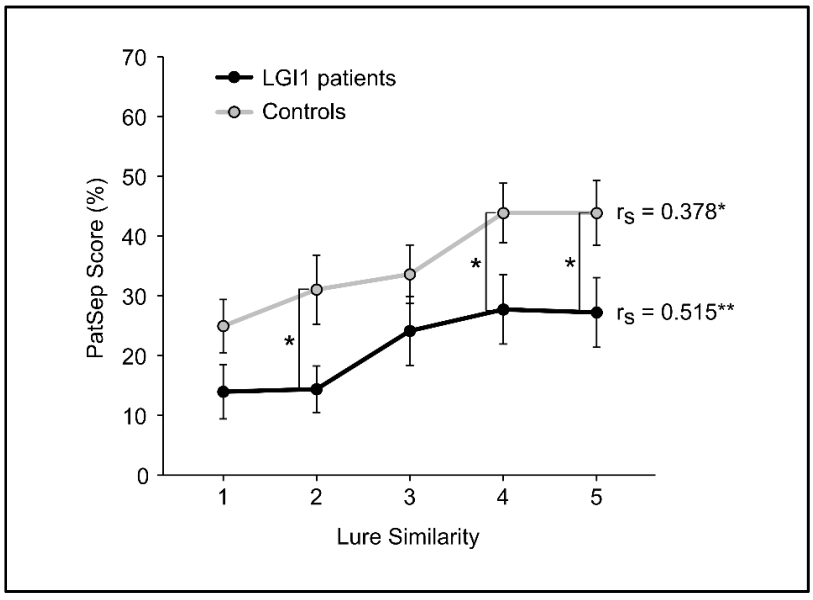

Figure 3-3 Pattern separation performance as a function of lure similarity from 1 (most similar) to 5 (least similar). There is a significant gradual increase in pattern separation performance from high to low similarity for both the patient and control group. However, controls show superior performance in pattern separation compared to LGI1 patients from highly similar lures to lures with low similarity to targets. * $p < 0.05$, ** $p < 0.01$.

3.4.2 Neuropsychological data

Patients were profoundly impaired in episodic verbal memory performance measured by the RAVLT (cf. Finke et al. (2017)). They memorized fewer words throughout the five learning trials (patients: 36.20 ± 4.46, controls: 60.67 ± 1.97, t(14) = 5.91, p = 0.0002, 95% CI [15.60, 33.34]), performed worse on the retention trial (patients: 6.33 ± 1.41, controls: 13.60 ± 0.46, Z = -3.08, p = 0.0021), as well as in delayed recall (patients: 5.87 ± 1.46, controls: 14.07 ± 0.36, t(14) = 5.41, p < 0.0001, 95% CI [4.95, 11.45]). Also, patients were impaired in recognizing the previously learned words compared to the healthy controls (patients: 10.00 ± 1.13, controls: 14.60 ± 0.16, t(14) = 4.24, p = 0.0002, 95% CI [2.27, 6.93]). Summarizing the results of the neuropsychological assessment, patients were also impaired in visuo-spatial and working memory, executive functions, as well as verbal fluency (Table 3-1). We did not find any correlation between the PatSep score and neuropsychological test variables in the patient group (all p's > 0.142), whereas the control group showed significant correlations between the PatSep score and the learning trials of the RAVLT (r = 0.636, p = 0.043), visuospatial memory (all p's < 0.05), and working memory (r = 0.625, p = 0.017).

Table 3-1. Neuropsychological data of LGI1 patients and controls (mean ± SEM)

	LGI1 patients	Controls	95% CI	t	Z	p
RCF copy	31.80 ± 1.20	29.40 ± 1.42	[-5.67, 0.87]	-1.57	-	0.138
RCF recall	15.78 ± 2.50	28.67 ± 1.38	[7.70, 18.10]	5.32	-	0.0001
TMT-A	57.13 ± 8.28	35.87 ± 4.22	[-33, -4]	-	-2.81	0.005
TMT-B	201.13 ± 47.94	95.23 ± 14.69	[-150, -14]	-	-2.67	0.008
MWT-B*	24.29 ± 2.50	29.53 ± 1.57	[-0.29, 10.29]	2.04	-	0.062
RWT-forenames	20.47 ± 1.98	30.60 ± 1.91	[3.61, 16.66]	3.33	-	0.005
RWT-S	12.40 ± 1.61	18.20 ± 1.32	[1.00, 10.60]	2.59	-	0.021
Digit span total	11.80 ± 1.21	16.47 ± 1.03	[1.81, 7.53]	3.50	-	0.004

t(df=14), * t(df=13); RCF, Rey-Osterrieth complex figure; TMT, Trail-making test; MWT, Mehrfach-Wortschatz-Intelligenztest; RWT, Regensburg word fluency test.

3.4.3 Hippocampal volumetry

A whole-brain analysis of normalized cortical gray matter volume showed no significant reduction in patients compared to controls ($t(14) = -1.83$, $p = 0.088$, 95% CI [-6.62, 0.52], patients: 39.71 ± 1.88, controls: 42.76 ± 1.40). Cortical gray matter volume was not correlated to behavioral measurements of the MST neither in the patient nor in the control group (all p's > 0.427). With regard to the whole bilateral hippocampal volume, we found a significant reduction for patients (Table 3-2). A three-way repeated measures ANOVA (group x side x subfield) revealed significant main effects of group ($F(1, 14) = 14.82$, $p < 0.01$) and subfield ($F(2, 18.06) = 1123.61$, $p < 0.0001$), but no effect of side ($F(1, 14) = 0.85$, $p = 0.373$). Among two way interactions only the group x subfield interaction was significant ($F(2, 28) = 10.11$, $p < 0.001$). The three way interaction between the included factors remained also non-significant ($F(2, 15.67) = 0.469$, $p = 0.525$). As we found no effects for the hippocampal sides, further analyses were based on collapsed left and right hippocampal volumes. Post-hoc pairwise comparisons showed that all analyzed subfields were significantly reduced in LGI1 patients (Table 3-2).

Table 3-2. Hippocampal volumetry (mm3) for each subfield for LGI1 patients (n=15) and controls (n=15)

	LGI1 patients	Controls	95% CI	t	p
CA1	544.88 ± 21.19	640.76 ± 18.06	[42.65, 149.12]	3.86	0.0029
CA2/3	182.14 ± 8.10	218.49 ± 6.15	[14.76, 57.93]	3.61	0.0029
DG	467.22 ± 19.74	554.44 ± 15.66	[36.00, 138.44]	3.65	0.003
Total hippocampal volume	2883.29 ± 112.32	3381.08 ± 95.82	[252.47, 743.09]	4.35	0.002

Volumes are presented as mean ± SEM (mm^3) averaged across sides and normalized for estimated total intracranial volume. $t(df = 14)$. Adjustment for multiple testing was done using Benjamini & Hochberg's False Discovery Rate.

Pattern separation performance depends on DG atrophy, whereas CA1 volume predicts recognition memory

The volumes of CA1, CA2/3, and DG were inserted into a stepwise multiple regression model to predict pattern separation performance. The backward stepwise regression demonstrated that only the volume of the DG was a significant predictor ($t(29) = 2.30$, $p = 0.029$, 95% CI [0.01, 0.17]) (Figure 3-4. a-d)A) in the statistically significant model ($F(1, 29) = 5.30$, $p = 0.029$) that accounted for approximately 16% of the variance of pattern separation performance. With regard to the RM score only the volume of CA1 earned entry to the prediction model ($t(29) = 4.75$, $p < 0.001$, 95% CI [0.001, 0.002]) (Figure 3-4. a-d)B). The resulting equation by removing insignificant CA2/3 and DG volume was able to explain nearly 45% of the variance of the RM score ($F(1, 29) = 22.54$, $p < 0.001$). All models with corresponding parameters from the backward stepwise regression analyses are presented in Table 3-3.

Table 3-3. Stepwise linear regression model to predict the PatSep and RM scores from variability in hippocampal subfield volume

PatSep	Model 1			Model 2			Model 3		
Variable	B	SE (B)	ß	B	SE (B)	ß	B	SE (B)	ß
CA1	-0.040	0.107	-0.201						
CA2/3	-0.401	0.330	-0.747	-0.394	0.324	-0.733			
DG	0.286	0.180	1.306	0.241	0.132	1.102	0.088	0.038	0.399*
R^2		0.207			0.203			0.159	
RM									
Variable	B	SE (B)	ß	B	SE (B)	ß	B	SE (B)	ß
CA1	0.002	0.001	0.783	0.002	0.001	0.762*	0.001	0.0003	0.668***
CA2/3	-0.0004	0.003	-0.076	-0.001	0.002	-0.104			
DG	-0.0001	0.002	-0.050						
R^2		0.448			0.448			0.446	

B, unstandardized coefficient; SE (B) standard error of the coefficient; ß, beta coefficient, *$p < 0.05$, *** $p < 0.001$.

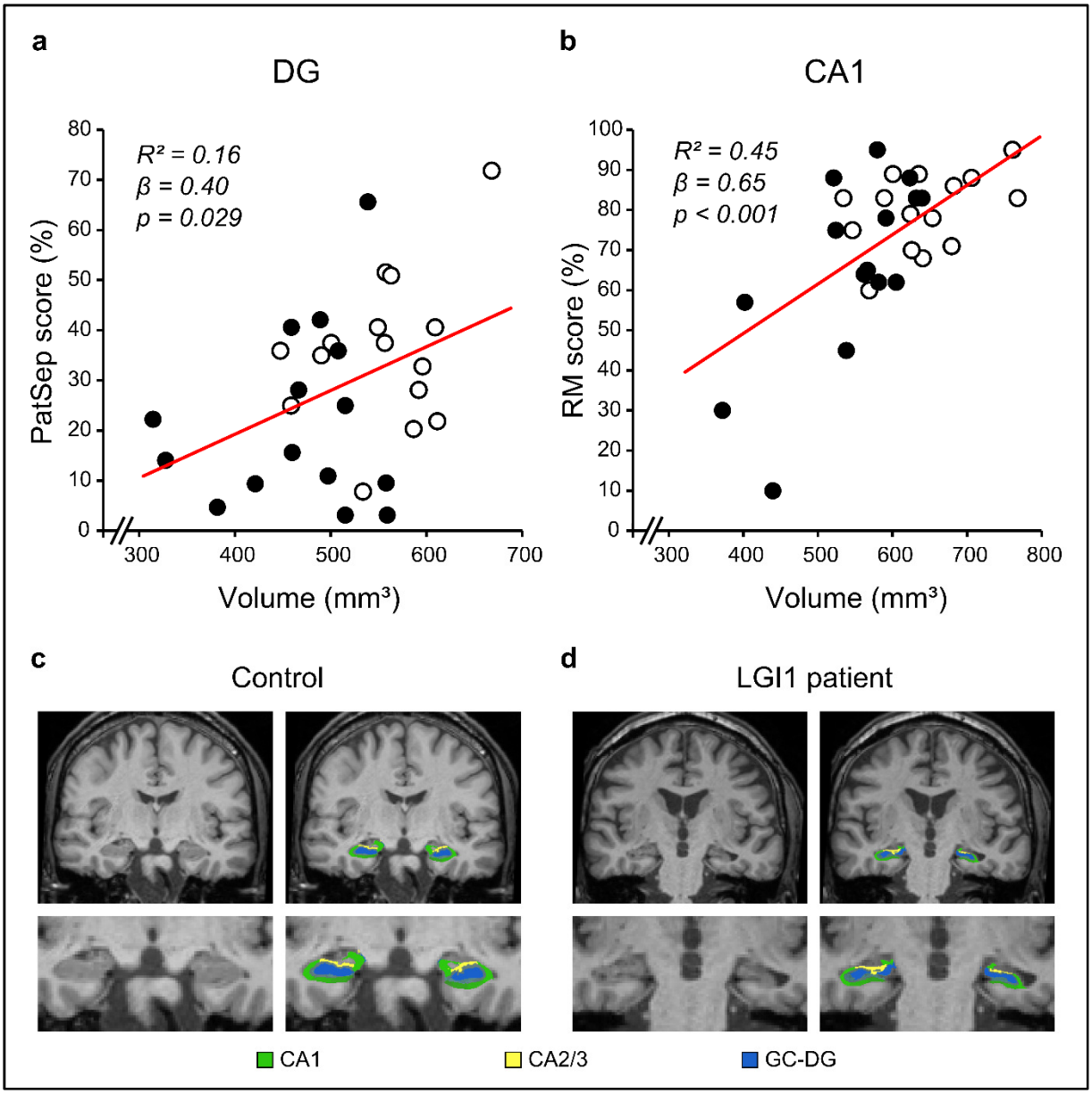

Figure 3-4. a-d) a) – b) Regression lines depict the predictive model of bilateral hippocampal subfield volumes (mm³) of the DG on pattern separation and CA1 on recognition memory. Higher volumes of the DG predict higher pattern separation performance across controls (white) and LGI1 patients (black), whereas higher volumes of CA1 predict higher recognition memory performance. c) – d) T1-weighted MR scans of representative subjects of both the control and patient group shows the hippocampal subfield segmentation. Note the higher hippocampal volume for the control participant. PatSep, pattern separation; RM, recognition memory CA, cornu ammonis; DG, dentate gyrus.

3.4.4 Clinical imaging

Follow-up routine MRI data were available for 14 patients and showed hippocampal atrophy in 13 patients (92.85%). In 9 of 14 patients (64.29%), hippocampal atrophy was accompanied by

T2/ FLAIR signal increase and loss of internal laminar architecture indicating hippocampal clerosis in the dentate gyrus region (Figure 3-5).

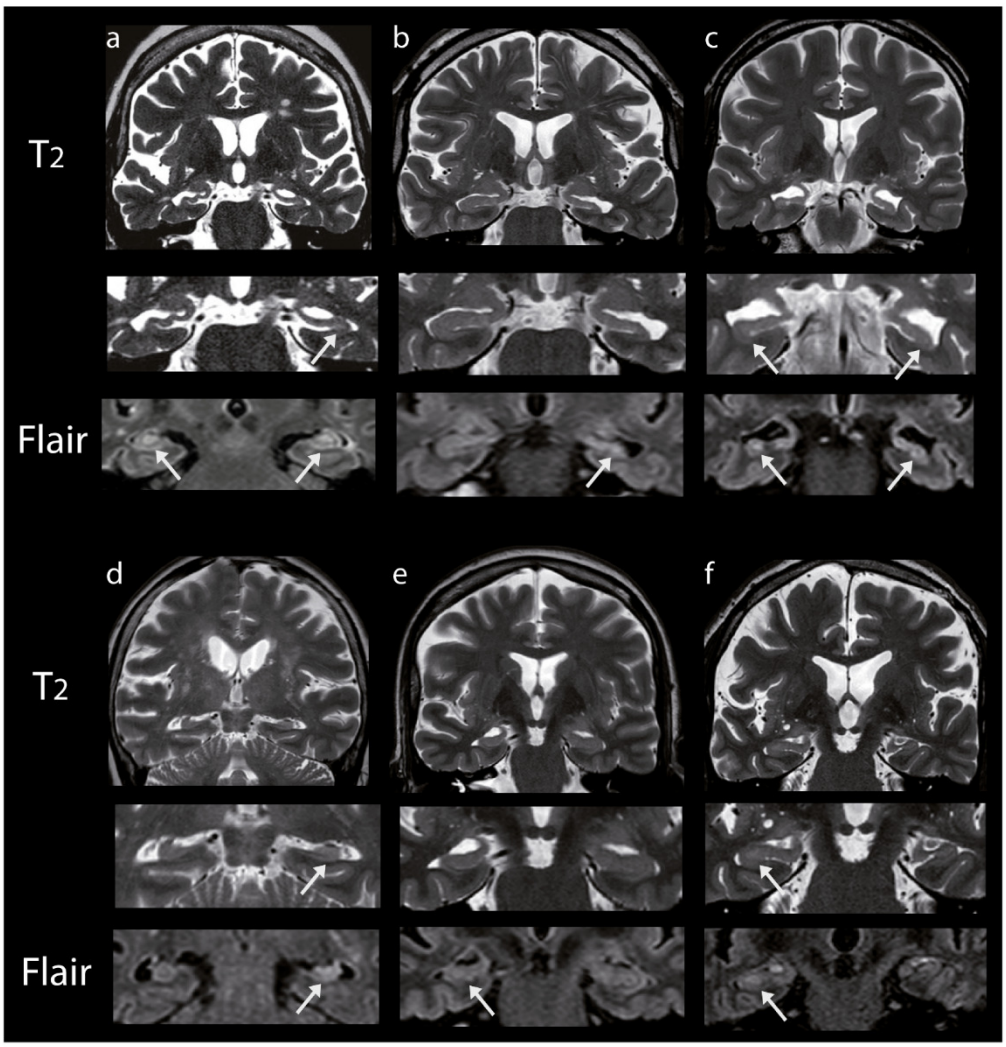

Figure 3-5. a – f): Representative clinical MR images of six patients with LGI1 encephalitis during follow-up and time point of testing. Top row: Coronal T2-weighted imaging showing bilateral (a, c, d) or unilateral (b, e, f) hippocampal atrophy. Magnification shows atrophy of all hippocampal cortical layers including CA1 and a predominant atrophy of the dentate gyrus region including loss of internal laminar architecture in CA4/DG. Coronal FLAIR imaging shows signal hyperintensities in hippocampal region CA4/DG (arrows).

3.5 Discussion

Our study demonstrates that patients with a LGI1 encephalitis compared to healthy controls show an impaired pattern separation and recognition memory performance in combination with a global hippocampal volume loss. However, despite the global volume reduction, we found a significant structure-function relationship for pattern separation performance for the DG. Compared to the areas CA2/3 and CA1, the DG proved to be the best predictor of pattern separation performance measured by a mnemonic similarity task. Our results thus corroborate the emerging findings of human studies that pattern separation performance is especially mediated by the hippocampal DG (Bakker et al., 2008; Lacy et al., 2011). Moreover, CA1 volume predicted recognition memory performance more than any other region of interest. These findings suggest a regional specialization of hippocampal functions involved in cognitive processing.

Using the MST in combination with magnetic resonance imaging, previous fMRI studies suggested the CA3 and DG regions to be associated with pattern separation performance (Bakker et al., 2008; Lacy et al., 2011). However, in these studies CA3/DG was collapsed and studied in a unitary way due to a limitation in the resolution of imaging. Of particular importance, a recent ultra-high resolution fMRI study with 7 Tesla showed that only the DG compared to other hippocampal subfields showed separation-like activity evoked by items presented by a mnemonic similarity task, supporting our finding of a preferential involvement of the DG in pattern separation performance in humans (Berron et al., 2016). In addition, a recent case study examining a patient with bilateral ischemic lesions in the DG further suggested a particular role for the DG in pattern separation as the impaired patient performed slightly worse on the MST compared to a healthy control group (Baker et al., 2016).

Data from recent human studies demonstrated deficits in behavioral pattern separation in patients with amnestic mild cognitive impairment (Stark et al., 2013; Yassa et al., 2010), Alzheimer's disease (Ally, Hussey, Ko, & Molitor, 2013), and traumatic brain injury (Kirwan et

al., 2012). Similarly, healthy aged humans showed deficits in pattern separation through a decline of pattern separation ability during aging (Holden, Toner, Pirogovsky, Kirwan, & Gilbert, 2013; Stark, Stevenson, Wu, Rutledge, & Stark, 2015; Stark et al., 2013; Stark, Yassa, & Stark, 2010; Toner, Pirogovsky, Kirwan, & Gilbert, 2009; Yassa, Lacy, et al., 2011). The present data thus complement the current view on pattern separation dependent on hippocampal integrity in the context of disease-related structural as well as age-related changes in humans. Moreover, we additionally studied mnemonic processing of stimuli with high or low similarity to a corresponding target. Our patient cohort showed impairments in pattern separation graded across all similarity levels, a finding that is reminiscent of the behavioral outcome found in healthy aging (Stark et al., 2013; Yassa, Lacy, et al., 2011). Indeed, a preferential degradation of DG function has been implicated in aging processes (Small, Tsai, DeLaPaz, Mayeux, & Stern, 2002; West, 1993; Yassa, Mattfeld, Stark, & Stark, 2011).

On a neural level, computational models suggest that within the hippocampal network, the DG performs pattern separation by a decorrelation of overlapping neural assemblies at encoding (Rolls, 2016; Treves & Rolls, 1994). This concept is supported by a variety of experimental rodent studies showing that the hippocampal DG is indeed involved in pattern separation, whereas the area CA3 performs pattern completion (J. K. Leutgeb et al., 2007; S. Leutgeb & Leutgeb, 2007; McHugh et al., 2007; Neunuebel & Knierim, 2014). In the process of pattern completion within CA3, the interconnection of pyramidal neurons functions as an auto-associative network so that a stored representation can be retrieved from an incoming partial cue (Norman & O'Reilly, 2003; O'Reilly & McClelland, 1994). Considering this, it has been suggested that the interplay of the DG and CA3 within the tri-synaptic circuit of the hippocampus is a reflection of a putative concomitant functional interdependence of pattern separation and completion as a result of a dynamic process-inherent trade-off depending on the current state of input-dependent system requirements (Lisman, 1999; O'Reilly & McClelland, 1994). In this

process, CA3 is assumed to be able to switch between pattern separation and completion based on input similarity (Knierim & Neunuebel, 2016; J. K. Leutgeb et al., 2007; Vazdarjanova & Guzowski, 2004). In this connection, we showed in another study that the process of pattern separation in the hippocampus is strongly influenced by oscillatory dynamics during sleep so that memory representations are stabilized (Hanert, Weber, Pedersen, Born, & Bartsch, 2017). Judging from the inherent network anatomy of the DG-CA3 networks in our patients, it seems plausible that a dysfunctional DG with its strong projections onto CA3 also affects the down-stream network functions of CA3 itself. Here, we assume that the hippocampal circuit disruption in our patient cohort caused the deficits in pattern separation compared to our healthy control group. However, regarding the DG-CA3-network embedded in the tri-synaptic circuit, the DG volume turned out to be a better predictor of pattern separation performance compared to CA2/3. Considering the dysfunctional and lesioned DG-CA3 network in our patients the CA2/3 region failed to reach significance in the model probably due to the strong dependency of CA3's pattern separation function on intact DG inputs.

Of note, we could not show a significant prediction of pattern separation performance by CA1. However, recent imaging findings in humans showed that CA1 exhibits pattern separation-like activity when the input similarity is low (i.e., when the change of the input increases) (Lacy et al., 2011). Recordings from CA1 and CA3 cells in rodents likewise suggest a linear transfer function of CA1, whereas CA3 responds in a non-linear fashion (i.e., with pattern separation like activity for both small and large environmental changes; Guzowski, Knierim, & Moser, 2004; J. K. Leutgeb et al., 2005). In that sense, both human and rodent studies showed that CA1 is able to exhibit pattern separation, provided that the change of the input was large (Lacy et al., 2011; Lee et al., 2004; J. K. Leutgeb et al., 2005). However, as we presented both large and small input changes, it is possible that the effect of CA1 variability on pattern separation was dampened. Overall, given the sequential processing of mnemonic information in the DG, CA3

and CA1 network in the hippocampal trisynaptic circuit, we show despite the global atrophic changes in hippocampal regions the highest prediction of pattern separation performance by the DG structure supporting the special role of the DG in pattern separation processes in humans.

In addition to impaired pattern separation, our patients showed decreased recognition memory. These results are in accordance with previous studies using mnemonic similarity tasks to consider hippocampal efficiency in memory impaired patients (Ally et al., 2013; Yassa et al., 2010). Given the fact that poor memory recovery due to hippocampal atrophy is common in patients with LGI1 encephalitis (Malter et al., 2014) this finding was actually not surprising. The persisting cognitive deficits are most likely a reflection of the severity of the encephalitis on hippocampal functions in our patients as also seen in the hippocampal atrophy. More importantly, in our study cohort, the volume of the hippocampal area CA1 was the best predictor of recognition memory performance. CA1 as the output relay area of the hippocampus receives input from CA3 via the Schaffer collaterals that converges with entorhinal input via the perforant path (Insausti & Amaral, 2004; van Strien, Cappaert, & Witter, 2009). It is assumed that CA1 compares the converging mnemonic representations from hippocampal CA3 and information about the actual present state carried by entorhinal input pattern (Hasselmo, 2005; Knierim & Neunuebel, 2016; Vinogradova, 2001). This ideal location of CA1 facilitates a full retrieval of memory traces and information that fully matches the actual state (Hasselmo & Eichenbaum, 2005; Hasselmo & Wyble, 1997). Accordingly, previous studies ascribed the function of novelty detection in the sense of a match/mismatch computation in memory processing to CA1 (Duncan, Ketz, Inati, & Davachi, 2012; Knierim & Neunuebel, 2016; Lisman, 1999; Reagh, Watabe, Ly, Murray, & Yassa, 2014). Thus, the position of CA1 that enables to retrieve a complete memory pattern due to an integration of mnemonic inputs from different subnetworks clearly explains the highly predictive value of the CA1 volume regarding recognition memory performance in our study. Notably, neither recognition memory nor any other neurocognitive

domain were correlated with pattern separation performance in LGI1 patients arguing against the possibility that the pattern separation impairment was secondary to cognitive deficits. Our results reflect a functional dissociation of pattern separation and recognition memory performance which might suggest that both computations are relayed by different hippocampal subnetworks. However, future experimental models have to further differentiate network-related mechanisms that affect distinct cognitive and behavioral outcome in humans.

Interestingly, Miller et al. (2017) showed a significant LGI1 encephalitis-induced hippocampal volume loss restricted to bilateral CA3, in contrast to the global volume reduction that was apparent in our data. The difference might be due to the segmentation method used to analyze ultra-high field 7 T MR images. However, it might be mentioned that, the examined LGI1 patients in the Miller study also showed a global hippocampal volume reduction, although not reaching significance levels. Hence, it is more likely that the global volume reduction that we observed may be better explained by a stronger noxious impact on the hippocampus due to stronger hippocampal inflammation in the acute phase leading to a greater disease severity of our study cohort. Indeed, we have shown a particular vulnerability of the hippocampus in encephalitis (Bartsch et al., 2015). Also, it is plausible that the epilepsy in the acute phase with subsequent hippocampal sclerosis in the DG further contributed to the structural sequelae in our cohort (Blümcke, Cross, & Spreafico, 2013; Blümcke, Thom, et al., 2013; Coras et al., 2014). It is, hence, important to note that pattern separation and completion deficits in the hippocampus may not exclusively be determined by atrophic changes but that memory processing deficits may also be the result of dysfunctional cellular and neuroplastic network alterations in the course of the disease process without leading to neurodegeneration and atrophy. In this vein, Coras et al. (2014) showed that cognitive deficits in patients with epilepsy due to hippocampal dysfunction and hippocampal sclerosis was not associated with atrophy but with cellular changes in hippocampal subfields. Thus, the dysfunction of memory processing can also be

caused by subregional network dysfunction that may not lead in atrophic sequelae in the hippocampus. In addition, the examination of damaged brain tissue can influence automated segmentation sensitivity. In patients with hippocampal sclerosis, automated segmentation by means of Freesurfer showed a greater difference to manual segmentation compared to the healthy brain (Pardoe et al., 2009). In this sense, our results may be confounded by a higher segmentation error in the patient group. However, supporting the reliability of our findings, it has been shown that Freesurfer's segmentation algorithm was sensitive to hippocampal atrophy in patients with mesial temporal lobe epilepsy; and, importantly, those results correlated with those of a manual segmentation technique (Morey et al., 2009; Pardoe et al., 2009). We have acknowledged the issue of a variability in segmentation due to an underlying pathology by visual inspection of the clinical MRI scans (Figure 3-5) that indicate the particular affection of the DG region thus corroborating the main effect of the DG lesioning on hippocampal functions. It has to be considered that a deficit in pattern separation as shown in our study cohort by diminished correct 'similar' response to lures – should be also reflected by a heightened false 'old' response to lures (i.e. a shift toward pattern completion; Ally et al., 2013; Yassa et al., 2010). However, our patient cohort showed an equal proportion of 'old' answers to lures compared with the healthy control group probably indicating that pattern completion processes were not affected by the present hippocampal atrophy. However, as a caveat and limiting the interpretation of pattern completion performance, the MST lacks specificity regarding partial cues that reactivate previously encoded mnemonic representations (Hunsaker & Kesner, 2013). We thus based our analysis and conclusions of behavioral data on pattern separation. Indeed, the assessment of pattern separation performance based on the MST has been shown in a variety of studies (Lacy et al., 2011; Stark et al., 2013; Yassa et al., 2010; Yassa, Mattfeld, Stark, & Stark, 2011).

In conclusion, our findings show that patients with LGI1 limbic encephalitis were impaired in pattern separation and recognition memory performance that can be traced back to hippocampal volume reduction and loss of hippocampal integrity. The facts that the LGI1 gene transcript is mainly expressed in DG and CA3 neurons (Herranz-Pérez et al., 2010; Kalachikov et al., 2002) and a deficiency of LGI1 selectively decreases synaptic transmission in the hippocampus (Fukata et al., 2010), emphasize the basic principle of the structure-function relationship between hippocampal subfields and memory processing. Specifically, the variability of the DG was predictive of behavioral pattern separation performance compatible with the current view on the DG to be involved in hippocampal pattern separation. By contrast, recognition memory was strongest predicted by the volume of CA1. These findings show that LGI1 encephalitis differentially targets distinct subregions of the hippocampal circuit and corroborate the framework of a regional specialization of hippocampal functions involved in cognitive processing.

3.6 References – Study II

Ally, B. A., Hussey, E. P., Ko, P. C., & Molitor, R. J. (2013). Pattern separation and pattern completion in Alzheimer's disease: evidence of rapid forgetting in amnestic mild cognitive impairment. *Hippocampus*, *23*, 1246–58. doi:10.1002/hipo.22162

Amaral, D. G., Scharfman, H. E., & Lavenex, P. (2007). The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). *Progress in Brain Research*, *163*, 3–22. doi:10.1016/s0079-6123(07)63001-5

Aschenbrenner, S., Tucha, O., & Lange, K. W. (2000). *Regensburger Wortflüssigkeitstest*. Göttingen: Testzentrale.

Baker, S., Vieweg, P., Gao, F., Gilboa, A., Wolbers, T., Black, S. E., & Rosenbaum, R. S. (2016). The human dentate gyrus plays a necessary role in discriminating new memories. *Current Biology*, *26*(19), 2629–2634. doi:10.1016/j.cub.2016.07.081

Bakker, A., Kirwan, C. B., Miller, M., & Stark, C. E. (2008). Pattern separation in the human hippocampal CA3 and dentate gyrus. *Science*, *319*, 1640–2. doi:10.1126/science.1152882

Bartsch, T., Döhring, J., Reuter, S., Finke, C., Rohr, A., Brauer, H., … Jansen, O. (2015). Selective neuronal vulnerability of human hippocampal CA1 neurons: lesion evolution, temporal course, and pattern of hippocampal damage in diffusion-weighted MR imaging. *Journal of Cerebral Blood Flow and Metabolism*, *35*, 1836–45. doi:10.1038/jcbfm.2015.137

Bartsch, T., Döhring, J., Rohr, A., Jansen, O., & Deuschl, G. (2011). CA1 neurons in the human hippocampus are critical for autobiographical memory, mental time travel, and autonoetic consciousness. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 17562–7. doi:10.1073/pnas.1110266108

Bartsch, T., Schönfeld, R., Müller, F. J., Alfke, K., Leplow, B., Aldenhoff, J., … Koch, J. M. (2010). Focal lesions of human hippocampal CA1 neurons in transient global amnesia impair place memory. *Science*, *328*, 1412–5. doi:10.1126/science.1188160

Bartsch, T., & Wulff, P. (2015). The hippocampus in aging and disease: From plasticity to vulnerability. *Neuroscience*, *309*, 1–16. doi:10.1016/j.neuroscience.2015.07.084

Berron, D., Schütze, H., Maass, A., Cardenas-Blanco, A., Kuijf, H. J., Kumaran, D., & Düzel, E. (2016). Strong evidence for pattern separation in human dentate gyrus. *Journal of Neuroscience*, *36*, 7569–79. doi:10.1523/JNEUROSCI.0518-16.2016

Bettcher, B. M., Gelfand, J. M., Irani, S. R., Neuhaus, J., Forner, S., Hess, C. P., & Geschwind, M. D. (2014). More than memory impairment in voltage-gated potassium channel complex encephalopathy. *European Journal of Neurology*, *21*, 1301–10. doi:10.1111/ene.12482

Blümcke, I., Cross, J. H., & Spreafico, R. (2013). The international consensus classification for hippocampal sclerosis: an important step towards accurate prognosis. *The Lancet. Neurology*, *12*(9), 844–846. doi:10.1016/S1474-4422(13)70175-3

Blümcke, I., Thom, M., Aronica, E., Armstrong, D. D., Bartolomei, F., Bernasconi, A., … Spreafico, R. (2013). International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: a Task Force report from the ILAE Commission on Diagnostic Methods. *Epilepsia*, *54*, 1315–29. doi:10.1111/epi.12220

Buckner, R. L., Head, D., Parker, J., Fotenos, A. F., Marcus, D., Morris, J. C., & Snyder, A. Z. (2004). A unified approach for morphometric and functional data analysis in young,

old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. *NeuroImage*, *23*(2), 724–738. doi:10.1016/j.neuroimage.2004.06.018

Butler, C. R., Miller, T. D., Kaur, M. S., Baker, I. W., Boothroyd, G. D., Illman, N. A., … Buckley, C. J. (2014). Persistent anterograde amnesia following limbic encephalitis associated with antibodies to the voltage-gated potassium channel complex. *Journal of Neurology, Neurosurgery and Psychiatry*, *85*(4), 387–391. doi:10.1136/jnnp-2013-306724

Coras, R., Pauli, E., Li, J., Schwarz, M., Rössler, K., Buchfelder, M., … Blümcke, I. (2014). Differential influence of hippocampal subfields to memory formation - insights from TLE patients. *Brain*, *137*(Pt 7), 1945–1957. doi:10.1093/brain/awu100

Döhring, J., Stoldt, A., Witt, K., Schönfeld, R., Deuschl, G., Born, J., & Bartsch, T. (2017). Motor skill learning and offline-changes in TGA patients with acute hippocampal CA1 lesions. *Cortex*, *89*, 156–168. doi:10.1016/j.cortex.2016.10.009

Duncan, K., Ketz, N., Inati, S. J., & Davachi, L. (2012). Evidence for area CA1 as a match/mismatch detector: a high-resolution fMRI study of the human hippocampus. *Hippocampus*, *22*, 389–98. doi:10.1002/hipo.20933

Finke, C., Prüss, H., Heine, J., Reuter, S., Kopp, U. A., Wegner, F., … Bartsch, T. (2017). Evaluation of cognitive deficits and structural hippocampal damage in encephalitis with leucine-rich, glioma-inactivated 1 antibodies. *JAMA Neurology*, *74*(1), 50–59. doi:10.1001/jamaneurol.2016.4226

Fischl, B., Salat, D. H., van der Kouwe, A. J. W., Makris, N., Ségonne, F., Quinn, B. T., & Dale, A. M. (2004). Sequence-independent segmentation of magnetic resonance images. *NeuroImage*, *23*, S69–S84. doi:10.1016/j.neuroimage.2004.07.016

Fukata, Y., Lovero, K. L., Iwanaga, T., Watanabe, A., Yokoi, N., Tabuchi, K., … Fukata, M. (2010). Disruption of LGI1-linked synaptic complex causes abnormal synaptic transmission and epilepsy. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(8), 3799–3804. doi:10.1073/pnas.0914537107

Guzowski, J. F., Knierim, J. J., & Moser, E. I. (2004). Ensemble dynamics of hippocampal regions CA3 and CA1. *Neuron*, *44*, 581–4. doi:10.1016/j.neuron.2004.11.003

Hanert, A., Weber, F. D., Pedersen, A., Born, J., & Bartsch, T. (2017). Sleep in humans stabilizes pattern separation performance. *Journal of Neuroscience*, *37*(50), 12238–12246. doi:10.1523/JNEUROSCI.1189-17.2017

Hasselmo, M. E. (2005). The role of hippocampal regions CA3 and CA1 in matching entorhinal input with retrieval of associations between objects and context: theoretical comment on Lee et al. (2005). *Behavioral Neuroscience*, *119*(1), 342–345. doi:10.1037/0735-7044.119.1.342

Hasselmo, M. E., & Eichenbaum, H. (2005). Hippocampal mechanisms for the context-dependent retrieval of episodes. *Neural Networks*, *18*(9), 1172–1190. doi:10.1016/j.neunet.2005.08.007

Hasselmo, M. E., & Wyble, B. P. (1997). Free recall and recognition in a network model of the hippocampus: simulating effects of scopolamine on human memory function. *Behavioural Brain Research*, *89*(1–2), 1–34.

Herranz-Pérez, V., Olucha-Bordonau, F. E., Morante-Redolat, J. M., & Pérez-Tur, J. (2010). Regional distribution of the leucine-rich glioma inactivated (LGI) gene family transcripts in the adult mouse brain. *Brain Research*, *1307*, 177–194. doi:10.1016/j.brainres.2009.10.013

Holden, H. M., Toner, C., Pirogovsky, E., Kirwan, C. B., & Gilbert, P. E. (2013). Visual object pattern separation varies in older adults. *Learning & Memory*, *20*, 358–62. doi:10.1101/lm.030171.112

Hunsaker, M. R., & Kesner, R. P. (2013). The operation of pattern separation and pattern completion processes associated with different attributes or domains of memory. *Neuroscience and Biobehavioral Reviews*, *37*(1), 36–58. doi:10.1016/j.neubiorev.2012.09.014

Iglesias, J. E., Augustinack, J. C., Nguyen, K., Player, C. M., Player, A., Wright, M., … Van Leemput, K. (2015). A computational atlas of the hippocampal formation using ex vivo , ultra-high resolution MRI: Application to adaptive segmentation of in vivo MRI. *NeuroImage*, *115*, 117–137. doi:10.1016/j.neuroimage.2015.04.042

Insausti, R., & Amaral, D. G. (2004). Hippocampal formation. In G. Paxinos (Ed.), *The human nervous system* (2nd ed., pp. 871–914). Amsterdam: Elsevier. doi:10.1016/B978-012547626-3/50024-7

Irani, S. R., Michell, A. W., Lang, B., Pettingill, P., Waters, P., Johnson, M. R., … Vincent, A. (2011). Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. *Annals of Neurology*, *69*(5), 892–900. doi:10.1002/ana.22307

Irani, S. R., Stagg, C. J., Schott, J. M., Rosenthal, C. R., Schneider, S. A., Pettingill, P., … Johnson, M. R. (2013). Faciobrachial dystonic seizures: the influence of immunotherapy on seizure control and prevention of cognitive impairment in a broadening phenotype. *Brain*, *136*, 3151–62. doi:10.1093/brain/awt212

Kalachikov, S., Evgrafov, O., Ross, B., Winawer, M., Barker-Cummings, C., Boneschi, F. M., … Gilliam, T. C. (2002). Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. *Nature Genetics*, *30*(3), 335–341. doi:10.1038/ng832

Kirwan, C. B., Hartshorn, A., Stark, S. M., Goodrich-Hunsaker, N. J., Hopkins, R. O., & Stark, C. E. L. (2012). Pattern separation deficits following damage to the hippocampus. *Neuropsychologia*, *50*, 2408–14. doi:10.1016/j.neuropsychologia.2012.06.011

Kirwan, C. B., & Stark, C. E. L. (2007). Overcoming interference: an fMRI investigation of pattern separation in the medial temporal lobe. *Learning & Memory*, *14*, 625–33. doi:10.1101/lm.663507

Knierim, J. J., & Neunuebel, J. P. (2016). Tracking the flow of hippocampal computation: Pattern separation, pattern completion, and attractor dynamics. *Neurobiology of Learning and Memory*, *129*, 38–49. doi:10.1016/j.nlm.2015.10.008

Lacy, J. W., Yassa, M. A., Stark, S. M., Muftuler, L. T., & Stark, C. E. L. (2011). Distinct pattern separation related transfer functions in human CA3/dentate and CA1 revealed using high-resolution fMRI and variable mnemonic similarity. *Learning & Memory*, *18*, 15–8. doi:10.1101/lm.1971111

Lee, I., Yoganarasimha, D., Rao, G., & Knierim, J. J. (2004). Comparison of population coherence of place cells in hippocampal subfields CA1 and CA3. *Nature*, *430*, 456–9. doi:10.1038/nature02739

Lehrl, S. (2005). *Mehrfachwahl-Wortschatz-Intelligenz-Test, MWT-B* (5th ed.). Balingen: Spitta Verlag.

Leutgeb, J. K., Leutgeb, S., Moser, M. B., & Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science*, *315*, 961–6. doi:10.1126/science.1135801

Leutgeb, J. K., Leutgeb, S., Treves, A., Meyer, R., Barnes, C. A., McNaughton, B. L., … Moser, E. I. (2005). Progressive transformation of hippocampal neuronal representations in “morphed” environments. *Neuron*, *48*, 345–58. doi:10.1016/j.neuron.2005.09.007

Leutgeb, S., & Leutgeb, J. K. (2007). Pattern separation, pattern completion, and new neuronal codes within a continuous CA3 map. *Learning & Memory*, *14*, 745–57. doi:10.1101/lm.703907

Leutgeb, S., Leutgeb, J. K., Treves, A., Moser, M. B., & Moser, E. I. (2004). Distinct ensemble codes in hippocampal areas CA3 and CA1. *Science*, *305*, 1295–8. doi:10.1126/science.1100265

Lisman, J. E. (1999). Relating hippocampal circuitry to function: recall of memory sequences by reciprocal dentate-CA3 interactions. *Neuron*, *22*, 233–42.

Malter, M. P., Frisch, C., Schoene-Bake, J. C., Helmstaedter, C., Wandinger, K. P., Stoecker, W., … Bien, C. G. (2014). Outcome of limbic encephalitis with VGKC-complex antibodies: relation to antigenic specificity. *Journal of Neurology*, *261*, 1695–705. doi:10.1007/s00415-014-7408-6

McClelland, J. L., McNaughton, B. L., & O’Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychological Review*, *102*, 419–57.

McHugh, T. J., Jones, M. W., Quinn, J. J., Balthasar, N., Coppari, R., Elmquist, J. K., … Tonegawa, S. (2007). Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science*, *317*, 94–9. doi:10.1126/science.1140263

Miller, T. D., Chong, T. T.-J., Aimola Davies, A. M., Ng, T. W. C., Johnson, M. R., Irani, S. R., … Rosenthal, C. R. (2017). Focal CA3 hippocampal subfield atrophy following LGI1 VGKC-complex antibody limbic encephalitis. *Brain*, *140*(5), 1212–1219. doi:10.1093/brain/awx070

Neunuebel, J. P., & Knierim, J. J. (2014). CA3 retrieves coherent representations from degraded input: direct evidence for CA3 pattern completion and dentate gyrus pattern separation. *Neuron*, *81*, 416–27. doi:10.1016/j.neuron.2013.11.017

Norman, K. A., & O'Reilly, R. C. (2003). Modeling hippocampal and neocortical contributions to recognition memory: a complementary-learning-systems approach. *Psychological Review*, *110*, 611–646. doi:10.1037/0033-295X.110.4.611

O'Reilly, R. C., & McClelland, J. L. (1994). Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off. *Hippocampus*, *4*, 661–82. doi:10.1002/hipo.450040605

Reagh, Z. M., Watabe, J., Ly, M., Murray, E., & Yassa, M. A. (2014). Dissociated Signals in Human Dentate Gyrus and CA3 Predict Different Facets of Recognition Memory. *The Journal of Neuroscience*, *34*(40), 13301–13313. doi:10.1523/JNEUROSCI.2779-14.2014

Reitan, R. M. (1979). *Trail Making Test (TMT)*. Göttingen: Hogrefe.

Rey, A. (1941). L'examen psychologique dans les cas d'encéphalopathie traumatique. *Archives de Psychologie*, *28*, 21.

Rolls, E. T. (2016). Pattern separation, completion, and categorisation in the hippocampus and neocortex. *Neurobiology of Learning and Memory*, *129*, 4–28. doi:10.1016/j.nlm.2015.07.008

Ségonne, F., Dale, A. M., Busa, E., Glessner, M., Salat, D., Hahn, H. K., & Fischl, B. (2004). A hybrid approach to the skull stripping problem in MRI. *NeuroImage*, *22*(3), 1060–1075. doi:10.1016/j.neuroimage.2004.03.032

Ségonne, F., Pacheco, J., & Fischl, B. (2007). Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Transactions on Medical Imaging*, *26*(4), 518–529.

Sled, J. G., Zijdenbos, A. P., & Evans, A. C. (1998). A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Transactions on Medical Imaging*, *17*(1), 87–97. doi:10.1109/42.668698

Small, S. A., Tsai, W. Y., DeLaPaz, R., Mayeux, R., & Stern, Y. (2002). Imaging hippocampal function across the human life span: is memory decline normal or not? *Annals of Neurology*, *51*(3), 290–295.

Stark, S. M., Stevenson, R., Wu, C., Rutledge, S., & Stark, C. E. L. (2015). Stability of age-related deficits in the mnemonic similarity task across task variations. *Behavioral Neuroscience*, *129*, 257–68. doi:10.1037/bne0000055

Stark, S. M., Yassa, M. A., Lacy, J. W., & Stark, C. E. L. (2013). A task to assess behavioral pattern separation (BPS) in humans: Data from healthy aging and mild cognitive impairment. *Neuropsychologia*, *51*, 2442–9. doi:10.1016/j.neuropsychologia.2012.12.014

Stark, S. M., Yassa, M. A., & Stark, C. E. L. (2010). Individual differences in spatial pattern separation performance associated with healthy aging in humans. *Learning & Memory*, *17*, 284–288. doi:10.1101/lm.1768110

Toner, C. K., Pirogovsky, E., Kirwan, C. B., & Gilbert, P. E. (2009). Visual object pattern separation deficits in nondemented older adults. *Learning & Memory*, *16*, 338–42. doi:10.1101/lm.1315109

Treves, A., & Rolls, E. T. (1994). Computational analysis of the role of the hippocampus in memory. *Hippocampus*, *4*, 374–91. doi:10.1002/hipo.450040319

Van Leemput, K., Bakkour, A., Benner, T., Wiggins, G., Wald, L. L., Augustinack, J., … Fischl, B. (2009). Automated segmentation of hippocampal subfields from ultra-high resolution in vivo MRI. *Hippocampus*, *19*(6), 549–557. doi:10.1002/hipo.20615

van Strien, N. M., Cappaert, N. L., & Witter, M. P. (2009). The anatomy of memory: an interactive overview of the parahippocampal-hippocampal network. *Nature Reviews Neuroscience*, *10*, 272–82.

Vazdarjanova, A., & Guzowski, J. F. (2004). Differences in hippocampal neuronal population responses to modifications of an environmental context: evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles. *Journal of Neuroscience*, *24*, 6489–96. doi:10.1523/jneurosci.0350-04.2004

Vinogradova, O. S. (2001). Hippocampus as comparator: role of the two input and two output systems of the hippocampus in selection and registration of information. *Hippocampus*, *11*(5), 578–598. doi:10.1002/hipo.1073

West, M. J. (1993). Regionally specific loss of neurons in the aging human hippocampus. *Neurobiology of Aging*, *14*(4), 287–293.

Yassa, M. A., Lacy, J. W., Stark, S. M., Albert, M. S., Gallagher, M., & Stark, C. E. L. (2011). Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults. *Hippocampus*, *21*, 968–79. doi:10.1002/hipo.20808

Yassa, M. A., Mattfeld, A. T., Stark, S. M., & Stark, C. E. L. (2011). Age-related memory deficits linked to circuit-specific disruptions in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 8873–8. doi:10.1073/pnas.1101567108

Yassa, M. A., & Stark, C. E. L. (2011). Pattern separation in the hippocampus. *Trends in Neurosciences*, *34*(10), 515–525. doi:10.1016/j.tins.2011.06.006

Yassa, M. A., Stark, S. M., Bakker, A., Albert, M. S., Gallagher, M., & Stark, C. E. L. (2010). High-resolution structural and functional MRI of hippocampal CA3 and dentate gyrus in patients with amnestic Mild Cognitive Impairment. *NeuroImage*, *51*, 1242–52. doi:10.1016/j.neuroimage.2010.03.040

4 Study III

Sleep in humans stabilizes pattern separation performance

A. Hanert, F. D. Weber, A. Pedersen, J. Born, and T. Bartsch

Published in *The Journal of Neuroscience*, *December 2017, 37(50):12238-12246,*

doi: 10.1523/JNEUROSCI.1189-17.2017, [Epub November 2017]

4.1 Abstract

Replay of hippocampal neural representations during sleep is thought to promote systems consolidation of declarative memory. How this reprocessing of memory during sleep affects the hippocampal representation itself, is unclear. Here we tested hippocampal stimulus processing (i.e., pattern separation) before and after periods of sleep and wakefulness in humans (female and male participants). Pattern separation deteriorated across the wake period but remained stable across sleep ($p = 0.013$) with this sleep-wake difference being most pronounced for stimuli with low similarity to targets ($p = 0.006$). Stimuli with the highest similarity showed a reversed pattern with reduced pattern separation performance after sleep ($p = 0.038$). Pattern separation performance was positively correlated with sleep spindle density, slow oscillation density, and theta power phase-locked to slow oscillations. Sleep, presumably by neural memory replay, shapes hippocampal representations and enhances computations of pattern separation to subsequent presentation of similar stimuli.

Keywords: consolidation; hippocampus; memory; pattern separation, sleep

4.2 Introduction

Whereas the wake state is optimal for the encoding of information, sleep following encoding is considered a brain state favoring the formation of long-term memory (Rasch & Born, 2013). In particular, sleep appears to benefit hippocampus-dependent (i.e., declarative) memory for episodes and facts (Inostroza & Born, 2013). The consolidation of hippocampus-dependent memories during sleep is causally related to the replay of patterns of neural activity that were present during encoding of the information during prior wakefulness (Ego-Stengel & Wilson, 2010; Girardeau, Benchenane, Wiener, Buzsáki, & Zugaro, 2009). In rats, firing patterns of hippocampal place cell assemblies evoked during navigating through a maze are replayed during subsequent slow-wave sleep (SWS; O'Neill, Pleydell-Bouverie, Dupret, & Csicsvari, 2010; Wilson & McNaughton, 1994). Such replay activity can be experimentally induced by presenting cues that were present during prior learning, and again during SWS after learning (Bendor & Wilson, 2012; Rasch, Büchel, Gais, & Born, 2007), which indeed enhanced the memory encoded before sleep, thus proving the causal role of neural reactivations for the consolidation of hippocampusdependent memory during sleep. The reactivated memory information is thought to be transmitted to extrahippocampal, mainly neocortical networks serving as long-term store (Diekelmann & Born, 2010). This process is supported by the phaselocking of the three prime rhythms of the EEG during SWS accompanying hippocampal replay, (i.e., hippocampal ripples that nest into thalamic 12-15 Hz spindle oscillations, which themselves nest into the up-states of the neocortical <1 Hz slow oscillations; Clemens et al., 2007; Sirota, Csicsvari, Buhl, & Buzsáki, 2003; Staresina et al., 2015).

Hippocampal memory reactivations, however, aside from promoting extrahippocampal changes, are expected to also change the features of the hippocampal representation per se, possibly resembling the effects of re-encoding (Inostroza & Born, 2013; Karpicke & Roediger, 2008). Pattern separation (PatSep) and pattern completion (PatComp) represent two principal

features of hippocampal memory processing (McNaughton & Morris, 1987; O'Reilly & McClelland, 1994). Pattern separation refers to the capability of the hippocampus to form nonoverlapping orthogonal neural representations from similar sequential episodic stimulus inputs (McClelland, McNaughton, & O'Reilly, 1995). Pattern completion refers to the recall of a memory based on the presentation of incomplete, noisy, or degraded stimulus patterns (Norman & O'Reilly, 2003). Based on the hippocampal circuit structure with its recurrent associative networks embedded into the unidirectional trisynaptic pathway, both processes have been linked to computations in different hippocampal subnetworks (Bartsch, Döhring, Rohr, Jansen, & Deuschl, 2011; Bartsch et al., 2010; O'Reilly & McClelland, 1994). Indeed, it has been shown that the hippocampal dentate gyrus (DG) is essential for pattern separation and that CA3 networks are involved in pattern completion (Guzowski, Knierim, & Moser, 2004; J. K. Leutgeb, Leutgeb, Moser, & Moser, 2007; Neunuebel & Knierim, 2014). In humans, high-resolution imaging studies confirmed the role of the DG and CA3 in pattern separation and determined that the CA1 area contributed to pattern completion, using mnemonic similarity recognition tasks (Bakker, Kirwan, Miller, & Stark, 2008; Berron et al., 2016; Lacy, Yassa, Stark, Muftuler, & Stark, 2011; Yassa, Mattfeld, Stark, & Stark, 2011; Yassa et al., 2010).

Here, we used the mnemonic similarity task (MST) to study how memory processing during sleep affects representations toward enhancing and/or diminishing pattern separation. We recorded sleep to identify EEG oscillations known to synchronize hippocampal memory replay activity (i.e., spindles) originating from thalamic networks and the neocortical slow oscillations.

4.3 Materials and Methods

4.3.1 Participants

Thirteen healthy students (mean age, 23.46 ± 0.5 years; age range: 21–26 years; 10 women) participated in the study. Participants were recruited via advertisement at the university and

received monetary compensation (€ 75) after completion. Participants were free of neurological or psychiatric disorders and did not use any medication. They kept a regular sleep/wake cycle and did not engage in any stressful activities (e.g., exams, night shift) for an interval of at least 6 weeks before the experiments. General sleep quality was assessed via the Pittsburgh Sleep Quality Index (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). None of the subjects had previously participated in an experiment in our laboratory. Participants had to refrain from drinking alcohol and caffeine, from stressful physical activities, and from napping on the experimental days. They were all vigilant and alert according to the subject's report at all time points tests were performed. Every participant gave written informed consent before the study, which was approved by the local ethics committee.

4.3.2 Mnemonic similarity task

The MST is an established recognition memory task that has been shown to tax pattern separation (Ally, Hussey, Ko, & Molitor, 2013; Bakker et al., 2008; Kirwan & Stark, 2007; Stark, Yassa, Lacy, & Stark, 2013; Toner, Pirogovsky, Kirwan, & Gilbert, 2009; Yassa, Lacy, et al., 2011; Yassa et al., 2010; http://faculty.sites.uci.edu/starklab/mnemonic-similarity-task-mst/). The MST comprises an encoding and a recall phase. For the encoding phase, the participant is asked to classify 256 items (i.e., everyday objects) as either an indoor or an outdoor object via button press on a keyboard. Objects are sequentially presented on a computer monitor each for 2 s with a 0.5s interstimulus interval. The two recall phases of the experiment (immediate and delayed) comprised 192 items (128 old plus 64 new items) each including (1) 64 exact repetitions of the previously seen objects ('targets'), (2) 64 objects that were similar to the previously seen objects ('lures'), and (3) 64 new objects that the participant had not seen before ('foils'). Half of the stimulus set used at encoding (128 items) was used for immediate recall testing, the other half for delayed recall testing. For testing recall, participants indicated (within the 2 s of item presentation) whether a presented object was 'old', 'similar', or 'new' by button press. In

this context, the answers to lure items were of substantial significance, implying a successful pattern separation when correctly responding ‘similar’ (Toner et al., 2009; Yassa et al., 2010). The order of sets across Wake and Sleep as well as for immediate and delayed recall was randomized, and the stimuli within each set followed a pseudorandomized order. Responses at recall enabled calculation of two different scores that comprise performance measures of pattern separation and recognition memory:

Pattern separation score.

Behavioral pattern separation was determined by the correct discrimination of a lure item from its target counterpart as follows: PatSep score = [p (correct similar response to lures) – p (false similar response to foils)]. The score was thus corrected for a possible response bias toward exhibiting a tendency to use the similar response (Bennett & Stark, 2016; Stark et al., 2013; Yassa, Lacy, et al., 2011; Yassa et al., 2010).

To assess the performance of pattern separation as a function of lure similarity, PatSep scores were calculated for the five degrees of lure similarity to a target object (1-5: most similar to least similar; Lacy et al., 2011; Yassa et al., 2010). The similarity bins were the same as in previous studies (Bennett & Stark, 2016; Stark et al., 2013).

Recognition memory score.

Recognition memory was assessed by the number of correct responses to targets. To correct for response bias to preferentially respond with the target button, the number of incorrect target responses to foils was subtracted: recognition memory (RM) score = [p (correct old response to targets) – p (false old response to foils)] (Stark et al., 2013).

4.3.3 Experimental design and procedure

Each participant attended both the Wake and Sleep conditions, following a within-subject crossover design. Participants were randomly assigned to one of the conditions to perform at first.

The second condition took place 42.54 ± 5.33 d later. Each condition started with encoding of the MST items (encoding phase) followed by an immediate recall test, followed by a 9 h retention interval covering either nocturnal sleep (Sleep condition) or daytime wakefulness (Wake condition). Thereafter, recall was tested again (delayed recall, Figure 4-1). Encoding and immediate recall testing lasted 30 min, and the delayed recall an additional 15 min. For the Sleep condition, subjects spent one adaptation night before the test night in the laboratory to habituate to sleeping under laboratory conditions including polysomnographic recordings. On experimental days, encoding and immediate recall took place in the evening from 9:00 to 10:00 P.M. Then, subjects were prepared for polysomnographic recordings, and lights were turned off at 11:00 P.M. Subjects were awakened at 07:00 A.M., and recall testing started at 7:30 A.M.

After delayed recall testing in the Sleep condition, participants completed a neuropsychological test battery evaluating (1) short-term memory by the Rey Auditory Verbal Learning Test (Rey, 1941), (ii) executive function by the Trail Making Test A and B (TMT; Reitan, 1979), (iii) verbal fluency by the Regensburg Word Fluency Test (Aschenbrenner, Tucha, & Lange, 2000), (iv) working memory by the digit span test (Wechsler, 1997), and (v) general intelligence by the Multiple Choice Vocabulary Intelligence Test (Lehrl, 2005). Also, handedness was assessed via the Edinburgh Handedness Inventory (Oldfield, 1971).

In the Wake condition, encoding and immediate recall testing took place in the morning between 8:00 and 9:00 A.M., and delayed recall was tested between 6:00 and 7:00 P.M. Participants were told to sleep at least 6 h, but at most 8 h, the night before testing. During the wake interval, participants were not engaged in stressful physical and emotional activities and demanding cognitive tasks like exam preparations.

4.3.4 Polysomnography, EEG power spectra, slow oscillations, and sleep spindles

Polysomnography included recordings of electroencephalogram (EEG) from F3, F4, C3, C4, O1, and O2 (international 10-20 system, referenced to electrodes at the mastoids, ground at AFz), electrooculogram (EOG) from electrodes around the eyes, and electrocardiogram (ECG). Signals were recorded using the SOMNOscreen™ EEG 10-20 system (Somnomedics) digitized at 128 or 256 Hz and filtered (EEG, 0.2-35 Hz; EOG, 0.2-10 Hz; ECG lowpass, 50 Hz). Sleep stages were scored off-line according to American Academy of Sleep Medicine criteria by a trained rater. For each subject, total sleep time, time spent in sleep stage 1 (S1), stage 2 (S2), SWS (i.e., stages 3 and 4), and rapid eye movement (REM) sleep and movement artifacts were detected. Sleep onset was defined with reference to lights off by the first occurrence of stage 1 sleep followed by stage 2 sleep. Analyses were based on the central channels.

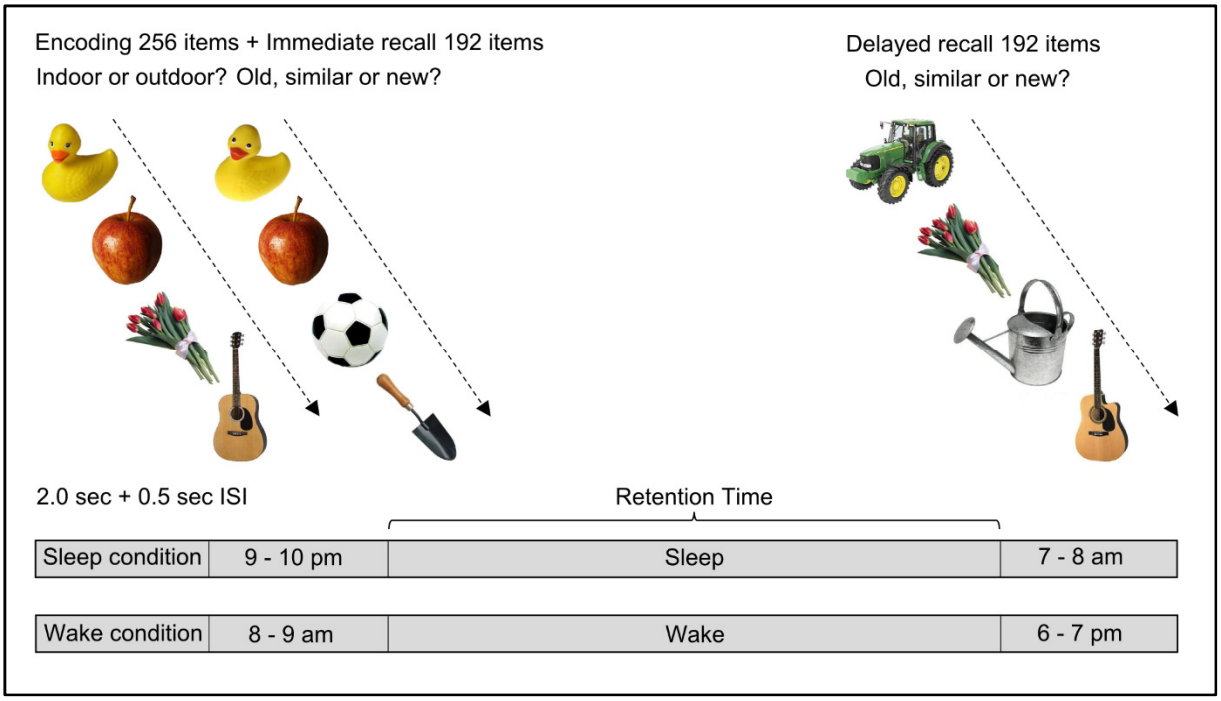

Figure 4-1. Design and procedures. Each participant performed on a Sleep and a Wake condition separated by at least 3 weeks. Conditions started with encoding of 256 MST items followed by an immediate recognition test (192 items), followed by a 9-hour retention interval covering nocturnal sleep (Sleep condition) or daytime wakefulness (Wake condition). Thereafter, delayed recall (192 items not used for immediate recall) was tested. Displayed pictures in the graph are taken from the original image database of the MST. ISI – interstimulus interval.

More fine-grained EEG analyses were performed to track the association of pattern separation and completion with specific sleep EEG oscillatory parameters during non-REM sleep (S2 and SWS; i.e., specifically with EEG power in different frequency band, slow oscillations, and sleep spindles). Analysis was based on epochs free of visually identified EEG artifacts and performed using the SpiSOP toolbox (http://www.spisop.org) based on MATLAB 2015a (MathWorks) and FieldTrip (Oostenveld, Fries, Maris, & Schoffelen, 2011). Additionally, EEG activity occurring phase-locked to the slow oscillations was explored. Before the analyses, EEG signals were down-sampled to 128 Hz. The algorithms are briefly described in the following sections.

Power spectral analyses.

Power spectra were calculated on consecutive 5s intervals of non-REM sleep, which overlapped in time by 4 s. Intervals were tapered by a single Hanning window before applying fast Fourier transformation that resulted in interval power spectra with a frequency resolution of 0.2 Hz. Power spectra were then averaged across all blocks (Welch's method) and normalized by the effective noise bandwidth to obtain power spectral density estimates for all data. Mean power density in the following frequency bands was determined for each EEG channel separately, as follows: slow oscillation (0.5–1 Hz); delta (1– 4 Hz); slowwave activity (0.5–4 Hz); theta (4–8 Hz); slow spindles (9–12 Hz); and fast spindles (12–15 Hz).

Slow oscillation detection.

Identification of slow oscillations was performed in non-REM sleep, separately for S2 and SWS. Detection was based on a previously published algorithm (Mölle, Marshall, Gais, & Born, 2002). For each EEG channel, the signal was filtered between 0.5 and 3.5 Hz (3 dB rolloff) using a digital finite impulse response filter (Butterworth filter, order of 4). Then, all time intervals with consecutive positive-to-negative zero crossings were marked as putative slow oscillation if their durations corresponded to a frequency between 0.5 and 1.11 Hz (Ngo, Martinetz, Born, & Mölle, 2013). Putative slow oscillations with lower amplitudes were immediately excluded when both negative and positive half-wave amplitudes were smaller than -15 and +10 μV, respectively. A slow oscillation was then identified if its negative half-wave peak potential was >1.25 times the mean of the negative half-wave peak of all putatively detected slow oscillations in the respective EEG channel, and also only if the amplitude of the positive half-wave peak was >1.25 times the mean positive half-wave amplitude of all other putatively detected slow oscillations within this channel. For each individual and channel, the number of slow oscillations, their density (per minutes of non-REM sleep), mean amplitude, and slope

(ratio between negative half-wave peak amplitude and the time between the negative peak to the next zero crossing; Riedner et al., 2007) were calculated.

Spindle detection.

Spindle identification focused on conventional (fast) spindles in central channels (C3, C4) as they typically occur in centroparietal brain regions (De Gennaro & Ferrara, 2003; Mölle, Bergmann, Marshall, & Born, 2011). Spindle frequency peaks were visually identified from non-REM power spectra according to their expected power maximum in the 12–15 Hz band (mean peak, 13.82 ± 0.11 Hz). For each EEG channel, the non- REM epochs signal was filtered with a bandpass of ±1 Hz (-3 dB cutoff, two filter passings) around the individual spindle frequency peaks. Then, using a sliding window with a size of 0.2 s the root mean square (RMS) was computed, and the resulting signal was smoothed in the same window with a moving average. A spindle was detected when the smoothed RMS signal exceeded an individual amplitude threshold 1.5 times the SD of the filtered signal for 0.5–3 s. The threshold crossings marked the beginning and the end of the spindle event. Spindles were excluded with amplitudes > 200 μV. For each subject and channel, absolute spindle counts, spindle density (per 30s non-REM epochs), mean amplitude, and mean length were calculated. For spindle analyses, data from one outlier (with a spindle density 2 SDs from the mean) were removed (resulting n = 12).

EEG activity phase-locked to slow oscillations.

Time-frequency power was calculated separately for each detected slow oscillation in frequency steps of 0.2 Hz and a range of 2–24 Hz using continuous Morlet wavelets with a length of 7 cycles that were applied to the EEG every 0.02 s, time-locked to the negative slow oscillation peak. Time-frequency data were then averaged for each subject. Averaged time-frequency power values were log transformed (in decibels) and, then, normalized by dividing them by the average power in ± 2 s window around the negative slow oscillation peak for each respective frequency bin. For statistical comparisons, for each subject the phase-locked power in two

frequency bands of interest (Ngo et al., 2013; Schreiner & Rasch, 2015) was averaged across specific time windows (i.e., theta activity; 4–8 Hz) occurring at the slow oscillation down state (0.5 to 0.25 s around the negative slow oscillation peak) and spindle activity (12-16 Hz) occurring at the subsequent slow oscillation up state (+0.25 to 1 s with reference to the negative slow oscillation peak).

4.3.5 Statistical analyses

To analyze differences between the Sleep and Wake conditions for MST parameters, difference values (delayed minus immediate recall scores) were calculated. A Shapiro-Wilk test for normality was applied to all parameters before statistical testing. Paired -samples t tests were used for the analysis of differences between Sleep and Wake conditions regarding PatSep, and RM scores. Also, one-sample t tests against zero were conducted to show a stabilization, decrease, or increase of the difference values of PatSep and RM scores. A two-factorial repeated -measures ANOVA was performed for examining sleep/wake differences regarding the different degrees of lure similarity with Sleep/Wake and lure similarity as within -subject factors. If the sphericity assumption was violated, degrees of freedom were reported according to a Greenhouse-Geisser correction. Post hoc paired -samples t tests (Fisher's least significant difference method) were performed to identify differences between the Sleep and Wake condition. For a further analysis to depict the relation between the PatSep score and lure similarity, Spearman's for immediate and delayed recall were separately calculated for every subject. One-sample Wilcoxon signed rank tests against zero were used to demonstrate graded decreases or increases of scores in relation to lure similarity. To examine differences between the correlational coefficients, Wilcoxon signed rank tests were calculated. To analyze the relationship between PatSep scores and sleep EEG parameters, Pearson productmoment correlations were computed for normal distributed and linearly related variables, for other variables Spearman's was used. Con-

sidering the exploratory nature of the correlational analysis, correction for multiple comparisons was excluded. All statistical analyses were performed using two-tailed tests. The significance level was set to $p < .05$. Data are expressed as the mean ± SEM.Results.

4.4 Results

Each of 13 healthy students was tested during a Sleep and a Wake condition (Figure 4-1, experimental design and procedure). On both conditions, the encoding of MST items (color photographs of everyday objects) was followed by an immediate recall, and a 9-hour retention interval covering either nocturnal sleep or daytime wakefulness. Thereafter, delayed recall of MST items was tested. At recall, subjects were required to judge presented objects as 'old target', 'similar lure' or 'new foil' items, enabling the calculation of individual scores of PatSep performance and recognition memory. The assessment of the effects of sleep was based on individual retention scores (i.e., delayed minus immediate recall).

4.4.1 Pattern Separation and Recognition Memory

PatSep scores were distinctly higher after sleep than after wakefulness ($t(12) = -3.08$, $p = .010$, Figure 4-2). Additionally, the delayed-immediate recall difference revealed that PatSep scores remained stable across sleep ($M = -3.32 \pm 2.98$, $t(12) = -1.16$ $p = .287$, for test against 0), whereas scores strongly decreased across wakefulness ($M = -20.82 \pm 4.24$, $t(12) = -4.91$, $p = .00004$). Regarding recognition memory, the scores were higher after sleep ($M = -7.54 \pm 2.71$) than wakefulness ($M = -23.77 \pm 3.12$; $t(12) = -3.603$, $p = .004$, Figure 4-2, Table 4-1).

Table 4-1. Overview of responses in the MST in Sleep and Wake condition.

Item type	Target			Lure			Foil		
Response	old	similar	new	old	similar	new	old	similar	new
Sleep									
Immediate Recall	83.54 (3.16)	13.54 (3.02)	3.00 (0.90)	37.92 (4.01)	54.69 (3.85)	7.23 (1.46)	1.31 (0.31)	19.23 (3.44)	79.77 (3.38)
Delayed Recall	79.08 (2.40)	15.77 (1.82)	5.23 (1.00)	32.69 (3.14)	50.69 (3.18)	16.62 (2.52)	4.38 (1.29)	18.46 (2.98)	77.15 (3.07)
Wake									
Immediate Recall	85.62 (2.86)	12.31 (2.62)	2.15 (0.82)	30.62 (3.99)	62.38 (3.72)	6.85 (1.43)	2.46 (0.31)	19.69 (4.43)	78.23 (4.47)
Delayed Recall	62.62 (4.89)	26.85 (4.48)	10.46 (1.97)	32.08 (3.52)	38.69 (3.25)	29.54 (2.69)	3.23 (0.84)	16.77 (2.77)	80.08 (2.93)

Mean ± SEM of response types relative to item types in percent.

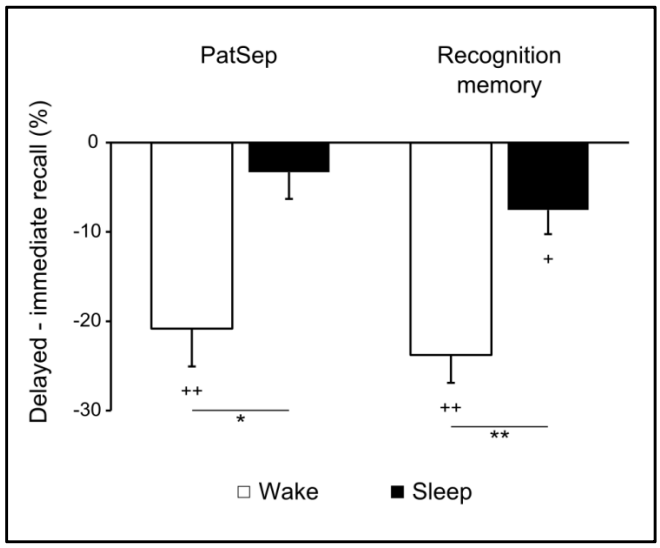

Figure 4-2. PatSep, and Recognition Memory performance separately for the Sleep (black) and Wake (white) conditions. Mean (±SEM) difference values (delayed-minus-immediate recall performance) are given. PatSep performance deteriorates across the wake interval but remains stable across sleep. * $p < .05$; ** $p < .01$, for pairwise comparisons between conditions. + $p < .05$; ++ $p < .01$, for test against zero.

We examined whether the effects of sleep on the PatSep score depended on the degree of the lure similarity (ranging from 1 to 5, from most similar to least similar to target). The stabilization of PatSep scores after sleep, compared with the decrease across wakefulness, was most pronounced for the least similar (5) lures ($F(4, 48) = 8.28$, $p = .00004$, Sleep/Wake x Similarity interaction, in a 2 x 5 repeated -measures ANOVA; Figure 4-3 a). The effect of sleep decreased with increasing lure similarity and, notably, was reversed for the most similar lures (lure 1) showing higher PatSep scores after wakefulness than after sleep ($t(12) = 2.33$, $p = .038$).

The diminished PatSep score for highly similar lures compared with wakefulness lead to the assumption that participants were here more prone to incorrectly responding 'old' to lures instead of 'similar'. To prove the hypothesis, we also calculated the 2x5 (Sleep/Wake x Similarity) ANOVA for the probability of incorrectly responding 'old' to lures (i.e., also corrected for the response bias, as follows: p (false old response to lures) – p (false old response to foils). As expected, the results mirrored the findings regarding the PatSep score. For the least similar lure 5, the old responses were more frequent after wakefulness than after sleep ($t(12) = 4.69$, $p = .001$). Conversely, for the most similar lure 1, they were more frequent after sleep than after wakefulness ($t(12) = -2.57$, $p = .025$).

To further analyze the dependency of sleep versus wake effects on lure similarity, we calculated correlations between lure similarity and the PatSep score, separately for the Sleep and Wake conditions for every participant. As expected, at immediate recall in both conditions the PatSep score was positively correlated with lure similarity (i.e., PatSep scores were the higher the less similar the lure was; Sleep: rs0rs = .747 ± 0.07, $Z = 3.22$, $p = .001$, for test against 0; Wake: rs = .735 ± 0.07, $Z = 3.19$, $p = .001$). The same positive correlation was found after sleep (rs = .733 ± 0.06, $Z = 3.18$, $p = .001$), but failed to reach significance after wakefulness (rs = .249 ± 0.14, $Z = 1.80$, $p = .071$). Comparing averaged correlations across participants at immediate

and delayed recall revealed that these coefficients significantly differed only in the Wake condition (Z = - 2.41, p = .016), whereas there was no difference in the Sleep condition (Z = - 0.31, p = .755;Figure 4-3 b).

4.4.2 Sleep Recordings, Slow Oscillations, and Spindles

Participants in the Sleep condition displayed normal sleep (total sleep time: 446.2 ± 7.1 min, sleep onset latency: 29.0 ± 5.1 min, sleep time in stage 1: 49.8 ± 6.9 min; sleep time in, stage 2: 196.2 ± 10.9 min; sleep time in, SWS: 105.1 ± 6.3 min; sleep time in, REM sleep 64.3 ± 8.0 min, movement time 10.3 ± 5.9 min). None of these parameters correlated with the PatSep score (all p values 0.28). Correlations between specific EEG oscillatory measures and the PatSep score are summarized in Table 4-2.

Table 4-2. Power Density, Slow Oscillations and Spindles - Correlations with Pattern Separation performance

		Correlations with PatSep retention score	
	Mean (SEM)	r	p
Power Density (μV²/Hz)			
0.5–1 Hz	329.58 (38.16)	.352	.238
0.5–4 Hz	112.27 (11.80)	.234	.441
1–4 Hz (#)	86.11 (9.24)	.143	.642
4–8 Hz (#)	7.55 (0.77)	-.236	.437
9–12 Hz (#)	3.18 (0.66)	-.352	.239
12–15 Hz (#)	3.53 (0.43)	-.104	.734
Slow Oscillations			
Count	1130.23 (90.91)	.408	.166
Density (/30 s)	1.93 (0.11)	.613*	.026
Amplitude (μV) (#)	169.02 (6.84)	.197	.519
Slope (μV/s)	401.16 (17.72)	.215	.480
Spindles			
Count	1659.91 (67.68)	.039	.909
Density (/30 s)	2.79 (0.05)	.683*	.014
Length (s)	0.85 (0.01)	-.055	.866
Amplitude (μV)	31.22 (1.46)	-.456	.159

Means (± SEM) for power within distinct EEG frequency bands, slow oscillation and spindle parameters. Right columns indicate correlations with the PatSep retention score and respective p-values. (#)

Correlations for frequency bands that were not normally distributed were calculated with Spearman's rho, all others with Pearson product-moment correlation. * $p < .05$.

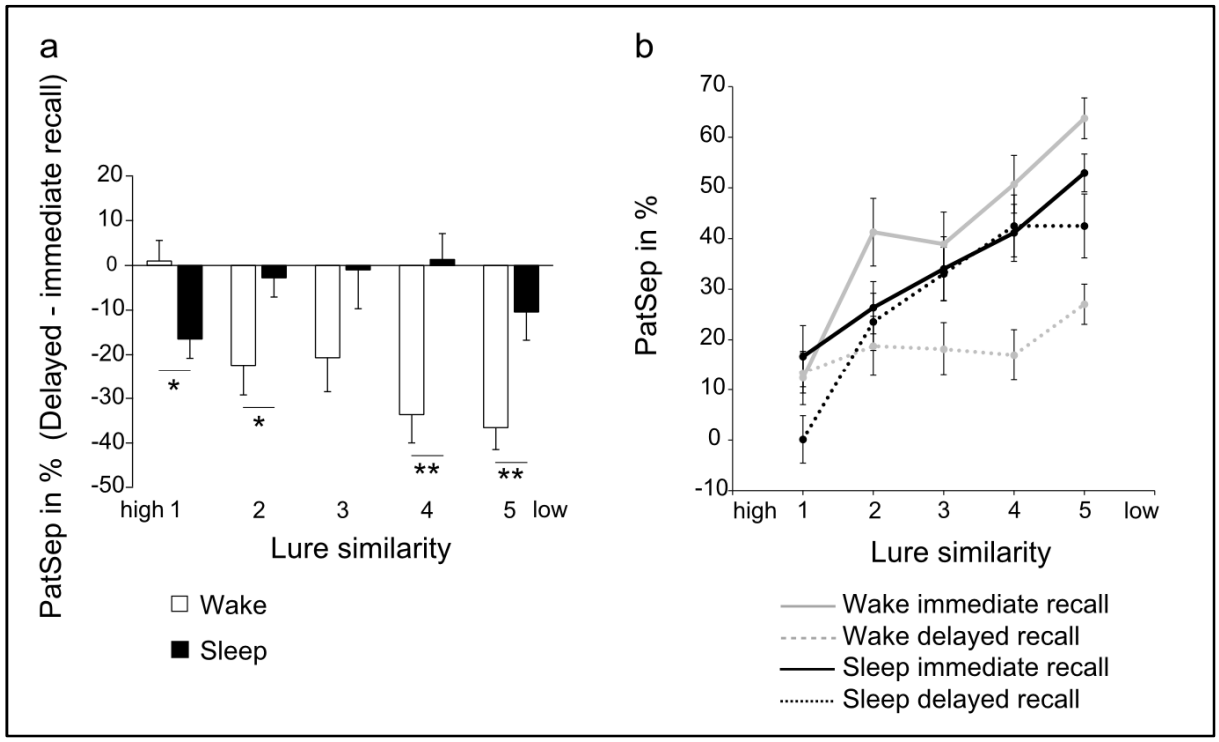

Figure 4-3. Pattern separation performance as a function of lure similarity. (a) Mean (±SEM) PatSep score across five degrees of lure similarity (1 – high, 5 – low similarity) separately for the Sleep (black bars) and Wake (white) conditions. * $p < .05$; ** $p < .01$ for pairwise comparisons between Sleep and Wake condition. Difference values (delayed-minus-immediate recall) are indicated. (b) Mean (±SEM) PatSep scores separately at immediate and delayed recall for the Sleep and Wake conditions depict graded decrease and increase in performance. Note, the typical increase in pattern separation performance with decreasing lure similarity seen before sleep and wakefulness intervals (immediate recall) is preserved after sleep, but deteriorates after wakefulness (delayed recall).

Robust relations were revealed for non-REM sleep spindles and slow oscillations. Spindle density as well as slow oscillation density during non- REM sleep were positively correlated with PatSep performance (for central electrode sites: spindles: $r = .683$, $p = .014$, slow oscillations: $r = .613$, $p = .026$; Figure 4-4 a,b). Considering evidence that the occurrence of hippocampal memory reactivations is synchronized to neocortical slow oscillation and thalamic spindle activity (Sirota et al., 2003; Staresina et al., 2015) we also explored the relationship of EEG activity occurring phase-locked to the slow oscillation (±1.5 s around the negative slow oscillation

peak) with PatSep performance. The analyses revealed a positive correlation between PatSep performance and phase-locked (4–8 Hz) theta power with a maximum at central channels (at C3: r = .589, p = .044; Figure 4-4 c). There was no correlation of PatSep performance with (12–16 Hz) spindle activity occurring phase-locked to the slow oscillation.

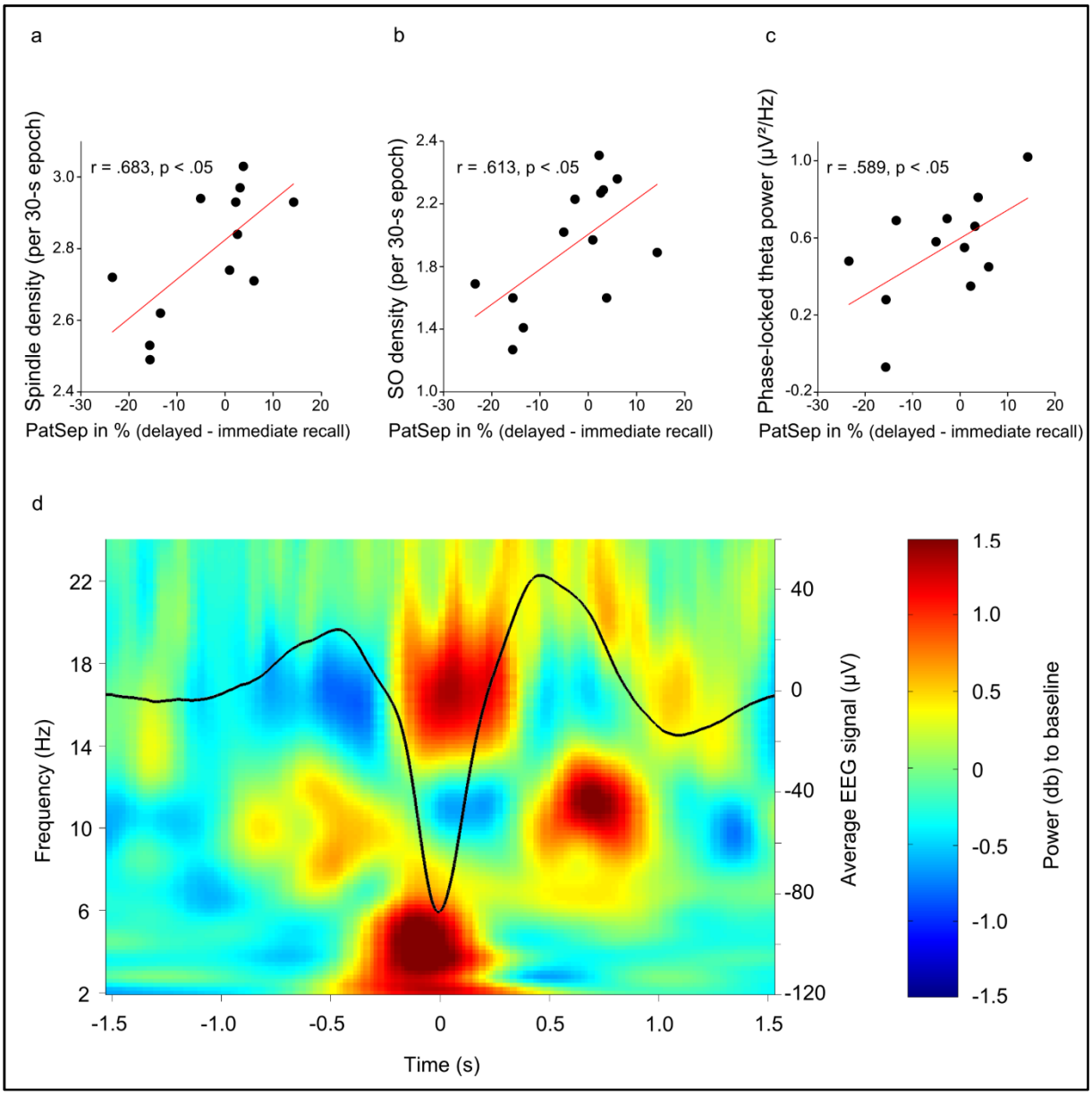

Figure 4-4. EEG spindle density, slow oscillation density and slow oscillation associated theta activity during NonREM sleep are related to pattern separation performance. Correlation between PatSep performance and a) spindle density (n = 12) and b) slow oscillation density (n = 13) during NonREM sleep (SWS and S2, recordings from C3/C4). c) Correlation between PatSep performance and phase-locked theta (4–8 Hz) power (at C3) during slow oscillation, i.e., in a -0.5 to 0.25-s window around the negative slow oscillation peak (n = 13). Slow oscillations were identified in NonREM sleep (SWS and S2). d)

Averaged time-frequency plot of EEG wavelet-power during slow oscillations (at C3) in a ±1.5-s time window around the negative slow oscillation peak (0 s) for a 2–24 Hz frequency band (n = 12).

4.5 Discussion

Our study in humans shows that pattern separation performance stabilized after sleep but deteriorated across a period of wakefulness. The stabilizing effect of sleep on pattern separation was most pronounced for lures with lowest similarity to the target stimulus. For stimuli with the greatest similarity to targets, pattern separation performance after postencoding sleep was significantly lower than after the wake period. Particularly, we found EEG spindle and slow oscillation density, as well as slow oscillation locked theta activity during non-REM sleep to be positively correlated with overnight changes in pattern separation performance. The picture arising from these findings is consistent with the notion that neural replay during sleep strengthens hippocampal representations such that similarity-dependent computations of pattern separation are stabilized.

Revealing superior recognition performance after sleep compared with wakefulness, our findings confirm previous studies indicating that sleep supports the formation of distinct representations for discriminative stimuli (Drosopoulos, Wagner, & Born, 2005; Ellenbogen, Hulbert, Jiang, & Stickgold, 2009; Fenn, Gallo, Margoliash, Roediger, & Nusbaum, 2009; Gais, Plihal, Wagner, & Born, 2000; Maurer et al., 2015; Stickgold, James, & Hobson, 2000). The present data go beyond these previous data by indicating that sleep changes recognition behavior strongly depending on the similarity of lures to target stimuli. The clear switch from similar to old responses regarding highly similar lures across sleep may suggest a predomination of pattern completion processes over pattern separation (Toner et al., 2009; Yassa et al., 2010). If this was the case, our results may indicate that reprocessing of representations during sleep simultaneously affects both modes of pattern separation and pattern completion. However, it should

also be mentioned here that the validity of the MST as a measure of hippocampal pattern separation and completion performance (Liu, Gould, Coulson, Ward, & Howard, 2016).

Specifically, the definition of pattern completion performance judging similar lures as old assuming a generalization process regarding target-lure pairs (Yassa & Stark, 2011) does not represent its theoretical construct as the reactivation of memory representations based on noisy or degraded input accurately (Hunsaker & Kesner, 2013). Consequently, since we could only show a shift toward pattern completion indirectly, our interpretation in this regard remains speculative. As the MST has already been validated in several studies for the assessment of pattern separation performance (Lacy et al., 2011; Yassa, Mattfeld, et al., 2011; Yassa et al., 2010) the paradigm is well suited for comparing our results with previous findings regarding pattern separation, so that we concentrated on those results. Indeed, the pattern separation performance assessed immediately after encoding in both the Sleep and Wake condition was comparable to those of corresponding age groups examined in previous studies (Stark et al., 2013; Toner et al., 2009; Yassa, Lacy, et al., 2011). While the MST has been used mainly in conjunction with an immediate recall test, we here use this task for assessing how reprocessing affects memory representations across longer retention periods of sleep and wakefulness. Across wakefulness, pattern separation particularly deteriorated, which is reminiscent of changes seen in aged humans (Stark et al., 2013; Yassa, Lacy, et al., 2011) and in patients with conditions including deficits in episodic memory such as Alzheimer's disease (Ally et al., 2013), Mild Cognitive Impairment (Yassa et al., 2010) and hippocampal damage (Kirwan et al., 2012). Recently, sleep deprivation was identified as another factor interfering with successful pattern separation at memory encoding (i.e., in an immediate recall test) whereby the impairment was restored after a recovery nap (Saletin et al., 2016).

Importantly, in comparison with wakefulness, sleep did not uniformly improve pattern separation performance at all levels of lure similarity, but for lures with the highest similarity to the

targets sleep diminished pattern separation. Evidence from studies of neural activity in rats indicates that the dentate gyrus and CA3 are simultaneously engaged in the processing of pattern separation and completion such that at a certain degree of similarity to a previously learned stimulus, acute stimulus input favors pattern completion operations (Guzowski et al., 2004; Lee, Yoganarasimha, Rao, & Knierim, 2004; S. Leutgeb, Leutgeb, Treves, Moser, & Moser, 2004; Vazdarjanova & Guzowski, 2004). Considering this switch from pattern separation to pattern completion with increasing target similarity is a hallmark of hippocampal information processing, the present observation that sleep enhanced pattern separation of dissimilar lures and diminished pattern separation of lures highly similar to the target corroborates the view of a direct impact of sleep on the hippocampal representation of the target stimuli. Neural assembly pattern reactivations during sleep might counter trace decay and, thus, keep the hippocampal representation shaped such that operations of pattern separation are enhanced to stimulus inputs of graded similarity, in comparison with the effects of post encoding wakefulness.

On the other side, the pattern of changes rules out a nonspecific effect of sleep generally enhancing discriminability of stimulus inputs. Other nonspecific confounding effects can likewise be excluded. There are hints that encoding and recall of declarative memory can be affected by circadian factors (Tilley & Warren, 1983). Indeed, in the Sleep and Wake conditions of the present experiments these processes took place at different circadian phases, to avoid stress-inducing effects of sleep deprivation impairing pattern separation (Saletin et al., 2016). However, morning and evening sessions in these conditions differed neither in immediate recall performance on the MST nor in vigilance and tiredness, rendering it unlikely that nonspecific changes in executive function substantially contributed to the differential recall pattern after sleep and wakefulness. Furthermore, participants achieved scores on immediate recall similar to those reported in previous studies (Stark et al., 2013) so that conditions are not only comparable to each other but also to findings beyond our experiment.

Also our exploratory analysis of relations to sleep EEG oscillatory parameters highlighted consistent positive correlations of spindle and slow-oscillation density as well as of theta activity occurring phase-locked to slow oscillations with pattern separation performance after sleep, which further corroborates the view of sleep, specifically non-REM sleep, being the primary factor mediating the effects on hippocampal memory. The correlation with sleep spindle density, a measure being independent of the duration of non-REM sleep, underscores the idea that spindles are genuinely involved in processes of memory consolidation. Indeed, a large body of findings suggests that spindle activity originating from thalamic networks enhances consolidation in different memory domains, including hippocampus-dependent spatial memories (Eschenko, Mölle, Born, & Sara, 2006; Gais, Mölle, Helms, & Born, 2002; Meier-Koll, Bussmann, Schmidt, & Neuschwander, 1999; Saletin, Goldstein, & Walker, 2011; Schabus et al., 2004). Spindles phase lock hippocampal ripples and co-occurring neural memory reactivations to the excitable trough of the spindle oscillation (Bergmann, Mölle, Diedrichs, Born, & Siebner, 2012; Clemens et al., 2007; Staresina et al., 2015). Concurrently, the slow oscillations drive hippocampal ripples and neural reactivations such that they preferentially occur during the depolarizing slow-oscillation up phase (Ji & Wilson, 2007; Sirota et al., 2003; Staresina et al., 2015). Against this backdrop, improved pattern separation performance associated with enhanced spindle and slow oscillation density might reflect that memory reactivation and ripples occurring in hippocampal networks during the excitable phase of the spindle and slow oscillation cycle, respectively, are more effective in keeping the respective memory representation. Enhanced EEG theta activity occurring in synchrony with the up-to-down transition of the slow oscillation might likewise be connected to a more effective hippocampal processing of memory information. In the wake state, theta oscillations are robust indicators of ongoing encoding and retrieval in hippocampal networks (Klimesch et al., 2006; Nyhus & Curran, 2010). Similarly,

during sleep, increases in frontal EEG theta activity were observed in humans when hippocampal memory representations were experimentally reactivated by presenting reminder cues during (Schreiner, Lehmann, & Rasch, 2015; Schreiner & Rasch, 2015). The cuing-evoked increase in theta activity might well reflect re-encoding of information during hippocampal reactivation, although the origin of scalp-recorded EEG theta activity in humans is obscure (Klimesch, 1996; Klimesch, Doppelmayr, Russegger, & Pachinger, 1996). In natural conditions, the neocortical slow oscillation synchronizes neural reactivations of memory information in hippocampal networks to the slow oscillation upstate (Diekelmann & Born, 2010; Ji & Wilson, 2007).

Against this backdrop, our observation of a positive correlation between pattern separation performance after sleep and theta activity occurring in synchrony with the slow oscillation during sleep fits well with the notion that hippocampal memory reactivations go along with a re-encoding and shaping of the hippocampal representations, thereby enhancing pattern separation and completion during the processing of stimuli with graded similarity. Altogether, our findings provide first-time evidence in humans supporting the notion that reactivation-based consolidation processes during sleep affect the hippocampal representation itself such that hippocampal computations of pattern separation are enhanced.

4.6 References - Study III

Ally, B. A., Hussey, E. P., Ko, P. C., & Molitor, R. J. (2013). Pattern separation and pattern completion in Alzheimer's disease: evidence of rapid forgetting in amnestic mild cognitive impairment. *Hippocampus*, *23*, 1246–58. doi:10.1002/hipo.22162

Aschenbrenner, S., Tucha, O., & Lange, K. W. (2000). *Regensburger Wortflüssigkeitstest*. Göttingen: Testzentrale.

Bakker, A., Kirwan, C. B., Miller, M., & Stark, C. E. (2008). Pattern separation in the human hippocampal CA3 and dentate gyrus. *Science*, *319*, 1640–2. doi:10.1126/science.1152882

Bartsch, T., Döhring, J., Rohr, A., Jansen, O., & Deuschl, G. (2011). CA1 neurons in the human hippocampus are critical for autobiographical memory, mental time travel, and autonoetic consciousness. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 17562–7. doi:10.1073/pnas.1110266108

Bartsch, T., Schönfeld, R., Müller, F. J., Alfke, K., Leplow, B., Aldenhoff, J., … Koch, J. M. (2010). Focal lesions of human hippocampal CA1 neurons in transient global amnesia impair place memory. *Science*, *328*, 1412–5. doi:10.1126/science.1188160

Bendor, D., & Wilson, M. A. (2012). Biasing the content of hippocampal replay during sleep. *Nat Neurosci*, *15*, 1439–1444.

Bennett, I. J., & Stark, C. E. L. (2016). Mnemonic discrimination relates to perforant path integrity: An ultra-high resolution diffusion tensor imaging study. *Neurobiology of Learning and Memory*, *129*, 107–112. doi:10.1016/j.nlm.2015.06.014

Bergmann, T. O., Mölle, M., Diedrichs, J., Born, J., & Siebner, H. R. (2012). Sleep spindle-related reactivation of category-specific cortical regions after learning face-scene associations. *Neuroimage*, *59*, 2733–42.

Berron, D., Schütze, H., Maass, A., Cardenas-Blanco, A., Kuijf, H. J., Kumaran, D., & Düzel, E. (2016). Strong evidence for pattern separation in human dentate gyrus. *Journal of Neuroscience*, *36*, 7569–79. doi:10.1523/JNEUROSCI.0518-16.2016

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res*, *28*, 193–213.

Clemens, Z., Mölle, M., Eross, L., Barsi, P., Halász, P., & Born, J. (2007). Temporal coupling of parahippocampal ripples, sleep spindles and slow oscillations in humans. *Brain*, *130*, 2868–78. doi:10.1093/brain/awm146

De Gennaro, L., & Ferrara, M. (2003). Sleep Spindles: an overview. *Sleep Medicine Reviews*, *7*, 423–440.

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nat Rev Neurosci*, *11*, 114–26. doi:10.1038/nrn2762

Drosopoulos, S., Wagner, U., & Born, J. (2005). Sleep enhances explicit recollection in recognition memory. *Learning & Memory*, *12*, 44–51.

Ego-Stengel, V., & Wilson, M. A. (2010). Disruption of ripple-associated hippocampal activity during rest impairs spatial learning in the rat. *Hippocampus*, *20*(1), 1–10. doi:10.1002/hipo.20707

Ellenbogen, J. M., Hulbert, J. C., Jiang, Y., & Stickgold, R. (2009). The sleeping brain's influence on verbal memory: boosting resistance to interference. *PLoS One*, *4*, e4117.

Eschenko, O., Mölle, M., Born, J., & Sara, S. J. (2006). Elevated sleep spindle density after learning or after retrieval in rats. *J Neurosci*, *26*, 12914–20. doi:10.1523/JNEUROSCI.3175-06.2006

Fenn, K. M., Gallo, D. A., Margoliash, D., Roediger, H. 3rd, & Nusbaum, H. C. (2009). Reduced false memory after sleep. *Learn Mem*, *16*, 509–513.

Gais, S., Mölle, M., Helms, K., & Born, J. (2002). Learning-dependent increase in sleep spindle density. *J Neurosci*, *22*, 6830–6834.

Gais, S., Plihal, W., Wagner, U., & Born, J. (2000). Early sleep triggers memory for early visual discrimination skills. *Nat Neurosci*, *3*, 1335–1339.

Girardeau, G., Benchenane, K., Wiener, S. I., Buzsáki, G., & Zugaro, M. B. (2009). Selective suppression of hippocampal ripples impairs spatial memory. *Nat Neurosci*, *12*, 1222–3. doi:10.1038/nn.2384

Guzowski, J. F., Knierim, J. J., & Moser, E. I. (2004). Ensemble dynamics of hippocampal regions CA3 and CA1. *Neuron*, *44*, 581–4. doi:10.1016/j.neuron.2004.11.003

Hunsaker, M. R., & Kesner, R. P. (2013). The operation of pattern separation and pattern completion processes associated with different attributes or domains of memory. *Neuroscience and Biobehavioral Reviews*, *37*(1), 36–58. doi:10.1016/j.neubiorev.2012.09.014

Inostroza, M., & Born, J. (2013). Sleep for preserving and transforming episodic memory. *Annu Rev Neurosci*, *36*, 79–102.

Ji, D., & Wilson, M. A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. *Nature Neuroscience*, *10*, 100–7. doi:10.1038/nn1825

Karpicke, J. D., & Roediger, H. 3rd. (2008). The critical importance of retrieval for learning. *Science*, *319*, 966–968.

Kirwan, C. B., Hartshorn, A., Stark, S. M., Goodrich-Hunsaker, N. J., Hopkins, R. O., & Stark, C. E. L. (2012). Pattern separation deficits following damage to the hippocampus. *Neuropsychologia*, *50*, 2408–14. doi:10.1016/j.neuropsychologia.2012.06.011

Kirwan, C. B., & Stark, C. E. L. (2007). Overcoming interference: an fMRI investigation of pattern separation in the medial temporal lobe. *Learning & Memory*, *14*, 625–33. doi:10.1101/lm.663507

Klimesch, W. (1996). Memory processes, brain oscillations and EEG synchronization. *Int J Psychophysiol*, *24*, 61–100.

Klimesch, W., Doppelmayr, M., Russegger, H., & Pachinger, T. (1996). Theta band power in the human scalp EEG and the encoding of new information. *Neuroreport*, *7*, 1235–1240.

Klimesch, W., Hanslmayr, S., Sauseng, P., Gruber, W., Brozinsky, C. J., Kroll, N. E., … Doppelmayr, M. (2006). Oscillatory EEG correlates of episodic trace decay. *Cereb Cortex*, *16*, 280–90. doi:10.1093/cercor/bhi107

Lacy, J. W., Yassa, M. A., Stark, S. M., Muftuler, L. T., & Stark, C. E. L. (2011). Distinct pattern separation related transfer functions in human CA3/dentate and CA1 revealed using high-resolution fMRI and variable mnemonic similarity. *Learning & Memory*, *18*, 15–8. doi:10.1101/lm.1971111

Lee, I., Yoganarasimha, D., Rao, G., & Knierim, J. J. (2004). Comparison of population coherence of place cells in hippocampal subfields CA1 and CA3. *Nature*, *430*, 456–9. doi:10.1038/nature02739

Lehrl, S. (2005). *Mehrfachwahl-Wortschatz-Intelligenz-Test, MWT-B* (5th ed.). Balingen: Spitta Verlag.

Leutgeb, J. K., Leutgeb, S., Moser, M. B., & Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science*, *315*, 961–6. doi:10.1126/science.1135801

Leutgeb, S., Leutgeb, J. K., Treves, A., Moser, M. B., & Moser, E. I. (2004). Distinct ensemble codes in hippocampal areas CA3 and CA1. *Science*, *305*, 1295–8. doi:10.1126/science.1100265

Liu, K. Y., Gould, R. L., Coulson, M. C., Ward, E. V., & Howard, R. J. (2016). Tests of pattern separation and pattern completion in humans-A systematic review: Tests of Pattern Separation and Pattern Completion. *Hippocampus*, *26*(6), 705–717. doi:10.1002/hipo.22561

Maurer, L., Zitting, K. M., Elliott, K., Czeisler, C. A., Ronda, J. M., & Duffy, J. F. (2015). A new face of sleep: The impact of post-learning sleep on recognition memory for face-name associations. *Neurobiol Learn Mem*, *126*, 31–38.

McClelland, J. L., McNaughton, B. L., & O'Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychological Review*, *102*, 419–57.

McNaughton, B. L., & Morris, R. G. (1987). Hippocampal synaptic enhancement and information storage within a distributed memory system. *Trends in Neurosciences*, 408-415.

Meier-Koll, A., Bussmann, B., Schmidt, C., & Neuschwander, D. (1999). Walking through a maze alters the architecture of sleep. *Perceptual and Motor Skills*, *88*, 1141–59.

Mölle, M., Bergmann, T. O., Marshall, L., & Born, J. (2011). Fast and slow spindles during the sleep slow oscillation: Disparate coalescence and engagement in memory processing. *Sleep*, *34*, 1411–1421.

Mölle, M., Marshall, L., Gais, S., & Born, J. (2002). Grouping of spindle activity during slow oscillations in human non-rapid eye movement sleep. *J Neurosci*, *22*, 10941–10947.

Neunuebel, J. P., & Knierim, J. J. (2014). CA3 retrieves coherent representations from degraded input: direct evidence for CA3 pattern completion and dentate gyrus pattern separation. *Neuron*, *81*, 416–27. doi:10.1016/j.neuron.2013.11.017

Ngo, H. V., Martinetz, T., Born, J., & Mölle, M. (2013). Auditory Closed-Loop Stimulation of the Sleep Slow Oscillation Enhances Memory. *Neuron*. doi:10.1016/j.neuron.2013.03.006

Norman, K. A., & O'Reilly, R. C. (2003). Modeling hippocampal and neocortical contributions to recognition memory: a complementary-learning-systems approach. *Psychological Review*, *110*, 611–646. doi:10.1037/0033-295X.110.4.611

Nyhus, E., & Curran, T. (2010). Functional role of gamma and theta oscillations in episodic memory. *Neurosci Biobehav Rev*, *34*, 1023–35. doi:10.1016/j.neubiorev.2009.12.014

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, *9*, 97–113.

O'Neill, J., Pleydell-Bouverie, B., Dupret, D., & Csicsvari, J. (2010). Play it again: reactivation of waking experience and memory. *Trends Neurosci*, *33*, 220–9. doi:10.1016/j.tins.2010.01.006

Oostenveld, R., Fries, P., Maris, E., & Schoffelen, J. M. (2011). FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Computational Intelligence and Neuroscience*, *2011*. doi:10.1155/2011/156869

O'Reilly, R. C., & McClelland, J. L. (1994). Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off. *Hippocampus*, *4*(6), 661–682. doi:10.1002/hipo.450040605

Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiol Rev*, *93*, 681–766. doi:10.1152/physrev.00032.2012.-Over

Rasch, B., Büchel, C., Gais, S., & Born, J. (2007). Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science*, *315*, 1426–9.

Reitan, R. M. (1979). *Trail Making Test (TMT)*. Göttingen: Hogrefe.

Rey, A. (1941). L'examen psychologique dans les cas d'encéphalopathie traumatique. *Archives de Psychologie*, *28*, 21.

Riedner, B. A., Vyazovskiy, V. V., Huber, R., Massimini, M., Esser, S., Murphy, M., & Tononi, G. (2007). Sleep homeostasis and cortical synchronization: III. A high-density EEG study of sleep slow waves in humans. *Sleep*, *30*, 1643–1657.

Saletin, J. M., Goldstein, A. N., & Walker, M. P. (2011). The role of sleep in directed forgetting and remembering of human memories. *Cereb Cortex*, *21*, 2534–41. doi:10.1093/cercor/bhr034

Saletin, J. M., Goldstein-Piekarski, A. N., Greer, S. M., Stark, S., Stark, C. E., & Walker, M. P. (2016). Human hippocampal structure: a novel biomarker predicting mnemonic vulnerability to, and recovery from, sleep deprivation. *J Neurosci*, *36*, 2355–63.

Schabus, M., Gruber, G., Parapatics, S., Sauter, C., Klosch, G., Anderer, P., … Zeitlhofer, J. (2004). Sleep spindles and their significance for declarative memory consolidation. *Sleep*, *27*, 1479–85.

Schreiner, T., Lehmann, M., & Rasch, B. (2015). Auditory feedback blocks memory benefits of cueing during sleep. *Nat Commun*, *6*, 8729. doi:10.1038/ncomms9729

Schreiner, T., & Rasch, B. (2015). Boosting vocabulary learning by verbal cueing during sleep. *Cereb Cortex*, *26*, 4169–79.

Sirota, A., Csicsvari, J., Buhl, D., & Buzsáki, G. (2003). Communication between neocortex and hippocampus during sleep in rodents. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 2065–9. doi:10.1073/pnas.0437938100

Staresina, B. P., Bergmann, T. O., Bonnefond, M., van der Meij, R., Jensen, O., Deuker, L., … Fell, J. (2015). Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nature Neuroscience*, *18*, 1679–86. doi:10.1038/nn.4119

Stark, S. M., Yassa, M. A., Lacy, J. W., & Stark, C. E. L. (2013). A task to assess behavioral pattern separation (BPS) in humans: Data from healthy aging and mild cognitive impairment. *Neuropsychologia*, *51*, 2442–9. doi:10.1016/j.neuropsychologia.2012.12.014

Stickgold, R., James, L., & Hobson, J. A. (2000). Visual discrimination learning requires sleep after training. *Nat Neurosci*, *3*, 1237–1238.

Tilley, A., & Warren, P. (1983). Retrieval from semantic memory at different times of day. *J Exp Psychol Learn Mem Cogn*, *9*, 718–24.

Toner, C. K., Pirogovsky, E., Kirwan, C. B., & Gilbert, P. E. (2009). Visual object pattern separation deficits in nondemented older adults. *Learning & Memory*, *16*, 338–42. doi:10.1101/lm.1315109

Vazdarjanova, A., & Guzowski, J. F. (2004). Differences in hippocampal neuronal population responses to modifications of an environmental context: evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles. *Journal of Neuroscience*, *24*, 6489–96. doi:10.1523/jneurosci.0350-04.2004

Wechsler, D. (1997). *WMS-III Administration and scoring manual*. San Antonio, TX: The Psychological Corporation. Harcourt Brace & Co.

Wilson, M. A., & McNaughton, B. L. (1994). Reactivation of hippocampal ensemble memories during sleep. *Science*, *265*, 676–9.

Yassa, M. A., Lacy, J. W., Stark, S. M., Albert, M. S., Gallagher, M., & Stark, C. E. L. (2011). Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults. *Hippocampus*, *21*, 968–79. doi:10.1002/hipo.20808

Yassa, M. A., Mattfeld, A. T., Stark, S. M., & Stark, C. E. L. (2011). Age-related memory deficits linked to circuit-specific disruptions in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 8873–8. doi:10.1073/pnas.1101567108

Yassa, M. A., & Stark, C. E. L. (2011). Pattern separation in the hippocampus. *Trends in Neurosciences*, *34*(10), 515–525. doi:10.1016/j.tins.2011.06.006

Yassa, M. A., Stark, S. M., Bakker, A., Albert, M. S., Gallagher, M., & Stark, C. E. L. (2010). High-resolution structural and functional MRI of hippocampal CA3 and dentate gyrus in patients with amnestic Mild Cognitive Impairment. *NeuroImage*, *51*, 1242–52. doi:10.1016/j.neuroimage.2010.03.040

5 General Discussion and Conclusions

Memories of the past form the essence of our identity, self, and mind. Remembering yesterday provides guidance for tomorrow and supports the understanding of who we are and the world we live in. Conscious recollection of events in the view of autobiographical memories encourages the reflection of past behavior leading to learning from mistakes, personal growth, and self-development (see Tulving (2005)). The biological substrates of memory can be assigned to different areas of the brain (Squire & Zola, 1996). Episodic memory content of our personal experiences is critically reliant on the hippocampus (McClelland, McNaughton, & O'Reilly, 1995; Scoville & Milner, 1957). An effective memory system (i.e. minimal interference and maximal capacity) must provide at least two cognitive functions: first, the rapid storage of experiences as individual events, and second, the effortless retrieval of those memories, when similar events are encountered (O'Reilly & McClelland, 1994). In this context, hippocampal pattern separation and pattern completion are essential cognitive processes for encoding and retrieval of episodes that can be assigned to specific hippocampal subnetworks (Marr, 1971; O'Reilly & McClelland, 1994). Theories regarding pattern separation and completion processes that derived from computational approaches have been consistently supported by studies in rodents (see Yassa and Stark (2011) for a review). The aim of the present thesis was to elucidate the role of the hippocampus in pattern separation and completion. In particular, we sought to determine the role of hippocampal subfield processing in pattern separation in humans. Specifically, we achieved this aim by investigating the behavioral outcome of a pattern separation task in two human hippocampal lesion models. First, the selective CA1 subfield lesions in TGA, and, second preferential neurodegeneration in DG/CA3 subfields in a patient cohort with rare LGI1 encephalitis, served as natural hippocampal lesion models to examine a causal relationship between anatomical structures and pattern separation performance. Furthermore, the investigation of pattern separation performance after post-encoding sleep in healthy humans was

performed to gain insight into the physiological neurobiological processes of memory consolidation regulated by the hippocampus.

5.1 The role of CA1 in pattern separation and recognition memory

In Study I, the natural lesion model of TGA supports the critical relay function of CA1 neurons in pattern separation performance. Additionally, by measuring hippocampal volume, Study II showed that the volume of CA1 was the best predictor of recognition memory. Information processing within CA1 is characterized by the comparison of dual afferent projections – from EC via the perforant path and from CA3 via the Schaffer collaterals (Knierim & Neunuebel, 2016; Lisman, 1999; O'Reilly & McClelland, 1994). The integration of those two projections within CA1 is assumed to be beneficial for immediate retrieval and consolidation in neocortical long-term stores (Treves & Rolls, 1994). With regard to the contribution of CA1 neurons to pattern separation processes in humans, our results complement the current picture arising from computational models and animal data (McClelland et al., 1995; Guzowski et al., 2004). Pattern separation is suggested to be facilitated by the DG that is in turn assumed to decorrelate overlapping memories by sparse coding of neural excitation from EC to CA3 (Rolls 2016, Treves rolls 1994). For the transfer to extra-hippocampal areas, CA3 projects to area CA1, the main hippocampal output (Insausti & Amaral 2004, Knierim neunuebel 2016). In Study I, the selective CA1 dysfunction caused a disruption in the transmission of separated information from the DG/CA3 network to the neocortex resulting in ineffective pattern separation performance on the behavioral level. This suggests that CA1 does not perform pattern separation on the neural level per se, but forwards memory information from previous DG/CA3 processes to neocortical areas. Connecting the results of Study I and II, this assumption applies to the result of the dependence of pattern separation performance on hippocampal DG volume, but a weaker association to the volume of CA1. The prediction of recognition memory by the CA1 volume can be

explained by the ideal position of CA1 as the functional readout of hippocampal circuit projections. The integration of the dual afferent projections from EC and CA3 facilitates the restoration of a memory trace and thus recognition of an environmental cue (Hasselmo & Eichenbaum 2005, Hassselmo & Wyble, 1997). Together, these results strengthen the view that CA1 with regard to its excitatory inputs is implicated in both pattern separation and recognition memory processes. The functional readout of the hippocampal circuit to neocortical areas involved in hippocampus-dependent memory formation, is thus highly dependent on the dynamics within the subnetworks.

5.2 The Dentate Gyrus in pattern separation

Study II showed that the DG volume was the best predictor of behavioral pattern separation compared to the regions CA2/3 and CA1. Theoretical models state that the DG performs pattern separation by the transformation of overlapping input patterns into distinct, non-overlapping representations (Marr, 1971; McClelland et al., 1995). This functional model of the DG in pattern separation processes has been confirmed by electrophysiological recordings in rodents (Leutgeb, Leutgeb, Moser, & Moser, 2007; Neunuebel & Knierim, 2014). Evidence for a separation-like activity within the DG in humans has been provided by high-resolution fMRI during a mnemonic similarity recognition paradigm (Berron et al., 2016). The results of Study II complement those findings by presenting a structure-function relationship between pattern separation and the DG. The findings are in accordance with previous studies that demonstrated a greater volume of the DG to be associated with a better discrimination of overlapping items (Dillon et al., 2017; Doxey & Kirwan, 2015; Stark & Stark, 2017).

Regarding the DG and pattern separation, attention has additionally focused on a neurobiological process of neurogenesis in the adult hippocampus (see França, Bitencourt, Maximilla, Bar-

ros, & Monserrat (2017) for a review). Adult neurogenesis is a special feature of the hippocampus, as the generation of new neurons in other parts of the brain are restricted to certain stages of development (Eriksson et al., 1998). In the mammalian brain, new neurons are generated from neural stem cells in the DG throughout adulthood and presumably integrated into the hippocampal circuits (Altman & Das, 1965; Eriksson et al., 1998). In rodents, there is evidence that the inhibition of neurogenesis in the DG results in deficient pattern separation (Clelland et al., 2009), whereas an increase strengthens the ability (Sahay et al., 2011). Although its existence in humans has recently started to be questioned (Sorrells et al., 2018), and direct evidence for a role of hippocampal neurogenesis in pattern separation in humans is lacking, investigations regarding neurodegenerative diseases associated with cognitive decline discuss an involvement of impaired hippocampal neurogenesis in cognition and memory (Deng, Aimone, & Gage, 2010; Zhao, Deng, & Gage, 2008).

5.3 Vulnerability and neuroplasticity in the hippocampus

With the clinical pictures of TGA and LGI1 the thesis presents two examples of the particular vulnerability of the hippocampus. Over the last twenty years, a large body of research has provided evidence for a differential affection of hippocampal subfields by pathology (see Small, Schobel, Buxton, Witter, & Barnes (2011) for a review). This vulnerability can be seen in the context of differences in regional vulnerability grounded on different cytoarchitectonic structures, as well as a differential metabolic vulnerability of the subfields. Generally speaking, the hippocampus is particularly susceptible to dysfunction caused by noxious vascular and metabolic influences where damage is most likely to occur in CA1 neurons (Bartsch et al., 2015; Small et al., 2011). With regard to the hippocampal vulnerability to chronic influences like neurodegenerative diseases, chronic epilepsy, and neuropsychiatric disorders, for instance, the

DG/CA3 network could be more susceptible as compared with acute hippocampal lesions (Bartsch & Wulff, 2015; Small et al., 2011).

Psychiatric diseases such as major depressive disorder, posttraumatic stress disorder, and schizophrenia display examples of clinical neurocognitive deficits associated with hippocampal impairment. Declarative memory has been shown to be the most commonly impaired cognitive function in schizophrenia (see Tamminga, Stan, and Wagner (2010) for a review). For instance, deficits in an old-new recognition memory paradigm were associated with hippocampal volume reduction in schizophrenic patients (Weiss et al., 2004). Schizophrenia has also been found to correlate with pattern separation deficits (Das, Ivleva, Wagner, Stark, & Tamminga, 2014). Histological examinations suggest that this deficit results from reduced adult neurogenesis in the DG (Das et al., 2014; Reif et al., 2006; Tamminga et al., 2010).

The strong vulnerability of the hippocampus to noxious impact is assumed to be a result of the high degree of neuroplasticity (McEwen, 1994). Neurogenesis as a form of enhanced plasticity in the hippocampal DG facilitates encoding of new information and retrieval of previously stored memories (Appleby, Kempermann, & Wiskott, 2011; Wiskott, Rasch, & Kempermann, 2006). Therefore, pattern separation abilities are dependent on the generation of new neurons (Sahay et al., 2011). It is assumed that stress-induced distortion of hippocampal neurogenesis in the DG causes pattern separation deficits that are likely mediating an overgeneralization of fear responses in posttraumatic stress disorders (Kheirbek, Klemenhagen, Sahay, & Hen, 2012). Therewith, decreased neurogenesis may also explain the involvement of hippocampal dysfunction in psychiatric diseases where fear generalization is involved in the pathology (Kheirbek et al., 2012).

Together those results support the formation of a framework, where the vulnerability of different hippocampal subfields result in differential malfunction of hippocampal memory pro-

cessing. This framework facilitates the differentiation of disorders that are related to hippocampal impairment and provides insight into the mechanisms of hippocampal pathology. Against this background, further investigations regarding hippocampal vulnerability and plasticity are of importance for the development of specific therapeutic interventions that can be applied early and more adapted to the patients' needs. (Bartsch & Wulff, 2015; Small et al., 2011).

5.4 Stabilization of pattern separation in the hippocampus: a role for sleep

Besides the investigation of the hippocampal subfield contribution to pattern separation in humans, the thesis aimed at showing how sleep is involved in the processes of pattern separation in the context of memory consolidation. Measuring pattern separation performance after post-encoding sleep by means of the MST Study III showed that pattern separation was stabilized after sleep, which was mostly pronounced for a large change of the input pattern (i.e. low similarity between target and lure). Additionally, the overnight change in pattern separation performance was significantly related to oscillatory dynamics in EEG signals that hallmark the process of sleep-dependent memory consolidation. The findings support the notion that pattern separation and sleep-dependent consolidation processes are linked by their shared underlying neurophysiological mechanisms. Both processes include critical transfer functions that extract essential information by reducing interference, stabilizing neural representations and integrating new information into preexisting memory networks (Diekelmann & Born, 2010; McClelland et al., 1995).

Recurrent connections within CA3 provide the basis for pattern completion: Attractor dynamics are able to reinstate a whole memory representation, even though only a fraction of cells begins to fire due to reactivation by a partial environmental cue (Treves & Rolls, 1994; Wills, Lever,

Cacucci, Burgess, & O'Keefe, 2005). Thus, it is assumed that pattern completion supports reactivation of memory engrams during sleep (O'Neill, Pleydell-Bouverie, Dupret, & Csicsvari, 2010). Likewise, as already stated, there is strong evidence that the interaction of CA3 and CA1 pyramidal cells generates sharp wave ripple events (Buzsáki, 1986, 1989; Csicsvari, Hirase, Mamiya, & Buzsáki, 2000). Here, CA3 is assumed to be the initiator of synchronous population bursts within all areas of the hippocampal circuitry during both SWS and non-exploratory wakefulness (Buzsáki, 1986). Importantly, sharp wave ripple events are associated with reactivation of hippocampal place cell ensembles (Pavlides & Winson, 1989; Wilson & McNaughton, 1994). Here, during sharp wave ripple activity, the reactivation of firing patterns of place cells induces synaptic plasticity in the form of long-term potentiation (Sadowski et al., 2016). As recurrent connections within CA3 drive the reactivation of firing patterns as well as promote the autoassociation essential for pattern completion, it is suggested that pattern completion processes may facilitate offline consolidation (McClelland & Goddard, 1996; O'Neill et al., 2010).

Discussing the results of Study III, we suggested that sleep promotes hippocampal pattern separation and completion depending on the degree of similarity of two input pattern: After sleep, the hippocampus performs pattern separation when the input similarity is low, whereas pattern completion is promoted when confronted with highly similar input pattern. A delay covered by wakefulness shows a complementary pattern. The mechanisms found within the CA3 system are comparable with those findings. As previously stated, the network within CA3 is biased towards pattern separation, when input overlap is low, but tends to perform pattern completion, when input similarity increases (Guzowski, Knierim, & Moser, 2004). The combination of those findings supports the hypothesis that neural mechanisms during sleep are highly connected to the computations within CA3. The initiation of high frequency oscillations in the form of sharp wave ripples that activate synaptic changes within the hippocampal network during sleep within CA3 (Buzsáki, 1986; Sadowski et al., 2016) highlight this assumption. Although

the detailed cellular mechanisms during sleep that bias CA3 toward pattern separation or completion remain to be clarified, the findings suggest that the function of CA3 could be strengthened in the sleeping brain.

5.5 Extraction of information during sleep

The findings of Study III complement an existing theory of schema formation during sleep suggested by Lewis and Durrant (2011). The theory draws upon the findings that sleep facilitates the integration of new information into preexisting memory representations (Ellenbogen, Hu, Payne, Titone, & Walker, 2007; Tamminen, Payne, Stickgold, Wamsley, & Gaskell, 2010) and provides insight into hidden rules (Wagner, Gais, Haider, Verleger, & Born, 2004). The authors suggest that overlapping elements of memory representations are selectively strengthened and more strongly consolidated in neocortical areas. Due to stronger reactivation of overlapping representations during SWS, connections of shared features are facilitated. In a long-term perspective of system consolidation, this process is thought to provide a framework for the formation of cognitive schemas as shared neural connections endure during SWS (Lewis & Durrant, 2011). On the conceptual level, in Study III pattern separation and completion mechanisms served as indicators for an active shaping of hippocampal representations within periods of SWS. A bias towards pattern separation after post-encoding sleep may indicate a refinement and sharpening of the neural representation with regard to a strengthening of details, whereas an extraction of essential features in terms of generalization during sleep may reflect pattern completion operations. This conception is in accordance with the theory of schema formation. Lastly, it also highlights the dependence of generalization and sharpening of neural representations on pattern separation and completion processes.

5.6 Methodological considerations

In humans, behavioral paradigms cannot directly report hippocampal computational processes on a neural level compared with rodent examinations that use single cell recordings (Liu, Gould, Coulson, Ward, & Howard, 2016). In this context, the inference from computational models to animal investigations as well as to human data can be erroneous and should be considered with caution (Deuker, Doeller, Fell, & Axmacher, 2014).

The MST has already been well validated regarding the measurement of behavioral pattern separation (Lacy et al., 2011; Stark et al., 2013; Yassa, Mattfeld, Stark, & Stark, 2011; Yassa et al., 2010). However, the MST as a measure of behavioral pattern completion has been questioned because the test lacks partial lure stimuli that can be used as retrieval cues in accordance with the requirement of its the theoretical background (Hunsaker & Kesner, 2013; Liu et al., 2016). To validate the thesis' findings with regard to pattern completion, future research should concentrate on tests that use incomplete lure stimuli. For instance, the 'memory image completion task' provides line-drawings of indoor scenes as encoding items that are shown in different levels of completeness at retrieval (Vieweg, Riemer, Berron, & Wolbers, 2018). The levels are explicitly parameterized that increase the sensitivity of the test in detecting differences (Liu et al., 2016) and enable an exact evaluation of pattern completion on different degrees of similarity. Moreover, the MST has been criticized with regard to its context-dependency: The use of everyday objects cannot exclude a familiarity regarding certain items. Associations to previously stored episodes can introduce a bias during encoding and retrieval (Deuker et al., 2014; Hunsaker & Kesner, 2013). Abstract and context-free stimuli during encoding may be more sensitive in the assessment of pattern separation (Deuker et al., 2014). Taken together, a refinement of paradigms with regard to the differentiation of pattern separation and pattern completion as well as the choice of items and retrieval cues may lead to a more nuanced assessment of the hippocampal functions by means of behavioral tests.

Another methodological constraint relates to the evaluation of structural MRI of patients with damaged brain tissue. The analysis of shrunk and deformed hippocampal subfields in patient cohorts requires higher quality standards. This issue may be addresses by using higher resolution and the application of manual segmentation protocols.

5.7 Outlook and future directions

The studies conducted within this thesis elucidated subfield specific contributions of the human hippocampus to the process of pattern separation as well as to neurobiological processes of consolidation using the example of sleep. With a behavioral pattern separation task (Stark, Yassa, Lacy, & Stark, 2013) and the assessment of EEG oscillatory dynamics during sleep, Study III used a combination of behavioral and neurophysiological markers of memory processing during sleep. However, these methods only allowed indirect conclusions regarding hippocampal activation, especially with limitations regarding the neural level. Future investigations that directly evaluate the involvement of the hippocampus are therefore suggested. First, this can be achieved by using high resolution fMRI techniques to clarify subfield specific activity of the hippocampus during learning, post-encoding sleep, and recall. Those methods may be useful to substantiate the presumption of an active shaping of hippocampal memory representations during sleep. Also, differences in the contributions of hippocampal regions concerning sleep-dependent memory consolidation could be described by means of this methodological supplement that allows individual mapping of hippocampal subfields. Second, a direct solution to measure the hippocampal involvement regarding enhancing effects of sleep on pattern separation is to address the single-cell level of neurons in animal studies. Applying those methods may be able to show a direct link between changes in the behavioral outcome after sleep and hippocampal replay during sleep. It is thus important to establish parallelized study protocols

that use similar behavioral paradigms to tax more comparable cognitive processes underlying the behavioral output in both rodents and humans for a valid cross-species comparison.

To further clarify the mechanisms of sleep-dependent memory consolidation on the hippocampal level, a direct manipulation of reactivation during sleep may determine how sleep affects the hippocampal representation with regard to pattern separation or completion tendencies after sleep. Reactivation of memories during sleep can serve as an efficient method to confirm the involvement of the hippocampus in memory consolidation, and to specifically analyze the underlying mechanisms that drive the hippocampus to efficient memory encoding, consolidation, and retrieval (Rudoy, Voss, Westerberg, & Paller, 2009).

5.8 Conclusions

The three studies conducted within this thesis aimed at clarifying the role of the human hippocampus and its specific subfield contributions to pattern separation and memory consolidation. We found that the hippocampal DG as well as intact CA1 neurons are essential for pattern separation in humans. We demonstrated that pattern separation was best predicted by the volume of the DG, whereas recognition memory was stronger associated with the volume of CA1. However, we also found that an impairment restricted to CA1 neurons, but intact remaining hippocampal subfields, complicated pattern separation performance. Collectively, these results refine the current view on hippocampus-dependent memory processing with the DG as a crucial 'pattern separator', and CA1 essentially involved in transferring the separated output to neocortical long-term stores. Importantly, we demonstrate the relevance of the hippocampal-neocortical transfer during SWS, in the stabilization of separated information. We propose the CA3 region as the common ground of both the generation of sharp-wave ripple events as well as pattern separation and completion computations. This may speak in favor of a fundamental link

between pattern separation and completion processes during sleep-dependent memory consolidation. As the stabilization effect was dependent on the degree of similarity and significantly correlated with EEG oscillatory markers of sleep-dependent memory consolidation, we suggest that sleep actively affects hippocampal memory representations. This novel finding serves as the basis for future research regarding the specific role of subfield activity during sleep. In conclusion, the thesis highlights the importance of the intriguing hippocampal subfield structure for pattern separation and the associated role of pattern separation in describing structure-function relationships in memory and consolidation.

6 Deutsche Zusammenfassung (German Summary)

6.1 Kurzzusammenfassung

In einer von stetigen Veränderungen geprägten Umwelt ist die Bildung von stabilen Gedächtnisinhalten unerlässlich, um unser Selbst sowie eine Identität zu entwickeln. Durch hohe kognitive Anforderungen im Alltag benötigen wir hierfür ein effizientes Gedächtnissystem. Um zwischen ähnlichen Ereignissen zu unterscheiden, aber auch Generalisierungen über ähnliche Ereignisse zu schaffen, bietet das Gedächtnis zwei entgegengesetzte, sich jedoch ergänzende Funktionen. Erstens werden durch die sogenannte Musterseparation ähnliche Gedächtnisrepräsentationen unabhängig voneinander gespeichert. Zweitens können durch den Prozess der Musterkomplettierung zuvor gespeicherte Ereignisse durch Hinweisreize aus der Umwelt erinnert werden. Die Abhängigkeit dieser Funktionen vom Hippocampus, insbesondere von dessen Subregionen Gyrus Dentatus und CA3, wurde bereits in einer Vielzahl von theoretischen Modellen beschrieben, sowie anhand von Tier- und Humanstudien untersucht. Allerdings fehlen den vergangenen Studien im Hinblick auf den menschlichen Hippocampus eine mechanistische Aussage zur Kausalität zwischen Anatomie und Funktion. Zudem ist es bisher unklar, in welcher Weise Musterseparation und -komplettierung im Hinblick auf die Bildung von Langzeitgedächtnis stabilisiert werden können. Diese Arbeit hatte zum Ziel, die Rolle des menschlichen Hippocampus bezüglich der Musterseparation sowie der Gedächtniskonsolidierung zu präzisieren. Hierfür wurden erstens Patienten mit einer selektiven Läsion in der CA1-Region des Hippocampus, und zweitens ein Patientenkollektiv, bei der eine Neurodegeneration bevorzugt im Subnetzwerk Gyrus Dentatus/CA3 auftritt, hinsichtlich der Musterseparation untersucht. Die Studien zeigten insgesamt, eine defizitäre Musterseparationsleistung bei selektiver Beeinträchtigung der CA1-Region. Außerdem ging hervor, dass die Musterseparation signifikant vom Volumen der Gyrus Dentatus-Region abhing, die Rekognitionsleistung hingegen vom Volumen

von CA1. Diese Ergebnisse sprechen für eine regionale Spezialisierung der Hippocampusfunktion. Eine weitere Studie wurde mit dem Ziel durchgeführt, Einblicke in die neurobiologischen Prozesse der hippocampusabhängigen Gedächtniskonsolidierung zu gewinnen. Diesbezüglich haben wir die Musterseparation nach einer Schlaf- und Wachphase bei gesunden Menschen untersucht. Hier fanden wir eine besondere Relevanz des Tiefschlafes bei der Stabilisierung von separierten Informationen. Insgesamt stellen die drei Studien die Bedeutung der einzigartigen Struktur des Hippocampus für die Musterseparation und für die Beschreibung von Struktur-Funktions-Beziehungen in Gedächtnis und Konsolidierung heraus.

6.2 Einleitung

Unser Gedächtnis bildet die Grundlage unseres Denkens, Handelns und Fühlens. Durch die fortwährende Speicherung persönlicher Erlebnisse und den möglichen Zugriff auf diese Erinnerungen schafft das Gedächtnis unsere Identität (Tulving, 2005). Angesichts der hohen kognitiven Anforderungen des Alltags sollte das Gedächtnis so effizient wie möglich genutzt werden können. Hierfür stehen dem Gedächtnissystem zwei wesentliche kognitive Prozesse zur Verfügung: Ähnliche Inhalte können getrennt voneinander gespeichert werden, um Interferenzen zu reduzieren und die Speicherkapazität zu maximieren. Darüber hinaus sollten innerhalb eines stabilen Langzeitgedächtnisses vergangene Ereignisse anhand von Hinweisreizen der Umwelt leicht abrufbar sein und verknüpft werden können. Diese beiden essentiellen Prozesse werden als Musterseparation und Musterkomplettierung bezeichnet. (Marr, 1971; McClelland, McNaughton, & O'Reilly, 1995; Yassa & Stark, 2011).

Beide Funktionen sind abhängig vom Hippocampus, einer Hirnstruktur im medialen Temporallappen, die für die Enkodierung, Speicherung, Konsolidierung und den Abruf von episodischem und semantischem Gedächtnis essentiell ist (Frankland & Bontempi, 2005; Squire,

1992). Das episodische Gedächtnis umfasst Inhalte bezüglich persönlicher Ereignisse und deren Integration in einem räumlichen und zeitlichen Zusammenhang, während das semantische Gedächtnis reines Faktenwissen enthält (Tulving, 1972). Diese beiden Arten von Gedächtnisinhalten werden unter dem deklarativen Gedächtnis zusammengefasst und stehen dem nicht-deklarativen Gedächtnis gegenüber. Unter nicht-deklarativen Gedächtnisinhalten werden implizite Formen des Gedächtnisses zusammengefasst und beinhalten vor allem das prozedurale Gedächtnis, Priming und Konditionierung (Milner, Squire, & Kandel, 1998; Squire, 1986; Squire & Wixted, 2011).

Ein initiales Ereignis ruft die Enkodierung neuronaler Muster der Wahrnehmungsinformation im primären und assoziativen Kortex hervor. Innerhalb des Hippocampus werden diese multiplen Informationen in einer einzelnen Gedächtnisspur integriert (Frankland & Bontempi, 2005; Morris et al., 2003). Dennoch ist die Gedächtnisrepräsentation in diesem Stadium eher labil und anfällig für Störungen (Müller & Pilzecker, 1900). Um langanhaltende Erinnerungen zu schaffen, müssen die Repräsentationen einen Konsolidierungsprozess durchlaufen, in dem stabilere und dauerhafte Gedächtnisspuren gebildet werden (Dudai, Karni, & Born, 2015; Müller & Pilzecker, 1900).

Diese Konsolidierungsprozesse finden auf zwei Ebenen statt: auf der Systemebene und der synaptischen Ebene (Frankland & Bontempi, 2005). Dadurch, dass Schädigungen des Hippocampus meist mit dem Verlust von kürzer zurückliegenden Erinnerungen einhergehen, weiter entfernte Ereignisse jedoch erinnert werden können, wird ein Zweistufenmodell der Gedächtniskonsolidierung angenommen. Innerhalb der sogenannten Systemkonsolidierung löst sich die Abhängigkeit der Gedächtnisrepräsentation vom Kurzzeitspeicher des Hippocampus hin zum Langzeitspeicher in neokortikalen Regionen (McClelland et al., 1995). Diese Reorganisation ist im Wesentlichen auf eine wiederholte Reaktivierung der Gedächtnisspur zurückzuführen

(Dudai, 2004). Es wird angenommen, dass die Reorganisation innerhalb des Konsolidierungsprozesses während des Schlafes am effektivsten ist, da kognitive Anforderungen und Interferenzen durch Umweltreize reduziert sind (Marr, 1971; O'Reilly & McClelland, 1994). Diese Systemebene ergänzend, tragen Restrukturierungen der synaptischen Konnektivität zur Formierung des Langzeitgedächtnisses bei (Dudai, 2004; Dudai et al., 2015). Langzeitpotentierung und Langzeitdepression auf zellulärer Ebene regulieren die Stärkung und Schwächung synaptischer Verbindungen und bilden die Basis für synaptische Plastizität (Bliss & Collingridge, 1993; Dudek & Bear, 1992; Hebb, 1949). Diese Form der Reorganisation unterstützt die Konsolidierung der Gedächtnisspur auf der Systemebene (Dudai et al., 2015).

Dem Schlaf wird eine wichtige Rolle in der Konsolidierung von hippocampus-abhängigen Gedächtnisinhalten zugesprochen (Marr, 1971; O'Reilly & McClelland, 1994). Spezifische Feldpotenzialschwankungen charakterisieren den Schlaf in unterschiedlichen Stadien. Die Hauptschlafstadien beinhalten den Tiefschlaf (engl. Slow Wave Sleep, SWS), der durch langsame Oszillationen (~ 0,75 Hz) gekennzeichnet ist, sowie den REM-Schlaf (REM, engl. Rapid Eye Movement), für dessen Hirnaktivität vor allem hippocampale Theta-Aktivität (~4-8 Hz) charakteristisch ist (Rasch & Born, 2013). Bezüglich der Gedächtnisfunktion des Schlafes unterstützt der SWS Hippocampus-abhängige Gedächtnisinhalte, wobei REM-Schlaf nicht-deklarative Gedächtnisinhalte verarbeitet (Maquet, 2001).

Der Hauptmechanismus der schlafabhängigen Gedächtniskonsolidierung von deklarativen Inhalten basiert auf dem Transfer vom hippocampalen Kurzzeitspeicher zum neocorticalen Langzeitspeicher während des SWS (McClelland et al., 1995). Dieser Transfer basiert auf einer zeitlichen Kopplung von neokortikalen langsamen Oszillationen (~ 0,75 Hz), thalamo-kortikalen Spindeln (~ 10-15 Hz) und hippocampalen Sharp-Wave-Ripple-Oszillationen (~ 150 -250 Hz) (Buzsáki, 1996; Mölle & Born, 2011; Steriade, 2006). Innerhalb dieses sogenannten hippocam-

palen-neocorticalen Dialoges regulieren die langsamen Oszillationen die Vernetzung von Hippocampus, Thalamus und Neocortex (Buzsáki, 1996). Da die Sharp-Wave-Ripple-Aktivität mit wiederholter Reaktivierung der Gedächtnisrepräsentationen verbunden ist, trägt der hippocampale-neocorticale Dialog zur Konsolidierung der Gedächtnisspur auf der Systemebene bei (Sirota, Csicsvari, Buhl, & Buzsáki, 2003; Wilson & McNaughton, 1994), welche als aktive Systemkonsolidierung bezeichnet wird (Rasch & Born, 2013). Aus energetischen Gründen wird während des SWS auf der synaptischen Ebene, die Aktivität durch homöostatische Prozesse reduziert (Tononi & Cirelli, 2003, 2006). Hierbei zerfallen nur schwach enkodierte Gedächtnisrepräsentationen, während stark enkodierte Inhalte auch der Reduzierung der synaptischen Aktivität standhalten (Vyazovskiy, Cirelli, Pfister-Genskow, Faraguna, & Tononi, 2008).

Die bedeutende Rolle des Hippocampus in der Verarbeitung neuer Gedächtnisinformationen und in der Bildung stabiler autobiographischer Erinnerungen beruht auf der komplexen zellulärmolekularen Struktur des Hippocampus sowie der speziellen Vernetzung seiner Subfelder (Amaral & Lavenex, 2007). Der sogenannte trisynaptische Schaltkreis wird durch unidirektionale Projektionen vom entorhinalen Kortex zum Gyrus Dentatus über CA3 zu CA1 charakterisiert (Amaral & Witter, 1989). Theoretische Modelle nehmen an, dass der Gyrus Dentatus und die CA3-Region besonders an der Ausführung von Musterseparationsprozessen beteiligt sind (Marr, 1971; McClelland et al., 1995; Rolls, 2016). Es wird vermutet, dass der Gyrus Dentatus ähnlichen Umweltreizen unterschiedliche neuronale Codes zuweist, wodurch die Ähnlichkeit zwischen neuronalen Mustern reduziert und damit die Separation vereinfacht wird (McClelland et al., 1995; Yassa & Stark, 2011). Hinsichtlich der Musterkomplettierung zeigen etablierte Modelle auf, dass innerhalb der CA3-Region ein rekurrentes Netzwerk am Prozess beteiligt ist. Hier wird selbst bei unvollständiger Reaktivierung eines bereits enkodierten Musters der Abruf der gesamten Repräsentation ermöglicht (O'Reilly & McClelland, 1994; Treves & Rolls, 1994).

Die theoretischen Modelle hinsichtlich der subfeldspezifischen Unterstützung der Musterseparations- und komplettierungsfunktionen konnten anhand von Tierstudien in Nagetieren bewiesen werden (Guzowski, Knierim, & Moser, 2004; Lee, Yoganarasimha, Rao, & Knierim, 2004; J. K. Leutgeb, Leutgeb, Moser, & Moser, 2007; S. Leutgeb, Leutgeb, Treves, Moser, & Moser, 2004; Neunuebel & Knierim, 2014). Auch zeigen unterschiedliche Humanstudien Evidenz für die Validität der Modelle anhand von funktioneller Magnetresonanztomografie (Bakker, Kirwan, Miller, & Stark, 2008; Berron et al., 2016; Lacy, Yassa, Stark, Muftuler, & Stark, 2011; Yassa et al., 2010).

Auf funktioneller Bildgebung begründet können Studien in Bezug auf den Hippocampus des Menschen ausschließlich korrelative Zusammenhänge zwischen der Subfeldaktivierung und der Musterseparation auf der Verhaltensebene aufzeigen. Zudem sind Läsionsmodelle, die eine kausale Interpretation der Struktur-Funktions-Beziehung zulassen würden, beim Menschen eine Seltenheit. Um die Funktion der einzelnen Subfelder des Hippocampus innerhalb der Musterseparation aufzuzeigen, haben wir die Musterseparationsleistung bei Patienten mit Hippocampusschädigungen untersucht. Ziel war außerdem aufzuzeigen, welche Rolle der schlafabhängigen Gedächtniskonsolidierung bei Musterseparation zukommt.

6.3 Studie I

Das Verständnis über die Rolle der CA1-Neurone bezüglich des Prozesses der Musterseparation und -komplettierung ist bezüglich des menschlichen Hippocampus lückenhaft. Hinsichtlich des Gyrus Dentatus und der CA3-Region ist die spezifische Wirkung innerhalb dieser Prozesse sowohl durch Nagetierstudien als auch in Humanstudien bereits belegt werden können. Um den Beitrag von CA1-Neuronen bezüglich der Musterseparation im menschlichen Hippocampus zu untersuchen, haben wir 14 Patienten (66,86 ± 2,29 Jahre, Spannweite 53 – 80, 50% weiblich) innerhalb einer akuten transienten globalen Amnesie (TGA), einer seltenen, selbstlimitierenden

Dysfunktion des Hippocampus mit spezifischen CA1-Läsionen, getestet. Die Musterseparationsleistung der Patienten wurde in der akut-amnestischen Phase sowie nach vollständiger Genesung innerhalb der Nachuntersuchung mittels des Mnemonic Similarity Task (MST) erfasst. Dieses Paradigma überprüft anhand der Darbietung von alten, ähnlichen und neuen Stimuli Musterseparation auf der Verhaltensebene, welche jedoch die hippocampale Musterseparationsleistung anspricht und damit anhand von Antwortverhalten auf Aktivität und Funktion des Hippocampus rückgeschlossen werden kann (Stark, Yassa, Lacy, & Stark, 2013). Dies konnte bereits anhand einer Vielzahl von Studien, auch mit Unterstützung funktioneller Magnetresonanztomografie, belegt werden (Bakker et al., 2008; Lacy et al., 2011; Yassa et al., 2010). In der Akutphase zeigten die Patienten ein starkes Defizit in der Musterseparation bezüglich der ähnlichen Stimuli ($p < 0.05$) sowie der Rekognitionsleistung bei alten Stimuli ($p < 0.001$). Zum Zeitpunkt der Nachuntersuchung erholten sich beide Funktionen. Wir konnten zudem zeigen, dass sich die Musterseparations- und Rekognitionsleistungen unterschiedlich in zeitlicher Abhängigkeit von läsionsassoziierten Hippocampusdefiziten regenerierten. Die Musterseparationsfunktion erholte sich im Vergleich zur Rekognition früher. Dies zeigte sich anhand des Vergleichs der Leistungen von frühen und späten Akutstadien der TGA. Diese Ergebnisse sprechen für einen Kausalzusammenhang zwischen der Funktion hippocampaler CA1-Neurone und der Musterseparationsleistung beim Menschen. Das Läsionsmodell zeigt die Auswirkungen selektiver CA1-Läsionen auf die Funktion des Netzwerkes. Auch wenn keine Aussage über Prozesse innerhalb der CA1-Region oder den Beitrag weiter vorgelagerter Netzwerkfunktionen in CA3 und Gyrus Dentatus möglich ist, können wir dennoch annehmen, dass CA1 eine essentielle integrative Funktion in der Verarbeitung von Musterseparationsprozessen hat.

6.4 Studie II

Bei der leucinreichen, gliominaktivierten 1 (LGI1) Enzephalitis konnte in Tiermodellen sowie in Bildgebungsstudien beim Menschen gezeigt werden, dass der Gyrus Dentatus sowie CA3-Neurone im Hippocampus bevorzugt in der Pathophysiologie involviert sind. Um die Struktur-Funktions-Beziehung und den Beitrag der hippocampalen Subfelder innerhalb der Musterseparation beim Menschen zu spezifizieren, haben wir 15 Patienten (64,47 ± 3,28 Jahre, Spannweite: 36-77 Jahre, 9 männlich) mit dieser seltenen Form der Hippocampusschädigung mithilfe des MST untersucht. Die Patienten wurden 3,53 ± 0,65 Jahre nach der akuten Phase der Erkrankung getestet. Folgeschädigungen der Neuroinflammation innerhalb des Hippocampus wurden anhand einer Volumetrie der Subfelder für den Gyrus Dentatus, CA1 und die CA2/3 Region mithilfe eines automatisierten Segmentierungsalgorithmus (Freesurfer 6.0.0; (http://surfer.nmr.mgh.harvard.edu/) anhand der T1-gewichteten Aufnahmen der Magnetresonanztomografie bestimmt. Die Patienten zeigten ein globales Gedächtnisdefizit einschließlich einer signifikanten Reduktion der Musterseparationsleistung im Vergleich zu einer gesunden altersgematchten Kontrollgruppe ($p = 0,016$). Die Hippocampusvolumetrie belegte eine signifikante Volumenreduktion in allen untersuchten Subfeldern. Eine schrittweise Regression zeigte, dass das Volumen des Gyrus Dentatus die beste Vorhersagekraft in Bezug auf die Musterseparationsleistung habe ($p = 0,029$), während die Reduktion des CA1-Volumens die Defizite in der Rekognitionsleistung am besten erklärte ($p < 0,001$). Vor dem Hintergrund, dass das LGI1-Gen-Transkript hauptsächlich im Gyrus Dentatus und der CA3-Region zum Ausdruck kommt (Herranz-Pérez, Olucha-Bordonau, Morante-Redolat, & Pérez-Tur, 2010; Kalachikov et al., 2002) und ein Mangel an LGI1 Proteinen selektiv die synaptische Übertragung innerhalb des Hippocampus verringert (Fukata et al., 2010), wird das Hauptprinzip der Struktur-Funktionsbeziehung zwischen Subfeldern und Gedächtnisprozessen verdeutlicht. Die besondere Vorhersagekraft des Gyrus Dentatus Volumens für die Musterseparation stimmt mit ursprünglichen

Gedächtnismodellen des Hippocampus überein, derer zufolge der Gyrus Dentatus die Musterseparation unterstützt (Marr, 1971; McClelland et al., 1995). Auch werden anhand dieses Ergebnisses Erkenntnisse aus Bildgebungsstudien am Menschen ergänzt, die die Funktion des Gyrus Dentatus innerhalb der Musterseparation hervorheben (Bakker et al., 2008; Berron et al., 2016). Die Rekognitionsleistung zeigte sich am besten durch das Volumen der CA1-Region, sodass die Ergebnisse insgesamt für eine regionale Spezialisierung der Hippocampusfunktionen sprechen.

6.5 Studie III

Stabilisierungsprozesse innerhalb der Musterseparation und -komplettierung sind weitgehend unklar. Aufgrund der Tatsache, dass Schlaf eine essentielle Rolle in der aktiven Systemkonsolidierung von hippocampusabhängigen Gedächtnisinhalten spielt (Marr, 1971; O'Reilly & McClelland, 1994), haben wir die neurobiologischen Grundlagen der Musterseparation innerhalb der schlafabhängigen Gedächtniskonsolidierung untersucht. Hierzu haben wir die hippocampale Stimulusverarbeitung anhand des MST vor und nach Schlaf- und Wachphasen bei 13 jungen, gesunden Probanden (23,46 ± 0,5 Jahre; Spannweite: 21–26 Jahre; 10 weiblich) untersucht. Jeder Proband nahm in randomisierter Reihenfolge sowohl an der Wach- als auch an der Schlafbedingung teil. Die zweite Bedingung erfolgte mindestens in einem Abstand von drei Wochen zu der ersten Bedingung. In beiden Bedingungen wurden zunächst die Items des MST gelernt und in einem sofortigen Abrufphase getestet. Nach einen 9-stündigen Intervall von entweder Schlaf oder Wachzustand wurde in einem verzögerten Abruf noch einmal die Musterseparationsleistung in Bezug auf die gelernten Stimuli ermittelt. Wir konnten zeigen, dass sich die Musterseparationsleistung über die Wachphase verschlechterte, jedoch über die Schlafphase hinweg stabil geblieben ist ($p = 0{,}013$). Die Diskrepanz zwischen der Leistung nach der

Wach- und Schlafphase hat sich am stärksten für Items manifestiert, die einen hohen Unterschied zum zuvor gelernten Stimulus aufwiesen ($p = 0{,}006$). Stimuli mit sehr hoher Ähnlichkeit zum zuvor Gelernten, zeigten ein umgekehrtes Ergebnismuster mit reduzierter Musterseparationsleistung nach der Schlafphase ($p = 0{,}038$). Die aufgezeichnete Polysomnografie brachte einen Nachweis dafür, dass für schlafabhängige Gedächtniskonsolidierung typische EEG Oszillationen positiv mit der Musterseparationsleistung korrelierten. Die Korrelation zeigte sich für die Spindeldichte, die Dichte der langsamen Oszillationen, sowie für Theta-Aktivität, die zeitlich an die langsamen Oszillationen gebunden waren. Die Ergebnisse unterstützen die Annahme, dass die neuronale Reaktivierung während aktiver Systemkonsolidierungsprozesse im Zuge des Schlafens die Gedächtnisrepräsentation im Hippocampus stärkt, sodass abhängig von der Ähnlichkeit des Stimulus die Musterseparationsleistung stabilisiert wird.

6.6 Diskussion

Das Ziel dieser Arbeit war es, die Rolle des Hippocampus und dessen subfeldspezifischen Verarbeitungsprozesse bei der Funktion der Musterseparation sowie bei der schlafabhängigen Gedächtniskonsolidierung beim Menschen zu spezifizieren. Wir konnten zeigen, dass sowohl der Gyrus Dentatus als auch intakte CA1-Neuron für die Musterseparation beim Menschen essentiell sind. Die Musterseparationsleitung konnte am besten durch das Volumen des Gyrus Dentatus vorhergesagt werden, wohingegen die Rekognitionsleistung stärker mit dem CA1-Volumen korrelierte. Eine selektive Beeinträchtigung von CA1-Neuronen führte jedoch auch zu einem Defizit in der Musterseparation. Insgesamt präzisieren diese Ergebnisse das aktuelle Verständnis von hippocampusabhängiger Gedächtnisverarbeitung: Der Gyrus Dentatus zeigte sich im Einklang mit der aktuellen Humanliteratur als entscheidend in der Musterseparation, wohingegen die CA1-Region im Wesentlichen an der Übertragung von separierter Information in neokortikale Langzeitspeicher beteiligt ist. Außerdem wird aus Studie III die Bedeutung des

hippocampalen-neocorticalen Transfers bei der Stabilisierung von separierten Informationen deutlich. Die CA3-Region stellt eine gemeinsame Ebene von Musterseparation und -komplettierung sowie der Generierung von Sharp-Wave-Ripple Oszillationen dar. Da der Stabilisierungseffekt durch SWS vom Grad der Ähnlichkeit der Stimuli abhing, sowie charakteristische EEG Oszillationen, die für schlafabhängige Gedächtniskonsolidierung sprechen, mit der Musterseparation korreliert sind, nehmen wir an, dass Schlaf aktiv auf hippocampale Repräsentationen einwirken kann. Diese neuen Ergebnisse dienen als Basis für die Planung von zukünftiger Forschung, die sich auf einen direkten Zusammenhang zwischen schlafabhängiger Gedächtniskonsolidierung und Aktivität der hippocampalen Subfelder konzentrieren sollte. Zuletzt betonen die drei Studien dieser Arbeit die Signifikanz der hippocampalen Subfeldstruktur für Musterseparationsprozesse in der Beschreibung der Struktur-Funktionsbeziehung im Hinblick auf Gedächtnis und Konsolidierung.

7 References

Abbott, L. F., & Nelson, S. B. (2000). Synaptic plasticity: taming the beast. *Nature Neuroscience*, *3 Suppl*, 1178–1183. doi:10.1038/81453

Altman, J., & Das, G. D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *The Journal of Comparative Neurology*, *124*(3), 319–335.

Amaral, D. G., Dolorfo, C., & Alvarez-Royo, P. (1991). Organization of CA1 projections to the subiculum: a PHA-L analysis in the rat. *Hippocampus*, *1*(4), 415–435. doi:10.1002/hipo.450010410

Amaral, D. G., Ishizuka, N., & Claiborne, B. (1990). Neurons, numbers and the hippocampal network. *Progress in Brain Research*, *83*, 1–11.

Amaral, D. G., & Lavenex, P. (2007). Hippocampal Neuroanatomy. In P. Andersen, R. Morris, D. Amaral, T. Bliss, & J. O'Keefe (Eds.), *The Hippocampus Book* (pp. 37–110). New York: Oxford University Press.

Amaral, D. G., & Witter, M. P. (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience*, *31*, 571–91.

Appleby, P. A., Kempermann, G., & Wiskott, L. (2011). The role of additive neurogenesis and synaptic plasticity in a hippocampal memory model with grid-cell like input. *PLoS Computational Biology*, *7*(1), e1001063. doi:10.1371/journal.pcbi.1001063

Bakker, A., Kirwan, C. B., Miller, M., & Stark, C. E. (2008). Pattern separation in the human hippocampal CA3 and dentate gyrus. *Science*, *319*, 1640–2. doi:10.1126/science.1152882

Bartsch, T., Alfke, K., Deuschl, G., & Jansen, O. (2007). Evolution of hippocampal CA-1 diffusion lesions in transient global amnesia. *Annals of Neurology*, *62*(5), 475–480. doi:10.1002/ana.21189

Bartsch, T., Alfke, K., Stingele, R., Rohr, A., Freitag-Wolf, S., Jansen, O., & Deuschl, G. (2006). Selective affection of hippocampal CA-1 neurons in patients with transient global amnesia without long-term sequelae. *Brain*, *129*(11), 2874–2884. doi:10.1093/brain/awl248

Bartsch, T., Döhring, J., Reuter, S., Finke, C., Rohr, A., Brauer, H., … Jansen, O. (2015). Selective neuronal vulnerability of human hippocampal CA1 neurons: lesion evolution, temporal course, and pattern of hippocampal damage in diffusion-weighted MR imaging. *Journal of Cerebral Blood Flow and Metabolism*, *35*, 1836–45. doi:10.1038/jcbfm.2015.137

Bartsch, T., Schönfeld, R., Müller, F. J., Alfke, K., Leplow, B., Aldenhoff, J., … Koch, J. M. (2010). Focal lesions of human hippocampal CA1 neurons in transient global amnesia impair place memory. *Science*, *328*, 1412–5. doi:10.1126/science.1188160

Bartsch, T., & Wulff, P. (2015). The hippocampus in aging and disease: From plasticity to vulnerability. *Neuroscience*, *309*, 1–16. doi:10.1016/j.neuroscience.2015.07.084

Berron, D., Schütze, H., Maass, A., Cardenas-Blanco, A., Kuijf, H. J., Kumaran, D., & Düzel, E. (2016). Strong evidence for pattern separation in human dentate gyrus. *Journal of Neuroscience*, *36*, 7569–79. doi:10.1523/JNEUROSCI.0518-16.2016

Bettcher, B. M., Gelfand, J. M., Irani, S. R., Neuhaus, J., Forner, S., Hess, C. P., & Geschwind, M. D. (2014). More than memory impairment in voltage-gated potassium channel complex encephalopathy. *European Journal of Neurology*, *21*, 1301–10. doi:10.1111/ene.12482

Bi, G. Q., & Poo, M. M. (1998). Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *The Journal of Neuroscience*, *18*(24), 10464–10472.

Blackstad, T. W., Brink, K., Hem, J., & Jeune, B. (1970). Distribution of hippocampal mossy fibers in the rat. An experimental study with silver impregnation methods. *The Journal of Comparative Neurology*, *138*(4), 433–449. doi:10.1002/cne.901380404

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*, *361*(6407), 31–39. doi:10.1038/361031a0

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *The Journal of Physiology*, *232*, 331–56.

Borbély, A. A., & Achermann, P. (1999). Sleep homeostasis and models of sleep regulation. *Journal of Biological Rhythms*, *14*(6), 557–568.

Borbély, A. A., Daan, S., Wirz-Justice, A., & Deboer, T. (2016). The two-process model of sleep regulation: a reappraisal. *Journal of Sleep Research*, *25*(2), 131–143. doi:10.1111/jsr.12371

Born, J., & Wilhelm, I. (2012). System consolidation of memory during sleep. *Psychological Research*, *76*(2), 192–203. doi:10.1007/s00426-011-0335-6

Butler, C. R., Miller, T. D., Kaur, M. S., Baker, I. W., Boothroyd, G. D., Illman, N. A., … Buckley, C. J. (2014). Persistent anterograde amnesia following limbic encephalitis associated with antibodies to the voltage-gated potassium channel complex. *Journal of Neurology, Neurosurgery and Psychiatry*, *85*(4), 387–391. doi:10.1136/jnnp-2013-306724

Buzsáki, G. (1986). Hippocampal sharp waves: their origin and significance. *Brain Research*, *398*, 242–252.

Buzsáki, G. (1989). Two-stage model of memory trace formation: a role for "noisy" brain states. *Neuroscience*, *31*(3), 551–570.

Buzsáki, G. (1996). The hippocampo-neocortical dialogue. *Cereb Cortex*, *6*, 81–92.

Buzsáki, G. (1998). Memory consolidation during sleep: a neurophysiological perspective. *J Sleep Res*, *7 Suppl 1*, 17–23.

Buzsáki, G., Horváth, Z., Urioste, R., Hetke, J., & Wise, K. (1992). High-frequency network oscillation in the hippocampus. *Science*, *256*, 1025–7.

Clark, R. E., & Squire, L. R. (2013). Similarity in form and function of the hippocampus in rodents, monkeys, and humans. *Proceedings of the National Academy of Sciences of the United States of America*, *110 Suppl 2*, 10365–10370. doi:10.1073/pnas.1301225110

Clelland, C. D., Choi, M., Romberg, C., Clemenson, G. D., Fragniere, A., Tyers, P., … Bussey, T. J. (2009). A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science*, *325*, 210–3. doi:10.1126/science.1173215

Clemens, Z., Mölle, M., Eross, L., Barsi, P., Halász, P., & Born, J. (2007). Temporal coupling of parahippocampal ripples, sleep spindles and slow oscillations in humans. *Brain*, *130*, 2868–78. doi:10.1093/brain/awm146

Clemens, Z., Mölle, M., Eross, L., Jakus, R., Rásonyi, G., Halász, P., & Born, J. (2011). Fine-tuned coupling between human parahippocampal ripples and sleep spindles. *The European Journal of Neuroscience*, *33*(3), 511–520. doi:10.1111/j.1460-9568.2010.07505.x

Cohen, N. J., & Squire, L. R. (1980). Preserved learning and retention of pattern-analyzing skill in amnesia: dissociation of knowing how and knowing that. *Science*, *210*, 207–10.

Csicsvari, J., Hirase, H., Czurkó, A., Mamiya, A., & Buzsáki, G. (1999). Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving Rat. *The Journal of Neuroscience*, *19*(1), 274–287.

Csicsvari, J., Hirase, H., Mamiya, A., & Buzsáki, G. (2000). Ensemble patterns of hippocampal CA3-CA1 neurons during sharp wave-associated population events. *Neuron*, *28*, 585–94.

Das, T., Ivleva, E. I., Wagner, A. D., Stark, C. E., & Tamminga, C. A. (2014). Loss of pattern separation performance in schizophrenia suggests dentate gyrus dysfunction. *Schizophr Res*, *159*, 193–7. doi:10.1016/j.schres.2014.05.006

Dash, M. B., Douglas, C. L., Vyazovskiy, V.-V., Cirelli, C., & Tononi, G. (2009). Long-term homeostasis of extracellular glutamate in the rat cerebral cortex across sleep and waking states. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *29*(3), 620–629. doi:10.1523/JNEUROSCI.5486-08.2009

De Gennaro, L., & Ferrara, M. (2003). Sleep Spindles: an overview. *Sleep Medicine Reviews*, *7*, 423–440.

Deller, T., Adelmann, G., Nitsch, R., & Frotscher, M. (1996). The alvear pathway of the rat hippocampus. *Cell and Tissue Research*, *286*(3), 293–303.

Deng, W., Aimone, J. B., & Gage, F. H. (2010). New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nature Reviews Neuroscience*, *11*, 339–50. doi:10.1038/nrn2822

Deuker, L., Doeller, C. F., Fell, J., & Axmacher, N. (2014). Human neuroimaging studies on the hippocampal CA3 region - integrating evidence for pattern separation and completion. *Front Cell Neurosci*, *8*, 64. doi:10.3389/fncel.2014.00064

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nat Rev Neurosci*, *11*, 114–26. doi:10.1038/nrn2762

Dillon, S. E., Tsivos, D., Knight, M., McCann, B., Pennington, C., Shiel, A. I., … Coulthard, E. J. (2017). The impact of ageing reveals distinct roles for human dentate gyrus and CA3 in pattern separation and object recognition memory. *Scientific Reports*, *7*(1), 14069. doi:10.1038/s41598-017-13853-8

Doxey, C. R., & Kirwan, C. B. (2015). Structural and functional correlates of behavioral pattern separation in the hippocampus and medial temporal lobe. *Hippocampus*, *25*(4), 524–533. doi:10.1002/hipo.22389

Drosopoulos, S., Wagner, U., & Born, J. (2005). Sleep enhances explicit recollection in recognition memory. *Learning & Memory*, *12*, 44–51.

Dudai, Y. (2004). The neurobiology of consolidations, or, how stable is the engram? *Annu Rev Psychol*, *55*, 51–86. doi:10.1146/annurev.psych.55.090902.142050

Dudai, Y., Karni, A., & Born, J. (2015). The consolidation and transformation of memory. *Neuron*, *88*, 20–32.

Dudek, S. M., & Bear, M. F. (1992). Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(10), 4363–4367.

Ellenbogen, J. M., Hu, P. T., Payne, J. D., Titone, D., & Walker, M. P. (2007). Human relational memory requires time and sleep. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 7723–8. doi:10.1073/pnas.0700094104

Eriksson, P. S., Perfilieva, E., Björk-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., & Gage, F. H. (1998). Neurogenesis in the adult human hippocampus. *Nature Medicine*, *4*(11), 1313–1317. doi:10.1038/3305

Finke, C., Prüss, H., Heine, J., Reuter, S., Kopp, U. A., Wegner, F., … Bartsch, T. (2017). Evaluation of cognitive deficits and structural hippocampal damage in encephalitis with leucine-rich, glioma-inactivated 1 antibodies. *JAMA Neurology*, *74*(1), 50–59. doi:10.1001/jamaneurol.2016.4226

França, T. F. A., Bitencourt, A. M., Maximilla, N. R., Barros, D. M., & Monserrat, J. M. (2017). Hippocampal neurogenesis and pattern separation: A meta-analysis of behavioral data. *Hippocampus*, *27*(9), 937–950. doi:10.1002/hipo.22746

Frankland, P. W., & Bontempi, B. (2005). The organization of recent and remote memories. *Nat Rev Neurosci*, *6*, 119–30. doi:10.1038/nrn1607

Fukata, Y., Lovero, K. L., Iwanaga, T., Watanabe, A., Yokoi, N., Tabuchi, K., … Fukata, M. (2010). Disruption of LGI1-linked synaptic complex causes abnormal synaptic transmission and epilepsy. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(8), 3799–3804. doi:10.1073/pnas.0914537107

Girardeau, G., & Zugaro, M. (2011). Hippocampal ripples and memory consolidation. *Current Opinion in Neurobiology*, *21*, 452–9. doi:10.1016/j.conb.2011.02.005

Graf, P., & Schacter, D. L. (1985). Implicit and explicit memory for new associations in normal and amnesic subjects. *Journal of Experimental Psychology. Learning, Memory, and Cognition*, *11*(3), 501–518.

Groch, S., Zinke, K., Wilhelm, I., & Born, J. (2015). Dissociating the contributions of slow-wave sleep and rapid eye movement sleep to emotional item and source memory. *Neurobiol Learn Mem*, *122*, 122–30.

Guzowski, J. F., Knierim, J. J., & Moser, E. I. (2004). Ensemble dynamics of hippocampal regions CA3 and CA1. *Neuron*, *44*, 581–4. doi:10.1016/j.neuron.2004.11.003

Haider, B., Duque, A., Hasenstaub, A. R., & McCormick, D. A. (2006). Neocortical network activity in vivo is generated through a dynamic balance of excitation and inhibition. *The Journal of Neuroscience*, *26*(17), 4535–4545. doi:10.1523/JNEUROSCI.5297-05.2006

Hebb, D. O. (1949). *The organization of behavior; a neuropsychological theory*. Oxford, England: Wiley.

Herranz-Pérez, V., Olucha-Bordonau, F. E., Morante-Redolat, J. M., & Pérez-Tur, J. (2010). Regional distribution of the leucine-rich glioma inactivated (LGI) gene family transcripts in the adult mouse brain. *Brain Research*, *1307*, 177–194. doi:10.1016/j.brainres.2009.10.013

Hodges, J. R., & Warlow, C. P. (1990). Syndromes of transient amnesia: towards a classification. A study of 153 cases. *Journal of Neurology, Neurosurgery and Psychiatry*, *53*(10), 834–843.

Holden, H. M., Hoebel, C., Loftis, K., & Gilbert, P. E. (2012). Spatial pattern separation in cognitively normal young and older adults. *Hippocampus*, *22*(9), 1826–1832. doi:10.1002/hipo.22017

Huber, R., Ghilardi, M. F., Massimini, M., & Tononi, G. (2004). Local sleep and learning. *Nature*, *430*(6995), 78–81. doi:10.1038/nature02663

Hunsaker, M. R., & Kesner, R. P. (2013). The operation of pattern separation and pattern completion processes associated with different attributes or domains of memory. *Neuroscience and Biobehavioral Reviews*, *37*(1), 36–58. doi:10.1016/j.neubiorev.2012.09.014

Insausti, R., & Amaral, D. G. (2004). Hippocampal formation. In G. Paxinos (Ed.), *The human nervous system* (2nd ed., pp. 871–914). Amsterdam: Elsevier. doi:10.1016/B978-012547626-3/50024-7

Irani, S. R., Michell, A. W., Lang, B., Pettingill, P., Waters, P., Johnson, M. R., … Vincent, A. (2011). Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. *Annals of Neurology*, *69*(5), 892–900. doi:10.1002/ana.22307

Irani, S. R., Stagg, C. J., Schott, J. M., Rosenthal, C. R., Schneider, S. A., Pettingill, P., … Johnson, M. R. (2013). Faciobrachial dystonic seizures: the influence of immunotherapy on seizure control and prevention of cognitive impairment in a broadening phenotype. *Brain*, *136*, 3151–62. doi:10.1093/brain/awt212

Isomura, Y., Sirota, A., Ozen, S., Montgomery, S., Mizuseki, K., Henze, D. A., & Buzsáki, G. (2006). Integration and segregation of activity in entorhinal-hippocampal subregions by neocortical slow oscillations. *Neuron*, *52*, 871–82. doi:10.1016/j.neuron.2006.10.023

Kalachikov, S., Evgrafov, O., Ross, B., Winawer, M., Barker-Cummings, C., Boneschi, F. M., … Gilliam, T. C. (2002). Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. *Nature Genetics*, *30*(3), 335–341. doi:10.1038/ng832

Kesner, R. P., & Rolls, E. T. (2015). A computational theory of hippocampal function, and tests of the theory: new developments. *Neurosci Biobehav Rev*, *48*, 92–147. doi:10.1016/j.neubiorev.2014.11.009

Kheirbek, M. A., Klemenhagen, K. C., Sahay, A., & Hen, R. (2012). Neurogenesis and generalization: a new approach to stratify and treat anxiety disorders. *Nat Neurosci*, *15*, 1613–20. doi:10.1038/nn.3262

Kirwan, C. B., & Stark, C. E. L. (2007). Overcoming interference: an fMRI investigation of pattern separation in the medial temporal lobe. *Learning & Memory*, *14*, 625–33. doi:10.1101/lm.663507

Knierim, J. J., & Neunuebel, J. P. (2016). Tracking the flow of hippocampal computation: Pattern separation, pattern completion, and attractor dynamics. *Neurobiology of Learning and Memory*, *129*, 38–49. doi:10.1016/j.nlm.2015.10.008

Lacy, J. W., Yassa, M. A., Stark, S. M., Muftuler, L. T., & Stark, C. E. L. (2011). Distinct pattern separation related transfer functions in human CA3/dentate and CA1 revealed using high-resolution fMRI and variable mnemonic similarity. *Learning & Memory*, *18*, 15–8. doi:10.1101/lm.1971111

Lavenex, P., & Amaral, D. G. (2000). Hippocampal-neocortical interaction: a hierarchy of associativity. *Hippocampus*, *10*, 420–30.

Lee, I., Yoganarasimha, D., Rao, G., & Knierim, J. J. (2004). Comparison of population coherence of place cells in hippocampal subfields CA1 and CA3. *Nature*, *430*, 456–9. doi:10.1038/nature02739

Leutgeb, J. K., Leutgeb, S., Moser, M. B., & Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science*, *315*, 961–6. doi:10.1126/science.1135801

Leutgeb, S., Leutgeb, J. K., Treves, A., Moser, M. B., & Moser, E. I. (2004). Distinct ensemble codes in hippocampal areas CA3 and CA1. *Science*, *305*, 1295–8. doi:10.1126/science.1100265

Lewis, P. A., & Durrant, S. J. (2011). Overlapping memory replay during sleep builds cognitive schemata. *Trends in Cognitive Sciences*, *15*, 343–51. doi:10.1016/j.tics.2011.06.004

Lisman, J. E. (1999). Relating hippocampal circuitry to function: recall of memory sequences by reciprocal dentate-CA3 interactions. *Neuron*, *22*, 233–42.

Liu, K. Y., Gould, R. L., Coulson, M. C., Ward, E. V., & Howard, R. J. (2016). Tests of pattern separation and pattern completion in humans-A systematic review: Tests of Pattern Separation and Pattern Completion. *Hippocampus*, *26*(6), 705–717. doi:10.1002/hipo.22561

Lorente de Nó, R. (1934). Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. *Journal Für Psychologie Und Neurologie*, *46*, 113–177.

Malter, M. P., Frisch, C., Schoene-Bake, J. C., Helmstaedter, C., Wandinger, K. P., Stoecker, W., … Bien, C. G. (2014). Outcome of limbic encephalitis with VGKC-complex antibodies: relation to antigenic specificity. *Journal of Neurology*, *261*, 1695–705. doi:10.1007/s00415-014-7408-6

Manahan-Vaughan, D. (1997). Group 1 and 2 metabotropic glutamate receptors play differential roles in hippocampal long-term depression and long-term potentiation in freely moving rats. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *17*(9), 3303–3311.

Maquet, P. (2001). The role of sleep in learning and memory. *Science*, *294*, 1048–52.

Markram, H., Lübke, J., Frotscher, M., & Sakmann, B. (1997). Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science (New York, N.Y.)*, *275*(5297), 213–215.

Marr, D. (1971). Simple memory: a theory for archicortex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *262*, 23–81.

McClelland, J. L., & Goddard, N. H. (1996). Considerations arising from a complementary learning systems perspective on hippocampus and neocortex. *Hippocampus*, *6*, 654–65. doi:10.1002/(SICI)1098-1063(1996)6:6<654::AID-HIPO8>3.0.CO;2-G

McClelland, J. L., McNaughton, B. L., & O'Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychological Review*, *102*, 419–57.

McEwen, B. S. (1994). The plasticity of the hippocampus is the reason for its vulnerability. *Seminars in Neuroscience.*, *6.*, 239–246.

McHugh, T. J., Jones, M. W., Quinn, J. J., Balthasar, N., Coppari, R., Elmquist, J. K., … Tonegawa, S. (2007). Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science*, *317*, 94–9. doi:10.1126/science.1140263

Miller, T. D., Chong, T. T.-J., Aimola Davies, A. M., Ng, T. W. C., Johnson, M. R., Irani, S. R., … Rosenthal, C. R. (2017). Focal CA3 hippocampal subfield atrophy following LGI1 VGKC-complex antibody limbic encephalitis. *Brain*, *140*(5), 1212–1219. doi:10.1093/brain/awx070

Milner, B., Squire, L. R., & Kandel, E. R. (1998). Cognitive neuroscience and the study of memory. *Neuron*, *20*, 445–68.

Mölle, M., & Born, J. (2011). Slow oscillations orchestrating fast oscillations and memory consolidation. *Progress in Brain Research*, *193*, 93–110. doi:10.1016/B978-0-444-53839-0.00007-7

Mölle, M., Marshall, L., Gais, S., & Born, J. (2004). Learning increases human electroencephalographic coherence during subsequent slow sleep oscillations. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(38), 13963–13968. doi:10.1073/pnas.0402820101

Mölle, M., Yeshenko, O., Marshall, L., Sara, S. J., & Born, J. (2006). Hippocampal sharp wave-ripples linked to slow oscillations in rat slow-wave sleep. *Journal of Neurophysiology*, *96*, 62–70. doi:10.1152/jn.00014.2006

Morris, R. G. M., Moser, E. I., Riedel, G., Martin, S. J., Sandin, J., Day, M., & O'Carroll, C. (2003). Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *358*(1432), 773–786. doi:10.1098/rstb.2002.1264

Müller, G. E., & Pilzecker, A. (1900). Experimentelle Beiträge zur Lehre vom Gedächtnis (Experimental Contributions to the Science of Memory). *Z Psychol Ergänzungsband I*, *1*, 1–300.

Muller, R. U., & Kubie, J. L. (1987). The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. *The Journal of Neuroscience*, *7*(7), 1951–1968.

Neunuebel, J. P., & Knierim, J. J. (2014). CA3 retrieves coherent representations from degraded input: direct evidence for CA3 pattern completion and dentate gyrus pattern separation. *Neuron*, *81*, 416–27. doi:10.1016/j.neuron.2013.11.017

Norman, K. A., & O'Reilly, R. C. (2003). Modeling hippocampal and neocortical contributions to recognition memory: a complementary-learning-systems approach. *Psychological Review*, *110*, 611–646. doi:10.1037/0033-295X.110.4.611

O'Neill, J., Pleydell-Bouverie, B., Dupret, D., & Csicsvari, J. (2010). Play it again: reactivation of waking experience and memory. *Trends Neurosci*, *33*, 220–9. doi:10.1016/j.tins.2010.01.006

O'Reilly, R. C., & McClelland, J. L. (1994). Hippocampal conjunctive encoding, storage, and recall: avoiding a trade-off. *Hippocampus*, *4*(6), 661–682. doi:10.1002/hipo.450040605

Pavlides, C., & Winson, J. (1989). Influences of hippocampal place cell firing in the awake state on the activity of these cells during subsequent sleep episodes. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *9*(8), 2907–2918.

Peyrache, A., Battaglia, F. P., & Destexhe, A. (2011). Inhibition recruitment in prefrontal cortex during sleep spindles and gating of hippocampal inputs. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 17207–12. doi:10.1073/pnas.1103612108

Plihal, W., & Born, J. (1997). Effects of early and late nocturnal sleep on declarative and procedural memory. *Journal of Cognitive Neuroscience*, *9*, 534–47. doi:10.1162/jocn.1997.9.4.534

Plihal, W., & Born, J. (1999). Effects of early and late nocturnal sleep on priming and spatial memory. *Psychophysiology*, *36*, 571–82.

Rasch, B., & Born, J. (2013). About sleep's role in memory. *Physiol Rev*, *93*, 681–766. doi:10.1152/physrev.00032.2012.-Over

Redondo, R. L., & Morris, R. G. M. (2011). Making memories last: the synaptic tagging and capture hypothesis. *Nature Reviews. Neuroscience*, *12*(1), 17–30. doi:10.1038/nrn2963

Reif, A., Fritzen, S., Finger, M., Strobel, A., Lauer, M., Schmitt, A., & Lesch, K.-P. (2006). Neural stem cell proliferation is decreased in schizophrenia, but not in depression. *Molecular Psychiatry*, *11*(5), 514–522. doi:10.1038/sj.mp.4001791

Rolls, E. T. (2007). An attractor network in the hippocampus: theory and neurophysiology. *Learn Mem*, *14*, 714–31. doi:10.1101/lm.631207

Rolls, E. T. (2013). The mechanisms for pattern completion and pattern separation in the hippocampus. *Front Syst Neurosci*, *7*, 74. doi:10.3389/fnsys.2013.00074

Rolls, E. T. (2016). Pattern separation, completion, and categorisation in the hippocampus and neocortex. *Neurobiology of Learning and Memory*, *129*, 4–28. doi:10.1016/j.nlm.2015.07.008

Rosene, D. L., & Van Hoesen, G. W. (1977). Hippocampal efferents reach widespread areas of cerebral cortex and amygdala in the rhesus monkey. *Science (New York, N.Y.)*, *198*(4314), 315–317.

Rudoy, J. D., Voss, J. L., Westerberg, C. E., & Paller, K. A. (2009). Strengthening Individual Memories by Reactivating them During Sleep. *Science*, *326*(5956), 1079. doi:10.1126/science.1179013

Sadowski, J. H. L. P., Jones, M. W., & Mellor, J. R. (2016). Sharp-Wave Ripples Orchestrate the Induction of Synaptic Plasticity during Reactivation of Place Cell Firing Patterns in the Hippocampus. *Cell Reports*, *14*(8), 1916–1929. doi:10.1016/j.celrep.2016.01.061

Sahay, A., Scobie, K. N., Hill, A. S., O'Carroll, C. M., Kheirbek, M. A., Burghardt, N. S., … Hen, R. (2011). Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature*, *472*, 466–70. doi:10.1038/nature09817

Scoville, W. B., & Milner, B. (1957). Loss of Recent Memory After Bilateral Hippocampal Lesions. *Journal of Neurology, Neurosurgery & Psychiatry*, *20*(1), 11–21. doi:10.1136/jnnp.20.1.11

Shu, Y., Hasenstaub, A., & McCormick, D. A. (2003). Turning on and off recurrent balanced cortical activity. *Nature*, *423*(6937), 288–293. doi:10.1038/nature01616

Siapas, A. G., & Wilson, M. A. (1998). Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron*, *21*, 1123–1128.

Sirota, A., & Buzsáki, G. (2005). Interaction between neocortical and hippocampal networks via slow oscillations. *Thalamus Relat Syst*, *3*, 245–259. doi:10.1017/s1472928807000258

Sirota, A., Csicsvari, J., Buhl, D., & Buzsáki, G. (2003). Communication between neocortex and hippocampus during sleep in rodents. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 2065–9. doi:10.1073/pnas.0437938100

Small, S. A., Schobel, S. A., Buxton, R. B., Witter, M. P., & Barnes, C. A. (2011). A pathophysiological framework of hippocampal dysfunction in ageing and disease. *Nat Rev Neurosci*, *12*, 585–601. doi:10.1038/nrn3085

Sorrells, S. F., Paredes, M. F., Cebrian-Silla, A., Sandoval, K., Qi, D., Kelley, K. W., … Alvarez-Buylla, A. (2018). Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. *Nature*, *555*(7696), 377–381. doi:10.1038/nature25975

Squire, L. R. (1986). Mechanisms of memory. *Science (New York, N.Y.)*, *232*(4758), 1612–1619.

Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychological Review*, *99*(2), 195–231.

Squire, L. R., Knowlton, B., & Musen, G. (1993). The structure and organization of memory. *Annual Review of Psychology*, *44*, 453–495. doi:10.1146/annurev.ps.44.020193.002321

Squire, L. R., & Wixted, J. T. (2011). The cognitive neuroscience of human memory since H.M. *Annual Review of Neuroscience*, *34*, 259–288. doi:10.1146/annurev-neuro-061010-113720

Squire, L. R., & Zola, S. M. (1996a). Ischemic brain damage and memory impairment: a commentary. *Hippocampus*, *6*, 546–52.

Squire, L. R., & Zola, S. M. (1996b). Structure and function of declarative and nondeclarative memory systems. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(24), 13515–13522.

Squire, L. R., & Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science*, *253*(5026), 1380–1386.

Staresina, B. P., Bergmann, T. O., Bonnefond, M., van der Meij, R., Jensen, O., Deuker, L., … Fell, J. (2015). Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nature Neuroscience*, *18*, 1679–86. doi:10.1038/nn.4119

Stark, S. M., & Stark, C. E. L. (2017). Age-related deficits in the mnemonic similarity task for objects and scenes. *Behavioral Brain Research*, *333*, 109–117. doi:10.1016/j.bbr.2017.06.049

Stark, S. M., Yassa, M. A., Lacy, J. W., & Stark, C. E. L. (2013). A task to assess behavioral pattern separation (BPS) in humans: Data from healthy aging and mild cognitive impairment. *Neuropsychologia*, *51*, 2442–9. doi:10.1016/j.neuropsychologia.2012.12.014

Steriade, M. (2006). Grouping of brain rhythms in corticothalamic systems. *Neuroscience*, *137*, 1087–106. doi:10.1016/j.neuroscience.2005.10.029

Steriade, M., Timofeev, I., & Grenier, F. (2001). Natural waking and sleep states: a view from inside neocortical neurons. *Journal of Neurophysiology*, *85*(5), 1969–1985. doi:10.1152/jn.2001.85.5.1969

Steward, O., & Scoville, S. A. (1976). Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. *The Journal of Comparative Neurology*, *169*(3), 347–370. doi:10.1002/cne.901690306

Stickgold, R. (2005). Sleep-dependent memory consoliodation. *Nature*, *437*, 1272–1278.

Swanson, L. W., & Cowan, W. M. (1977). An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. *The Journal of Comparative Neurology*, *172*(1), 49–84. doi:10.1002/cne.901720104

Swanson, L. W., Wyss, J. M., & Cowan, W. M. (1978). An autoradiographic study of the organization of intrahippocampal association pathways in the rat. *The Journal of Comparative Neurology*, *181*(4), 681–715. doi:10.1002/cne.901810402

Tamamaki, N., & Nojyo, Y. (1995). Preservation of topography in the connections between the subiculum, field CA1, and the entorhinal cortex in rats. *The Journal of Comparative Neurology*, *353*(3), 379–390. doi:10.1002/cne.903530306

Tamminen, J., Payne, J. D., Stickgold, R., Wamsley, E. J., & Gaskell, M. G. (2010). Sleep spindle activity is associated with the integration of new memories and existing knowledge. *J Neurosci*, *30*, 14356–60. doi:10.1523/JNEUROSCI.3028-10.2010

Tamminga, C. A., Stan, A. D., & Wagner, A. D. (2010). The hippocampal formation in schizophrenia. *Am J Psychiatry*, *167*, 1178–93. doi:10.1176/appi.ajp.2010.09081187

Tononi, G., & Cirelli, C. (2003). Sleep and synaptic homeostasis: a hypothesis. *Brain Research Bulletin*, *62*(2), 143–150.

Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep Medicine Reviews*, *10*(1), 49–62. doi:10.1016/j.smrv.2005.05.002

Treves, A., & Rolls, E. T. (1992). Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. *Hippocampus*, *2*(2), 189–199. doi:10.1002/hipo.450020209

Treves, A., & Rolls, E. T. (1994). Computational analysis of the role of the hippocampus in memory. *Hippocampus*, *4*, 374–91. doi:10.1002/hipo.450040319

Tulving, E. (1972). Episodic and semantic memory. In E. Tulving & W. Donaldson (Eds.), *Organization of memory* (pp. xiii, 423–xiii, 423). Oxford, England: Academic Press.

Tulving, E. (1991). Concepts in human memory. In L. R. Squire, N. M. Weinberger, G. Lynch, & J. L. McGaugh (Eds.), *Memory: Organization and Locus of Change* (pp. 3–32). New York: Oxford University Press.

Tulving, E. (2005). Episodic Memory and Autonoesis: Uniquely Human? In H. S. Terrace & J. Metcalfe (Eds.), *The Missing Link in Cognition: Origins of self-reflective consciousness* (pp. 3–56). New York, NY, US: Oxford University Press.

Vazdarjanova, A., & Guzowski, J. F. (2004). Differences in hippocampal neuronal population responses to modifications of an environmental context: evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles. *Journal of Neuroscience*, *24*, 6489–96. doi:10.1523/jneurosci.0350-04.2004

Vieweg, P., Riemer, M., Berron, D., & Wolbers, T. (2018). Memory Image Completion: Establishing a task to behaviorally assess pattern completion in humans. *Hippocampus*. doi:10.1002/hipo.23030

Vyazovskiy, V. V., Cirelli, C., Pfister-Genskow, M., Faraguna, U.-, & Tononi, G. (2008). Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nature Neuroscience*, *11*(2), 200–208. doi:10.1038/nn2035

Wagner, U., Gais, S., Haider, H., Verleger, R., & Born, J. (2004). Sleep inspires insight. *Nature*, *427*, 352–5.

Weiss, A. P., Zalesak, M., DeWitt, I., Goff, D., Kunkel, L., & Heckers, S. (2004). Impaired hippocampal function during the detection of novel words in schizophrenia. *Biological Psychiatry*, *55*(7), 668–675. doi:10.1016/j.biopsych.2004.01.004

Wills, T. J., Lever, C., Cacucci, F., Burgess, N., & O'Keefe, J. (2005). Attractor dynamics in the hippocampal representation of the local environment. *Science (New York, N.Y.)*, *308*(5723), 873–876. doi:10.1126/science.1108905

Wilson, M. A., & McNaughton, B. L. (1994). Reactivation of hippocampal ensemble memories during sleep. *Science*, *265*, 676–9.

Wiskott, L., Rasch, M. J., & Kempermann, G. (2006). A functional hypothesis for adult hippocampal neurogenesis: avoidance of catastrophic interference in the dentate gyrus. *Hippocampus*, *16*(3), 329–343. doi:10.1002/hipo.20167

Witter, M. P. (1993). Organization of the entorhinal-hippocampal system: a review of current anatomical data. *Hippocampus*, *3 Spec No*, 33–44.

Witter, M. P. (2007). Intrinsic and extrinsic wiring of CA3: Indications for connectional heterogeneity. *Learning & Memory*, *14*(11), 705–713. doi:10.1101/lm.725207

Witter, M. P., & Amaral, D. G. (1991). Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. *The Journal of Comparative Neurology*, *307*(3), 437–459. doi:10.1002/cne.903070308

Yassa, M. A., Lacy, J. W., Stark, S. M., Albert, M. S., Gallagher, M., & Stark, C. E. L. (2011). Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults. *Hippocampus*, *21*, 968–79. doi:10.1002/hipo.20808

Yassa, M. A., Mattfeld, A. T., Stark, S. M., & Stark, C. E. L. (2011). Age-related memory deficits linked to circuit-specific disruptions in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 8873–8. doi:10.1073/pnas.1101567108

Yassa, M. A., & Stark, C. E. L. (2011). Pattern separation in the hippocampus. *Trends in Neurosciences*, *34*(10), 515–525. doi:10.1016/j.tins.2011.06.006

Yassa, M. A., Stark, S. M., Bakker, A., Albert, M. S., Gallagher, M., & Stark, C. E. L. (2010). High-resolution structural and functional MRI of hippocampal CA3 and dentate gyrus

in patients with amnestic Mild Cognitive Impairment. *NeuroImage*, *51*, 1242–52. doi:10.1016/j.neuroimage.2010.03.040

Zhao, C., Deng, W., & Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell*, *132*(4), 645–660. doi:10.1016/j.cell.2008.01.033

Zola-Morgan, S., Squire, L. R., & Amaral, D. G. (1986). Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. *The Journal of Neuroscience*, *6*, 2950–67.

Acknowledgements

First and foremost, I would like to express my special appreciation and thanks to my supervisor Professor Dr. Thorsten Bartsch for all his support and trust in my work. Beside taking time for all my questions and concerns, I am very thankful for providing me the opportunities needed for making progress in my academic career.

I am also pleased to say thank you to my supervisor Professor Dr. Anya Pedersen for the excellent professional support and making my doctoral studies at the philosophical faculty possible in the first place.

I would also like to express a special thanks to Professor Dr. Daniela Berg and Professor Dr. Günther Deuschl for providing the best working conditions to carry out my research in the Department of Neurology.

I wish to thank Prof. Dr. Jan Born and the members of the SFB 654 Sleep and Plasticity initiative for showing me to the interesting field of sleep research, especially Frederik D. Weber who kindly introduced me to sleep data analysis.

I also thank Professor Dr. Martin Ziegler, Professor Dr. Christian Kaernbach, Professor Dr. Hermann Kohlstedt, Nick Diederich, and all members of the FOR 2093 research group for providing new insights and perspectives on memory research.

Special thanks go to my colleagues from the ‘Memory Disorders and Plasticity Group’, Dr. Juliane Döhring, Julius Rave, Sarah Schulze, and Isabel Schneider. I would also like to thank my colleagues from the ‘Imaging Lab’ Oliver Granert, Dr. Inken Rothkirch, Elisa Pawlitzki, Adrian Lehrke, and Alexander Baumann for discussions, interesting conversations and an always nice working atmosphere.

I would also like to thank all the patients and participants who patiently performed all tests within the study protocols. My research would have been impossible without them.

My heartful thanks at this point to my wonderful family and lovely friends for their emotional support and all my gratitude to Martin and his endless helpfulness. You are the best.

Curriculum Vitae

Annika Katrin Hanert

Schulische und akademische Ausbildung

10/2015 – 06/2017	**Promotion** Philosophische Fakultät der Christian-Albrechts-Universität zu Kiel
10/2012 – 09/2014	**Master of Science Psychologie** Universität Hamburg
10/2009 – 09/2012	**Bachelor of Science Psychologie** Universität Hamburg
07/2009	**Abitur** Dahlmannschule, Gymnasium der Stadt Bad Segeberg

Berufserfahrung

07/2015 -	**Wissenschaftliche Mitarbeiterin** Klinik für Neurologie Universitätsklinikum Schleswig-Holstein Campus Kiel