Functional segregation of hippocampal subdivisions in learning and memory

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Preface

Out of day-to-day experiences, we are forming memories of places, events and people, which bear meaning to us. Remembering recent and long past events shapes how we perceive our environment and how we behave in similar or new situations. From our experiences, which comprise what happened and where and when, we extract information that becomes general knowledge to us, facts we know about places and people. We can set them in relation to each other and form associations between them. One of the core structures involved in these declarative memory processes is the hippocampus, which is well known for its function in spatial navigation and episodic memories. It also plays a role in semantic memories, which refers to the formation of general knowledge about facts. The hippocampus has been the focus of extensive research on memory processing, but still its precise role in learning and memory remains controversial. It is a long C-shaped structure, which shows strikingly different features along its longitudinal axis form dorsal to ventral hippocampus. Distinct functionalities have been assigned to subdivisions along this hippocampal axis, which will be described in detail in the following sections.

In this thesis, I explore whether hippocampal subdivisions exhibit different but complementary functions in declarative memories. I use chemogenetic silencing to locally interfere with memory processes in dorsal and ventral hippocampus, respectively, in order to study their contributions in learning and memory in various paradigms. First, I compare the functions of dorsal and ventral hippocampal subdivisions in single-trial learning. Then, I am addressing their roles in the formation of associations to previously acquired memories. Moreover, applying chemogenetic silencing and powerful recently developed techniques to genetically target learning-related neuronal populations, I study the localization of single-trial and association memories within the hippocampus, thereby gaining new insights into hippocampal memory processing. I will show how the different hippocampal subdivisions

encode distinct memory components of the same task. Thus, they provide a mechanism to recall previously acquired memories and to form associations to them without interference of memories, but instead with the possibility to independently use the distinct memory components. In a supplementary part, I have started to investigate the function of the transversal hippocampal axis, in particular the dentate gyrus, in association learning. This study allows a first insight into a possible mechanism that might shape memory assemblies to form associations.

1. Introduction

The hippocampus

The hippocampus is a C-shaped structure situated bilaterally within the medial temporal lobe. It is a highly conserved brain area across all mammals and has been implicated in a wide range of memory formation, storage and retrieval processes. Navigating in space and learning from experiences, thereby forming memories of events and facts are major functions of the hippocampus. I will describe in detail the hippocampal anatomy and function as well as principles of memory formation and retrieval.

1.1. Anatomy of the hippocampus

1.1.1. Local hippocampus circuit along the transversal axis

Already the first drawings by Golgi in 1886 revealed the beautiful characteristic composition of the hippocampus proper, which comprises the dentate gyrus (DG), the cornu ammonis (CA) regions CA1, CA2, CA3 and CA4, and the subiculum. The information flow through the hippocampus is mainly unidirectional, whereby each station serves a specific function to process the information (Basu & Siegelbaum, 2015; Amaral & Witter, 1989). It receives highly processed sensory input from entorhinal cortex (EC) layer II

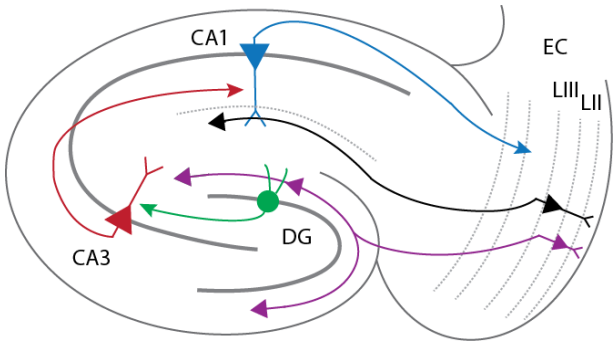

Figure 1.1. Hippocampus anatomy along the transversal axis. Entorhinal cortex projects to hippocampus, where information flows unidirectional through the trisynaptic pathway, from DG to CA3 to CA1 and then back to the entorhinal cortex, thereby forming a closed loop. Each subpart of the hippocampus receives direct EC input (Modified from Basu & Siegelbaum, 2015).

via the perforant path, which innervates the granule cells in the DG (Fig.1.1). The granule cells send their mossy fiber axons to CA3, where they target the pyramidal cells of CA3 with large mossy fiber terminals. The CA3 pyramidal cells form a highly interconnected autoassociative network through its recurrent collaterals and send projecting axons, the Schaffer collaterals, to CA1 pyramidal cells. This circuit is the classical trisynaptic pathway, the best-described information path through the hippocampus. CA1 pyramidal cells then connect to subiculum and EC layer V, thereby completing the EC-hippocampus-EC loop. Beside the trisynaptic pathway, the EC also directly accesses CA3 (from EC layer II via perforant path) and CA1 (from EC layer III via temporoammonic path), all of which have different roles in information processing, as will be explained later (section 1.2.4).

1.1.2. Hippocampal connectivity along the dorsoventral axis

Specialized connectivity and function along the longitudinal axis of the hippocampus has been the focus of much research. Mainly three subdivisions, the dorsal, intermediate and ventral hippocampus, are distinguished. Notably, there are no demarcated anatomical boundaries, but rather smooth transitions between subdivisions. Classification is based on functional characteristics, differential connectivity as well as other features, which lead to the hypothesis that even further smaller subdivisions may exist (see section 1.2.3; Strange, 2014; Risold & Swanson, 1996; Thompson, 2008) Despite the regular circuitry along its transverse axis, the longitudinal axis from dorsal to ventral hippocampus exhibits major differences in connectivity (Fig.1.2) (Amaral & Witter, 1989). Overall, hippocampal connectivity to cortical areas is topographically organized (Dolorfo & Amaral, 1998; van Strien, 2009). The main cortical input arrives from entorhinal cortex, with dorso-lateral to ventro-medial entorhinal cortex projecting in a gradient from dorsal to ventral hippocampus. Furthermore, inputs from medial and lateral entorhinal cortex arrive in different strata on principle cell dendrites of the hippocampus. The hippocampus-EC connectivity is reciprocal hence hippocampal innervation of EC follows the same principle. Other cortical areas are also

differentially connected to hippocampus, for example dorsal hippocampus primarily connects to retrosplenial cortex (RSC), a cognitive part of cingulate cortex (Jones & Witter, 2007). By contrast, ventral hippocampus retrieves (via EC and nucleus reuniens) and sends input to prelimbic and infralimbic cortices (Jay & Witter, 1991; Ferino, 1987; Thierry, 2000; Strange, 2014). The topography principle extends to subcortical structures as well. For example the septum - functioning as relay station for hippocampus output to hypothalamic nuclei – is innervated by dorsal hippocampus in its dorsal parts and gradually more ventral parts of hippocampus project to more ventral parts of septum (Risold & Swanson, 1996 & 1997). This topography is maintained by further projections from septum to hypothalamus, resulting in matching fornix connections of ventral hippocampus to anterior hypothalamic nuclei – medial preoptic nucleus and periventricular zone (endocrine nuclei of the hypothalamus) – and dorsal hippocampus to posterior hypothalamic nuclei such as the mammillary body, which is involved in memory processing (Strange, 2014; Canteras & Swanson, 1992). Furthermore, the nucleus accumbens is gradually innervated by dorsal and ventral hippocampus

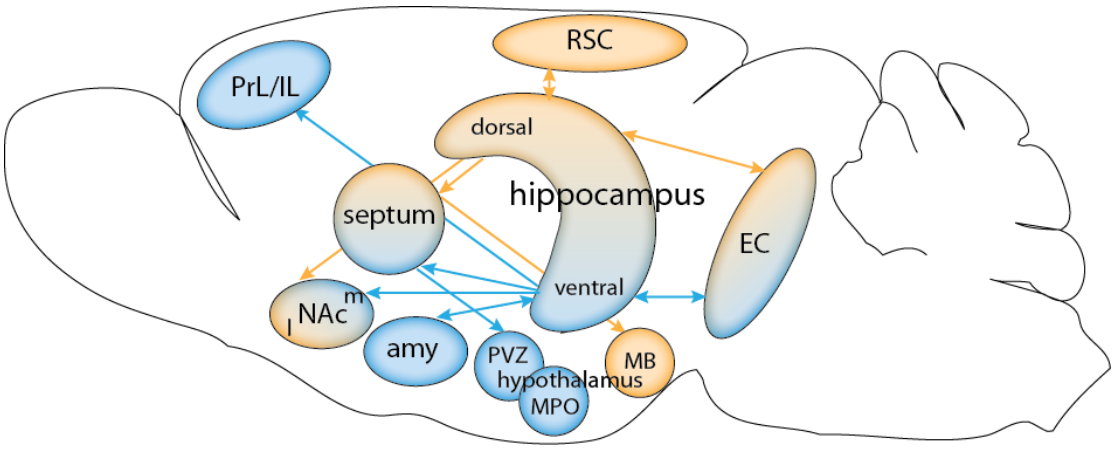

Figure 1.2. Differential connectivity of the hippocampus dorsoventral axis with cortical and subcortical areas. Connectivity to entorhinal cortex, septal nuclei and nucleus accumbens are topographically organized along the dorsoventral axis. Several cortical and subcortical areas specifically connect with either dorsal or ventral subdivisions. Dorsal connections are depicted in orange, ventral connections in blue. (Based on Strange, 2014; Tannenholz & Kheirbek, 2014; Fanselow & Dong, 2010).

projecting to lateral and medial accumbens regions, respectively (Groenewegen, 1987). Interestingly, only the ventral hippocampus directly connects with amygdala (Pikkarainen, 1999; Pitkänen, 2000; Kishi, 2006). Importantly, in view of the different connectivity along its longitudinal axis with cortical and subcortical brain areas, it has been proposed that hippocampal subdivisions might have distinct functional roles (see section 1.2.3).

1.2. Hippocampus function

Earlier studies have shown that highly processed sensory information arriving from entorhinal cortex passes through the trisynaptic hippocampal loop and is sent back to the entorhinal cortex. What does this closed loop through the hippocampus contribute to processing of information? Since the famous case of patient H.M., who lost his ability to retrieve recent memories and to form new ones after the surgical removal of the hippocampus and adjacent medial temporal lobe structures owing to his seizures (Scoville & Milner, 1957), the hippocampus has received tremendous attention in memory research. It has been implicated in various fundamental processes of memory formation, storage and retrieval (Squire, 2004).

1.2.1. Hippocampus in spatial memories

The hippocampus has been intensively studied for its role in spatial memory and navigation. The investigations started when O'Keefe and Dostrovski (1971) found cells in the hippocampus, which fire whenever the animal is in a certain location in the environment. The discovery of these place cells, together with hippocampal lesion studies revealing deficits in spatial learning, led to the conclusion that hippocampus serves to create a cognitive map of the environment (O'Keefe & Nadel, 1978). It has been shown that the entire hippocampus, including all subfields along the transversal axis contain place cells. Interestingly, place field properties differ along the dorsoventral axis. Dorsal place cells are tuned to small place fields (around 1m), whereas

ventral cells cover large place fields (up to 10m), leading to a graded representation with different resolution of space along the hippocampal axis (Kjeltrup, 2008). Furthermore, place cells function together with entorhinal grid cells, head direction cells and border cells to ensure navigation in space (Hafting, 2005; Sargolini, 2006; Solstad, 2008; Taube, 1990; McNaughton, 2006; Moser, 2008a/b). A prevailing exciting idea is that spatial navigation might be the evolutionary basis for memory formation. Learning to navigate implies remembering past locations and related events, which could underlie the mechanism for elaborated memory processes (Buzsaki & Moser, 2013).

1.2.2. Hippocampus in declarative memories

The hippocampus is involved in processing declarative memories, which can be subdivided into episodic and semantic memories (Tulving, 1972; Burgess, 2002). Episodic memories are defined as long-term memories for events or episodes that can be consciously recalled. They are perceived as our personal experiences. Based on episodes, associative memories are formed. In this type of memories, relationships between items and concepts are learned and remembered (Suzuki, 2008). Hence the formation of associative memories requires linking (related) elements, such as the context in which they are encoded.

In addition, the hippocampus is also implicated in the formation of semantic memories, which consist of facts accessible to conscious recall (Schacter, 1999; Davachi, 2006; Chua, 2007). Notably, those facts are not specifically related to personal experiences, but rather comprise information extracted from experience (O'Reilly & Rudy, 2001). They could have possibly evolved by combination and/or categorization of different episodic memories, which can be recalled from partial input cues (Eichenbaum, 1999 and 2004; O'Reilly and Rudy, 2001; Buzsaki & Moser, 2013).

All types of declarative memories share the element of linking information – setting them in relation to each other or binding them into time, context or

concept. Is it the core function of hippocampus to create and recall links to give “sense” to the highly processed sensory information arriving from cortex? Recalling memories can be divided into two processes, recollection and familiarity detection (Suzuki, 2014). As an example, cortical regions upstream of hippocampus, such as the perirhinal cortex (directly projecting to hippocampus or indirectly via entorhinal cortex) have been shown to preferentially detect familiarity in form of altered activity patterns at repeated stimuli presentations (Brown & Aggleton, 2001; Brown, 2010), without detecting the context in which the stimulus occurred. In addition, the hippocampus is thought to process recollection of contextual details of events and episodes, thereby setting stimuli into relation to other memories (contexts, events). However, how different structures contribute to familiarity and recollection is still under debate (Suzuki, 2014).

1.2.3. Distinct functions along the dorsoventral hippocampal axis: Current view

A widely accepted view is that dorsal hippocampus – being connected to retrosplenial cortex and theta-rhythm generating mammillary bodies - is required for cognitive and spatial memory functions, while ventral hippocampus with its connection to limbic areas of prefrontal cortex, amygdala and endocrine nuclei of hypothalamus is involved in emotional learning and stress responses (Fanselow & Dong, 2010; Strange, 2014; Bannerman, 2003 and 2004; Gray and McNaughton, 2000; Trivedi & Coover, 2004). This view has been further supported by lesions studies, specifically showing a ventral but not dorsal involvement in unconditioned fear behavior (Kjelstrup, 2002; Bannerman, 2002). Also theta rhythm coherence, an indicator of functional connectivity, is strong within but less pronounced across hippocampal subdivisions (Strange, 2014). However, it needs to be kept in mind that there are no demarcated boundaries between subdivisions and a distinction into three main parts (dorsal, intermediate and ventral) along the longitudinal axis is rather a useful simplification for experimental accessibility to the system. Several characteristics, such as gene expression

profiles, topographically graded connectivity to cortical areas (entorhinal cortex, nucleus accumbens) and place cell properties (size of cells and place fields) have proposed multiple smaller subdivisions or even gradual functionality along the axis (Strange, 2014; Kjelstrup, 2008; Thompson, 2008). Notably, place cells have been detected in the entire hippocampus, with gradually increasing place fields along the axis from dorsal to ventral. Thus leading to a different view of hippocampal function, which assigns a general role of the entire hippocampus in spatial navigation and learning as well as cognitive processes. Within these processes the hippocampal subdivisions might contribute different computations (e.g. different scale). In this regard, the ventral hippocampus was proposed to function in large scale spatial processing, leading to generalization across different context (Komorowski, 2013) and potentially forming higher-order connections. This hypothesis (still

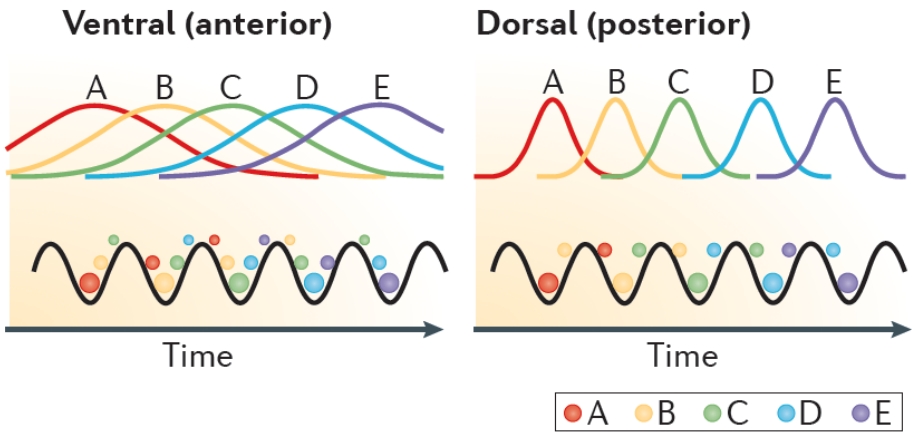

Figure 1.3. Forming episodic sequences and higher-order connections in the hippocampus. Interleaved firing of neuronal assemblies in ventral and dorsal hippocampus. Colored lines depict place fields, which could represent locations or items (A-E) that are broader tuned in ventral compared to dorsal hippocampus. Below, size of circles represents spiking activity of each cell assembly, representing any given location or item in the sequence with peak firing activity at trough of theta. Note that firing of each assembly extends into previous and following theta cycle by weaker but repeated firing. Thereby, cell assemblies are imbedded into sequences, representing subsequent locations or items within each theta cycle. Due to larger place fields in ventral hippocampus, longer sequences are incorporated, which could form connections between non-consecutive locations or items, hence create higher-order-connections (Strange, 2014; based on Buzsaki & Moser, 2013; Buzsaki, 2010).

not proven) follows the notion that large place field firing in ventral hippocampus could span firing of many dorsal sequences of episodes (within theta synchrony), thereby linking them into semantic memories (Fig.1.3) (Bunsey, 1996; Buzsaki & Moser, 2013; Buzsaki, 2010). This is supported by human fMRI studies indicating a ventral function in semantic memories by testing associative memory retrieval (Chua, 2007). Similarly, also attributing a cognitive function to the entire hippocampus, it was proposed that dorsal hippocampus processes detailed information, whereas ventral hippocampus forms rather “gist-like” memories (Poppenk, 2013). Further evidence stems from again mainly human fMRI studies finding vH specifically responding to task novelty (Strange, 1999; Duzel, 2003; Daselaar, 2006). In contrast, dorsal hippocampus responded to detection of familiar events after long-term training. This could, on the one hand, be interpreted as encoding and retrieval processes, but this appears to be problematic since encoding and retrieval are unlikely to occur in distinct areas of the brain. On the other hand, they could be again seen as formation and retrieval of “gist-like” and detailed information in ventral and dorsal hippocampus, respectively (Strange, 2014). Whether these seemingly partially opposing proposals on dorsal and ventral functions might converge into a general concept still needs to be investigated.

1.2.4 Transversal axis: Dentate gyrus, CA3, CA1 function

Along the transversal axis, distinct functional roles have been assigned to the subparts of the hippocampus. It is comprised of dentate gyrus and the cornu ammonis (CA) regions CA1, CA2 and CA3, each of which can be directly accessed by entorinal cortex inputs. In the most studied trisynaptic pathway through DG, CA3 and CA1 each subpart is thought to contribute specific computations to memory processing.

Dentate gyrus function

The dentate gyrus calculates the transformation of a dense cortical signal into a sparse hippocampal code. Thus, it is proposed to function as pattern separator (Acsady & Kali, 2007). It seems critical to encode memory

representations, which are similar but not identical, helping to reduce interference between similar memories (Treves and Rolls, 1994; Leutgeb, 2007; Treves et al., 2008). This notion is supported by several unique anatomical features of the dentate gyrus. First, the large cell number in the DG compared to input and output areas (EC:DG:CA3 = 1:5:1 approximately). Entorhinal inputs arrive on a multitude of relatively weak synapses on granule cells, the principle cells of the dentate gyrus. The local microcircuit is dominated by inhibition, resulting in very low background activity. Moreover, only the strongest convergent entorhinal inputs overcome inhibition and drive action potentials in a very sparse population of granule cells. This, together with the absence of direct granule cell interactions, is thought to de-correlate activity patterns (Acsady & Kali, 2007). As a single output, the mossy fiber projections of granule cells form large mossy fiber terminals onto CA3 pyramidal cells. Those terminals have been considered as detonator synapses due to their high reliability to trigger spiking, whereby they enforce a new well-separated activity pattern onto CA3 pyramidal cells (Kobayashi & Poo, 2004). Interestingly, they do not show Hebbian plasticity and hence might serve a selective role in learning (Nicoll, 2005). Also remarkably, as one of the only two areas with the ability of adult neurogenesis, the dentate gyrus generates new granule cells throughout life. Adult-born granule cells have been assigned a function in learning and memory, especially in pattern separation (Kheirbek, 2012; Danielson, 2016).

Of note, the hippocampus is an evolutionary old brain area, which is conserved in function across different evolutionary lineages, but the dentate gyrus was added or expanded dramatically in mammals (Acsady & Kali, 2007; Striedter, 2016). Why the dentate has gained this importance raises an intriguing question towards its specific function. One of the most consistent findings on DG function stems from lesion studies, which assign an important role to the DG in acquisition of spatial memories in Morris water maze (Sutherland, 1983; McNaughton, 1989; Acsady, 2007). Another interesting aspect of DG function arises from studies on dentate place cells, which points to roles beyond its function in encoding and pattern separation. Granule cells often have several place fields, which can change firing rates separately with

small changes in the environment, thus pointing to a higher coding density in DG compared to other hippocampal subparts (Treves, 2008; Leutgeb, 2007; Jung & McNaughton, 1993). In contrast, place cells in CA3 and CA1 have one sharp place field to represent the animal's position in the local environment. Furthermore, similar to a functional segregation along the longitudinal axis of the entire hippocampus, a recent study proposed a DG function in encoding spatial memories and controlling anxiety behavior, corresponding to its relative position along the axis from dorsal to ventral hippocampus (Kheirbek, 2013).

CA3 function

The principal cells of CA3, the pyramidal neurons, receive convergent input from three main routes: highly separated granule cell input via mossy fiber terminals, entorhinal cortex inputs via perforant path and input from the recurrent collaterals of other pyramidal cells. Thereby, the different types of input arrive stratified by layer and each appears to involve a specific function. Mossy fiber input may enforce new patterns onto CA3 to encode new memories, whereas direct entorhinal context innervation is thought to be more important for retrieval of memories (Treves & Rolls, 1992). Of particular interest are the recurrent collaterals, which form a highly interconnected auto-association network of CA3 pyramidal cells. It serves pattern completion, also referred to as an auto-associative memory function, in which partial cues, arriving as entorhinal input, can restore entire memory representations (Rolls, 2013; Nakazawa, 2002; Treves & Rolls, 1992). Furthermore, the CA3 network is thought to be the first area within the sequence of information processing regions, which may store information in the form of memory representations. This notion is supported by the fact that, starting in CA3, the hippocampus can autonomously reactivate memory assemblies without external cues, which in turn reactivates complete memory representations in the cortex during so called offline states, for example slow-wave sleep (Buzsaki, 1992; Diba & Buzsaki, 2007). This process might underlie the consolidation and maintenance of episodic and semantic memories (Kali & Dayan, 2004; Girardeau, 2009).

CA1 function

CA1 receives its main inputs from CA3 via Schaffer collaterals and from the entorhinal cortex. Considering its position within the hippocampus, CA1 serves as the main output area of the hippocampus, projecting to subiculum and back to entorhinal cortex, and thereby playing an important role in memory retrieval (Witter & Amaral, 2004; van Groen, 1990). Furthermore, CA1 has been proposed to function as a "novelty detector" (Lisman, 2005). This model suggests that newly arriving sensory information starts processes in DG and CA3, which calculate predictions of future events based on their stored memory representations. The CA1 receives these predictions via Schaffer collateral and compares them with directly arriving novel information from entorhinal inputs. The detection of input discrepancy triggers a signaling loop, via nucleus accumbens and ventral pallidum to VTA that then releases dopamine into the hippocampus, which in turn enhances learning (Lisman, 2005). Thus this model raises the question of how learning and retrieval of memories can be discriminated within one memory representation. As a possible solution, theta oscillations might provide temporal processing units, in which signals arriving at peaks and troughs can be distinguished, either shaping dendritic synaptic plasticity or triggering somatic spiking (Hasselmo, 2002; Hasselmo & Stern, 2014).

1.3 What is learning and memory?

1.3.1 Synaptic rearrangements underlying memory assembly formation

The process of learning is thought to form neuronal assemblies, which can be recruited together, thus representing a certain memory. Studying learning can therefore be targeted at different stages and levels: how are such assemblies formed, maintained and/or modified as well as retrieved? Which molecular (genetic), synaptic, cellular, microcircuits and network-wide mechanisms drive these processes? The first proposal of where memories could be stored was made by Cajal, suggesting contacts between neurons as site of memory storage (Ramon y Cajal, 1893). Hebb's famous postulate then provided a

potential mechanism by stating that neuronal connections are strengthened by correlated activity (Hebb, 1949). By now known as spike-time-dependent plasticity, the principle that synapses strengthen when a presynaptic neuron persistently takes part in firing the postsynaptic neuron is generally accepted to underlie the formation of cell assemblies. Initial work on aplysia has provided first evidence for Hebb's rule in learning, which was extended to a general principle in learning (Kandel, 2014).

More detailed mechanisms on molecular events to strengthen synapses have been described since the discovery of synaptic long-term potentiation (LTP) in the 1970s. Hereby, calcium levels increase via NMDA receptors in the post-synapse, leading to insertion and clustering of AMPA receptors and hence to an increase in synaptic efficiency. This mechanism has indeed been shown to underlie learning, since blocking AMPA receptor trafficking to synapses impaired memory formation (Nabavi, 2014; Kessels, 2009).

Furthermore, learning has been linked to structural changes in form of increased synapse rearrangements and spine turnover (Caroni, 2012 and 2014). Thus, synapses and spines are not only strengthened, but also new ones are formed and pruned, whereas others are weakened during learning (Hill, 2013; Trachtenberg, 2002; Holtmaat, 2005). Increased turnover of spines might allow for selecting specific connections during the learning process. Often spine plasticity appears clustered along dendrites, which could indicate dendritic domains contributing to the formation of a certain memory assembly (Chen, 2012; Hofer, 2009). In line with this, spine formation during learning has been linked specifically to the newly learnt task memory (Fu, 2012; Hayashi-Takagi, 2015).

1.3.2 Windows of consolidation/plasticity

Memories can be subdivided into short- (minutes), intermediate- (hours) and long-term (days, years) memories. Short-term memory does not depend on transcription and synthesis of new proteins, whereas those processes are

required for long-term memories (Bekinschtein, 2007). Learning leading to long-term memories has been shown to follow a sequence of plasticity events to orchestrate the formation and consolidation of long-term memories. First beautifully shown for single-trial learning paradigms, many mechanisms also hold true for incremental learning. Triggered by learning, a program of consolidation processes is initiated to form stable memories by strengthening of pre-existing synapses and formation of new ones (Caroni, 2014; De Roo, 2008; Holtmaat & Svoboda, 2009; Takeuchi, 2013; Xu, 2009). In a first window of consolidation directly following memory acquisition, as mentioned above, early LTP (lasting for minutes) and a late protein-dependent LTP (lasting for hours) strengthens synapses in learning-related assemblies by insertion and clustering of AMPA receptors, followed by the production and incorporation of new synaptic proteins and receptors, respectively.

Importantly, the expression of immediate early genes (IEG), such as cFos, Arc, Zif268 have been linked to long-term consolidation (Katche, 2010 and 2013; Nakayama, 2015; Caroni, 2014). They are expressed with a delay of at least 45 minutes after onset of learning and can remain upregulated for up to four hours as shown for cFos (Karunakaran, 2016). So far, it has remained unknown whether all cells or which of those cells active at learning later express IEGs. It also remains elusive which plasticity processes are triggered by IEGs. Very likely they play a role in strengthening and forming new synapses through epigenetic changes, gene expression and synthesis of new synaptic proteins e.g. glutamate receptor subunits and scaffolding proteins (Holtmaat & Caroni, 2016). Therefore, IEGs can rather be seen as markers for cells undergoing learning-induced plastic changes (instead of general activity markers). A functional role of IEGs in plasticity during learning has been demonstrated in several studies (Bozon, 2003; Plath, 2006).

Interestingly, a second wave of IEGs and other transcription factors was detected at 12-15h after learning, a time point in which long-term memory consolidation is completed (Katche, 2010; Trifilieff, 2006). The precise role of this second window of memory consolidation/plasticity is still unclear. Notably, besides the described synaptic and neuronal plasticity, replay processes play

a very important role in memory consolidation. Memory cell assemblies can be recruited again without sensory information as cues during so-called offline states of the brain for up to several hours after the acquisition of the memory, for example during slow-wave sleep and quiet wakefulness. Hereby, the assemblies are reactivated in sequences corresponding to their initially acquired order, as beautifully shown for replay of spatial memory episodes in the hippocampus (Acsady, 2007; Carr, 2011; Davidson, 2009; Buzsaki, 2015). Interestingly, replay happens during sharp-wave ripple activity, which was shown to be functionally linked to learning-induced plasticity of inhibitory PV basket cells (Karunakaran, 2016; Girardeau, 2009). The function of interneuron microcircuits in learning will be introduced in detail later. However, consolidation processes such as replay might have crucial roles in selecting appropriate cell assemblies for long-term memory formation and could potentially underlie mechanisms of flexible use of memory cell assemblies.

1.3.3 Memory allocation: Where are memories stored in the brain?

Specific strengthening of connections between neurons underlies the formation of neuronal assemblies to create representations of memories. However, such assemblies encoding a certain memory might be part of neuronal representations that can span across networks including different brain regions. This whole population defined as physical location for storage and retrieval of a memory is called an engram, a term first coined by Semon in 1908. Recently, impressive advances have been achieved in search for the localization of neuronal assemblies representing memories (Hübener & Bonhoeffer, 2010; Josselyn, 2015; Holtmaat & Caroni, 2016).

First, investigations to localize specific functions in memory processing were restricted to applying targeted lesions and studying the effect on memory recall. To then study localization in more detail, molecular markers for activity and plasticity served to visualize potential memory assemblies. These experiments were based on the assumption that neuron active during learning encode the memory (which has been shown to hold true for hippocampal

place cells (Pfeiffer, 2015; de Lavilléon, 2015; Holtmaat & Caroni, 2016). Immediate early genes served as markers for memory assemblies during learning, consolidation and recall, but for a long time no direct evidence existed showing that learning and recall use the same assemblies. Very recently, new techniques to genetically access learning-related cells (by TRAPing) opened new opportunities to study memory cell assemblies (Guenthner, 2013; Reijmers, 2007; Luo, 2008; Rogerson, 2014; Garner, 2012). More specifically, fluorescent markers, ion channels or GPCRs have been coupled to promoters of learning-related IEGs cFos and Arc as well as CREB, thus allowing for visualization and manipulation of the cells that expressed these transcription factors during learning. Importantly, cell assemblies expressing cFos and Arc have been found to exhibit many characteristics of Hebb's memory engrams, such as increased synaptic strength and spine density (Holtmaat & Caroni, 2016; Ryan, 2015). Hence, memory assemblies can now be defined as those populations whose reactivation triggers memory recall, whereas inhibition of these assemblies prevents recall (Han, 2009; Tanaka, 2014; Liu, 2012). This principle was shown for different memory types and systems, for example hippocampus and BLA (Gore, 2015). But still, experimental access is limited. On the one hand, it might only target a fraction of the entire memory ensemble, which likely spreads across networks in different brain areas (Hübener & Bonhoeffer, 2010; Josselyn, 2015). On the other hand, individual (targeted) neurons can be part of several distinct assemblies, a mechanism thought to underlie large memory storage capacities. However, with the new genetic tagging tools many questions have become tractable concerning memory cell allocation. For example, using the transcription factor CREB (cAMP response element-binding protein), which is enhanced in active populations during learning, it was shown for the first time how neurons might be selected into memory assemblies (Han, 2007; Reijmers, 2007; Kim, 2014). Overexpression of CREB before learning enhanced neuronal excitability and thereby increased the likelihood of the CREB-overexpressing neurons to be recruited into the memory assembly. In line with this, selective ablation of the CREB-overexpressing neurons erased the memory (demonstrated in BLA neurons participating in fear memories, Han 2009). Seemingly, neuronal excitability is

the main determinant for recruitment, as shown by using different techniques to enhance excitability (optogenetics, chemogenetics in piriform cortex and BLA) (Yiu, 2014; Zhuo, 2009; Choi, 2011; Gore, 2015). Such mechanisms might exist endogenously, for example, place cells participating in preplay were shown to be more likely to be recruited in subsequent learning (Dragoi, 2011). Recruitment by excitability raises the question of how specific memories are acquired without interference of memory assemblies. Indeed, when tagged cFos neurons were experimentally reactivated in a context unrelated to the initial memory, then a false memory was formed (Ramirez, 2013). To solve the problem of memory interference, highly excitable neurons were proposed to serve as nodes for cell assemblies (Yassin, 2010; Grosmark, 2016; Holtmaat & Caroni, 2016), to which related information can be added or removed. The flexible use of memory assemblies still needs to be investigated. First indications propose that memory assemblies can be used in distinct manners, for example by gaining a new value (Redondo, 2014). Of particular interest will be the flexible use of memory assemblies in incremental learning and the formation of associative memories, which rely on the addition of information to previously formed memory assemblies. It has been postulated that shared neuronal ensembles can link distinct memories, particularly those encoded close in time (Cai, 2016). Potentially, this is due to enhanced excitability of recently used cells in acquisition of one memory, which increases their probability to be recruited again in another memory assembly (Yiu, 2014; Zhuo, 2009). Furthermore, it is needed to be kept in mind that not necessarily all neurons active during memory acquisition will become a permanent part of the memory assembly. Memories could also be localized transiently to certain populations while later assemblies are modified and/or other assemblies of a distributed memory engram gain importance, which could even be localized to other brain areas (Denny, 2014; Poirier, 2008; Rashid, 2016). The dynamics of memory assemblies, their use and interactions with each other are exciting open fields for future research.

1.4. Excitation/inhibition balance in the hippocampus: Role of PV basket cells in learning

The brain is made of a large repertoire of distinct cell types, organized into dedicated microcircuits to perform complex computations as encoding, consolidating and retrieving memory representations. In general, the hippocampus (like most cortical areas) consists of around 80% excitatory neurons and 20% GABAergic interneurons, which provide inhibition and thereby regulate neuronal activity (Meinecke & Peters, 1987; Kullmann, 2011; Kepecs & Fishell, 2014; Klausberger & Somogyi, 2008). Spatially and temporally localized inhibition and disinhibition has been shown to underlie learning (Letzkus, 2011; Wolff, 2014; Fu, 2015) and regulate plasticity processes (Hensch, 2005), thus ultimately defining neuronal assemblies. Based on morphology, layer occupancy and synaptic connectivity, firing properties, molecular expression profiles and other features, there exist around 20 different interneuron types, each contributing in a distinct fashion to shape cell and network activity (Klausberger & Somogyi, 2008; Ascoli, 2008). Among inhibitory cell types, fast-spiking PV basket cells are the most numerous ones. They provide powerful local feedforward and feedback inhibition onto the perisomatic region of principal cells (Freund & Katona, 2007). They have been shown to synchronize network activity, supporting different types of neuronal network oscillations, such as gamma and theta oscillation, ripple and spindle activity (Amilhon, 2015; Royer, 2012; Stark, 2012; Lapray, 2012; Cardin, 2009). Thereby, they play an important role in the stable formation and consolidation of cell assemblies (Karunakaran, 2016; Jadhav, 2015). Moreover, learning-related plasticity of PV basket cells has been reported to transiently shift PV cell networks into configurations either supporting or suppressing further plasticity and learning (Donato, 2013). These configurations are mediated by two distinct subpopulations of PV cells, which are differentially regulated by excitation and inhibition, respectively (Donato, 2015), showing that the excitatory-inhibitory microcircuit functions bi-directionally. PV interneurons regulate learning processes as well as undergo plasticity themselves.

1.5. Aim and rational of the thesis

The hippocampus is well known for its function in declarative memories, but its precise role in learning and memory remains controversial. Considering the different connectivity along its longitudinal axis with cortical and subcortical brain areas, gene expression profiles, place cell properties and many other strikingly distinct features, it has been proposed that hippocampal subdivisions might have distinct functional roles. According to a current view, the dorsal hippocampus is required for cognitive functions, such as spatial navigation and episodic memories, without involvement of emotional components. Less consensus exists on ventral hippocampus, which has been proposed to function in emotional learning and stress responses, detection of novelty, spatial navigation and generalization of memories across contexts, to name a few. Whether these proposals on dorsal and ventral functions might converge into a general concept requires further investigations. Thus, in my opinion, the detailed analysis of the functional organization along the hippocampal longitudinal axis seems essential to understand the role of hippocampus in memory processing.

In this thesis, I address the question of whether the hippocampal subdivisions exhibit distinct but complementary functions in declarative memories. I am using targeted chemogenetic silencing, thereby exploiting the fundamental role of PV basket cells in shaping network activity as tool (chemogenetic silencing) to locally interfere with memory processing in dorsal and ventral hippocampus, respectively, in order to understand their contribution in learning and memory. First, I am comparing their function in single trial learning paradigms, in particular in recalling memories at different time points. Later, I study the formation of associations to previously acquired memories and ask whether the hippocampal subdivisions might have distinct roles in association learning and retrieval. Furthermore, I aim to localize distinct memory components to the hippocampal subdivisions. To this end, I monitor the induction of the immediate-early gene product cFos and genetically target its expression, thereby identifying learning-related neuronal assemblies for different types of memories in dorsal and ventral hippocampus.

In a supplementary part of the thesis, I am exploring the transversal axis of the hippocampus in association learning. Thereby, I particularly focus on the function of the dentate gyrus in the formation of associations, comparing its distinct functional features in the dorsal and ventral hippocampus.

2. Results

Specific requirement for vH in long-term retrieval of single-trial learning

To investigate specific contributions of dorsal (dH) and ventral hippocampal (vH) subdivisions in learning and memory, these areas were transiently bilaterally silenced during learning or recall by local pharmacogenetic activation of PV interneurons (Magnus et al., 2011; Karunakaran et al., 2016). To this end, Cre-dependent PSAM was virally delivered in PV-Cre mice in either dH or vH, resulting in strong and selective expression of excitatory PSAM receptor in PV interneurons in the area of injection, spanning the transversal subdivisions DG, CA3 and CA1 (Fig.2.1a). I.p. application of the ligand molecule PSEM308 activated PV interneurons, thus transiently inactivating the target area.

To begin testing dH and vH contributions in learning and memory, such silencing was applied in classic single-trial learning paradigms. We first investigated contextual fear conditioning (cFC), a form of Pavlovian association learning known to involve hippocampal function (Fanselow & Dong, 2010; Bast, 2001; Maren, 1997; Philips and LeDoux, 1992). To confirm efficient local silencing during behavior, induction of the IEG cFos was monitored upon fear memory retrieval with or without silencing. In the target area, absence of retrieval-induced increase in contents of cFos expressing cells confirmed silencing (Fig.2.1b), whereas unaffected cFos induction outside the target area provided evidence for specificity. Silencing vH during recall of fear memory 24h after acquisition (will be referred to throughout as time point +xh, i.e. in this case +24h) suppressed freezing response, whereas silencing dH during next-day retrieval did not affect freezing to context (Fig.2.1c). Since fear conditioning involves a strong emotional response and vH has been implicated in emotional responses, we next investigated a context-dependent familiar object recognition task as a hippocampus-dependent single-trial learning protocol without emotional component/valence. Notably, local inactivation of vH again specifically impaired memory recall at +24h, whereas silencing dH left object recognition unaffected (Fig.2.1d). These findings provided evidence for a specific requirement for vH and not dH in hippocampus-dependent long-term memory recall.

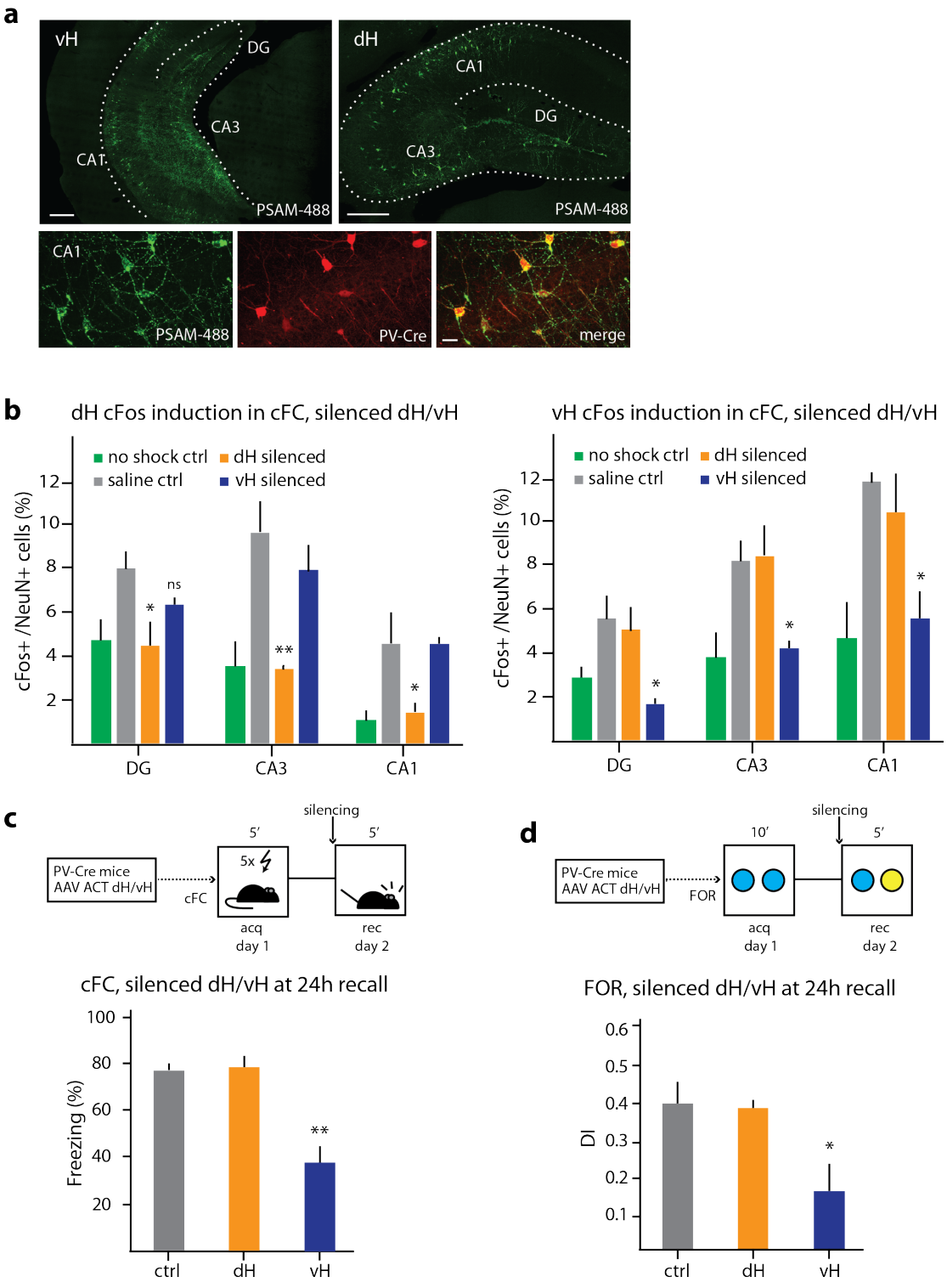

Figure 2.1. Specific requirement of vH for long-term retrieval of single-trial learning. (a) Cre-dependent expression of PSAM in PV interneurons. Example labeling, using bungaroxin-488 for PSAM visualization, spanning the transversal subdivisions DG, CA3 and CA1 in vH (left) and dH (right). Bottom: example labeling showing specific Cre-dependent expression of PSAM in PV interneurons, in PV-Cre/Rosa-tdTomato mice. Bars: 500 (left), 400 (right), 20

(bottom) mm (b) Absence of recall-induced increase in cFos contents in cFC upon local pharmacogenetic silencing of dH and vH, respectively. N=3 each. (c,d) Schematic of the experiment and impact of dH and vH silencing on cFC (c) and FOR (d). Critical role of vH, but not dH, at +24h recall of fear and object memory. N=5-6 each (c,d). *p<0.05, **p<0.01.

Memory recall during first 5-6h after acquisition depends on dH, not vH

Having demonstrated a vH, but not dH, requirement in long-term memory retrieval raised the question of what might be the contribution of dH in single-trial learning. We therefore investigated the dependency of memory recall during early time points after acquisition, in particular within the first window of consolidation. Interestingly, we found that inactivating dH strongly impaired memory retrieval at early time points (+0-5h) after acquisition (Fig2.2a,b), although such silencing leaves long-term memory recall unaffected (Fig.2.1). This early requirement of dH in recall holds true for both contextual fear memory as well as object memory in familiar object recognition. Remarkably, silencing vH did not affect the recall of memory at early time points in both single-trial learning paradigms. A switch of recall dependence from dH to vH occurred between +5h to +7h after acquisition, indicating a sequential requirement of the hippocampal subdivisions in memory recall, depending on time but not on the emotional valence of the learning task.

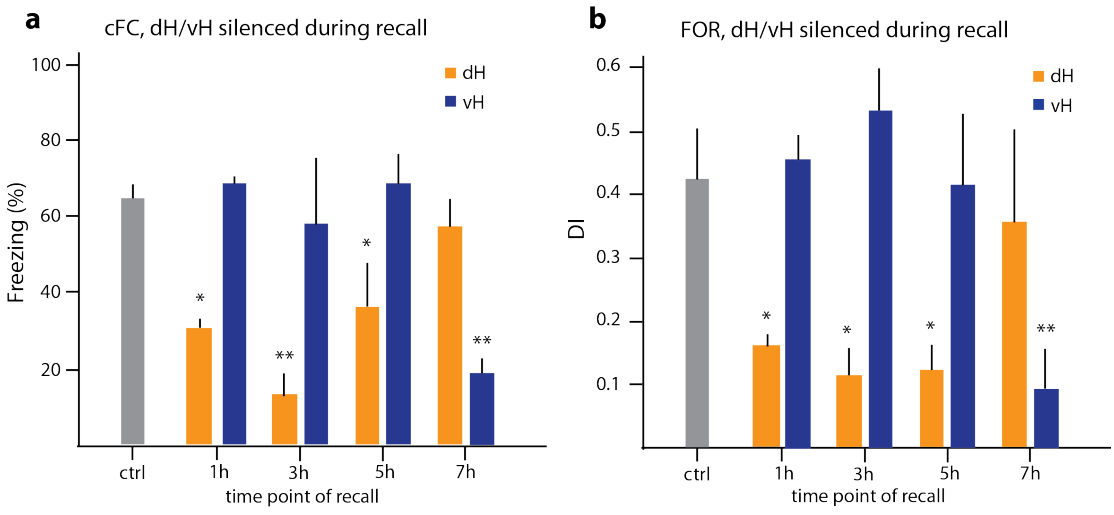

Figure 2.2. Memory recall during first 5-6h after acquisition depends on dH, not vH. (a,b) Time course of dH and vH dependence of memory recall in cFC (a) and FOR (b). Silencing dH at +1h, +3h and +5h, but not +7h, suppressed fear and object memory recall. Silencing vH at +1h, +3h and +5h had no effect on memory recall, but silencing vH at +7h impaired memory recall. N=4-5 each (b). *p<0.05, **p<0.01.

Specific requirement of dH to associate information in learning

As single-trial memory recall depends first on dH and later on vH, this principle could extend to encoding as well, creating a general concept, in which each new learning has a dH-dependent early window. Therefore, to better understand the sequential requirement of the hippocampal subdivisions, especially the role of dH in the early window, new learning was performed in addition to previously acquired single-trial memory.

Extinction of a contextual fear memory was chosen as learning paradigm. Here, the fear memory is long-term retrieved in the fear context, but the absence of foot shocks causes animals to learn to alter their behavior and stop freezing. According to current views, extinction forms a new memory in addition to the original fear memory based on associative networks (Dunsmoor, 2015; Orsini & Maren, 2012). Hence, retrieval and learning can be distinguished by dividing the 30 min extinction protocol into a 10 min retrieval session (insufficient to extinguish) and a 20 min session 3 h later, within the early window, to continue the extinction experience and learn to unfreeze. Silencing during the second 20 min session revealed that only dH inactivation and not vH suppressed extinction learning (Fig.2.3a). Notably, vH was necessary for initial recall of the fear memory (Fig.2.1c), and vH silencing delayed the onset of extinction, without affecting learning. This data indicates a requirement of dH in associative learning within a task, specifically, to add information and edit previously acquired memories.

To further confirm the specific contribution of dH and vH in learning and memory, mice were trained in MWM, an incremental spatial learning task, which has been demonstrated to depend on hippocampus (Ruediger, 2012;

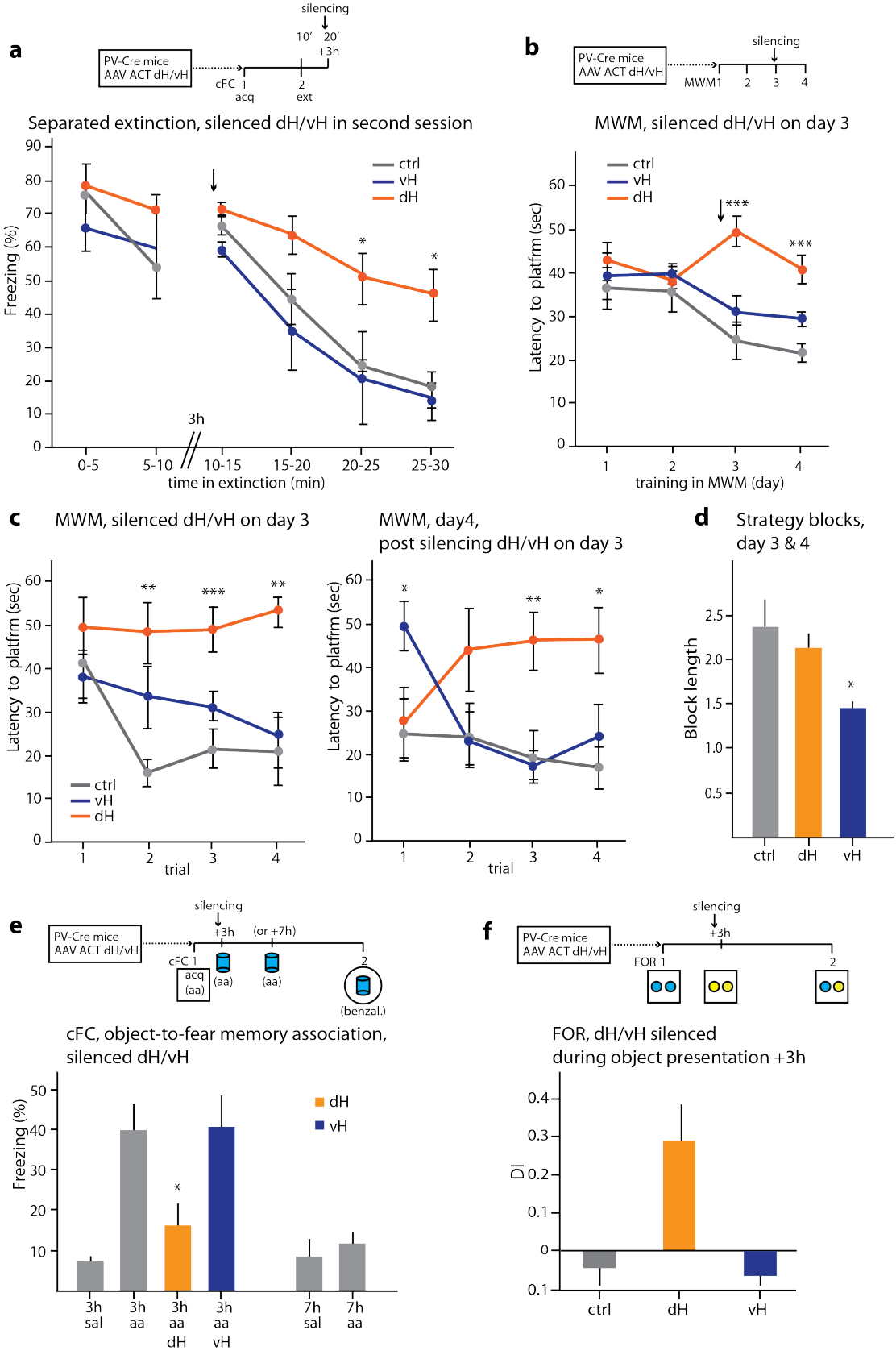

Figure 2.3. Specific requirement of dH to associate information in learning. (a) Extinction learning in the separated extinction protocol with schematic of the experiment. Silencing dH, but not vH, impaired extinction

learning. N=4-5 each. (b) Schematic of the experiment and MWM learning until day 4. Silencing dH on day 3 impaired MWM learning on day 3 and day 4. Silencing vH showed no difference in overall performance. N=6-8 each. (c) Analysis of individual trials on day 3 and day 4 revealed impaired memory recall upon vH silencing, whereas dH silencing impaired learning across trials. (d) Learning in search strategy blocks was impaired by vH inactivation, but not dH. (e, f) Schematic of association learning in cFC (e) and FOR (f). Silencing dH, but not vH, prevented the formation of associations. N=4-5 each (e) and N=3 each (f). *p<0.05, **p<0.01, ***p<0.001.

Moser, 1995; Morris, 1982). In MWM, mice swim in a pool with opaque water, where they learn to find a hidden escape platform using distant spatial cues. Four trials of one minute each (with inter-trial interval of 5 min) were performed per day, in which a spatial map of the environment is formed over the course of several days, helping the animals to improve performance. It was therefore hypothesized that performance on each day requires the retrieval of long-term memory and subsequent associative learning to add information across trials, thereby improving behavior. To investigate this hypothesis along with the contributions of the hippocampal subdivisions, a day in the middle phase of maze learning was chosen. Silencing dH on day 3 of MWM strongly impaired performance, as shown by increasing latencies to find the platform, whereas vH silencing only slightly, but not significantly, slowed the learning curve (Fig.2.3b). In order to better distinguish the relative roles of dH and vH in MWM learning, we monitored the performance across individual trials on day 3 (silenced) and 4 (post silencing) (Fig.2.3c). On day 3, control mice started the first trial with latencies like at end of day 2, showing the retrieval of the memory. In subsequent trials mice improved their performance, hence shortened escape latencies. Animals with inactivated dH started with the same latency like controls in the first trial, but failed to improve across trials, showing that new learning was strongly impaired. This effect was still visible on the day post silencing. In comparison, vH silenced animals also started with latency like controls in the first trial, showed still high latencies in the next trial, then improved performance and reached control levels at the end of day3. On the day following vH silencing, animals were unable to find the platform in the first trial, then showed normal additional learning. This finding provides further evidence that vH functions in long-term

retrieval, while specifically dH plays a crucial role in associative learning within the task.

We then analyzed the search strategies that the animals apply to find the platform (Ruediger, 2012). Controls learn in characteristic strategy blocks, using the same strategy in consecutive trials, then switch mainly to blocks of more advanced strategies. Remarkably, in the absence of vH, mice switched strategies more frequently (Fig. 2.3d), seemly unable to select the best option they have learnt. Silencing dH left strategy selection unaffected. This finding suggests that vH learns and retrieves the concept of the task, reflected in appropriate selection of strategies and thus learning in strategy blocks.

Since new learning within a task can be interpreted as adding information to previously acquired memory, the dH might play a general role in associating any information, even simple information units, to any given previously defined task. This was investigated in a modified version of the fear conditioning paradigm, which was based on the formation of an object-to-fear memory association. In this task, mice underwent the acquisition of cFC in the presence of an odor, thereby encoding a fear to context memory. Then at +3h, within the window for dorsal-dependent memory recall, mice were exposed to an object with matching odor and tested next day for freezing to the object in a novel context (Ananya Chowdhury, unpublished). Control mice showed a robust freezing response to the object, when the object was presented at +3h, but not at +7h, having associated the previously unrelated object to the fear memory via matching odor (Fig.2.3e). Notably, these time points are matching with the dorsal-dependence of memory recall (Fig.2.2). Upon inactivation of dH at object exposure at +3h, mice showed strongly reduced freezing to the object when tested next day, demonstrating that no object-to-fear memory association was formed. By contrast, silencing vH had no effect on the formation of object-to-fear memory associations.

Next, the same principle was examined in a modified version of FOR, which allows to study the formation of an association between different sets of objects. In this task, a first acquisition, in which mice were exposed to two

objects (AA), was followed by a second acquisition at +3h, in which mice were given two new objects (BB) for exploration in the same context. Next day, mice were presented with one of each objects (AB) again in the same context and exploration ratios were monitored. Control mice explored both objects equally and less than at acquisition. Mice with silenced dH during the second acquisition, explored object B like an unknown object at testing next day (Fig.2.3f), indicating that object B was not associated to the memory representation of the context with A. These data further support the notion that dH is specifically required for forming associations within a context-dependent task, ranging from single item-to-context associations up to complex new associative learning in extinction and MWM. Likely, this dH-dependent association process occurs during the early window of dH-dependent memory recall.

Time window for association learning defines duration of dH-dependent recall

Since windows for association learning and for dH-dependent retrieval are closely matching in time (up to +6h), we investigated whether these two windows are functionally linked. It has been shown previously in the lab (Ananya Chowdhury, unpublished) that association learning depends on upregulated cFos activity in the hippocampus. Hence local stabilization of the cFos protein beyond +6h via application of a proteasome inhibitor extended the window for association learning. To confirm this strategy, a proteasome inhibitor was injected into dH, resulting in an elongated window to form an object-to-fear memory association (Fig.2.4a). To test for correspondingly elongated recall dependence, the proteasome inhibitor was injected locally into dH and subsequently, the hippocampal subdivisions were silenced at +7h recall. As for associative learning, the treatment also shifted the retrieval dependence, as dH silencing at +7h retrieval now suppressed the freezing response (Fig.2.4b), while vH silencing had no effect anymore. This finding provides strong evidence that the time window for association learning defines the window of dorsal-dependent memory recall.

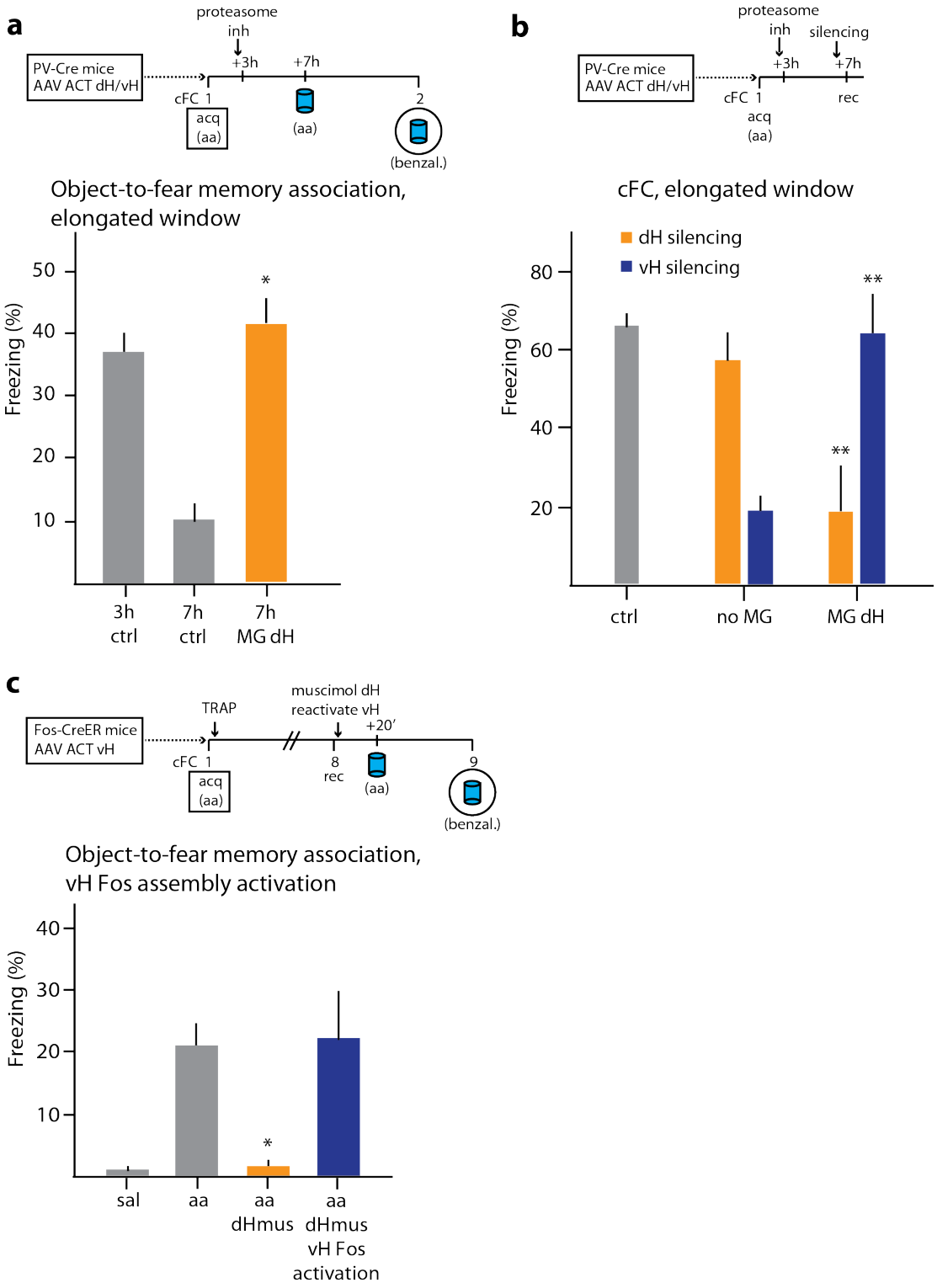

Figure 2.4. Time window for association learning defines duration of dH-dependent recall. (a) Elongation of the window for association learning in cFC. Schematic of the experiment and window elongation by application of proteasome inhibitor to dH. (b) Likewise, proteasome inhibitor applied to dH extended the window for dorsal dependent memory recall in cFC beyond +6h.

(c) Increased activity in vH Fos+ assemblies is sufficient to form association memories, even in absence of dH activity. N=4 each. T-test (a), one-way ANOVA followed by Bonferroni post-hoc test (b). *p<0.05, **p<0.01.

Interestingly, object-to-fear memory associations could also form post fear memory recall. Like association learning during the early window, the formation of associations to recalled memories was dependent on dH (Fig.2.4c). Therefore, we next aimed to understand whether vH has the potential to form associated memories or it is a selective function of dH. This we investigated by artificially enhancing activity in learning-related cell assemblies in vH. Having found the importance of cFos activity in association learning, we genetically targeted cFos expressing cells (via TRAP) in vH at fear memory acquisition. Then, the fear memory was recalled, followed by inactivation of dH by muscimol injection and at the same time reactivation of vH Fos assemblies during object presentation. Indeed, selective reactivation of vH cFos assemblies induced an object-to-fear memory association in the absence of dH. This finding suggests that vH is sufficient but not required for association learning.

Separate learning and memory processes in vH and dH

When do the hippocampal subdivisions start implementing their specific roles? The relative contributions of dH and vH in learning and recall raise the question whether they already encode functionally distinct memories at acquisition, which then cause/contribute to their specific functions (or whether they are both encoding the same event, then selective network recruitment at different time points is underlying their contributions in learning). Therefore, local dH or vH silencing was performed during acquisition of cFC. Subsequently, the effect on fear memory recall and on further association learning was investigated. Inactivation of dH and vH, respectively, left next-day recall intact, while silencing both dH and vH together strongly impaired recall (Fig.2.5a). Interestingly, analysis of recall at +10d revealed decreased freezing levels, indicating the formation of a less stable memory when one of the subdivisions is not functional during acquisition. Likewise, vH silencing

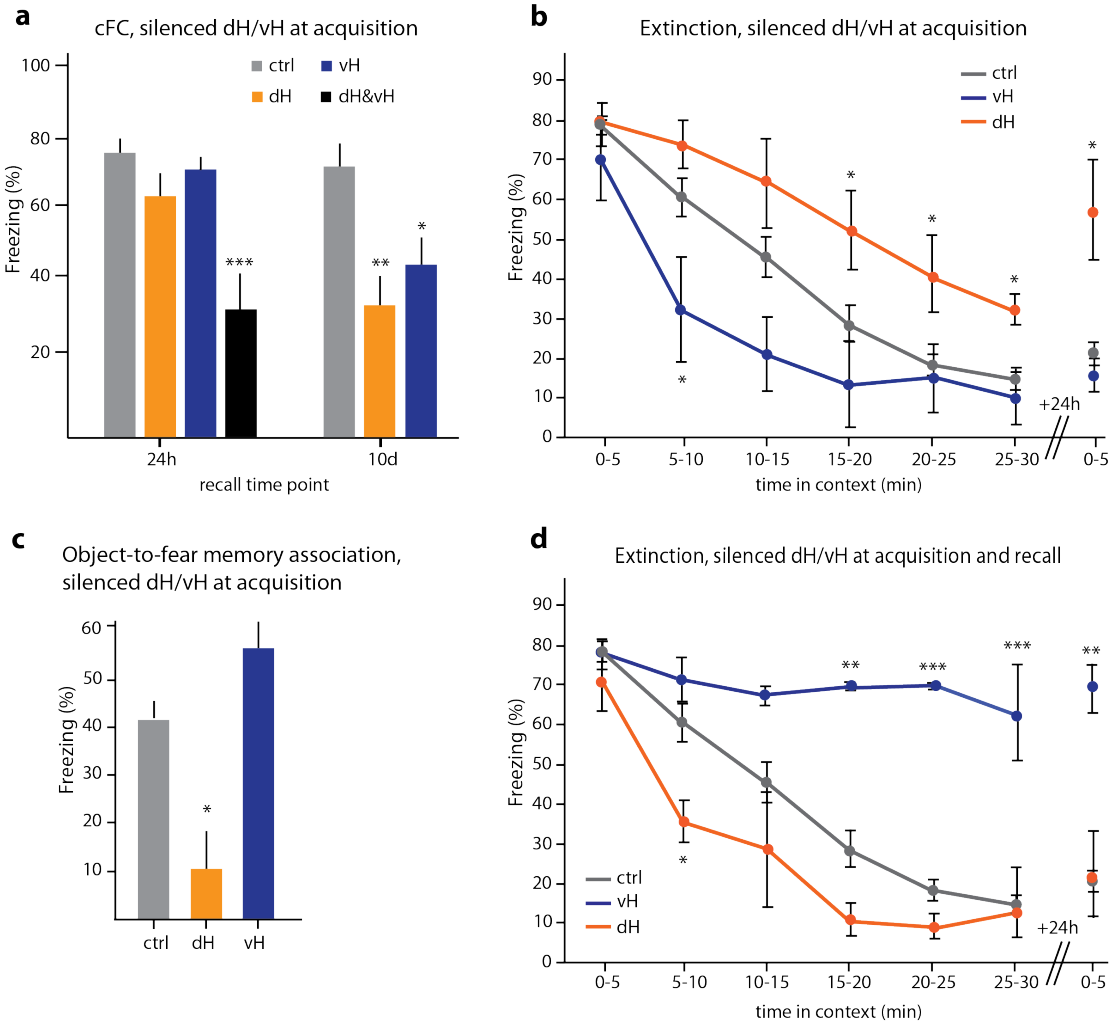

Figure 2.5. Separate learning and memory processes in vH and dH.
(a) Formation of stable memory impaired upon silencing dH and vH, respectively, at acquisition of cFC. Silencing at acquisition left +24h recall intact, but decreased freezing response at +10d recall. Silencing both dH and vH together at acquisition suppressed freezing response at +24h. (b) Silencing dH at acquisition of cFC impaired subsequent extinction learning, whereas silencing vH accelerated extinction. (d) Likewise, silencing dH at acquisition of cFC prevented subsequent formation of an object-to-fear memory association, whereas vH silencing left association learning unaffected. (d) Silencing at acquisition and again at recall of cFC. Double silencing of vH prevented extinction, whereas double silencing of dH accelerated extinction. N=4-5 each. *p<0.05, **p<0.01, ***p<0.001.

accelerated extinction, also pointing to a less stable memory (Fig2.5b). On the contrary, silencing dH at cFC acquisition impaired subsequent extinction learning. This is in accordance with dH function in new association learning and suggests that association learning is only possible if a memory trace of the original memory was encoded in dH. In line with this notion, also the

formation of an object-to-fear memory association was prevented by dH inactivation at cFC acquisition, whereas vH silencing had no effect on association learning (Fig.2.5c). These data provide evidence that both hippocampal subdivisions encode a memory at acquisition of a task, which is subsequently used to accomplish their specific functions. Regarding this notion, having found that the formation of a vH memory at acquisition is not necessary for +24h recall seems surprising, compared to the initial results on vH requirement for +24h recall. We therefore asked, whether dH forms a memory at acquisition in the absence of vH, which is later used by vH for recall. To this end, silencing vH at acquisition and at +24h recall was performed. Surprisingly, memory recall was still intact, indicating that dH is sufficient to recall memory at +24h upon vH silencing (Fig. 2.5d). Moreover, under these conditions the dH memory is not extinghuished. Since vH is not required for extinction learning itself, this result suggests that vH is required for the onset of extinction (possibly extinction is not identified as novel event with novel valence). On the contrary, silencing dH at acquisition and again at +24h recall caused accelerated extinction, likely due to formation of a less stable memory at acquisition.

Recall of associated memories specifically depending on dH

So far, we have investigated how associated memories are formed by dH. Since this is an encoding process, we further explored whether these associated memories remain localized to dH or whether a transfer of information occurs to vH for long-term recall and integration of associations into the general task. First, learning to unfreeze in the extinction paradigm is based on the formation of associations and required the dH. Does the memory of extinction learning remain in dH for long-term recall? Mice were therefore fear conditioned, followed three days later by extinction of the contextual fear memory. Next day, mice were again exposed to the fear context, testing the retention of extinction. Surprisingly, silencing dH at long-term retention lead to a freezing response to the context comparable to mice which had not undergone extinction (Fig.2.6a). This finding shows that the extinction memory is localized to dH for long-term recall, whereas the fear

memory is not recalled via dH. Contrarily, inactivation of vH resulted in low freezing levels, comparable to control mice, suggesting that vH is not involved in the formation and storage of extinction memory. But of note, low freezing response upon vH silencing could also reflect impaired fear memory recall, masking an effect on extinction learning.

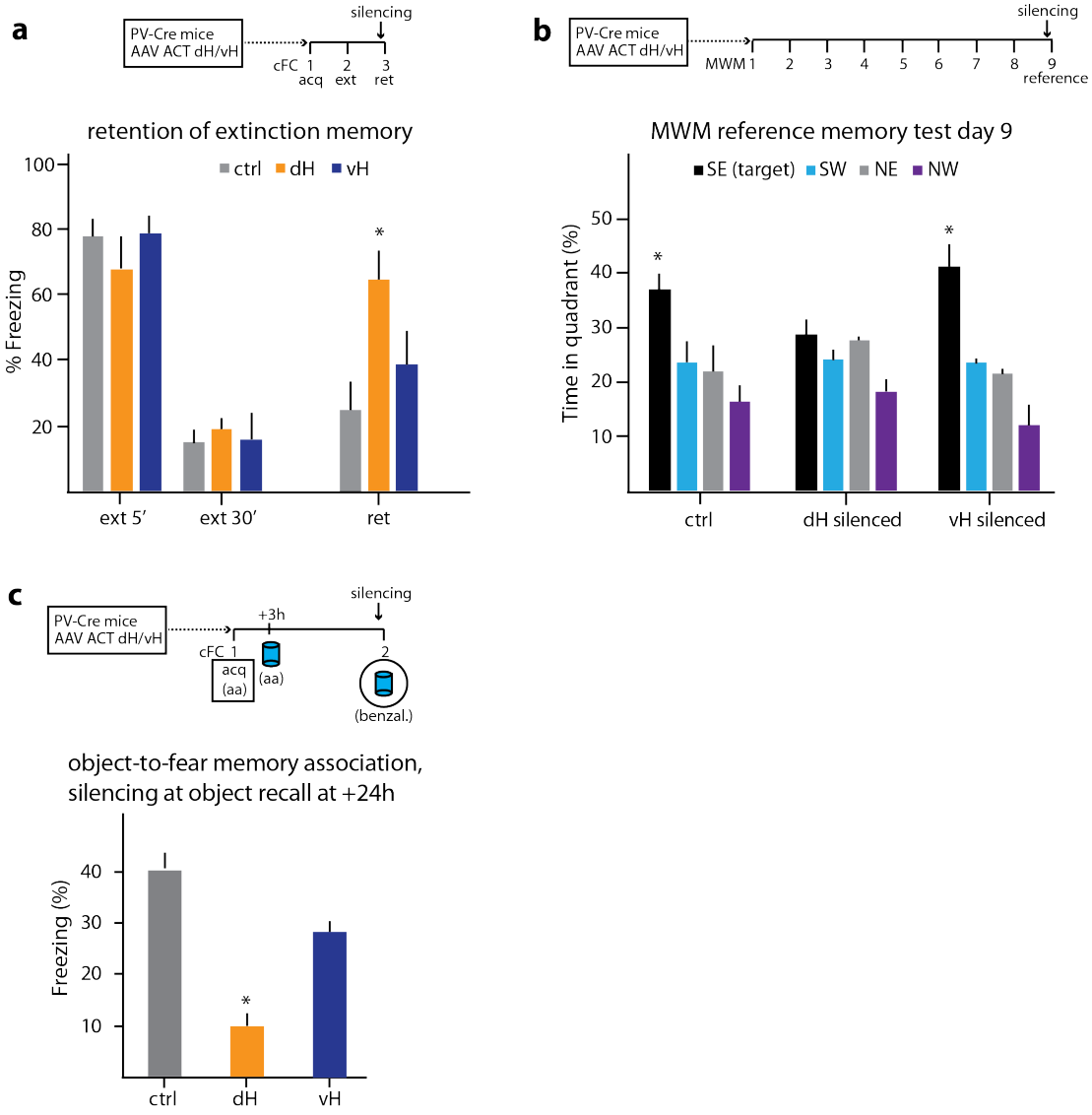

Figure 2.6. Recall of associated memories specifically depending on dH. (a) Retention of extinction learning with schematic of the experiment. Suppressed retention of extinction memory upon silencing dH, but not vH. N=4-5 each. (b) Spatial reference memory test in MWM with schematic of the experiment. Silencing dH, but not vH, prevented preference for target quadrant in reference memory test. N=6 each. (c) Recall of object-to-fear memory association impaired upon dH, but not vH silencing. N=4 each. *p<0.05.

Therefore, the potential concept of specifically dH-dependent recall of associated memories was further studied in MWM. In this task, spatial learning occurs across trials and days, resulting in the formation of a spatial reference memory. Having shown that dH is necessary for daily learning across trials (while vH is required to recall memory from previous days, tested on day 3), we investigated whether the spatial reference memory is subsequently recalled via dH or whether it has a vH component. After 8 days of maze learning, the platform was removed and the time spent in the target quadrant, previously containing the platform, was monitored. Inactivation of dH during reference memory test suppressed any preference for the target quadrant (Fig.2.6b), while vH silenced animals preferred the target quadrant like control animals. Hence, reference memory dependence on dH, but not vH, further supports the notion that associated memories are not only specifically encoded but also specifically retrieved from dH.

Applying this logic to single item associations, we performed fear conditioning together with object presentation at +3h, and then silenced the hippocampal subdivisions at +24h retention of the object-to-fear memory association. dH inactivation suppressed the recall of the associated memory, as detected in low freezing response to the object (Fig.2.6c). In comparison, vH silencing only slightly, but not significantly impaired object-to-fear memory retrieval. To summarize, in all behavior paradigms analyzed, the associated memory was specifically recalled by dH, but not vH.

More insight into the localization of a memory can be gained by studying neuronal assemblies that are active in memory formation and recall, via genetic or immunohistochemical targeting of cFos expression (Guenthner, 2013). To unravel the cellular counterparts of associated memories within the hippocampal subdivisions, contents of cFos+ neurons were compared in dorsal and ventral CA1 in contextual fear memory acquisition, recall (+24h), extinction (+48h) and retention of extinction memory (+72h). In vCA1 a strong induction of cFos contents of similar magnitude was detected across all conditions compared to baseline (Fig.2.7a). In dCA1, fear memory acquisition,

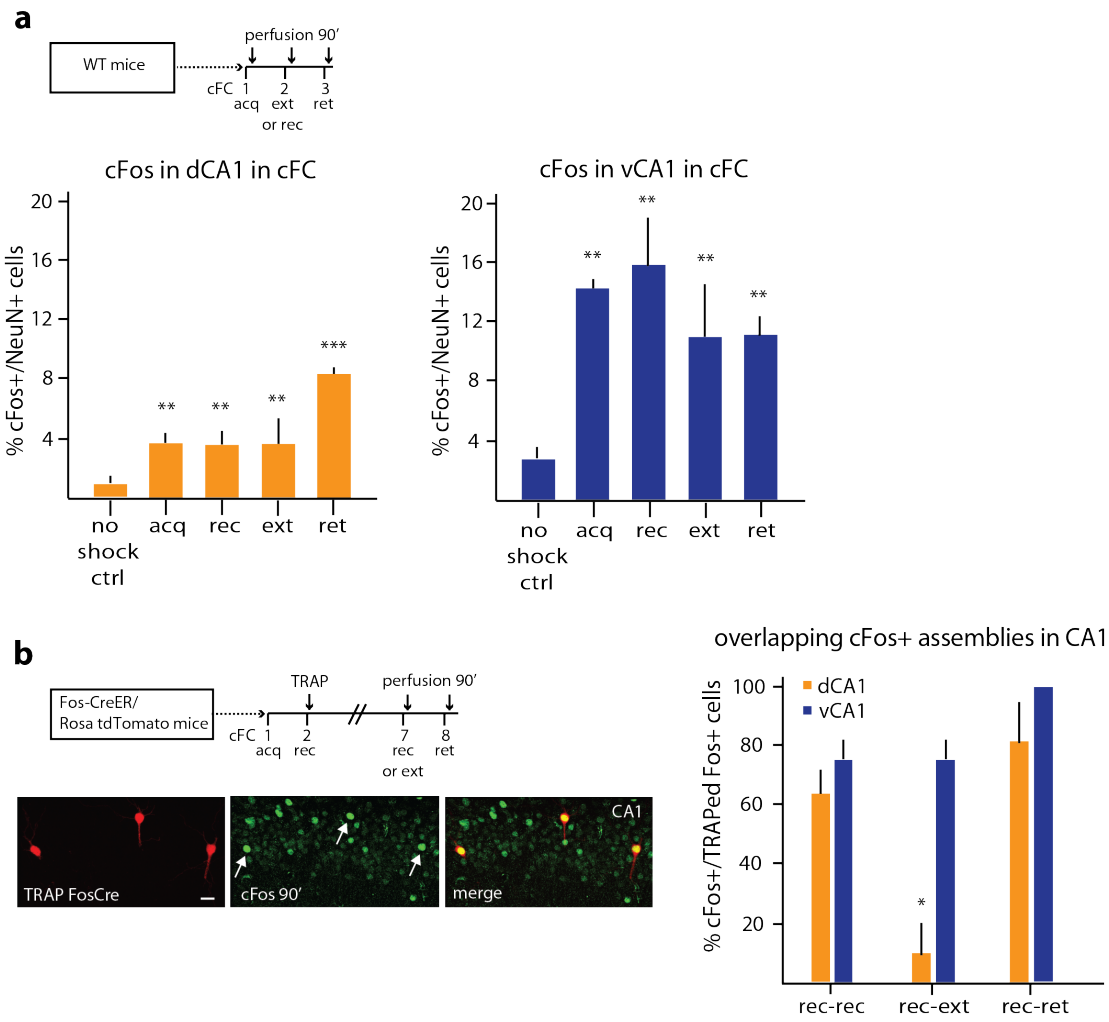

Figure 2.7. Localization of associated memory assemblies specifically in dH. (a) cFos induction in dorsal CA1 (left) and ventral CA1 (right) upon acquisition, recall, extinction and retention of extinction memory in cFC. (b) Overlapping Fos assemblies in cFC and associated extinction memory with schematic of the experiment and example images of Fos+ neurons TRAPed at recall and cFos+ immunoreactivity (left). Arrows indicate cFos+ immunoreactivity/TRAP double-labeled cells. Comparison of cFos+/TRAP double-labeled cells in second recall, extinction or retention of extinction (right), revealed overlap in fear- and extinction-induced Fos+ assemblies in vCA1, but no overlap in dCA1. Bar: 20μm. N=3-4 each. *p<0.05, **p<0.01, ***p<0.001.

recall and extinction robustly elevated contents of cFos-expressing cells, which further increased upon retention of extinction.

To determine whether similar or distinct cell assemblies are recruited in fear memory recall compared to extinction and retention of extinction, overlapping neuronal cFos expression was investigated. Fos-CreER mice underwent

contextual fear conditioning and cells active (cFos+) at recall (+24h) were genetically labeled via TRAPing (targeted recombination of active populations) (Guenthner, 2013). Subsequent fear memory recall, extinction and retention of extinction, respectively, re-induced cFos expression, which was visualized by immunohistochemistry. Analysis of overlapping assembly activity in vCA1 revealed that neurons active at fear memory recall were reactivated in a second recall, in extinction and retention of extinction, respectively (Fig.2.7b), suggesting that vH recruits the same cell assemblies in any recall related to the initial fear memory. By contrast, in dCA1, recall-to-recall cell assemblies overlapped, while recall-to-extinction assemblies showed very few reactivated cells, reflecting the recruitment of a new set of neurons in extinction compared to recall. Interestingly, comparing recall assemblies and those active in retention of extinction, again an overlap was detected. Of note, the overall cFos contents at retention were about doubled compared to fear memory recall and extinction, leading to the assumption that at retention both the fear memory assembly and the extinction assembly were reactivated in dCA1. In summary, the results clearly show that fear memory and extinction memory recruit distinct neuronal assemblies in dH, but not in vH, thereby providing further evidence that associated memories are specifically localized in dH within the hippocampus.

Complementary roles of dH and vH in declarative learning

In order to test whether the different memory components provided by dH and vH play complementary roles within the same task, we investigated the memory dependence after successive formation of two distinct associations to one fear memory. Therefore, mice underwent cFC acquisition, followed by the first object-to-fear memory association, based on a matching odor as linking cue. Next day, mice were exposed to the fear context in presence of the odor to recall the fear memory. Subsequently, a different object was presented with matching odor to allow for the formation of a second association to the same fear memory. Testing for recall of the association memory was performed each time in a novel context with a novel odor. Silencing dH at object-to-fear memory recall, suppressed freezing response to both objects, while vH

silencing left recall of both object-to-fear memory associations intact. Remarkably, subsequent test for fear memory revealed that only silencing vH decreased the freezing to the fear context, which is unaffected by dH silencing. This data shows that dH forms associations without interfering with the original memory to which the association was formed.

To further test whether the dH- and vH-dependent memory components can be used independently and flexibly, mice were trained in a modified version of MWM. In the standard version of the task, on the one hand, mice learn the concept of the task, which involved vH processing and on the other hand, they acquire a dH-dependent spatial reference memory. To test independency of the two memory components, on MWM day 8, when the spatial memory had been formed, the distant reference cues surrounding the MWM pool were replaced by a new set of cues. This new maze requires the acquisition of a new spatial map to reach the platform. Does the vH provide a memory component that has conceptualized the task independent of the previous dorsal reference memory and thus leads to an enhanced performance in the new maze? Indeed, control mice learn the new maze faster than the first maze and silencing vH slowed this new learning curve, whereas dH silenced mice performed like controls. Interestingly, the vH-silenced mice were impaired in choosing appropriate search strategies to reach the platform. Controls and dH-silenced mice preferred a global search strategy not involving the spatial reference cues (chaining is based on the distance of the platform from wall of the pool), while vH-silencing lead to large scale scanning. Although inefficient, vH-silenced mice did not switch strategies. This data further shows that vH plays a role in defining the concept of a task, to be used as basis for subsequent learning. The vH is able to extend previously acquired knowledge to a new context in a conceptually related task, a characteristic feature of declarative memories. Furthermore, the vH-dependent memory can be used independent of dH memories, previously acquired within the same task, showing that the hippocampal subdivisions have complementary roles within the formation of a memory of the same task.

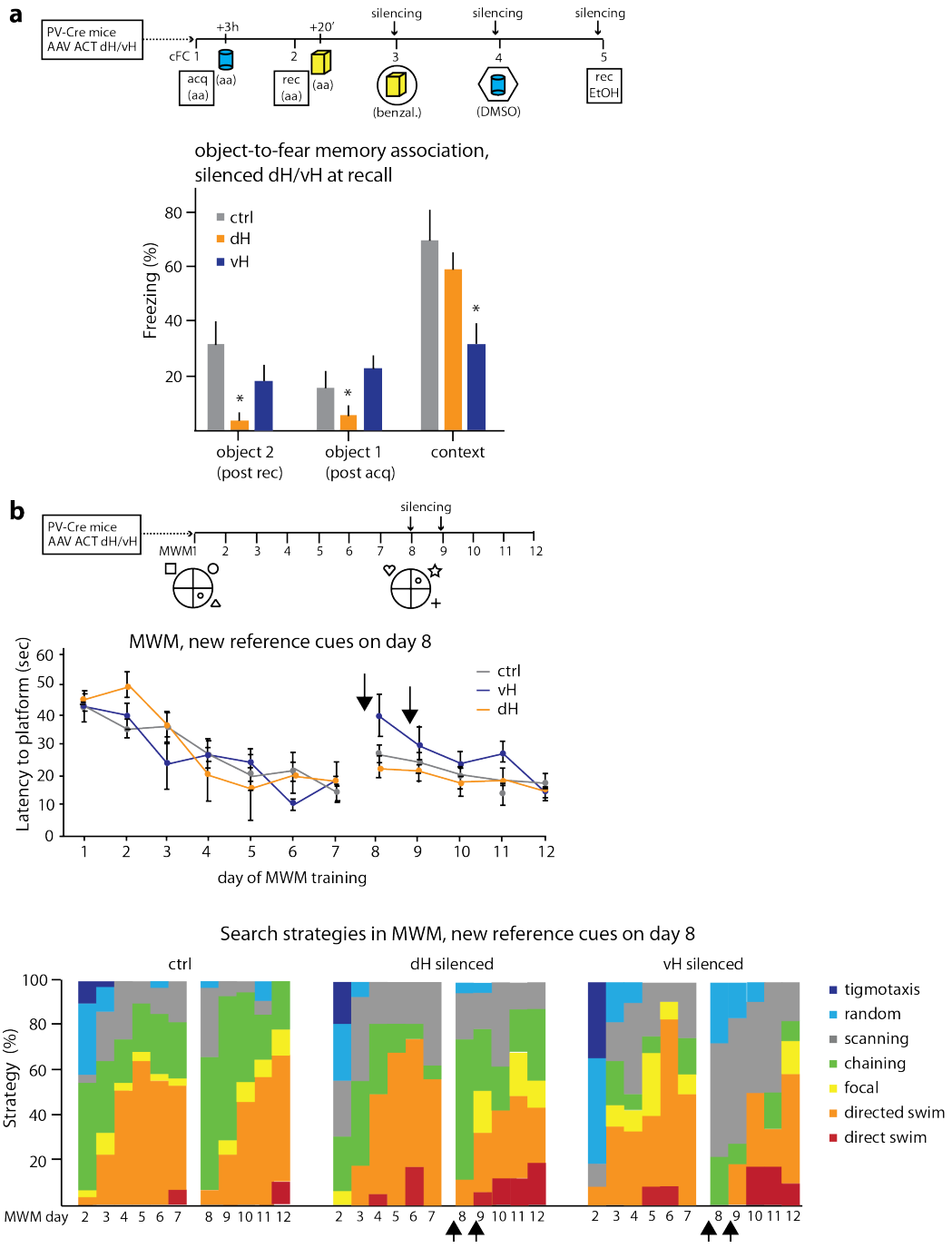

Figure 8. Complementary roles of dH and vH in declarative learning.
(a) Schematic of the experiment of successive association learning steps in cFC. Recall of each association memory is suppressed by dH silencing, but not vH. Contrarily, recall of the fear memory is only suppressed by vH silencing, but not dH. N=4-5 each. (b) MWM with new reference cues on day 8 with schematic of the experiment. Substitution of reference cues on day 8 first increases the latency to find the platform, followed by fast learning of the new position of the platform and appropriate application of search strategies

from global to more spatially defined strategies. Silencing vH on day 8 and 9 further increased latencies to platform and prevented adaptation to new appropriate learning strategies. Contrarily, dH silencing had no effect on latencies and strategies. Arrows indirect start of silencing. N=3-6 each. *$p<0.05$.

2.1. Supplementary results

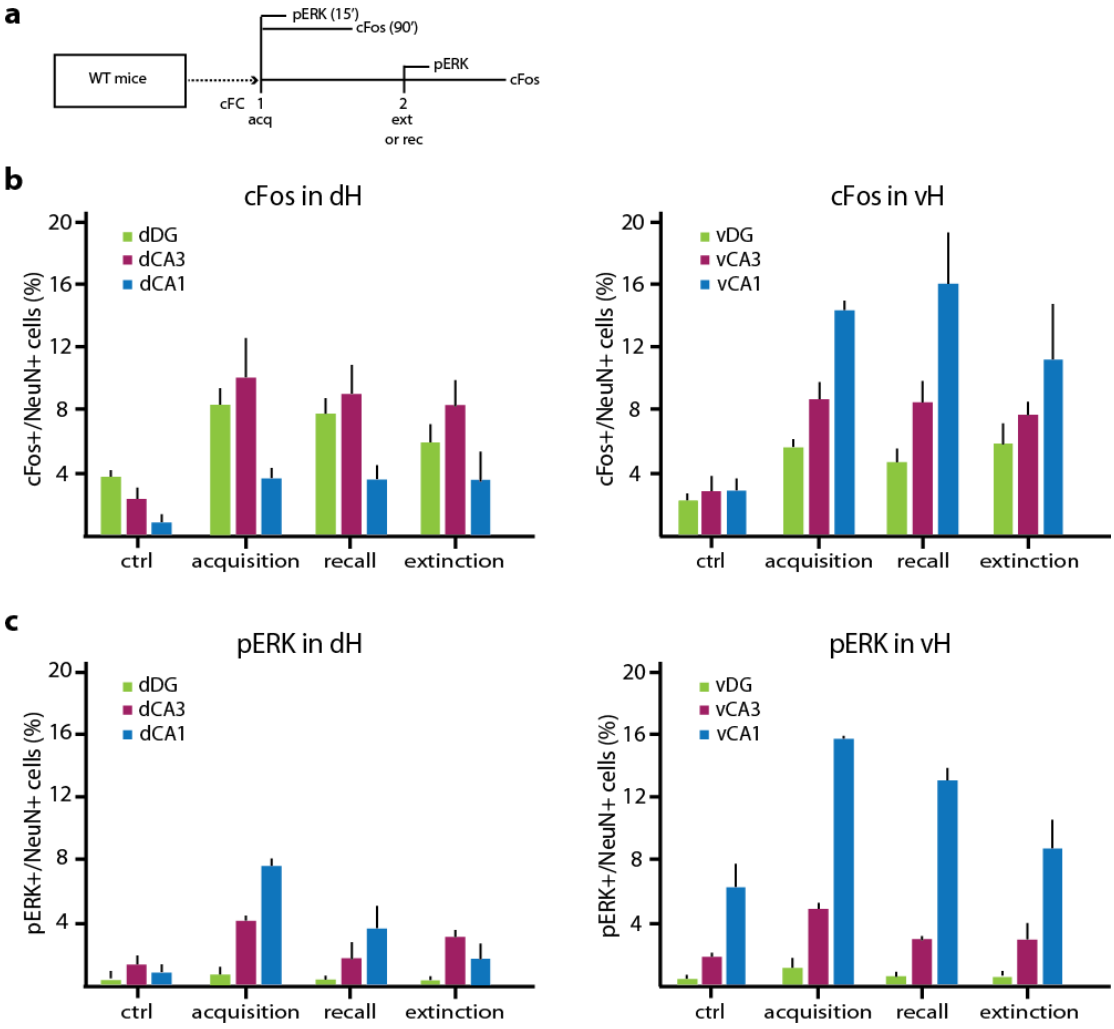

Figure S1. Distinct pattern of induction of cFos and pERK in hippocampal subdivisions along the transversal axis in dH and vH
(a) Schematic of the experiment, including cFC and perfusion for immunohistochemistry post acquisition, recall and extinction. (b) Induction of cFos+ contents (+90 min) in dH and vH, comparing the transversal hippocampal subdivisions DG, CA3 and CA1. In dH, predominant increase in cFos+ contents in DG and CA3, whereas vH showed predominant induction of cFos+ in CA3 and CA1. (c) In comparison, another activity marker, pERK (+15 min), revealed increased activity in the same subdivisions, predominantly CA3-CA1 in dH and vH. Distinct patterns of activity taken as hypothesis for specific role of cFos assemblies in dorsal DG (dDG) post acquisition, possibly during early window, when cFos is upregulated. N=3 each.

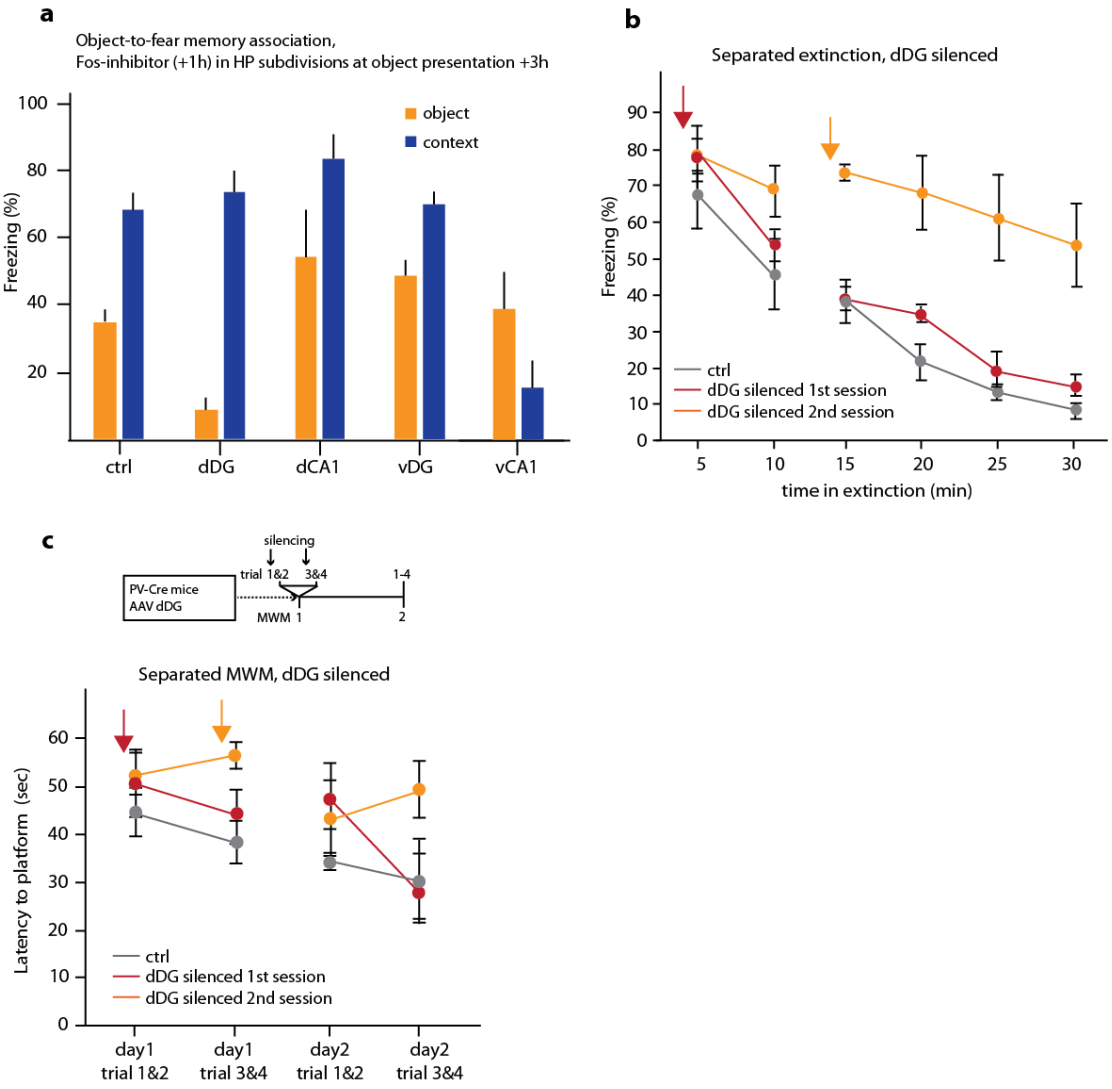

Figure S2. Requirement of cFos activity in dDG (but not vDG or CA1) for association learning (a) Inhibition of cFos activity at +1h in dDG, but not dCA1, vDG or vCA1 suppressed the formation of an object-to-fear memory association, leaving the fear memory intact. Only interfering with cFos activity in vCA1 prevented fear memory formation. (b) In a separated extinction protocol, silencing of dDG suppressed extinction learning (silencing during second session), while recall of the fear memory remained unaffected (silencing during first session). (c) Separated MWM protocol, consisting of 2 sessions of 2 trials each per day, spaced by 3h. Silencing dDG during second trial suppressed MWM learning on this day and day 2, whereas recall was not impaired (latency compared to controls in first session on day 2). Silencing dDG during first session on day 1 had no effect on learning or recall, indicating a specific function of dDG during the early window after memory acquisition, particularly in association learning. Arrows indicate start of silencing dDG. N=3-4 each.

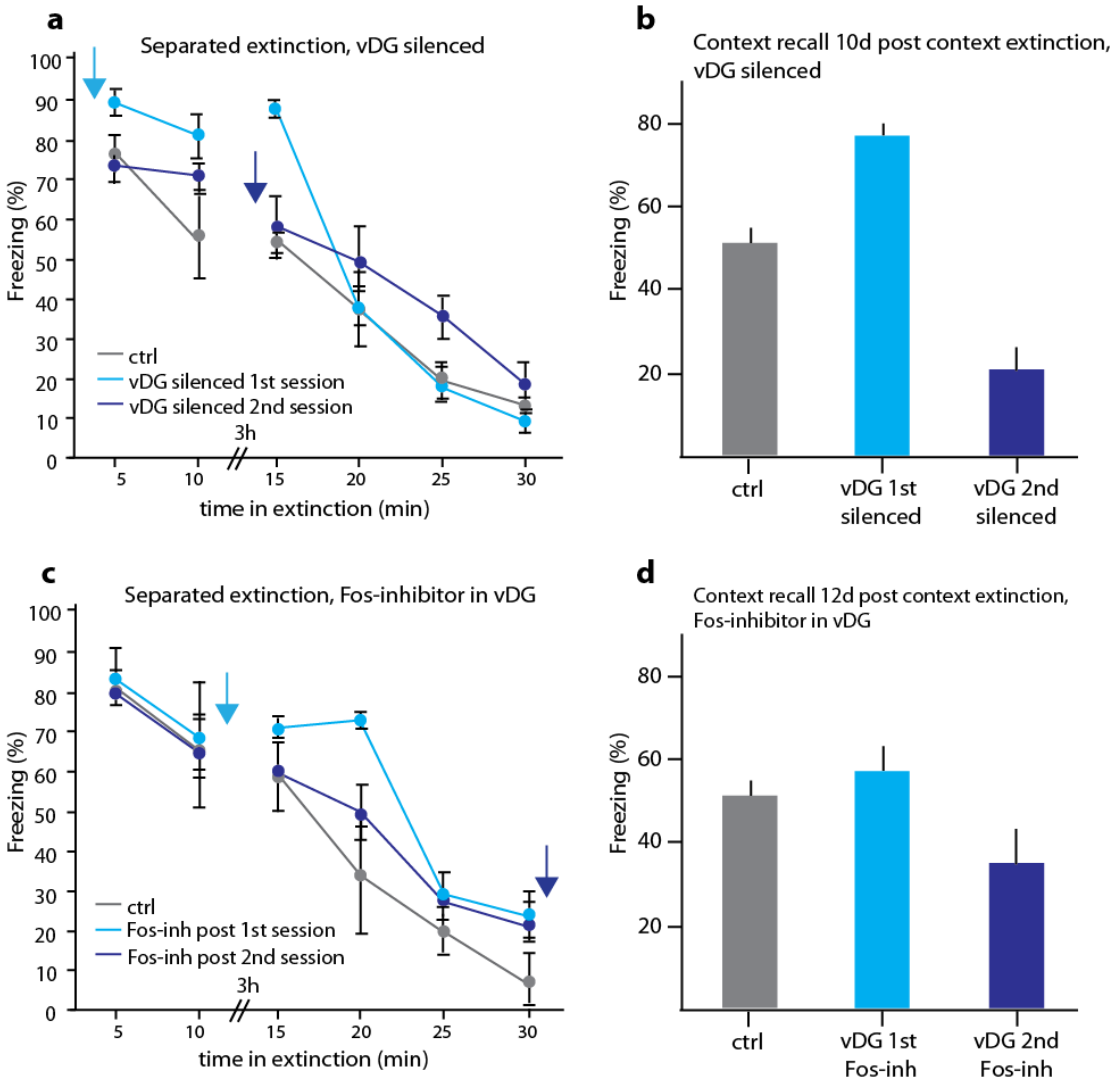

Figure S3. Requirement of vDG in task definition and onset of learning. (a) In a separated extinction protocol, vDG silencing during the first session slightly increased the freezing response. Silencing vDG during the second session delayed the onset of extinction learning, but the learning process remained comparable to controls, shown by equally low freezing responses after 20 min to 30 min of extinction in silenced vDG and control condition. (b) But however, when tested for recovery of the fear memory, mice with silenced vDG during first session froze like not having undergone extinction. Opposite, vDG silencing during second session prevented fear recovery. (c) Likewise, blocking Fos activity after first session in vDG delayed onset of new learning. Learning process seemed unaffected by suppressed cFos activity. (d) In subsequent test for fear memory recovery, Fos inhibition after first session resulted in stronger freezing, indicating that memory of task definition was not accessible during second session. Contrarily, Fos inhibition after second training decreased freezing in recovery test. Arrows indicate start of silencing and Fos-inhibitor application, respectively. N=3-4 each.

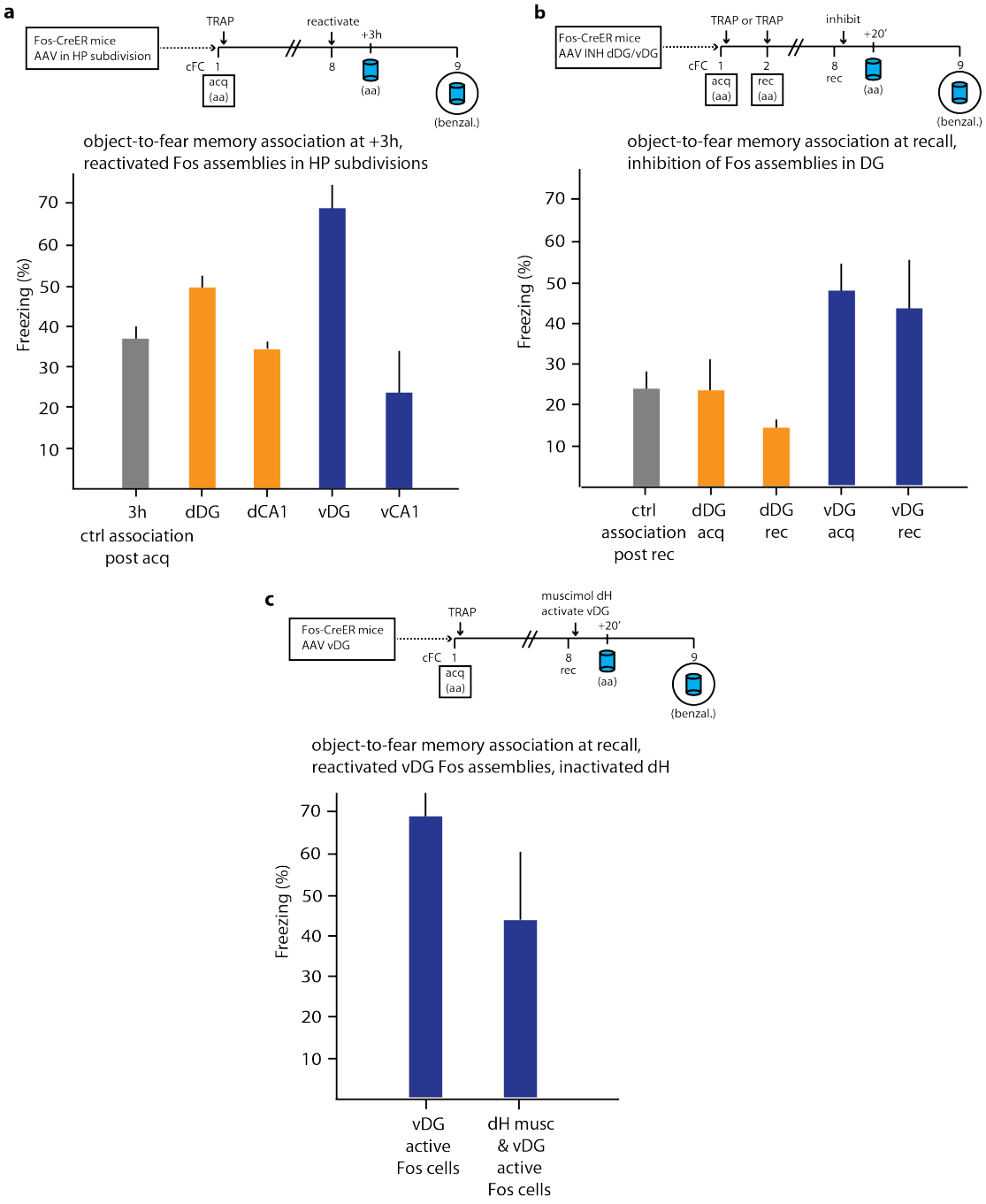

Figure S4. Fear memory-related Fos assemblies in DG required for object-to-fear memory association learning. (a) Reactivation of DG TRAPed Fos cells in association learning with schematic of the experiment. Reactivation of fear memory-related Fos assemblies in dDG and vDG, respectively, and object presentation at +3h lead to strong formation of an object-to-fear memory association. Reactivation of dCA1 and vCA1 Fos assemblies caused weaker association memory compared to DG reactivation (likely due to activation of the hippocampal-entorhinal loop, and thus increased activity in DG). (b) Inhibition of DG TRAPed Fos cells during association learning (to recalled fear memory) with schematic of the

experiment. Inactivation of fear memory-related Fos assemblies in dDG TRAPed at recall, but not acquisition, prevented the formation of an association of the object to recalled fear memory, showing requirement of specific Fos populations in dDG for association learning. In contrast, inactivation of vDG Fos assemblies did not impair the formation of association memory, but instead caused a comparably strong association memory. (c) Reactivation of vDG TRAPed Fos cells in association learning (to recalled fear memory) in the absence of dH activity with schematic of the experiment. Reactivation of fear memory-related vDG Fos assemblies was sufficient to form an object-to-fear memory association. N=3-4 each.

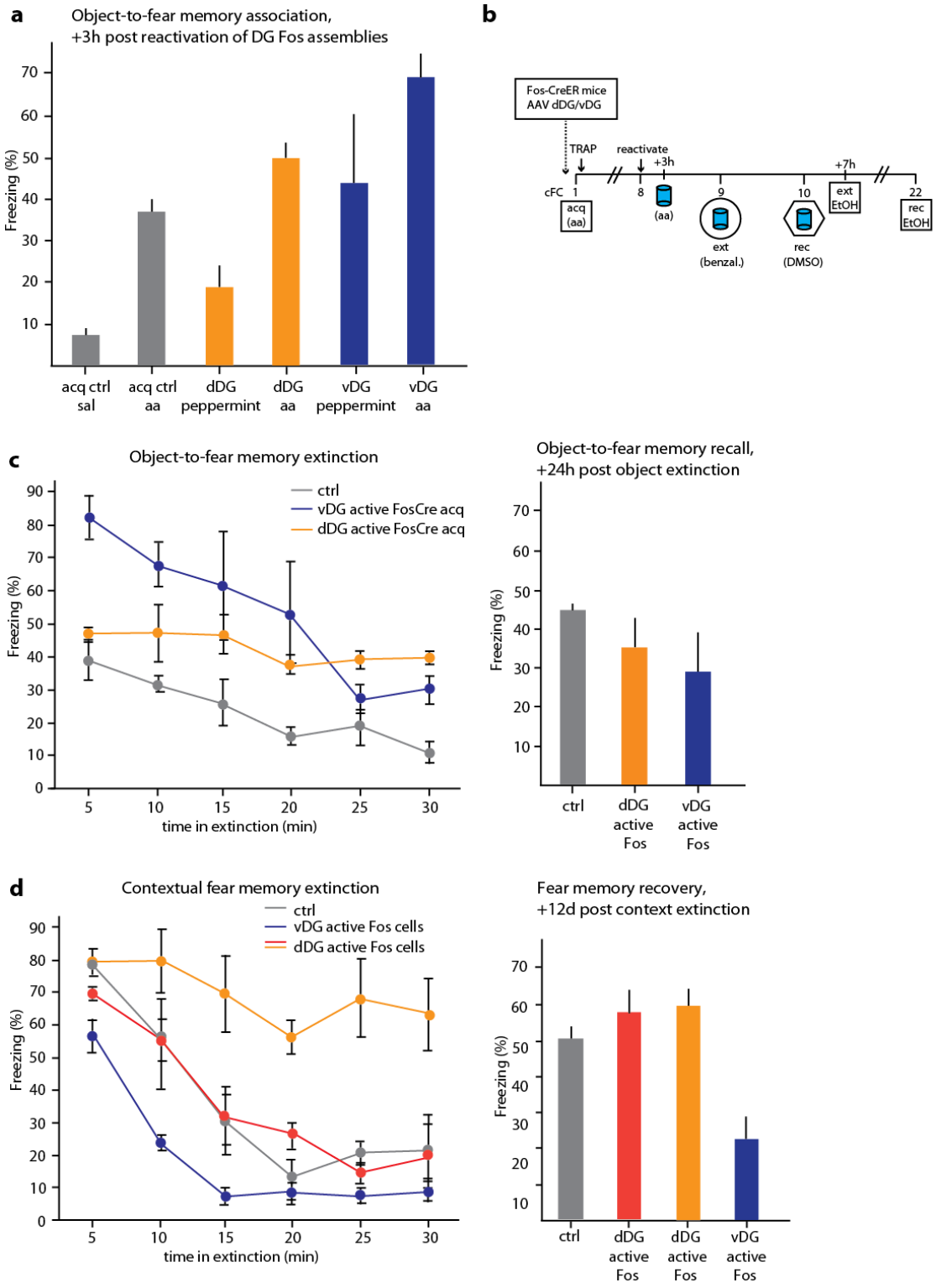

Figure S5. Differential role of Fos assemblies in dDG and vDG in forming associations and combining memories. Fos assemblies in dDG form associations with distinct memories for fear and object, vDG Fos assemblies combine object and fear memory into one memory. (a) Schematic of the experiment. Fos cells TRAPed at cFC acquisition trigger object-to-fear memory association, followed by elongated exposure to object (potential

extinction of the association memory) and subsequent contextual fear memory extinction. (b) Formation of object-to-fear memory association required odor as matching cue. In the absence of a matching odor, the formation of an association by reactivation of dDG Fos assemblies was impaired, while reactivation of Fos assemblies in vDG still caused strong association. (c) Association memories cannot be extinguished by prolonged object exposure. Although potentially adaptive response released freezing during 30 min object exposure, freezing response was strong next day. Association memory triggered by reactivation of dDG Fos assemblies followed the same pattern. In contrast, reactivation of vDG Fos assemblies caused very strong object-to-fear memory association, following the dynamics of contextual extinction learning. Next day, freezing to object was decreased to levels at the end of object extinction. (d) Subsequent context extinction of the (contextual) fear memory followed normal extinction dynamics in controls (comparable to controls without association learning, not shown here) and normal recovery of the fear memory at +12d post extinction. Fear memory extinction following dDG-triggered association learning was either comparable to controls or memory did not extinguish (possibly due to massive over-excitation of Fos assemblies in pharmacogenetic approach). Recovery of the freezing response to context was comparable to controls. In contrast, fear memory extinction following vDG-triggered association learning and extinction was accelerated and did show recovery of the fear memory, indicating that the previous object extinction has weakened the fear memory, thus the object and fear memory have become part of the same memory. N=3-4 each.

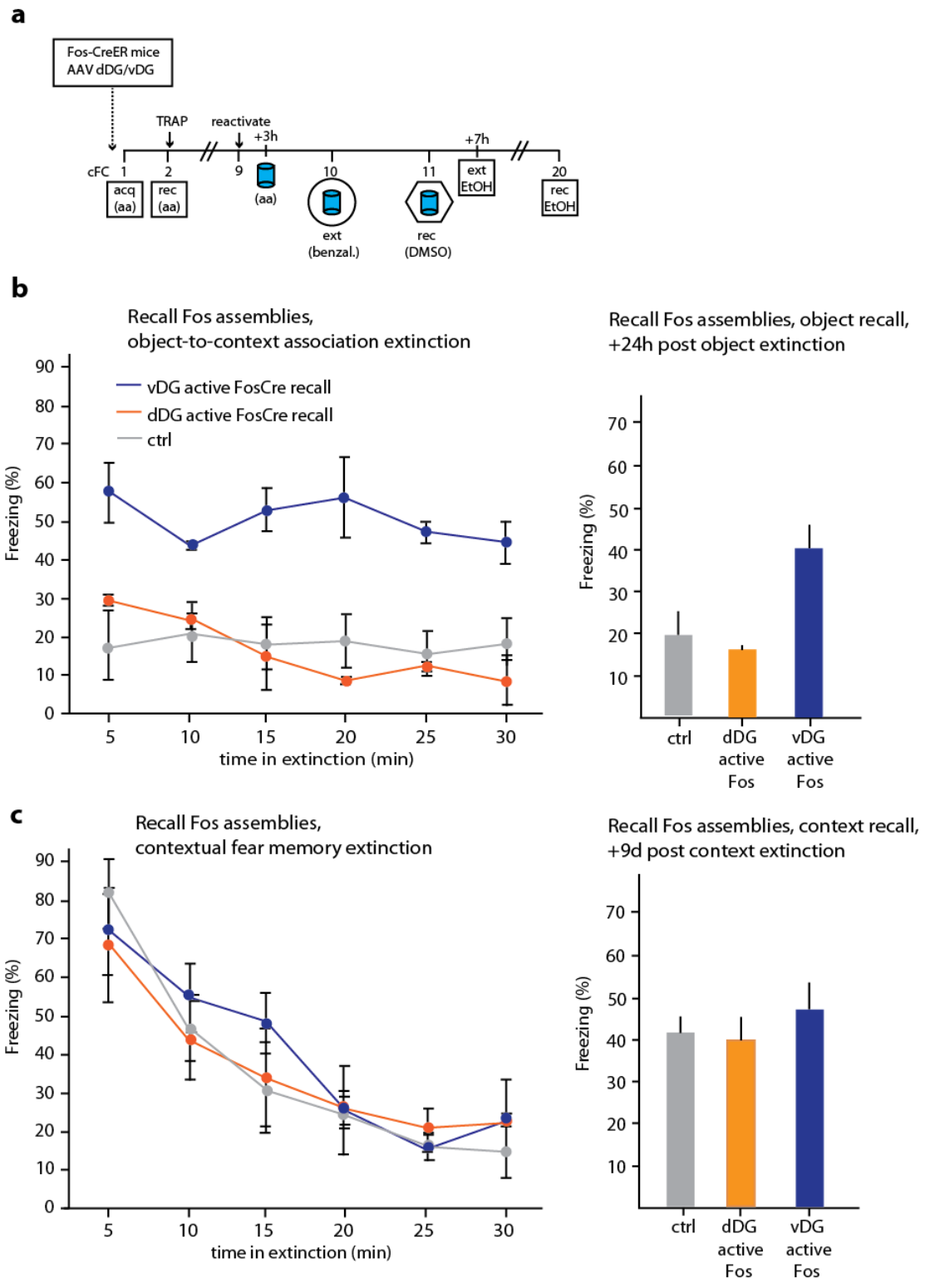

Figure S6. Recall Fos assemblies in dDG and vDG trigger formation of distinct memories for recalled and associated memories. (a) Schematic of the experiment, same as Figure 5, but TRAPing at cFC recall. (b) Object-to fear memory association triggered by reactivation dDG Fos assemblies TRAPed at recall is weak and non-extinguishable. Likewise, reactivation of

vDG Fos assemblies TRAPed at recall caused stronger, but non-extinguishable association memory. (c) Elongated exposure to the object did not affect the contextual extinction of the fear memory and its recovery, indicating that Fos assemblies in dDG and vDG at long-term recall form associations with distinct memories for the recalled memory and the subsequent association. N=3 each.

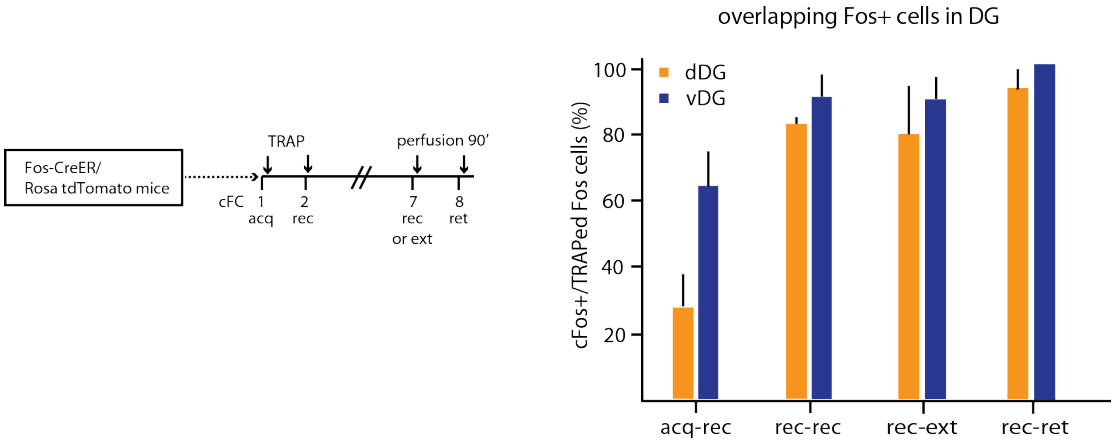

Figure S7. Overlapping Fos assemblies in DG in long-term memory and association learning. Schematic of the experiment. Comparison of cFos+/TRAP double-labeled cells in cFC recall to second recall, extinction or retention of extinction, revealed overlap in all conditions in dDG and vDG. Very few overlap detected only in dDG comparing cFC acquisition and recall. N= 3-4 each.

3. Discussion

Summary

The results presented in this thesis provide evidence for a novel functional diversification of hippocampal subdivisions along the dorsoventral axis. The vH subdivision identifies tasks, forms a memory of the task concept/context and is essential for recalling the memory from 6h post acquisition onwards. On the other hand, within the first 6h post acquisition, memories are recalled by dH. In this early time window the dH can add new details of the task, which are encoded as distinct task-associated memories and are recalled as such specifically through dH. Furthermore, it was shown that dH and vH contribute differently to memory processes from the acquisition of the task onwards. At acquisition, both subdivisions form separate memories essential for their respective functions at later time points of learning and for the formation of stable long-term memories. This was further confirmed by genetic targeting of learning-related neuronal populations, which demonstrated the localization of learning- and recall-related neuronal assemblies of the distinct memory components in dH and vH within the same task. Neuronal assemblies encoding associations to the task were specifically detected in dH, whereas vH contained neuronal assemblies representing the general task. Thus, this physical separation of neuronal memory assemblies provides a mechanism to recall previously acquired conceptual memories via vH and form associations to them in dH without interference of memories. At the same time, this system creates an opportunity to independently and flexibly use the distinct memory components according to task demands.

Sequential requirement of hippocampal subdivisions

This study provides a novel view on hippocampal learning, assigning different roles to the hippocampal subdivisions in learning processes. The first novel principle involves sequential recruitment of hippocampal subdivisions during recall of classical single-trial learning paradigms like cFC and FOR. Memories recalled in the first 6h after acquisition were dependent on the dH while recall

at all subsequent time points was ventral-dependent. Such temporal recruitment had not been noted by previous studies. On the contrary and according to the general view, dH was expected to be involved in cognitive processes in FOR and vH to play a role in strong emotional memories such as fear learning (Fanselow & Dong, 2010; Strange, 2014; Bannerman, 2014). Surprisingly, I found both hippocampal subdivisions essential in both learning tasks, depending on the time of memory retrieval but not on the valence of the task.

To better understand the logic behind recruitment of the hippocampal subdivisions I further concentrated on their specific functions at different time points. Note that the mechanism directing the switch from dH to vH dependence of retrieval has not been investigated here. Possible options could be addressed in future studies: 1) dH and vH are part of a network whose recruitment is decided by an extra-hippocampal brain area or 2) intra-hippocampal molecular mechanisms serve as timekeeper, defining the 5-6h window. How the switch of retrieval dependence might be linked to functional specifications in dH and vH will be discussed in detail in the following sections.

Requirement of vH in retrieval of long-term memories

This study provides evidence that vH is specifically involved in single-trial learning and recalling single-trial memory after 6h. In particular, this was shown to hold true for both fear learning and FOR, thereby challenging the current dominant concept of distinct roles of dorsal and ventral subdivision in cognitive and emotional responses, respectively (Fanselow and Dong, 2010). This study shows that both subdivisions are involved in cognitive processing as well as memories containing a strong emotional valence. To further elaborate, vH contribution to fear learning is in line with previous ideas, whereas its involvement in FOR was unexpected, as this incidental learning paradigm does not involve any emotional response. However, cFC and FOR, both being single-trial learning paradigms, share a common feature of novelty

detection. This can be defined as identification of task parameters (spanning novel context, novel task/event, encoding within limited time). In fact and highly interestingly, functional MRI data from mainly human studies proposed a functional segregation of anterior (vH) in novelty detection and posterior hippocampus (dH) in familiarity detection after long-term training (Strange, 1999; Duzel, 2003; Daselaar, 2006). These studies point out that both dH and vH might play a cognitive role within the same task and are in line with the presented data on vH defining the task (novelty). dH familiarity detection could reflect association learning in long-term training paradigms, see next section.

Furthermore, the presented data on vH reveal that vH does not only identify the task context and concept, but also recalls it (after 6h) in both, single–trial and incremental learning. In particular, MWM learning curves showed the requirement of vH in retrieval of the task concept every day prior to additional learning, which improves performance. In order to improve, mice apply search strategies, whereby the same strategy is used repeatedly across consecutive trials before more advanced strategies are applied (Ruediger, 2012). Such strategy selection depended on vH, suggesting that vH provides a conceptual framework of the task that is used to optimize strategies in order to solve the task. This vH function of task identification and conceptualization extends previous knowledge on vH, from detecting contexts and generalizing across multiple contexts (McKenzie, 2014; Kjelstrup, 2008; Buzsaki & Moser, 2008) to detecting new concepts of the task. This new idea on task conceptualization puts the vH in an ideal position for formation of semantic memories, as will be discussed later. The exact features of task conceptualization, e.g. context and emotional valence are yet to be determined. The requirement of vH for valence switch was demonstrated in extinction learning, in which silencing vH delayed or suppressed the onset of extinction. Such a switch maybe dependent on specific connectivity between vH and emotional response centers like amygdala nuclei (Pikkarainen, 1999; Pitkänen, 2000; Strange, 2014). Considering this as well as other specific connectivity of the vH, such as endocrine hypothalamic nuclei, one might also speculate that the identification or recognition of a context and task by vH

could trigger an emotional state in the brain and body to adjust behavioral responses to the identified situation. In addition to these considerations, this study suggests that the identification of the task by vH opens a window of 5-6h in which dH forms associations, thereby adding information to the task.

Requirement of dH in association memories

This study demonstrates that all new learning has a dH-dependent early window of 5-6h, in which information is associated to the initially vH-identified task. These association memories can vary in complexity, as shown here, from single items to forming a spatial map (MWM) or learning to stop freezing as seen in fear extinction. The presented findings are in good agreement with current ideas on dH function. The dH has long been thought to be the “cognitive part” of the hippocampus, particularly involved in spatial learning and formation of episodic memories. Separating extinction learning and MWM into recall and new learning phases demonstrated that dH is essential in learning new associated memories, but is not involved in recall of the task concept. Along similar lines, in extinction and MWM, recalling the newly associated memory only depended on dH, but not vH. Previous reports had suggested an exclusive role of dH in extinction memory as well as in remote spatial memory (Zelikowski, 2012; Corcoran & Maren, 2001). But remarkably, in the learning phase of both paradigms the performance over time once again showed a functional diversification of the hippocampal subdivisions. vH identified and recalled the task concept, while dH formed new associations leading to unfreezing during fear extinction or to the formation of a spatial map in MWM. The results further suggest that incremental learning is particularly dependent on the associated information. This also holds true in the case of associated memories that trigger a behavioral switch like in extinction (explaining why initially these learning paradigms were thought to be specifically depending on dH, but not vH).

Does the window of dH-dependent recall correspond to the window for formation of association memories? Indeed, using the object-to-context-fear

memory association, I see a direct functional link of both windows. A potential mechanism underlying this result might be found in neuronal excitability (Josselyn, 2015; Han, 2007; Yiu, 2014; Zhou, 2009), which directs memories into cell assemblies, and in the widely accepted view that encoding and retrieval share the same physical basis in form of memory assemblies. Thus memory recall within one brain area creates the possibility to physically link newly arriving information to the reactivated assembly and result in (partially) overlapping assemblies. According to current views, associative learning always requires a direct link, likely to create an overlap in the neuronal representations of associated memories. In agreement with the above, in this study, either context or odor provided a link for associating memories. Distinct contributions of odor, context and other cues to link memory representations still require further investigations. However, the element of timed activation of neuronal assemblies to form links between them has been reported before. Thus memories were proposed to have a higher likelihood to share neuronal assemblies, when they were encoded close in time, as demonstrated for assemblies in the amygdala (Cai, 2016). The observed timings are matching well with the early window for dH memory dependence presented here in this study. However, in this regard, it needs to be mentioned that the formation of associated memories might be specifically attributable to dH concerning contextual associations, due to the specific role of hippocampus in general in contextual memories. Nonetheless, similar functional diversifications might exist in other brain areas, for example in striatum in habitual learning and motor learning systems. A potential mechanism by which associated memories could be formed in the hippocampus might include DG activity to shape neuronal assemblies. This possibility will be discussed briefly below.

Separate learning processes in dH and vH

Silencing experiments have shown that both dH and vH contribute to learning in different ways from the onset of the task itself. Both dH and vH are required during the acquisition of the task, here cFC, to form a long-lasting stable memory. Furthermore, these memory processes starting at acquisition are

essential for the function of each subdivision in subsequent learning processes. In more detail, first, in the absence of vH, dH encodes a memory, which can be retrieved at long-term recall. Notably, this dH memory is less stable, as shown by accelerated extinction next day and by reduced freezing at 10d recall. Surprisingly, silencing vH twice, at acquisition and again at recall, shows that the dH memory is not transferred to or recruited by vH for long-term recall. At the same time, in the absence of vH at acquisition and recall, dH-acquired fear memories cannot be extinguished. This finding is consistent with the interpretation that vH is essential to identify extinction as a novel task with altered valence. Without this novelty detection the fear memory is recalled and persists.

In related experiments, dH was silenced at acquisition of cFC, leaving vH unaffected. This revealed that the dH subdivision encodes a fear memory trace, which is essential for subsequent formation of an object-to-fear memory association as well as for fear extinction learning. This result not only confirms that dH is required for association learning, but also suggests a requirement for localizing representations of the original memory and the according association to the same brain area (dH). Associated memories might be characterized by an overlap of neuronal assemblies – thus a representation of the fear memory and the object or extinction memory must both be localized in dH to allow for formation of an overlap, hence to form an association. This finding, together with the proposal of allocation of memories to cell populations by neuronal excitability might be underlying the functional link of dH-dependency of memory retrieval and formation of associations in the early 5-6h window. Increased neuronal activity has been shown to direct memories into those active populations (Yiu, 2014; Zhou, 2009). Applying this principle to this study, recalling an event by dH during the early window (in context or via cue) could increase excitability within the neuronal representation of the recalled memory, creating a physical basis to form overlapping ensembles by directing the newly encoded association into the initial memory.

Recall of associated memories

This study shows that associated memories are localized and recalled from dH, whereas the general memory containing the task concept localizes to vH for recall. First, silencing experiments demonstrated specific dH requirement for recall of associated memories. This holds true for single items associated to fear memory as well as extinction memory. The initially acquired fear memory remained vH-dependent even after additional associations were formed. Remarkably, in MWM the spatial reference memory test depended only on dH, indicating that in incremental learning additional details/ associated information are more important for task performance than the general task concept.

In a second approach, memory localization was defined by immuno-histochemical and genetic targeting of learning-related cFos expression in neuronal assemblies. In dH, distinct but overlapping neuronal populations were active at recall and extinction of a fear memory, demonstrating the formation of an associated memory. Interestingly, both ensembles have the same size but contain mainly non-overlapping neurons with only a 20% overlap, similar to previous findings (Cai, 2016). By contrast, seemingly both fear and extinction ensembles were activated at recall of extinction, thereby possibly creating an opportunity to selectively recruit each of the ensembles for behavioral output and to further modify the ensembles or create further associations.

In the case of vH, the same neuronal ensembles were active throughout the whole learning task, spanning fear learning, formation of an association, extinction learning and retention. This result provides further evidence that vH provides a general identification of the task (and generalizes across the whole task). Recognition of extinction as a novel task could not be detected at a cellular level since active assemblies were highly similar with 80% overlap. How this switch in valence is detected and accomplished by vH is an interesting question for future studies.

Novel concept of dH and vH in declarative learning

vH was demonstrated to identify a task, to which dH can then associate new memories within a limited time window of 6h. The retrieval of the general task (context and concept of the task) remains dependent on vH, whereas task-associated memories are recalled via dH. This new model of subdivision functionalities is in contrast with previous leading ideas on cognitive versus emotional processing in dH and vH, respectively (Fanselow & Dong, 2010; Bannerman, 2014). On the other hand, these results are in accordance with other ideas, for example those defining dH and vH as processing details versus gist-like memories (Proppenk, 2013). With regards to functional specialization of dH, all literature including the presented study converge onto cognitive processing and in particular in the formation of associative/episodic memories, as described above. Concerning vH function, less convergence exists. This study promotes notions, which assign a cognitive function to vH, e.g. detecting novelty and generalization across contexts. The presented results furthermore support the hypothesis that vH memory processing is ideally suited to underlie semantic/relational/integrative memories (Eichenbaum, 1999; McKenzie, 2014), especially considering the fact that dH and vH share the same trisynaptic pathway, including the CA3 associative network. The idea would be that semantic memories link representations in vH in a mechanism resembling how dH links established episodic/associative memories, but on a different scale. Previous studies have already shown that vH can generalize across contexts, shown by combination of different contexts within one task paradigm, whereby memories acquired in one context can be used in another context via vH function. As an underlying mechanism, integration across context might be achieved by different place cell properties along the dorsoventral axis (Kjelstrup, 2008). Small place field sizes in dH might allow for rapid encoding of detailed environmental representation and many episodes. Large place fields in vH could serve as integrators, spanning activity across many such episodes within one large place field. Furthermore, the hippocampus has been shown to be implicated in switching valence of a context memory (Redondo, 2014). It is therefore tempting to speculate that novel task detection in the vH, or assigning novel

valence to the task opens a network state (via connectivity to emotional centers like amygdala and/or hypothalamic nuclei), in which memories can be associated to better define tasks and adjust behavioral output (new learning within task).

This study further extends the ideas outlined above by showing that vH identifies and recalls not only the context but also the general concept of a task. Hence, I propose that vH memory processing is ideally suited to form higher-order memory representations to create semantic memories which are long-term memories of facts, ideas and concepts, accumulated to result in general knowledge that can be retrieved consciously (McRae, 2013). Semantic memories might have evolved from linking and integrating episodes until seemingly out of context recruitment is also possible (Buzsaki & Moser, 2013). However, memories are never pure context-free facts, and instead involve formation of higher-order representations. Thereby, information might be rather linked by logic instead of context. One might therefore speculate that the conceptualization of the task in vH could serve integrating dH-acquired associations in a later time point of consolidation, possibly the second window of consolidation at +12h (Karunakaran, 2016; Katche, 2010).

An indication supporting this hypothesis comes from the MWM data presented here. In MWM, recalling the task identity depended on vH, whereas the spatial map was formed as an associated trace in dH. Furthermore, during daily learning sessions animals applied different search strategies to find the location of the hidden platform (Ruediger, 2012). The selection of appropriate strategies was dependent only on vH, but not on dH. This could be an output of higher-order memory connections, linking the spatial map and possible actions (episodes of previous behavior) to the task concept. Notably, in MWM the effect of vH silencing on performance was stronger on the day following silencing than during silencing itself. This suggests that in the absence of vH, there is a dysfunction in the integration of newly acquired spatial details to the task concept.

The hypothesis on vH function in semantic memory formation is furthermore supported by a potential mechanism involving vDG Fos assembly activity to drive memories into the same representations (supplementary results and section in discussion below). To clarify the vH contribution to semantic memories, future studies could use transitive learning paradigms, where the formation of indirect associations can be addressed. It has been shown that learning a logical sequence of events (if event A causes B and B causes C, then A causes C) depends on the hippocampus (Bunsey, 1996). It would be interesting to test whether the vH is specifically required for such a conceptual linking of memories (A to C) into a higher-order representation.

Advantage of distributed functionality across dorsoventral axis

The findings in this thesis suggest a general mechanism by which the hippocampus is optimized to acquire complex memories, starting from simple tasks, which can then elaborate and gain complexity. Sequential recruitment might be a possible mechanism to separate processing of information at different levels. This might involve first establishing general concepts of the task, then forming associations to the task, which can later be generalized across tasks and concepts (possibly into semantic memories).

Associative memories formed in dH allow for rapid encoding of many overlapping but distinct cells assemblies. These assemblies contain detailed information and many episodes of behavioral possibilities, which can be retrieved separately to guide behavioral response. Initially, encoding and recall require a link to the context/task, in which the memory is embedded. While the general context and concept of the task is stored in vH, associated memories are formed in dH. Such dissociation permits memory processing to occur independently in the two hippocampal subdivisions. This further allows for recruitment of dH or vH memories separately, resulting in the following possibilities: a) dH traces can be formed, allowing for many different associations within one task, creating the possibility of fine tuning or switching behavior without interfering with the general concept of the task stored in vH

and b) the vH or dH memory can be re-used independently in similar tasks/concepts/contexts and c) higher-order representations can be formed (linking concepts or details independent of each other).

The first possibility has been tested by forming consecutive object-to-fear/context associations, which are all acquired and recalled from dH without interfering with the fear memory in vH. The second notion was investigated in MWM. Here, the initially learned vH-dependent concept of the task can be re-used to learn a new maze faster. Behavioral performance is optimized, since relatively short latencies to platform are already achieved by adjusting search strategies, without having formed a precise map of environment. Whether spatial map is learned faster in the second maze has not been investigated so far, but provides an interesting paradigm to further disentangle dH and vH contributions in learning and memory. The idea of vH playing a role in the formation of higher-order representations has not been investigated here and provides an interesting topic for future studies.

Potential mechanism to form associated memories

In a supplementary part, I have started to investigate the function of the transversal hippocampal axis, in particular the dentate gyrus in association learning. These preliminary results allow a first insight into a possible mechanism how dentate gyrus activity might shape hippocampal memory assemblies to form associations. In line with dH and vH functional segregation, I have found that dDG learning-related Fos assemblies direct the formation of associations comprising several features typical for association learning. This includes the necessity of linking cues, not extinguishable association memories and no interference with the memory to which the association was created. I further found that reactivation of vDG Fos assemblies, which were active at memory acquisition combines memories with each other into one memory representation, potentially to create one representation of the task concept. By contrast, reactivation of vDG Fos assemblies, which were active at long-term recall of a memory triggers the

formation of distinct associated memories. These findings lead to the exciting hypothesis that the DG might serve as pattern generator to shape neuronal assemblies in hippocampal CA3 and CA1, in order to create overlaps in representation for associations or combinations of memories. Taking together both parts of my thesis, this could be a possible mechanism underlying the formation of episodic memories in dH and semantic memories in vH by conceptually linking memory assemblies, hence forming higher-order memory representations. This is a completely new concept on dentate gyrus function, which will require further investigations for additional support and further refinement.

Conclusion and outlook

As a result of this thesis I am proposing a novel functional segregation of the hippocampal subdivisions in which vH identifies a task, its context and concept and is essential to recall these features after 6h. Within the first 6h after initial memory acquisition, dH recalls the memories and learns additional details of the task at different levels of complexity. It stores and recalls this additional associated information as distinct task-associated memory traces. This defines the hippocampal subdivisions as specialized for associated (episodic) memories (dH) versus conceptual memories and potentially integration of memory traces into semantic memories (vH). Accordingly, dH and vH form complementary memory components of the same task, which can be used independently and flexibly as a function of task demands.

The flexible use of memory representations is a fundamental process, about which hardly anything is known to date. Future studies are therefore required to understand how memory representations are shaped, linked to each other and combined into higher-order representations. A very exciting open question is the mechanism by which semantic memories are created and which precise role the vH plays in this process. Future studies might also address mechanisms, which direct the memory dependence on dH and vH, particularly what triggers the switch of memory dependence at 6h post

acquisition. It would also be interesting to determine whether the functional segregation shown for vH and dH in task identification and additional learning also exists in other systems, such as habitual learning in striatum.

4. Material and Methods

Mice

PV-Cre mice were from Jackson laboratories (129P2-Pvalbtm1 (cre)Arbr/J), Fos-CreER (B6.129(Cg)-Fostm1.1 (Cre/ERT2)Luo/J) and Rosa-td-Tomato reporter mice (B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J) were a kind gift from S. Arber (Friedrich Miescher Institute). All animal procedures were approved and performed in accordance with the Veterinary Department of the Kanton Basel-Stadt.

Behavioral procedures

All behavioral experiments were carried out with male mice that were 2-3 months old at onset of the experiment.

Single-trial learning paradigms:
Contextual fear conditioning (cFC) and familiar object recognition (FOR) were performed as described in Donato, 2013. Briefly, in cFC mice underwent an acquisition session of 5 min, in which they first freely explored the fear conditioning chamber for 2.5 min, then received 5 shocks (each 1 s duration and 0.8 mA, inter-shock intervals of 30 s). Freezing was tested for 5 min in the fear conditioning chamber.
In familiar object recognition, mice explored two identical objects in an open chamber for 10 min. To test object recognition, mice were placed back into the context for 5 min, but now one of the objects was replaced by a new one. Exploration of the novel and the familiar object was scored and discrimination indices were calculated as $(t_{novel} - t_{familiar})/(t_{novel} + t_{familiar})$. Behavioral performance was tested at time points as indicated in the results.

Contextual fear memory extinction:
For extinction of contextual fear memory, mice were exposed to the fear context for 30 min without shocks, in spaced extinction the protocol was split into a first 10 min session and a second 20 min session spaced by 3 h. The freezing responses were analyzed in 6 consecutive bins of 5 min each.

Retention of extinction memory was tested 24 h after extinction. Therefore, mice were placed back into the fear conditioning/extinction chamber for 5 min. Recovery of fear memory was tested at time points indicated in results, mainly around 10 d post extinction by placing the mice back into the fear conditioning chamber for 5 min.

Morris water maze:

Morris water maze experiments were carried out as described in Ruediger, 2012. The maze consists of a pool (140 cm in diameter), which is filled with opaque water. An escape platform (10 cm in diameter) is above (day 1) or hidden below the surface (from day 2 onwards) for visible and invisible trials, respectively. Mice were trained to find the platform in four trials per day, each lasting for maximum 1 min, spaced by 5 min. Three different distal cues placed around the pool served as reference for spatial orientation. On day 1, during visible platform training, the platform was shown to the mice if they were not able to find it. From the second day onwards, the platform was kept invisible and located in the quadrant opposite to day 1. Latency to the platform was scored. In probe trials, the platform was removed and time spent in the quadrant previously containing the platform was measured. Data collection and analysis was performed using Viewer2 software (Biobserve, Bonn, Germany). Search strategies were analyzed as described in Ruediger, 2012. The following types of strategies were distinguished: tigmotaxis, random swim, scanning, chaining, focal search, directed and direct swim. For analysis of strategy blocks on day 3, all strategy blocks lasting from day 2 to 3, those on day 3 and those lasting from day 3 to 4 were considered.

Object associations:

For object association in cFC, mice were fear conditioned in the presence of an odor, then 3 h later an unrelated object wiped with the matching odor was introduced in the home cage of the animal. Freezing to the object was tested the next day without the odor in a novel context. In a related experiment, the protocol was extended to a second association, in which another object was presented 20 min after fear memory recall, all done with an odor matching to the fear conditioning acquisition. For testing the object-to-fear/context

associations, mice were first presented 24 h later to the object introduced after recall and then again 24 h later to the object introduced after cFC acquisition. During testing, each object presentation was performed in a novel context with a novel odor.
For object binding in FOR, a pair of objects A was introduced to mice in the FOR context, then separated by 3 h animals explored a second pair of objects B in the same context. Object memory of both objects was tested at 24 h by showing one of each A and B together, again in the same context.

Combined extinction paradigm:
To study DG function in association learning, object association and extinction experiments were combined. First, animals underwent fear conditioning in the presence of an odor, followed by TRAPing of Fos+ assemblies using rAAV9-CAG-flox-PSAM(L41F,Y116F)5HT3-WPRE. Animals were left for construct expression in the home cage for seven days. Fos+ assemblies were reactivated by i.p. injection of the ligand and an object was presented with matching odor 3 h later. Next day, the object was presented in a novel context with novel odor for 30 min, for possible extinction of the object-fear memory association. Next day, mice first tested for object-fear association in another novel context and another novel odor. Seven hours later, they were re-exposed to the fear context for 30 min to test for context extinction. Recovery of the fear memory was tested for 5 min in the fear context 10d-12d after extinction.

Stereotaxis surgery

Surgeries were performed under aseptic conditions using a small animal stereotaxic instrument (David Kopf Instruments). For virus delivery glass pipettes connected to a picospritzer (Parker Hannifin Corporation) were used. To target hippocampal subdivisions separately and covering whole dH and vH, respectively, we targeted two injection sites per subdivision with the following coordinates relative to bregma: dH (anteroposterior (AP) -1.7 mm, mediolateral (ML) $\pm$1.8 mm, dorsoventral (DV, relative to dura) -2.0 mm and

-1.6 mm); vH (AP -3.0 mm, ML ±3.1 mm, DV -3.5 mm and -3.0 mm). To target a specific subpart, DG and CA1, respectively, one injection site was used per condition: dDG (AP -1.7 mm, ML ±1.25 mm, DV -2.0 mm), dCA1 (AP -1.7 mm, ML ML ±1.8 mm, DV -1.6 mm), vDG (AP -3.0 mm, ML ML ±2.3 mm, DV -2.0 mm) and vCA1 (AP -3.1 mm, ML ±3.5 mm, DV -2.6 mm). All injections into hippocampal subdivisions were bilateral. Viral suspensions were delivered at the rate of 50 nl/min to a final volume of ~200 nl/injection site (if two injection sites) or ~300 nl/injection site (if single injection site). The pipette was kept in place for 5-10 min after injection to allow for diffusion and avoid backflow or spreading of the virus outside the target area.
For drug delivery, cannula guides (plastics one, 26G) were inserted according to above described coordinates. Mice were kept in home cages for minimum seven days to allow for recovery from the surgical procedure. During subsequent behavior tests, mice were anesthetized for drug delivery and drugs were injected at a rate of 100 nl/min to a total volume of ~300 nl/injection site. All injections were paired with saline-injected control animals to account for any effect due to the surgical procedure.

Pharmacology in vivo

Drugs were used as follows: MG132 (100 μM in 1% DMSO, Calbiochem, proteasome inhibitor), T-5224 (1 mg/side in 20% PVP and 10% DMSO, MedChemExpress, inhibitor of cFos-AP1 TF complex; Aikawa, 2008) and muscimol (0.2 μg/μl in saline, Tocris, $GABA_A$ receptor agonist).

Pharmacogenetics in vivo

For local silencing by activation of PV interneurons, rAAV9-CAG-flox-PSAM(L41F,Y116F)5HT3-WPRE was injected bilaterally into dH or vH of PV-Cre mice or PV-Cre/Rosa-td-Tomato mice (Magnus et al., 2011; Donato et al., 2013; Karunakaran et al., 2016). Mice were kept under control conditions for

8-10 days to allow for transgene expression before onset of the behavior procedure. PSAM agonist PSEM308 was injected i.p. at 5 mg/kg of animal weight at various time points during behavior experiments as indicated in results, each time 20-30 min before behavior onset.

Genetic targeting of active populations

For double labeling of potential memory assemblies, TRAPing (targeted recombination in active populations) was performed using Fos-CreER/tdTomato mice. Mice which had been fear conditioned underwent a recall session, directly followed by i.p. injection of 4-Hydroxytamoxifen to label neurons activated by behavior. Mice were kept under control conditions for 5-7 days to allow for construct expression, then the behavior protocol continued. Subsequently, mice were perfused and processed for immunohistochemistry.

To artificially reactivate learning-related Fos assemblies, rAAV9-CAG-flox-PSAM(L41F,Y116F)5HT3-WPRE was bilaterally injected into DG and CA1, respectively, of either dorsal or ventral hippocampus in Fos-CreER mice. Mice underwent cFC acquisition, followed by i.p. injection of 4-Hydroxytamoxifen to label active neurons. Mice were kept under control conditions minimum 7 days to allow for construct expression. Mice were then used for behavioral procedures as indicated in the results.

Immunohistochemistry

Mice were transcardially perfused with 4% PFA in PBS (pH7.4) either 90 min (for cFos analysis) or 15 min (for pERK analysis) after the end of the behavioral protocol. Brains were collected and kept for overnight fixation in 4% PFA at 4 °C, followed by another overnight incubation in 30% sucrose, also at 4 °C to prepare the tissue for cryo-sectioning. For immuno-histochemistry, 40 mm coronal sections were cut at the cryostat. The following

primary antibodies and respective concentrations were used: rabbit anti-cFos (Santa Cruz), 1:7000; rabbit anti-pERK (Cell signaling), 1:500; mouse anti-NeuN (Millipore), 1:1000. Bungarotoxin-488 or -555 (Molecular Probes), 1:500, was used to label Cre-dependent expression of rAAV9-CAG-flox-PSAM(L41F,Y116F)5HT3-WPRE. The standard procedure for immunostainings was as follows: sections were blocked for one hour at room temperature with 10% BSA in PBS-T (0.3% Triton X-100 in PBS). Incubation in primary antibody was done overnight in the antibody solution containing 3% BSA and 0.3% PBS-T. After three washing steps, sections were incubated in secondary antibody solution (also in 3% BSA and 0.3% PBS-T, Alexa Flour secondary antibodies, 1:500) at room temperature for two hours. After another three washing steps, sections were mounted in Prolong Gold antifade reagent (Molecular probes) and kept at 4 °C until imaging.

Imaging

Images were taken at 40x using a Zeiss LSM 700 confocal microscope equipped with ZEN2010 (Zeiss). For intensity analysis, all samples of one experimental set were processed in parallel, using the same imaging settings. Image analysis was performed using the Imaris 7.0.0 software (Bitplane AG, expected radius 10 mm). XUV tools served for stitching images. For cFos analysis, cells were detected automatically by signal intensities using spot detection in Imaris. cFos+ cells were counted above an intensity threshold (>800 arbitrary units) and numbers were normalized to total NeuN+ cells. For pERK quantification, all labeled cells were counted.

Statistical analysis

All statistical analyses were performed using one-way ANOVA followed by Dunnet's post-hoc test; $P < 0.05$ in post hoc comparisons, if not otherwise described in results. All tests were two-tailed. For all analyses, the software GraphPad Prism 6 was used. Results are presented as mean ± s.e.m. All

experimental mice were compared to saline-injected controls. Therefore, mice of comparable age were assigned randomly to the different groups. Mice with silenced dH and vH are always processed in parallel in all experiments.

5. Abbreviations

aa	Acetic acid
AAV	Adeno-Associated Virus
benzal.	Benzaldehyde
BSA	Bovine Serum Albumin
CA	Cornu Ammonis
cFC	Contextual Fear Conditioning
d	Dorsal
DG	Dentate Gyrus
dH	Dorsal Hippocampus
DMSO	Demethylsulfoxide
EC	Entorhinal Cortex
EtOH	Ethanol
ext	Extinction
FOR	Familiar Object Recognition
GABA	Gamma Amino Butyric Acid
GC	Granule Cell
HP	Hippocampus
IEG	Immediate Early Gene
MWM	Morris Water Maze
PBS	Phosphate Buffered Saline
pERK	Phosphorylated Extracellular Signal-Regulated Kinases
PFA	Paraformaldehyde
PP	Perforant Path
PSAM	Pharmacologically Selective Actuator Module
PSEM	Pharmacologically Selective Effector Molecule
PV	Parvalbumin
rec	Recall
ret	Retention (of extinction)
sal	Saline
TRAP	Targeted Recombination in Active Populations
v	Ventral
vH	Ventral Hippocampus

6. Bibliography

Acsády, L., Káli, S. Models, structure, function: the transformation of cortical signals in the dentate gyrus. *Prog Brain Res* 163, 577-99. (2007).

Amaral, D. and Witter, M. The there dimensional orga nization of the hippocampal formation: a review of anatomical data. *Neuroscience* 31, 571–591 (1989).

Amilhon, B., Huh, C.Y., Manseau, F., Ducharme, G., Nichol, H., Adamantidis, A., Williams, S. Parvalbumin interneurons of hippocampus tune population activity at theta frequency. *Neuron.* 86, 1277-1289 (2015).

Ascoli, G. A. et al. Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat Rev Neurosci 9, 557-568 (2008).

Bannerman, D.M. *et al.* Double dissociation of function within the hippocampus: spatial memory and hyponeophagia. *Behav. Neurosci.* 116, 884–901 (2002).

Bannerman, D.M., Matthews, P., Deacon, R.M., Rawlins, J.N. Medial septal lesions mimic effects of both selective dorsal and ventral hippocampal lesions. *Behav Neurosci.* 118, 1033-41 (2004).

Bannerman, D.M., Sprengel, R., Sanderson, D.J., McHugh, S.B., Rawlins, J.N., Monyer, H., Seeburg, P.H. Hippocampal synaptic plasticity, spatial memory and anxiety. *Nat Rev Neurosci* 15, 181–192 (2014).

Basu, J., Siegelbaum, S.A. The Corticohippocampal Circuit, Synaptic Plasticity, and Memory. *Spring Harb Perspect Biol* (7) a021733 (2015).

Bast, T., Zhang, W. N. & Feldon, J. The ventral hippocampus and fear conditioning in rats. Different anterograde amnesias of fear after tetrodotoxin inactivation and infusion of the GABAA agonist muscimol. *Exp. Brain Res.* 139, 39–52 (2001).

Bekinschtein, P. et al. Persistence of long-term memory storage requires a late protein synthesis- and BDNF- dependent phase in the hippocampus. *Neuron* 53, 261–277 (2007).

Bozon, B., Davis, S., Laroche, S. A requirement for the immediate early gene zif268 in reconsolidation of recognition memory after retrieval. *Neuron* 40, 695-701 (2003).

Brown, M.W., Aggleton, J.P. Recognition memory: What are the roles of the perirhinal cortex and hippocampus? *Nat. Rev. Neurosci*. 2, 51–61 (2001).

Brown, M.W., Warburton, E.C., Aggleton, J.P. Recognition memory: material, processes, and substrates. *Hippocampus* 20, 1228–44 (2010).

Bunsey, M. & Eichenbaum, H. Conservation of hippocampal memory function in rats and humans. *Nature* 379, 255–257 (1996).
Burgess, N., Maguire, E.A., O'Keefe, J. The human hippocampus and spatial and episodic memory. *Neuron* 35, 625-41 (2002).

Buzsáki, G. Neural syntax: cell assemblies, synapsembles, and readers. *Neuron* 68, 362–385 (2010).

Buzsáki, G. Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning. *Hippocampus* 25, 1073–1188 (2015).

Buzsáki, G., Horvath, Z., Urioste, R., Hetke, J., and Wise, K. High-frequency network oscillation in the hippocampus. *Science* 256,1025-1027 (1992).

Buzsaki, G., Moser, E.I. Memory, navigation and theta rhythm in the hippocampal-entorhinal system. *Nat Neurosci* 16: 130–138. (2013)

Canteras, N.S., Swanson, L.W. Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: a PHAL anterograde tract-tracing study in the rat. *J Comp Neurol*. 324, 180-94. (1992).

Cardin, J. A. et al. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* 459, 663-667 (2009).

Caroni, P., Chowdhury, A., Lahr, M. Synapse rearrangements upon learning: from divergent-sparse connectivity to dedicated sub-circuits. *Trends Neurosci*. 37, 604-14 (2014).

Caroni, P., Donato, F. & Muller, D. Structural plasticity upon learning: regulation and functions. *Nat. Rev. Neurosci*. 13, 478–490 (2012).

Carr, M.F., Jadhav, S.P. & Frank, L.M. Hippocampal replay in the awake state: a potential substrate for memory consolidation and retrieval. *Nat. Neurosci.* 14, 147–153 (2011).

Corcoran, K.A. and Maren, S. Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *J. Neurosci.* 21, 1720-1726. (2001).

Danielson, N.B., Kaifosh, P., Zaremba, J.D., Lovett-Barron, M., Tsai, J., Denny, C.A., Balough, E.M., Goldberg, A.R., Drew, L.J., Hen, R., Losonczy, A., Kheirbek, M.A. Distinct Contribution of Adult-Born Hippocampal Granule Cells to Context Encoding. *Neuron* 90, 101-12 (2016).

Daselaar, S.M., Fleck, M.S., Cabeza, R. Triple dissociation in the medial temporal lobes: recollection, familiarity, and novelty. *J Neurophysiol*. 96, 1902-11 (2006).

Davidson, T.J., Kloosterman, F. & Wilson, M.A. Hippocampal replay of extended experience. *Neuron* 63, 497–507 (2009).

Chen, J.L., Villa, K.L., Cha, J.W., So, P.T., Kubota, Y., Nedivi, E. Clustered dynamics of inhibitory synapses and dendritic spines in the adult neocortex. *Neuron* 74, 361-73 (2012).

Choi, G.B. et al. Driving opposing behaviors with ensembles of piriform neurons. *Cell* 146, 1004–1015 (2011).

Chua, E. F., Schacter, D. L., Rand‐Giovannetti, E., Sperling, R. A. Evidence for a specific role of the anterior hippocampal region in successful associative encoding. *Hippocampus* 17, 1071–1080 (2007).

Davachi, L. Item, context and relational episodic encoding in humans. Curr. *Opin. Neurobiol*. 16, 693–700 (2006).

Debiec, J., LeDoux, J.E., Nader, K. Cellular and systems reconsolidation in the hippocampus. *Neuron* 36, 527-538 (2002).

Denny, C.A., Kheirbek, M.A., Alba, E.L., Tanaka, K.F., Brachman, R.A., Laughman, K.B., Tomm, N.K., Turi, G.F., Losonczy, A., Hen, R. Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. *Neuron* 83, 189–201 (2014).

De Roo, M. et al. LTP promotes a selective long-term stabilization and clustering of dendritic spines. *PLoS Biol.* 6, e219 (2008).

Diba, K., Buzsáki, G. Forward and reverse hippocampal place-cell sequences during ripples. *Nat Neurosci.* 10, 1241-2 (2007).

Dolorfo, C.L., Amaral, D.G. Entorhinal cortex of the rat: topographic organization of the cells of origin of the perforant path projection to the dentate gyrus. *J Comp Neurol.* 398, 25-48 (1998).

Donato, F., Chowdhury, A., Lahr, M., Caroni, P. Early- and late-born parvalbumin basket cell subpopulations exhibiting distinct regulation and roles in learning. *Neuron* 85, 770-86 (2015).

Donato, F., Rompani, S.B., Caroni, P. Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. *Nature* 504, 272–276 (2013).

Dragoi, G. & Tonegawa, S. Preplay of future place cell sequences by hippocampal cellular assemblies. *Nature* 469, 397–401 (2011).

Dunsmoor, J.E., Niv, Y., Daw, N., Phelps, E.A. Rethinking Extinction. *Neuron* 88, 47-63 (2015).

Duzel, E. *et al.* Human hippocampal and parahippocampal activity during visual associative recognition memory for spatial and nonspatial stimulus configurations. *J. Neurosci.* 23, 9439–9444 (2003).

Eichenbaum, H., Dudchenko, P., Wood, E., Shapiro, M., Tanila, H. The hippocampus, memory, and place cells: Is it spatial memory or a memory space? *Neuron* 23, 209–26 (1999).

Eichenbaum, H. Hippocampus: cognitive processes and neural representations that underlie declarative memory. *Neuron* 44,109-20 (2004).

Fanselow, M.S., Dong, H.W. Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* 65, 7–19 (2010).

Ferino, F., Thierry, A., Glowinski, J. Anatomical and electrophysiological evidence for a direct projection from Ammon's horn to the medial prefrontal cortex in the rat. *Exp Brain Res.* 65, 421-426 (1987).

Freund, T. F. & Buzsaki, G. Interneurons of the hippocampus. *Hippocampus* 6, 347-470 (1996).

Freund, T.F., Katona, I. Perisomatic inhibition. *Neuron* 56, 33-42 (2007).

Fu, M., Yu, X., Lu, J., Zuo, Y. Repetitive motor learning induces coordinated formation of clustered dendritic spines in vivo. *Nature* 483, 92-5 (2012).

Fu, Y., Kaneko, M., Tang, Y., Alvarez-Buylla, A. & Stryker, M.P. A cortical disinhibitory circuit for enhancing adult plasticity. *Elife* 4, e05558 (2015).

Garner, A.R., Rowland, D.C., Hwang, S.Y., Baumgaertel, K., Roth, B.L., Kentros, C, Mayford, M. Generation of a synthetic memory trace. *Science* 335, 1513–1516 (2012).

Girardeau, G., Benchenane, K., Wiener, S.I., Buzsáki, G., Zugaro, M.B. Selective suppression of hippocampal ripples impairs spatial memory. *Nat Neurosci.* 12, 1222-3. (2009).

Golgi, C. Opera omnia, Volume 1, Milan (1903).

Gore, F. et al. Neural representations of unconditioned stimuli in basolateral amygdala mediate innate and learned responses. *Cell* 162, 134–145 (2015).

Gray, J. A. & McNaughton, N. The Neuropsychology of Anxiety 2nd edn *Oxford Univ. Press* (2000).

van Groen, T., Wyss, J.M. Extrinsic projections from area CA1 of the rat hippocampus: olfactory, cortical, subcortical, and bilateral hippocampal formation projections. *J Comp Neurol.* 302, 515-28 (1990).

Groenewegen, H.J., der Zee, E.V.-V., te Kortschot, A., Witter, M.P. Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of *Phaseolus vulgaris* leucoagglutinin. *Neuroscience* 23, 103-120 (1987).

Grosmark, A.D. & Buzsáki, G. Diversity in neural firing dynamics supports preexisting and experience-dependent hippocampal sequences. *Science* 351, 1440–1443 (2016).

Guenthner, C.J., Miyamichi, K, Yang, H., Heller, H.C., Luo, L. Permanent genetic access to transiently active neurons via TRAP: Targeted Recombination in Active Populations. *Neuron* 78, 773-784 (2013).

Hafting, T., Fyhn, M., Molden, S., Moser, M.-B., Moser, E.I. Microstructure of a spatial map in the entorhinal cortex. *Nature* 436, 801-806 (2005).

Han, J. H. et al. Neuronal competition and selection during memory formation. *Science* 316, 457–460 (2007).

Han, J.H. et al. Selective erasure of a fear memory. *Science* 323, 1492–1496 (2009).

Hasselmo, M.E., Bodelon, C., Wyble, B.P. A proposed function for hippocampal theta rhythm: separate phases of encoding and retrieval enhance reversal of prior learning. *Neural Comput.* 14, 793–817 (2002).

Hasselmo, M.E., Stern, C.E. Theta rhythm and the encoding and retrieval of space and time. *Neuroimage* 85, 656-66 (2014).

Hebb, D.O. The Organization of Behavior: A Neuropsychological Approach *John Wiley & Sons*, New York (1949).

Hensch, T. K. Critical period plasticity in local cortical circuits. *Nat Rev Neurosci.* 6, 877-888 (2005).

Hill, T.C., Zito, K. LTP-induced long-term stabilization of individual nascent dendritic spines. *J Neurosci*. 33, 678-86 (2013).

Holtmaat, A.J. et al. Transient and persistent dendritic spines in the neocortex in vivo. *Neuron* 45, 279–291 (2005).

Holtmaat, A. & Caroni, P. Functional and structural underpinnings of neuronal assembly formation in learning. *Nat Neurosci*. doi: 10.1038/nn.4418 [Epub ahead of print] (2016).

Holtmaat, A. & Svoboda, K. Experience-dependent structural synaptic plasticity in the mammalian brain. *Nat. Rev. Neurosci.* 10, 647-658 (2009).

Hofer, S.B., Mrsic-Flogel, T.D., Bonhoeffer, T. & Hübener, M. Experience leaves a lasting structural trace in cortical circuits. *Nature* 457, 313–317 (2009).

Hübener, M. & Bonhoeffer, T. Searching for engrams. *Neuron* 67, 363–371 (2010).

Jadhav, S.P., Kemere, C.P., German, P.W., Loren, M.F. Awake Hippocampal Sharp-Wave Ripples Support Spatial Memory. *Science* 336, 1454–1458 (2012).

Jay, T., Witter, M. Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. *J Comp Neurol.* 313, 574-586 (1991).

Jones, B.F. & Witter, M.P. Cingulate cortex projections to the parahippocampal region and hippocampal formation in the rat. *Hippocampus* 17, 957-76 (2007).

Josselyn, S.A., Köhler, S., Frankland, P.W. Finding the engram. *Nat Rev Neurosci.* 16, 521-34 (2015).

Jung, M.W. & McNaughton, B.L. Spatial selectivity of unit activity in the hippocampal granular layer. *Hippocampus* 3,165–182 (1993).

Káli, S. & Dayan, P. Off-line replay maintains declarative memories in a model of hippocampal-neocortical interactions. *Nat Neurosci.* 7, 286-94 (2004).

Kandel, E.R., Dudai, Y., Mayford, M.R. The molecular and systems biology of memory. *Cell* 157,163–186 (2014).

Karunakaran, S., Chowdhury, A., Donato, F., Quairiaux, C., Michel, C.M., Caroni, P. PV plasticity sustained through D1/5 dopamine signaling required for long-term memory consolidation. *Nat. Neurosci.* 19, 454–464 (2016).

Katche, C. et al. Delayed wave of c-Fos expression in the dorsal hippocampus involved specifically in persistence of long-term memory storage*. Proc. Natl. Acad. Sci.* USA 107, 349–354 (2010).

Katche, C., Cammarota, M. & Medina, J.H. Molecular signatures and mechanisms of long-lasting memory consolidation and storage. *Neurobiol. Learn. Mem.* 106, 40–47 (2013).

Keinath, A.T., Wang, M.E., Wann, E.G., Yuan, R.K., Dudman, J.T., Muzzio, I.A. Precise spatial coding is preserved along the longitudinal hippocampal axis. *Hippocampus* 24,1533–1543 (2014).

Kepecs, A., Fishell, G. Interneuron cell types are fit to function. *Nature* 505, 318-326 (2014).

Kessels, H.W. & Malinow, R. Synaptic AMPA receptor plasticity and behavior. *Neuron* 61, 340–350 (2009)

Kheirbek, M.A., Tannenholz, L., Hen, R. NR2B-dependent plasticity of adult-born granule cells is necessary for context discrimination. *J. Neurosci.* 32, 8696–8702 (2012).

Kheirbek, M.A., Drew, L.J., Burghardt, N.S., Costantini, D.O., Tannenholz, L., Ahmari, S.E., Zeng, H., Fenton, A.A., Hen, R. Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus. *Neuron* 77, 955–968 (2013).

Kim, J., Kwon, J.T., Kim, H.S., Josselyn, S.A., Han, J.H. Memory recall and modifications by activating neurons with elevated CREB. *Nat. Neurosci*. 17, 65–72 (2014).

Kishi, T., Tsumori, T., Yokota, S., Yasui, Y. Topographical projection from the hippocampal formation to the amygdala: A combined anterograde and retrograde tracing study in the rat. *The Journal of Comparative Neurology.* 496, 349-368 (2006).

Kjelstrup, K.G., Tuvnes, F.A., Steffenach, H.A., Murison, R., Moser, E.I., Moser, M.B. Reduced fear expression after lesions of the ventral hippocampus. *Proc Natl Acad Sci U S A*. 99, 10825-30 (2002).

Kjelstrup, K. B. *et al*. Finite scale of spatial representation in the hippocampus. *Science* 321, 140–143 (2008).

Klausberger, T., Somogyi, P. Neuronal diversity and temporal dynamics: The unity of hippocampal circuit operations. *Science* 321: 53–57 (2008).

Kobayashi, K., Poo, M.M. Spike train timing-dependent associative modification of hippocampal CA3 recurrent synapses by mossy fibers. *Neuron* 41, 445-54 (2004).

Komorowski, R.W., Garcia, C.G., Wilson, A., Hattori, S., Howard, M.W., Eichenbaum, H. Ventral hippocampal neurons are shaped by experience to represent behaviorally relevant contexts. *J. Neurosci*. 33, 8079–8087 (2013).

Kullmann, D. M. Interneuron networks in the hippocampus. *Curr Opin Neurobiol* 21, 709-716 (2011).

Lapray, D., Lasztoczi, B., Lagler, M., Viney, T.J., Katona, L., Valenti, O., Hartwich, K., Borhegyi, Z., Somogyi, P., Klausberger, T. Behavior-dependent specialization of identified hippocampal interneurons. *Nat. Neurosci.* 15, 1265-1271 (2012).

de Lavilléon, G., Lacroix, M.M., Rondi-Reig, L., Benchenane, K. Explicit memory creation during sleep demonstrates a causal role of place cells in navigation. *Nat. Neurosci*. 18, 493–495 (2015).

Ledoux, J. *The Emotional Brain: The Mysterious Underpinnings of Emotional* Life (Simon & Schuster, 1998).

Letzkus, J.J. et al. A disinhibitory microcircuit for associative fear learning in the auditory cortex. *Nature* 480, 331–335 (2011).

Leutgeb, J.K., Leutgeb, S., Moser, M.B., Moser E.I. Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science* 315, 961–966 (2007).

Lisman, J.E., Grace, A.A. The hippocampal-VTA loop: Controlling the entry of information into long-term memory. *Neuron* 46, 703–713 (2005).

Liu, X., Ramirez, S., Pang, P.T., Puryear, CB, Govindarajan, A, Deisseroth, K., Tonegawa, S. Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature* 484: 381–385 (2012).

Luo, L., Callaway, E.M., and Svoboda, K. Genetic Dissection of Neural Circuits. *Neuron* 57, 634–660 (2008).

Magnus, C.J. et al. Chemical and genetic engineering of selective ion channel-ligand interactions. *Science* 333, 1292–1296 (2011).

Maren, S., Aharonov, G. & Fanselow, M. S. Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behav. Brain Res*. 88, 261–274 (1997).

McKenzie, S., Frank, A.J., Kinsky, N.R., Porter, B., Rivière, P.D., Eichenbaum, H. Hippocampal representation of related and opposing memories develop within distinct, hierarchically organized neural schemas. *Neuron* 83, 202-15 (2014).

McNaughton, B.L., Battaglia, F.P., Jensen, O., Moser, E.I., Moser, M.-B. Path integration and the neural basis of the “cognitive map.” *Nat. Rev. Neurosci*. 7, 663–78 (2006).

McNaughton, B.L., Barnes, C.A., Meltzer, J. and Sutherland, R.J. Hippocampal granule cells are necessary for normal spatial learning but not for spatially-selective pyramidal cell discharge. *Experimental brain research. Experimentelle Hirnforschung* 76, 485–496 (1989).

Meinecke, D. L. & Peters, A. GABA immunoreactive neurons in rat visual cortex. *J Comp Neurol* 261, 388-404 (1987).

Morris, R.G.M., Garrud, P., Rawlins, J.N.P., O'Keefe, J. Place navigation impaired in rats with hippocampal lesions. *Nature* 297, 681-683 (1982).

Moser, E.I., Kropff, E., Moser, M.B. Place cells, grid cells, and the brain's spatial representation system. *Annu Rev Neurosci.* 31, 69-89 (2008a).

Moser, E.I., Moser, M.B. A metric for space. *Hippocampus* 18, 1142-56 (2008b).

Moser, M.B., Moser, E.I., Forrest, E., Andersen, P., Morris, R.G. Spatial learning with a minislab in the dorsal hippocampus. *Proceedings of the National Academy of Sciences.* 92, 9697-9701 (1995).

Nabavi, S. *et al.* Engineering a memory with LTD and LTP. *Nature* 511, 348–352 (2014).

Nakazawa, K., Quirk, M.C., Chitwood, R.A., Watanabe, M., Yeckel, M.F., Sun, L.D., Kato, A., Carr, C.A., Johnston, D., Wilson, M.A., and Tonegawa, S. Requirement for hippocampal CA3 NMDA receptors in associative memory recall. *Science* 297, 211–218 (2002).

Nakayama, D. et al. Long-delayed expression of the immediate early gene Arc/Arg3.1 refines neuronal circuits to perpetuate fear memory. *J. Neurosci.* 35, 819–830 (2015).

Nicoll, R.A., Schmitz, D. Synaptic plasticity at hippocampal mossy fibre synapses. *Nat Rev Neurosci.* 6, 863-76 (2005).

O'Keefe, J., Dostrovsky, J. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely moving rat. *Brain Res* 34, 171–175 (1971).

O'Keefe, J. & Nadel, L. *The Hippocampus as a Cognitive Map*, Clarendon (1978).

Orsini, C.A. & Maren, S. Neural and cellular mechanisms of fear and extinction memory formation. *Neurosci Biobehav Rev*. 36, 1773-802 (2012).

Pfeiffer, B.E. & Foster, D.J. Place cells. Autoassociative dynamics in the generation of sequences of hippocampal place cells. *Science* 349, 180–183 (2015).

Pikkarainen, M., Rönkkö, S., Savander, V., Insausti, R., Pitkänen, A. Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. *The Journal of Comparative Neurology* 403, 229-260 (1999).

Pitkänen, A., Pikkarainen, M., Nurminen, N., Ylinen, A. Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat: A review. *Annals of the New York Academy of Sciences* 911, 369-391 (2000).

Plath, N., Ohana, O., Dammermann, B., Errington, M.L., Schmitz, D., Gross, C., Mao, X., Engelsberg, A., Mahlke, C., Welzl, H., Kobalz, U., Stawrakakis, A., Fernandez, E., Waltereit, R., Bick-Sander, A., Therstappen, E., Cooke, S.F., Blanquet, V., Wurst, W., Salmen, B., Bösl, M.R., Lipp, H.P., Grant S.G., Bliss, T.V., Wolfer, D.P., Kuhl, D. Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. *Neuron* 52, 437-44 (2006).

Poirier, G.L., Amin, E., Aggleton, J.P. Qualitatively different hippocampal subfield engagement emerges with mastery of a spatial memory task by rats. *J Neurosci.* 28, 1034-45 (2008).

Poppenk, J., Evensmoen, H.R., Moscovitch, M., Nadel, L. Long-axis specialization of the human hippocampus. *Trends Cogn Sci.* 17, 230-40 (2013).

McRae, K., Jones, M. Semantic Memory. *The Oxford Handbook of Cognitive Psychology*. New York: Oxford University Press. 206–216 (2013).

Ramirez, S., Liu, X., Lin, P.A., Suh, J., Pignatelli, M., Redondo, R.L., Ryan, T.J., Tonegawa, S. Creating a false memory in the hippocampus. *Science* 341, 387–391 (2013).

Ramon y Cajal, S. Neue Darstellung vom histologischen Bau des Centralnervensystems. *Arch. Anat. Physiol.* 17, 9–428 (1893).

Rashid, A.J., Yan, C., Mercaldo, V., Hsiang, H.L., Park, S., Cole, C.J., De Cristofaro, A., Yu, J., Ramakrishnan, C., Lee, S.Y., Deisseroth, K., Frankland, P.W., Josselyn, S.A. Competition between engrams influences fear memory formation and recall. *Science* 353, 383-7 (2016).

Redondo, R.L., Kim, J., Arons, A.L., Ramirez, S., Liu, X., Tonegawa, S. Bidirectional switch of the valence associated with a hippocampal contextual memory engram. *Nature* 513, 426-30 (2014).

Reijmers, L.G., Perkins, B.L., Matsuo, N., Mayford, M. Localization of a stable neural correlate of associative memory. *Science* 317, 1230–1233 (2007).

Risold, P. Y. & Swanson, L. W. Structural evidence for functional domains in the rat hippocampus. *Science* 272, 1484–1486 (1996).

Risold, P. & Swanson, L. Connections of the rat lateral septal complex. *Brain Res. Rev.* 24, 115–195 (1997).

Rogerson, T. et al. Synaptic tagging during memory allocation. *Nat. Rev. Neurosci.* 15, 157–169 (2014).

Rolls, E.T. A quantitative theory of the functions of the hippocampal CA3 network in memory. *Front Cell Neurosci.* 7, 98 (2013).

Royer, S., Zemelman, B.V., Losonczy, A., Kim, J., Chance, F., Magee, J.C., Buzsáki, G. Control of timing, rate and bursts of hippocampal place cells by dendritic and somatic inhibition. *Nat Neurosci.* 15, 769-75 (2012).

Rudy, J. W. & O'Reilly, R. C. Conjunctive representations, the hippocampus, and contextual fear conditioning. *Cogn Affect Behav Neurosci* 1, 66-82 (2001).

Ruediger, S., Spirig, D., Donato, F., Caroni, P. Goal-oriented searching mediated by ventral hippocampus early in trial-and-error learning. *Nature Neuroscience* 15, 1563-1571 (2012).

Ryan, T.J., Roy, D.S., Pignatelli, M., Arons, A. & Tonegawa, S. Memory. Engram cells retain memory under retrograde amnesia. *Science* 348, 1007–1013 (2015).

Schacter, D. L. & Wagner, A. D. Medial temporal lobe activations in fMRI and PET studies of episodic encoding and retrieval. *Hippocampus* 9, 7–24 (1999).

Sargolini, F., Fyhn, M., Hafting, T., McNaughton, B.L., Witter, M.P., Moser, M.B., Moser, E.I. Conjunctive representation of position, direction, and velocity in entorhinal cortex. *Science* 312, 758-762 (2006).

Scoville, W. B. & Milner, B. Loss of recent memory after bilateral hippocampal lesions. *J. Neurol. Neurosurg. Psychiatry* 20, 11–21 (1957).

Solstad, T., Boccara, C.N., Kropff, E., Moser, M.B., Moser, E.I. Representation of geometric borders in the entorhinal cortex. *Science* 322, 1865-1868 (2008).

Striedter, G.F. Evolution of the hippocampus in reptiles and birds. *J Comp Neurol.* 524, 496-517 (2016).

van Strien, N.M., Cappaert, N.L.,Witter, M.P. The anatomy of memory: An interactive overview of the parahippocampal- hippocampal network. *Nat Rev Neurosci.* 10, 272–282 (2009).

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

Squire, L.R., Stark, C.E., Clark, R.E. The medial temporal lobe. *Annu Rev Neurosc*i 27, 279–306 (2004).

Stark, E., Koos, T., Buzsáki, G. Diode probes for spatiotemporal optical control of multiple neurons in freely moving animals. *Journal of Neurophysiology* 108, 349-363 (2012).

Strange, B. A., Fletcher, P. C., Henson, R. N. A., Friston, K. J., Dolan, R. J. Segregating the functions of human hippocampus. *Proc. Natl Acad. Sci. USA* 96, 4034–4039 (1999).

Strange, B.A., Witter, M.P., Lein, E.S., Moser E.I. Functional organization of the hippocampal longitudinal axis. *Nat Rev Neurosci.*15, 655-69 (2014).

Sutherland, R.J., Whishaw, I.Q. and Kolb, B. A behavioural analysis of spatial localization following electrolytic, kainate- or colchicine-induced damage to the hippocampal formation in the rat. *Behav. Brain Res*. 7, 133–153 (1983).

Suzuki, W.A. Associative learning signals in the brain. *Prog Brain Res.* 169, 305-20 (2008).

Suzuki, W.A., Naya, Y. The perirhinal cortex. *Annu Rev Neurosci 3*7, 39-53 (2014).

Tanaka, K. Z. et al. Cortical representations are reinstated by the hippocampus during memory retrieval. *Neuron* 84, 347–354 (2014).

Takeuchi, T. et al. The synaptic plasticity and memory hypothesis: encoding, storage and persistence. *Philos. Trans. R. Soc. Lond. B: Biol. Sci*. 369 (2013)

Tannenholz, L., Jimenez, J.C., Kheirbek, M.A. Local and regional heterogeneity underlying hippocampal modulation of cognition and mood. *Front Behav Neurosci*. 8, 147 (2014).

Taube, J.S., Muller, R.U., Ranck, J.B., Jr. Head-direction cells recorded from the postsubiculum in freely moving rats. II. Effects of environmental manipulations. *J. Neurosci.* 10, 436–447 (1990).

Thompson, C. L. et al. Genomic anatomy of the hippocampus. *Neuron* 60, 1010–1021 (2008).

Trachtenberg, J.T. et al. Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* 420, 788–794 (2002).

Treves, A. and Rolls, E.T. Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. *Hippocampus* 2, 189–199 (1992).

Treves, A., Tashiro, A., Witter, M.P., Moser, E.I. What it the DG good for? *Neuroscience* 154,1155-72 (2008).

Trifilieff, P., Herry, C., Vanhoutte, P., Caboche, J., Desmedt, A., Riedel, G., Mons, N., Micheau, J. Foreground contextual fear memory consolidation requires two independent phases of hippocampal ERK/CREB activation. *Learning & Memory* 13, 349-358 (2006).

Trivedi, M.A., Coover, G.D. Lesions of the ventral hippocampus, but not the dorsal hippocampus, impair conditioned fear expression and inhibitory avoidance on the elevated T-maze. *Neurobiol Learn Mem.* 81, 172-84 (2004).

Tulving, E. Organization of Memory. 381-403 (1972).

Thierry, A. M., Gioanni, A. M., Degenetais, E., Glowinski, J. Hippocampo-prefrontal cortex pathway: anatomical and electrophysiological characteristics. *Hippocampus* 10, 411–419 (2000).

Treves, A. and Rolls, E.T. Computational analysis of the role of the hippocampus in memory. *Hippocampus* 4 374–391 (1994).

Witter, M.P., & Amaral, D.G. Hippocampal formation. In The Rat Nervous System, Third Edition, G. Paxinos, ed. (San Diego, CA: Academic Press) 637–703, Chapter 21 (2004).

Wolff, S.B. et al. Amygdala interneuron subtypes control fear learning through disinhibition. *Nature* 509, 453–458 (2014).

Xu, T. et al. Rapi formation and selective stabilization of synapses for enduring motor memories. *Nature* 457, 313-317 (2009).

Yassin, L. et al. An embedded subnetwork of highly active neurons in the neocortex. *Neuron* 68, 1043–1050 (2010).

Yiu, A.P., Mercaldo, V., Yan, C., Richards, B., Rashid, A.J., Hsiang, H.L., Pressey, J., Mahadevan. V., Tran, M.M., Kushner, S.A., Woodin, M.A., Frankland, P.W., Josselyn, S.A. Neurons are recruited to a memory trace based on relative neuronal excitability immediately before training. *Neuron* 83, 722-35 (2014).

Zelikowsky M, Bissiere S, Fanselow MS. Contextual fear memories formed in the absence of the dorsal hippocampus decay across time. *J Neurosci.* 32, 3393-7 (2012).

Zhou, Y. et al. CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. *Nat. Neurosci.* 12, 1438–1443 (2009).

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