Interactions between the entorhinal cortex and hipocampal formation
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Citation for published version (APA):
Chapter 1

General introduction

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The nervous system is by far the most complex organ in the human body, containing an enormous number of nerve cells ('neurons') that communicate with each other through a multitude of connections. For today's neuroscience, it is a major challenge to understand how these interconnected networks of neurons give rise to the cognitive functions of the brain and how behavior emerges from concerted activity within and between these networks.

Our brain endows us with the amazing capacity to learn about our environment and to acquire new skills (e.g., learning to ride a bike), such that we may use this new information to predict what will happen in the near future and respond appropriately. The information that is stored in our brain ('memories') can be retrieved shortly after the moment it has been acquired or even years later. In recent years, it has become clear that learning and memory is not a single phenomenon, but that several 'memory systems' may be recognized (Squire and Zola, 1996), as is summarized in figure 1.1. Each of the memory systems is associated with a set of brain structures that are essential for that kind of memory (as depicted in fig. 1.1).

However, this doesn't mean that these structures are completely devoted to a given memory system, or that other regions are not involved at all. In general, it is thought that those areas that are involved in processing specific information (e.g., visual stimuli in the visual cortex or motor patterns in the motor cortex and cerebellum) are also the areas that participate in
memory of that information.

The memory that is referred to as ‘memory’ in every day life (‘declarative memory’), i.e. memory of events (‘episodic memory’) and the acquisition of factual knowledge (‘semantic memory’) is critically dependent on a set of structures that reside in the medial temporal lobe. This was dramatically shown in the famous patient HM, who underwent brain surgery to relieve him from frequent and invalidating epileptic seizures (Scoville and Milner, 1957). In this patient, large parts of the medial temporal lobe in both hemispheres were removed. After recovery from the surgery, epileptic seizures did not recur, but it was found that HM was not able to create new long-term declarative memories (anterograde amnesia) and he had lost memories of an extended period before the surgery (retrograde amnesia).

In order to understand how the structures in the medial temporal lobe support the formation of declarative memory and how this memory formation is affected in pathologies such as epilepsy, brain lesions and dementias, like Alzheimer’s disease, it is imperative to understand the organization of this memory system and to elucidate the contribution of these structures to different memory processes. In this respect, the information obtained from patients with brain lesions, such as HM, from neuropsychological studies, as well as from modern brain imaging studies, using fMRI, PET, MEG, etc., to study neurocognitive functions, are very useful. The imaging techniques, for example, can provide clues on which brain areas become active during a memory task. Most fundamental questions regarding how individual cells or small neuronal networks interact and how they operate during behavior, however, cannot be investigated in healthy human subjects, but can only be fully explored using animal models. Animal models have the advantage that one can relatively easily intervene in the system, for example by local injection of neurotransmitter receptor (ant)agonists or reproducible lesions of a chosen brain area or fiber pathway. The choice of an animal model is determined by the degree it mimics the human situation and by its applicability in practice. The most widely used animal models in neuroscience, and also in this thesis, are rodents (i.e. rats and mice). This raises the question whether the results obtained with these animals can be generalized to humans. There is no doubt that structural differences exist between the brains of rodents and humans and that rodents do not share many of the cognitive capabilities that humans possess. In spite of these differences, however, for many human brain regions a homologue can be found in the rodent brain and in most cases these appear to exert similar functions. Still, one should keep in mind that a rodent brain is not the same as a human brain and not even a simpler version of a human brain, since the brains of rodents and humans reflect differential adaptation to the environment during evolution. Rodents, for example, make extensive use of olfactory and tactile (e.g. via their whiskers) cues when exploring their environment and the neocortical areas devoted to processing this sensory information therefore occupy a large portion of the rodent brain. Humans, in contrast, are much more visually oriented and accordingly the visual cortices are
relatively large. Therefore, one should always be careful when extrapolating results obtained in rodents to the human situation.

The structures in the human medial temporal lobe do have their counterparts in rodents, but since rodents do not have a well-defined temporal lobe, the set of brain regions that is believed to be critical for memory processes, is called 'the hippocampal memory system', after one of its main components, the hippocampal formation (HPF). The other main component of the hippocampal memory system is the parahippocampal region (PHR). In the next paragraphs, an introductory description will be given of the brain areas included in the hippocampal memory system. Several experimental articles and reviews have appeared in recent years, which describe what is presently known about the cyto-architecture and connectivity of the structures in the HPF and PHR (Amaral and Witter, 1995, Burwell et al., 1995, Burwell, 2001, Insausti et al., 1997, Lopes da Silva et al., 1990, Witter et al., 1989, Witter, 2002). Here, I will summarize several of the main organizational principles and for most details I will refer to the articles cited above, unless these details are important for the chapters that follow. The emphasis will be put on one of the main sub-fields of the parahippocampal region, i.e. the entorhinal cortex, since all studies described in the following chapters focus on this structure.

COMPONENTS OF THE RODENT HIPPOCAMPAL MEMORY SYSTEM

The hippocampal formation

In the rat brain, the HPF is a curved structure extending from dorsal-septal to ventral-temporal sites and this axis is referred to as the longitudinal axis of the HPF (fig. 1.2B). In a cross-section, stained for the neuronal marker NeuN, several sub-fields can be distinguished (fig. 1.2D): the dentate gyrus, areas of the Cornu Ammonis (CA1-3) and subiculum (Scharfman et al., 2000). All these sub-fields of the HPF have in common a single layer of principal cells: pyramidal neurons in CA1-3 and subiculum and granule neurons in the dentate gyrus. In contrast to the cell layers of the dentate gyrus and areas CA1-3, the subicular cell layer is not very compact and this marks the transition to the sub-fields of the PHR (i.e. pre- and parasubiculum in fig. 1.2D). The principal neurons are the main source of intrinsic hippocampal projections (see sections about connectivity below) and of projections to brain structures residing outside the HPF via the fiber tracts in the alveus, angular bundle or fimbria (fig. 1.2D). In each hippocampal sub-field, in addition to the cell-layer, one or more layers are defined which mainly contain the dendrites of the principal neurons and harbor relatively few neuronal somata. In figure 1.2D, these layers are indicated for area CA1, subiculum and the dentate gyrus. Principal neurons in the hippocampal formation generally use the excitatory neurotransmitter glutamate for synaptic communication. A smaller, but diverse group of
neurons exist which are scattered throughout all layers and which use the inhibitory neurotransmitter GABA (γ-amino butyric acid) (Freund and Buzsaki, 1996). Since it was originally believed that the axonal plexi of these neurons remain within sub-field boundaries, they were called ‘interneurons’, however it is now known that GABAergic neurons may in fact have axon collaterals that reach extra-hippocampal structures (Ceranik et al., 1997, Toth et al., 1993, van Haeften et al., 1995).

In addition to the longitudinal axis defined above, the axis that runs parallel to the cell layers of areas CA1-3 and subiculum and is more or less perpendicular to the longitudinal axis, is called the transverse axis (fig. 1-2D). By definition, the side of this axis that is closest to the dentate gyrus is ‘proximal’ and the other side is ‘distal’, e.g. the part of CA1 that borders CA3 is proximal and the part that borders subiculum is distal.

The parahippocampal region

The PHR comprises several brain areas that occupy most of the caudal-lateral side of the rat cortex (fig. 1.2C), i.e. the entorhinal cortex, the peri- and postrhinal cortices and the pre- and parasubiculum (Witter, 2002). The entorhinal cortex can be further subdivided into the lateral entorhinal area (LEA) and the medial entorhinal area (MEA) (fig. 1.2C). These subdivisions differ with respect to some cyto-architectonic characteristics and in addition they display strikingly different patterns of connectivity as will be discussed below. The pre- and parasubiculum are adjacent to the subiculum and therefore are part of the transition between the HPF and PHR. The sub-fields of the PHR differ from those of the HPF in that they have multiple cell layers. For detailed information about the cyto-architecture of several sub-fields of the PHR the reader is referred to the articles written by Burwell (2001), Burwell and Witter (2002) and Insausti et al. (1997). In the remainder of this section, I will focus on the entorhinal cortex.

Usually, the entorhinal layers are grouped into superficial layers (I-III, close to the pial surface) and deep layers (V-VI, close to the underlying white matter). A cell-sparse layer, called the lamina dissecans (layer IV), separates deep and superficial layers. This arrangement of layers is indicated in figure 1.2D and a more detailed picture is shown in figure 1.2E. The appearance of a laminated structure reflects the differences in cell packing as well as the size and shape of the cell somata. For example, neurons in layer II are relatively large and densely packed, whereas neurons in layer III are smaller and more dispersed. Layer I and the lamina dissecans, on the other hand, contain relatively few cell somata. Several different cell-types have been described in the entorhinal cortex (Hamam et al., 2000, Hamam et al., 2002, Soriano et al., 1993, Wouterlood, 2002). The principal cell-types in layer II are stellate neurons (see example in fig. 1.2E), whereas in layers III and V the neurons are predominantly pyramidal-shaped (see examples in fig. 1.2E). These principal cells utilize the excitatory neurotransmitter glutamate and their axons are distributed within the entorhinal cortex.
Chapter 1

A. Rat brain
- cortical hemispheres
- cerebellum
- olfactory bulbs
- brainstem
- rhinal sulcus

B. Hippocampal formation
- septal
- temporal
- hippocampal formation

C. Parahippocampal region
- POR
- pre-/parasubiculum
- cerebellum
- PER
- LEA
- MEA
- (caudal-lateral view)
- brainstem

(figure 1.2)
D. Cyto-architecture HPF & EC

![D. Cyto-architecture HPF & EC](image)

E. Cyto-architecture EC

![E. Cyto-architecture EC](image)

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**Figure 1.2** Localization and cyto-architecture of the hippocampal formation and parahippocampal region in the rat brain. **A.** Three-dimensional reconstruction of the rat brain, showing several major subdivisions. **B.** Location of the hippocampal formation (purple, combination of dentate gyrus, CA1-3 and subiculum) in the rat brain. Orientation is similar to A, but the left hemisphere is largely removed to reveal the hippocampal formation. **C.** Location of the structures of the parahippocampal region in a caudal-lateral view of the rat brain. Notice that parts of the midbrain, cerebellum and brainstem are removed to give unrestricted view on the parahippocampal region. Pre- and parasubiculum (green) are located at the medial aspect of the cortical mantle and only a small part is visible in this view. **D.** Horizontal section of the hippocampal formation and parahippocampal region illustrating their cyto-architecture. The section was stained for NeuN to visualize neuronal cell somata. Notice the single cell-layer in dentate gyrus, CA1-CA3 and the dispersed cell-layer in subiculum. For the dentate gyrus, area CA1 and subiculum also the dendritic layers are indicated. In the entorhinal cortex, multiple layers can be recognized (I-VI). Notice the light band between layers III and V, which is the lamina dissecans (layer IV). **E.** Detailed picture of the cyto-architecture of the entorhinal cortex in a horizontal section stained for Nissl substance. Notice the distinct appearance of the cell-layers, due to difference in cell-size and packing density. Three major cell types are shown for layer II (stellate neuron) and layers III and V (pyramidal neurons). Notice that all these cells have dendrites that extend into more superficial layers. For abbreviations, see list.
(Dolorfo and Amaral, 1998b, Köhler, 1986, 1988) and project also to extrinsic structures (see sections about connectivity below). Several types of inhibitory interneurons with a variety of neurochemical signatures have also been described, and these are particularly abundant in layers II and III (Wouterlood, 2002). Examples of these interneurons are chandelier cells (Soriano et al., 1993) and basket-like neurons (Jones and Buhl, 1993), which may have a strong inhibitory influence on the local principal neurons.

It should be stressed, however, that the several cell layers only refer to the cell somata, whereas the dendrites and axons of these cells are not necessarily restricted to these layers and in fact in many instances they cross layer boundaries (as is clear for the neurons depicted in figure 1.2E). Generally, dendrites extend into layers more superficial to the layer of origin, but they do not extend into deeper layers. This important observation will return in the discussion about the connectivity in the hippocampal memory system, since it means that it cannot always be assumed that axon terminals that are restricted to one particular layer only synapse onto neurons having their cell somata in that same layer.

**CONNECTIVITY IN THE RODENT HIPPOCAMPAL MEMORY SYSTEM**

The extensive network of connections by which neurons communicate makes the brain a tremendously versatile system. These connections are not laid out randomly, but are organized in specific patterns that determine the anatomical framework within which the system operates.

At a global scale, the organization of the PHR and HPF may be described as a cascade of connections from sensory association cortices to peri- and postrhinal cortices and to the entorhinal cortex and subsequently to the HPF, and from here back to the entorhinal cortex, peri- and postrhinal cortices and finally to the association cortices where it started (fig. 1.3A). Generally, information flow towards the HPF is referred to as the ‘input’ and information flow in the reverse direction is referred to as the ‘output’ of the system.

Rather than a merely serial linkage of structures, the scheme in figure 1.3A shows that a characteristic of HPF and PHR connectivity is the existence of parallel pathways (Naber et al., 2000). Considering the input-side first, one can see that CA1 and subiculum, which may be regarded as the areas where all inputs congregate, can receive sensory information along at least three parallel pathways. The first is a direct projection from peri- and postrhinal cortices to CA1 and subiculum (Kosel et al., 1983, McIntyre et al., 1996, Naber et al., 1997, Naber et al., 1999, Naber et al., 2001b), the second is an indirect projection to CA1 and subiculum via the entorhinal cortex (Steward and Scoville, 1976, Witter et al., 1992) and the third pathway consist of the projection from the entorhinal cortex to the dentate gyrus and CA3 via the classic perforant path (Dolorfo and Amaral, 1998a, Tamamaki, 1997).
A. Scheme of connections in the hippocampal memory system

**NEOCORTEX** | **PARAHIPPOCAMPAL REGION** | **HIPPOCAMPAL FORMATION**

- Sensory and association cortices
  - 'output side'
  - PER
  - LEA
  - MEA
  - POR
  - MOSSY FIBERS
  - Perforant path
  - Schaffer collaterals

- 'input side'

B. Segregation & convergence of inputs

C. Cortical in- & outputs

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**Figure 1.3** Connectional principles of the hippocampal memory system. **A.** Major connections between areas of the hippocampal formation and parahippocampal region. Direct connections between the peri- and postrhinal cortices and the hippocampal formation are dashed because they are not as strong as the connections between the entorhinal cortex and hippocampal formation. **B.** Scheme illustrating segregation and convergence of inputs in the hippocampal memory system. Each area is represented by a box. Segregation is attained by the two topographical patterns of the input projections: one is illustrated by the red and blue colors, the second is illustrated by the different shades of red and blue. Purple colors indicate sites of convergence (e.g. in the dentate gyrus and area CA3). **C.** Illustration of the connections between the parahippocampal region and sensory and association cortices. Notice that perirhinal cortex and LEA are connected to a largely distinct set of cortical areas (yellow-orange areas; e.g. gustatory/visceral, insular and piriform cortices), as compared to postrhinal cortex and MEA (blue areas; e.g. visual, parietal and retrosplenial cortices). Some association cortices project to both perirhinal/LEA and postrhinal/MEA cortices (purple areas; e.g. auditory and temporal cortices). Connections are also present with frontal cortices, but these are not shown here. For abbreviations, see list.
The latter two areas, in turn, connect to CA1 and subiculum via intra-hippocampal pathways (i.e. mossy fibers and Schaffer collaterals, fig. 1.3A).

Along the parallel pathways both segregation and convergence of information flows can occur. Segregation is attained by the topographical organization of most of the input connections present in the hippocampal memory system. Topographical matching means that the location of the terminal field in the receiving area is related to the location of the cells of origin in the projecting area. Two clear topographical patterns can be recognized for the input projections in the HPF and PHR and these are illustrated in figure 1.3B. The first is related to the LEA/MEA subdivisions of the entorhinal cortex and the transverse axis in the HPF: LEA projects to the distal part of CA1 and proximal part of subiculum, whereas MEA projects to the proximal part of CA1 and distal part of subiculum (Steward, 1976, Witter and Amaral, 1991, Witter, 1993). The connection from CA1 to subiculum also displays a clear-cut topography, such that proximal CA1 project to distal subiculum and distal CA1 projects to proximal subiculum (Amaral et al., 1991). Regarding the connections from peri- and postrhinal cortices to the entorhinal cortex, CA1 and subiculum, it was shown that these are in accordance with the above-described pattern. Thus, perirhinal cortex preferentially projects to LEA (Burwell and Amaral, 1998b), the distal part of CA1 and the proximal part of subiculum (Naber et al., 1999). The postrhinal cortex, in contrast, preferentially projects to MEA (Burwell and Amaral, 1998b), the proximal part of CA1 and the distal part of subiculum (Naber et al., 1997, Naber et al., 2001b). This means that inputs mediated by perirhinal cortex and relayed to LEA are transmitted to a different group of neurons in CA1 and subiculum than those inputs mediated by postrhinal cortex and relayed to MEA (i.e. red vs. blue areas and arrows in fig. 1.3B). The organization of the connections between CA1 and subiculum assures that this segregation is maintained (Amaral et al., 1991, Naber et al., 2001a) in the communication between these areas.

The second topographical pattern is related to the lateral-to-medial axis in the entorhinal cortex (not to be confused with LEA/MEA subdivisions), which is mapped onto the septal-temporal axis of the HPF (these axes are represented by three different shades in fig. 1.3B) (Dolorfo and Amaral, 1998a, Witter, 1993). Interestingly, the projections from peri- and postrhinal cortices terminate predominantly in the lateral aspect (i.e. close to the rhinal sulcus) of the entorhinal cortex (Burwell and Amaral, 1998b) and thus influence mainly the septal part of the HPF. This second topographical organization, however, is much more gradual than the first one and there is a significant amount of divergence along the hippocampal septo-temporal axis of projections originating in the entorhinal cortex. Therefore it is less likely that this topography leads to prominent segregation.

In addition to segregation there exists convergence or integration (purple areas and arrows in fig. 1.3B). This may occur, for example, through the interconnections between perirhinal cortex and postrhinal cortex (Burwell and Amaral, 1998b) or the associational connections within the entorhinal cortex (Dolorfo and Amaral, 1998b, Köhler, 1986, 1988).
Another site at which convergence occurs is the projection from the entorhinal cortex to the dentate gyrus and CA3. Both LEA and MEA project to the same groups of cells in the dentate gyrus and CA3 (Canning et al., 2000, Dolorfo and Amaral, 1998a, Tamamaki, 1997).

On the output side, parallel pathways are also evident since both CA1 and subiculum project to peri- and postrhinal cortices directly (Deacon et al., 1983, Swanson and Cowan, 1977) and indirectly via the entorhinal cortex (Beckstead, 1978, Köhler, 1985, Naber et al., 2001a, Tamamaki and Nojyo, 1995). An important question here, of course, is whether the segregation of the parallel input pathways is continued in the output pathways. This issue was investigated using anatomical tracing studies and the results are described in chapter 2.

Segregation of information flow is only functionally relevant if there is a difference between the segregated pathways, either because of differential processing or because the segregated pathways receive qualitatively distinct sensory information. There is indeed anatomical evidence that the latter is the case. This becomes clear if we analyze the processing pathways of sensory information entering the brain. Sensory impulses are conveyed from the receptors in the eyes, ears, skin, etcetera, to the uni-modal primary sensory cortices, in most cases through thalamic relay nuclei. In subsequent association cortices, sensory information is integrated, both within and across modalities. Integrated information from several sources then reaches peri- and postrhinal cortices. These cortices receive qualitatively different inputs from adjacent association cortices (Burwell and Amaral, 1998b), as is illustrated in figure 1.3C. The perirhinal cortex receives its inputs mainly from ventro-laterally located cortices that subserve all sensory modalities, i.e. piriform cortex, insular cortex and temporal association cortex (fig. 1.3C). In contrast, cortical inputs to the postrhinal cortex originate mainly in visual association cortex and visuo-spatial areas, such as the parietal cortex and retrosplenial cortex, which are located dorsally (fig. 1.3C). The entorhinal cortex also receives sensory information directly from uni- and multi-modal association cortices (Burwell and Amaral, 1998b). These direct connections are organized such that LEA receives inputs from those cortical areas that also project to perirhinal cortex, and MEA receives inputs from cortical areas that project to the postrhinal cortex. The reverse connections from the peri- and postrhinal cortices and from the entorhinal cortex to the association cortices is in register with the input projections (Insausti et al., 1997). This underscores the concept of segregation of sensory information flow in the hippocampal memory system.

Next to the connections with cortical areas, HPF and PHR also project to and receive projections from a variety of subcortical structures (i.e. brainstem, basal forebrain, amygdala, (hypothalamus), which are important for a proper functioning of the system. It is, however, outside the scope of this thesis to discuss these connections in detail. For further information about these connections, the reader is referred to several recent articles that summarize these connections (Amaral and Witter, 1995, Gaykema et al., 1990, Lopes da Silva et al., 1990, Pitkänen et al., 2000, Vertes et al., 1999, Witter et al., 1989).
Detailed connectivity of the entorhinal cortex

As is clear from the scheme in figure 1.3A, the entorhinal cortex is the main interface between the HPF and neocortex. Since all following chapters deal with the entorhinal cortex and its interactions with the hippocampal formation, some more detail about the cellular elements involved and their interconnections is provided here. In the entorhinal cortex the cells that project via the perforant path to the sub-fields of the HPF are located in the superficial layers II and III. The stellate shaped neurons in layer II provide an excitatory input to the dendrites of dentate granule cells in the outer two-third of the dentate molecular layer. Entorhinal layer II stellate neurons also project to area CA3. The entorhinal projections to CA1 and subiculum, however, originate from the pyramidal-shaped neurons in layer III. Terminations are exclusively found in stratum (lacunosum-)moleculare and are segregated from the terminals of the Schaffer collateral pathway originating in CA3, which are located in stratum radiatum. Next to the excitatory projection to principal cells of the hippocampal formation, entorhinal neurons also target local interneurons (Desmond et al., 1994), possibly providing a feed-forward inhibitory input to the principal cells.

Pyramidal neurons in CA1 and subiculum are the main source of hippocampal formation output to the entorhinal cortex. They mainly target spiny, presumed excitatory pyramidal neurons, and non-spiny, presumed inhibitory interneurons in deep layers, particularly in layer V (van Haefsten et al., 1995). The layer V pyramidal neurons, in turn, are the main source of entorhinal output projections to peri- and postrhinal cortices and other cortical areas (Insauti et al., 1997). Peri- and postrhinal inputs as well as other cortical inputs to the entorhinal cortex predominantly terminate in superficial layers (Burwell and Amaral, 1998a). The exact postsynaptic targets are not known, but it is generally assumed that layer II stellate neurons and layer III pyramidal neurons are among those targets. It is possible, however, that also the apical dendrites of layer V pyramidal neurons that extend into superficial layers (i.e. see fig. 1.2E), receive peri- and postrhinal inputs.

Most, if not all entorhinal neurons also have axon collaterals that remain within the entorhinal cortex and form local circuits. Presently, little is known, however, about the synaptic connections in these circuits. There is some evidence that principal neurons, particularly those in layers V and III, form extensive horizontal (i.e. intra-laminar) association connections (Dolorfo and Amaral, 1998b, Köhler, 1986, 1988), which could be part of a local recurrent circuit (Dhillo and Jones, 2000). For some interneuron types (i.e. Chandelier cells and basket interneurons) it has been demonstrated that they synapse onto local principal neurons (Jones and Buhl, 1993, Soriano et al., 1993).

An important issue that follows from the description above is that the population of entorhinal neurons that give rise to projections to the HPF (i.e. layers II and III) does not overlap with the population of neurons that receive projections from the HPF (i.e. layer V). Anatomical studies, however, have shown that axon collaterals of entorhinal deep layer...
neurons also distribute to superficial layers (Dolorfo and Amaral, 1998b, Köhler, 1986, 1988, Ramón y Cajal, 1955). Recently, we learned more about the synaptic interactions of the projection from entorhinal deep layers to superficial layers, as described by van Haeften et al. (2003). This study showed that these inter-laminar projections are mainly excitatory, targeting both presumed principal neurons and interneurons. In contrast to the ascending connections in the entorhinal cortex, projections from neurons in superficial layers to deep layers are rather sparse (Dolorfo and Amaral, 1998b).

**FUNCTIONAL OPERATION OF THE HIPPOCAMPAL MEMORY SYSTEM**

How do HPF and PHR operate in the formation of declarative memories? One widely supported view is that the HPF and presumably also the PHR have only a time-limited involvement in memory formation (Squire and Zola-Morgan, 1991). The first argument for this view came from the observation that patients with lesions to the temporal lobe had temporally graded retrograde amnesia, i.e. ‘old’ memories were better remembered than more recent memories. Subsequently, this effect was also observed in animal models. It is believed that memory traces, after being acquired by the hippocampal memory system, are then transferred to their final location in the neocortex (a process termed systems consolidation). After consolidation memories are relatively resistant to lesions of the hippocampal formation (Eichenbaum and Cohen, 2001).

An important question is also whether all areas in the hippocampal memory system always operate together and can be considered a ‘single unit’ or that separate areas may also act independently from the other areas. A related question is whether specific functions can be assigned to individual sub-fields in the hippocampal memory system. The results of some lesion studies suggested that for some memory tasks the HPF is not crucial, but that the same task was performed poorly if the structures in the PHR were damaged (Allen et al., 2002, Eichenbaum and Cohen, 2001, Kaut and Bunsey, 2001, Shohamy et al., 2000). For many memory tasks, however, the HPF as well as its intimate connections with the entorhinal cortex are indispensable (Burgess et al., 2002, Eichenbaum, 2001, Suzuki and Clayton, 2000).

This description of the possible operation of the hippocampal memory system still does not explain how exactly neuronal networks in the HPF and PHR mediate the acquisition, storage, retrieval and consolidation of memory traces. For this it is imperative to know how individual neurons and neuronal networks act and interact during these processes. A vast amount of literature has been devoted to identify the activities of single neurons and populations of neurons using electrophysiological techniques. Importantly, distinct activity patterns – oscillatory potentials and other mass potentials – have been described during different behavioral states. For example, rhythmic 4-12 Hz oscillations (rhythmic slow activity or theta oscillations) are dominant in the HPF and entorhinal cortex during waking.
and rapid-eye-movement (REM) sleep (Alonso and Garcia-Austt, 1987, Buzsaki, 2002, Leung, 1998). Theta oscillations are found in each sub-field of the HPF and in the superficial layers of the entorhinal cortex. Concurrent with theta oscillations, fast field oscillations (>30 Hz, gamma activity) can be recorded, which represent temporally organized discharge of hippocampal and entorhinal neurons leading to population volleys at gamma frequencies (Bragin et al., 1995, Chrobak and Buzsaki, 1998).

During some stages of sleep (i.e. slow-wave-sleep (SWS)), low frequency (<4 Hz) potentials and spindle waves (12-15 Hz) characterize the neocortical electroencephalogram (EEG) (Steriade, 2000). A dominant low frequency oscillation in the neocortex, called the ‘slow oscillation’, has been described in detail by Steriade and co-workers (Steriade et al., 1993a, b). This slow oscillation is generated in the cortex itself and it does not rely on interactions with the thalamus, in contrast to spindle waves (Sanchez-Vives and McCormick, 2000, Steriade et al., 1993b, Timofeev and Steriade, 1996). Nevertheless, slow oscillations are likely to influence communication between neocortex and thalamus. The slow oscillation has also been observed in the perirhinal cortex and entorhinal cortex (Collins et al., 1999, Collins et al., 2001), but much less is known about where these oscillations are generated and how they interact with other local activity patterns.

In the hippocampal formation, CA1 population activity during SWS is characterized by transient bursts of activity (sharp waves), associated with temporal structured discharge of CA1 pyramidal neurons and interneurons (‘ripples’) (Buzsaki, 1986, Suzuki and Smith, 1987, Ylinen et al., 1995). These sharp waves may also be transmitted to the entorhinal cortex (Chrobak and Buzsaki, 1994). In addition another pattern of activity has been reported in the entorhinal cortex, as well as in the perirhinal cortex, which were called ‘sharp potentials’ (Collins et al., 1999, Paré et al., 1995). The origin of sharp potentials is presently unknown, but sharp potentials have been linked to CA1 sharp waves, as well as to amygdalar inputs (Chrobak and Buzsaki, 2002, Paré et al., 1995).

The coordinated activity patterns described above are likely important for selective and effective transfer of information between brain structures. Therefore it is imperative to know the organization of these activity patterns within the HPF and entorhinal cortex, and the relation between the two regions during these oscillations and mass potentials.

Concerning the actual storage of memories it is widely accepted that in the brain information is stored in changes of the strength of the transmission between neurons (i.e. synaptic plasticity). It was the seminal idea of Hebb that strengthening of a connection would occur if both the sending and receiving neurons were simultaneously active. Both long-lasting enhancement (long-term potentiation, LTP) and reduction (long-term depression, LTD) of synaptic responses have been reported, using high-frequency stimulation or more physiological stimulus patterns. Long-term potentiation was first described in the HPF and a great deal of research has been devoted to elucidating its cellular and molecular mechanisms (Bi and Poo, 2001, Braunewell and Manahan-Vaughan, 2001, Frey, 2001, Martin et al., 2000, 22
O'Mara et al., 2001, Paulsen and Sejnowski, 2000, Silva, 2003). Synaptic plasticity has also been found in the PHR (Alonso et al., 1990, Cheong et al., 2002, de Curtis and Llinas, 1993, Ivanco and Racine, 2000, Kourrich and Chapman, 2003, Yun et al., 2000, Ziaiopoulos et al., 1999) and it is probably a property that is common to most brain structures. These forms of synaptic plasticity are very sensitive to the frequency at which the neurons are activated. For example, activation at the theta frequency has been shown to promote LTP, whereas activation at low frequencies may favor LTD (Bear and Malenka, 1994, Morgan and Teyler, 2001). In addition, precise timing of pre- and postsynaptic activation (Magee and Johnston, 1997, Markram et al., 1997) and the phase relation of synaptic inputs to population oscillatory activities (Orr et al., 2001) appear to be important parameters for induction of synaptic plasticity.

The process of storing information in neuronal circuits may be mediated by a temporary enhancement of cell firing. This persistent activity is sometimes referred to as ‘dynamic memory’, since it does not involve the lasting structural changes that accompany synaptic plasticity. Persistent activity may involve distinct neuronal groups which are located in different cortical areas, and as such these groups may be functionally linked to form cell assemblies, as first proposed by Hebb (1949). Since cells belonging to the same assembly are simultaneously active, their connections may be strengthened, leading to a further establishment of the assembly. Cell assemblies have been proposed as a mechanism by which remote cortical areas, encoding different aspects of a sensory stimulus are bound together, forming a distributed representation of the stimulus. This binding has been proposed to occur by means of transient synchronization of fast gamma activities (Engel and Singer, 2001, Fell et al., 2003, Varela et al., 2001). This implies that coordinated gamma oscillations between different brain areas may be important for the processing of information necessary for memory formation.

Persistent activity can be turned on and maintained in several ways (Wang, 2001), and one possibility is that it can occur in re-entrant loops. As is clear from the previous sections and from the scheme in figure 1.3A, an important characteristic of anatomical organization in the hippocampal memory system is the presence of reciprocal connections between HPF and PHR, as well as within the PHR, which form the basis for re-entrant loops in the hippocampal memory system. The intrinsic entorhinal connections between deep and superficial layers (Dolorfo and Amaral, 1998b, Köhler, 1986, 1988, van Haeften et al., 2003) are an important link in the establishment of such loops. Persistent activity has indeed been found in the parahippocampal region as well as in the subiculum during the delay phase of a memory task (Hampson et al., 2000, Hampson and Deadwyler, 2003, Suzuki et al., 1997, Young et al., 1997), although the involvement of re-entrant loops has not been established yet. Besides the induction of persistent activity, re-entrant loops in the hippocampal memory system could provide a means by which processed output is combined with information flow in input.
Chapter 1

pathways. The nature of the processing that occurs in such loops, however, is far from understood.

AIMS AND OUTLINE OF THIS THESIS

In the previous sections, an overview was presented of the organization of the hippocampal memory system and it was argued that the intimate connections between the entorhinal cortex and the sub-fields of the hippocampal fields are essential for memory processes. The studies described in this thesis were carried out to elucidate the interactions between deep and superficial layers of the entorhinal cortex, particularly in the view of the intimate relations between the entorhinal cortex and hippocampal formation. To this end, both anatomical and electrophysiological techniques were employed (see Box). In particular, the studies described in this thesis focused on the presence of re-entrant circuits in this system with the aim to elucidate both the anatomical specificity as well as the functionality of these circuits, without addressing here explicitly how these circuits may mediate memory processes.

In the first study (chapter 2), the question was addressed whether the organization of output projections from the HPF to the PHR matched the organization of the input projections. Anatomical tracing techniques (see Box) were applied to reveal the topographical organization of the connections from subiculum to the PHR. This study demonstrated that indeed input and output organizations were matched, which implies that the segregation that is present in the input pathways (see section about connectivity above) is maintained at the output stage.

In chapter 3, the output from the HPF to the entorhinal cortex was investigated by means of in vivo electrophysiology (see Box). We were especially interested to see whether superficial entorhinal layers could be activated by electric stimulation of hippocampal output and to determine how deep layers were involved in this process. The results clearly demonstrated an entorhinal deep layer mediated excitation of entorhinal layer III and we were able to determine the dynamics of this inter-laminar communication.

Even though the study in chapter 3 showed entorhinal deep-to-superficial layer communication, it did not provide an answer to the question if entorhinal layer II or III neurons that project to the HPF actually are activated via deep entorhinal layers. Therefore, in the following study (chapter 4), simultaneous recordings were performed in the entorhinal cortex and HPF to solve this issue. Repetitive stimulation of hippocampal output was able to induce long-latency 're-entrant' potentials in both CA1 and dentate gyrus. An important new finding is that these two parallel re-entrant pathways differ in their sensitivity to anesthetic agents. We may speculate that in freely moving animals both re-entrant loops can be regulated differentially, possibly related to the behavioral state of the animal.
The studies described in chapters 3 and 4 utilized artificial activation of hippocampal output by electrical stimulation to probe hippocampal-entorhinal interactions. Very useful information can be extracted from these studies, but it is unlikely that such a massive recruitment of neurons would take place under physiological conditions. For that reason hippocampal-entorhinal interactions were further investigated in chapter 5 by analyzing spontaneous activity patterns in the local EEG of the HPF and entorhinal cortex. In particular, we focused on slow oscillations present in the entorhinal cortex and hippocampal formation and how these are organized in and between these structures. We also demonstrated a clear relation between the slow oscillations and other activity patterns.

In the last chapter a brief summary is given of the relevant results and conclusions presented in chapters 2-5. Finally, the new findings put forward in this thesis are discussed and particularly attention is given to the possible role of entorhinal deep-to-superficial layer communication in the information processing in the hippocampal-entorhinal system.
Anatomical tracing techniques are commonly used to reveal the organization of connections between brain areas. Tracers are injected into the brain and either are taken up by dendrites and somata and transported via the axon to the terminal field (anterograde tracer, fig. A, see chapter 2) or are taken up by axon terminals and transported to the cell soma (retrograde tracer). The labeled axonal plexus or cell somata can then be visualized and inspected by light microscopy.

Neurons use electrical signals for very fast communication. Each neuron maintains an electrochemical gradient across its membrane. The electrical resistance offered by the membrane can undergo changes due to the opening/closing of ion-permeable channels, leading to the generation of trans-membrane currents. The currents induce measurable changes of intracellular and extra-cellular potentials. In figure B, the generated current flow is shown for a neuron receiving an excitatory synaptic input on its dendrite. The extra-cellular currents and potentials generated by a single cell are generally small. However, in the case the dendrites of a group of neurons are orderly arranged and these neurons simultaneously receive synaptic inputs on a similar part of their dendrites, extra-cellular potentials may summate, resulting in an easily measurable population (field) potential. One disadvantage of extra-cellular potentials is that they can be measured at large distances from the actual site of generation, a property generally referred to as volume conduction. An estimate of the local trans-membrane currents can be made using current source density (CSD) analysis. The principle of one-dimensional CSD analysis is shown in figure B. If potentials are recorded at regular intervals along a line, the first order...
difference will give estimates of the currents flowing between the voltage sensors, and the second order difference will give estimates of the current that is ‘lost’ (sink) or ‘gained’ (source) at a particular location. Under the assumption that the major extra-cellular current flow is oriented parallel to the recording track, these current sinks and sources are proportional to the trans-membrane currents. Generally, in the calculation of the CSD the electrical resistance of the tissue between voltage sensors is assumed constant. Identification of current sinks and sources alone is generally not sufficient to allow an interpretation of the underlying cellular and synaptic events. For example, the same current flow in figure B could be generated by an inhibitory synapse located at the soma. Additional information can be gained by recording neuronal firing, and by taking the microstructure of the neurons and circuits into consideration. Neuronal discharges recorded extra-cellularly are referred to as unit activity. In the case the action potentials of a single neuron are recorded (single unit activity) using a glass micropipette, it is possible to label the neuron so that it can be morphologically characterized (juxta-cellular labeling, see chapters 3 & 5).

The electrophysiological studies in this thesis were performed in vivo, in anesthetized rats. This preparation has both advantages and disadvantages, but it is a reasonable trade-off between how well it compares to the behaving animal (e.g. all connections are intact) and how easily the preparation can be manipulated (e.g. pharmacological interventions). One should keep in mind, however, that anesthesia can have profound effects on the cellular and network physiology.

Exploration of neuronal circuits and systems can be done by measuring the responses to a stimulus, for example by artificial electrical stimulation of a brain area (see chapters 3 & 4), or by analyzing the ‘spontaneous’ activity in these circuits. In the latter case, cross-correlation analysis as well as spectral analysis may be performed to reveal the properties of the signals and the relations between different brain areas (see chapter 5). Another technique we used to explore spontaneous activity patterns was by decomposing the signal into statistically independent components (i.e. independent component analysis, ICA), which will be further explained in chapter 5.