IS THERE BEHAVIORAL RELEVANCE FOR THE
TOPOGRAPHICAL ORGANIZATION OF MAMMALIAN
HIPPOCAMPAL FORMATION?

BY

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A thesis submitted in conformity with the requirements for the
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Is there behavioral relevance for the topographical organization of mammalian hippocampal formation?

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Abstract

The idea that memory is constituted of a number of fundamentally different processes has been investigated using the concept of memory systems. Memory subsystems are hierarchically subordinate components distinguished on two criteria: kinds of information they process, and neuroanatomical substrates. It is generally accepted that the hippocampal formation is the site of a memory system dedicated to learning relationships among cues of various kinds. Anatomical, neurochemical, and physiological organization of this structure suggests that it may be subdivided into several memory subsystems. The work presently described investigated this possibility by studying the function of septal vs. temporal hippocampal areas in spatial learning in the water task, context conditioning, and conditioned place preference. Results indicated that both dorsal and ventral hippocampus are involved in spatial navigation, although the former is a better substrate for this process. In contrast, these networks were equally involved in comparing contexts with different affective valence. Third, dorsal hippocampal lesions impaired, while ventral hippocampal lesions enhanced performance in conditioned place preference. Taken together, these data demonstrate a dissociation between the functions of dorsal and
ventral hippocampal areas. This distinction is made according to behavioral paradigms rather than kinds of information processed. Acquisition of spatial information, stimulus-affective response associations, and comparison among cues connect the three tasks employed, but the dorsal/ventral hippocampal lesions affected them differentially. This suggests that a more useful criterion for defining memory subsystems may be task requirements. From this perspective, the hippocampal formation may include three different memory subsystems. The first, important for spatial navigation, is based on activity of dorsal hippocampal area, and is connected to medial entorhinal cortex, septum and lateral nucleus accumbens. The second, involved in modulation of interaction with amygdala memory system, is based on activity of ventral hippocampal and is connected to medial nucleus accumbens. The third is relevant for comparison among cues presented simultaneously, utilizes the hippocampal longitudinal network, and is likely connected to lateral entorhinal cortex.
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1. INTRODUCTION

The idea that memory is not a unitary phenomenon, but rather a collection of fundamentally different kinds of processes is relatively new. The history of debate on this issue can be traced back to Tolman (1948), who argued that representations of stimuli encountered in the environment are used in forming cognitive maps. These in turn represent the basis on which behavioral responses are performed. In Tolman's view, animals can learn either stimulus-response or stimulus-stimulus associations. This argument was against Hull's proposal (1933) that learning is a homogenous process involving formation of stimulus-response associations. History eventually proved Tolman right. Scoville and Milner (1957) published a now famous paper showing that bilateral resection of medial temporal lobes resulted in severe and permanent inability of acquiring new information. This finding prompted a series of studies whose general conclusion is that hippocampal function is involved in some, but not all learning processes.

A well-developed theoretical framework for learning and memory research is far from being achieved, as empirical data add not only knowledge, but also puzzlement. One reason for this situation was the absence of clearly formulated criteria for defining and dissociating among different kinds of memories, processes associated with them, and their neurological substrate. A recently formulated concept that originates in Tolman's perspective and has shaped the debate within the learning and memory literature is the one of memory systems. A review of the definitions given by various authors to this term (Schacter and Tulving,
1994) reveals the importance of a number of factors such as type of information input, principles of operation on this information, neural structures involved, phylogenetic development, and forms of representation. Building on a series of previous attempts (Hirsh, 1974; Gaffan, 1974; O'Keefe and Nadel, 1978; Olton et al., 1979 among others), Schacter and Tulving (1994) argued that memory systems should be distinguished based on three main characteristics:

Class-inclusion operations. Systems perform a large number of tasks of a particular category, regardless of the type of information input.

Properties and relations. The properties (rules of operation, kind of information processed, and neural substrate) define the nature of the memory system’s identity and the relationships in which it engages with other memory systems.

Convergent dissociations. Independent memory systems contribute differentially to behavioral performance in different tasks.

This approach allows distinction between the concept of memory system on one hand and kind of memory (e. g., explicit/implicit) and neuropsychological disorders on the other hand. Schacter and Tulving also postulate the existence within each memory system of hierarchically subordinate memory subsystems. The latter are distinguished in terms of the kind of information they process and their neuroanatomical basis. Subsystems share the operational principles of the memory system to which they belong.

A large body of empirical data suggests that the hippocampal formation is the functional center of a specific memory system. Despite accumulating evidence, a heated debate
continues to take place regarding the rules of operation and the kind of information this system employs. Some argue that the hippocampal formation encodes and processes exclusively spatial information. Others see learning about space as a subset of the more general ability of acquiring relationships among cues (Memory Systems 1994; O'Keefe and Nadel, 1978; Hippocampus – Forum, 1991; Squire and Zola-Morgan, 1991; Rudy and Sutherland, 1995; Sutherland and Rudy, 1989; Eichenbaum et al., 1992; Shapiro and Eichenbaum, 1999; Eichenbaum et al., 1999; O'Keefe, 1999). One reason why this debate is still so heavily laced with controversy is that the hippocampally-based memory system may be composed of a series of subsystems whose relevance for behavior is differentially contingent upon specificities of behavioral tasks.

Increasingly, anatomical, pharmacological, and electrophysiological studies suggest that the organization of the hippocampal formation follows certain topographical principles. The work presented in this thesis investigated the behavioral relevance of distinct neural circuits along the longitudinal axis of this structure. I will first summarize anatomical, neurochemical, neurophysiological and behavioral evidence arguing for the presence of memory subsystems within the hippocampal formation. I will then briefly review the current debate on hippocampal function. This will provide the conceptual framework within which the present experiments have been designed. Then I will present the empirical evidence constituting the body of this thesis (chapters 2, 3, 4), and finally I will consider the relevance of this work to the issues of hippocampal function and memory subsystems in the mammalian brain (chapter 5).
1.1 Anatomical organization of parahippocampal region and hippocampal formation

Anatomical data clearly and coherently indicate that the hippocampal formation and its inputs/outputs are topographically organized. The parahippocampal region (PHR) and the hippocampal formation are both major parts of the limbic system. The term 'limbic' has been initially coined by Broca who derived it from the Latin word *limbus* (rim). However, a description of its specific circuits and a theory regarding their function was not developed until later by Papez (for a detailed discussion, see LeDoux, 1996, pp. 85-103 and Witter et al., 1989, pp. 162-163) who hypothesized that the hippocampal formation plays a role in generating experiences and controlling emotional reactions. Prompted by the reports of Klüver and Bucy (1937, 1939) that medial temporal lobectomies in the monkey resulted in modifications of affective response, MacLean (1952) introduced the term *limbic system*. He later described the limbic system as the second of three levels of organization existent in the human brain: reptilian, mammalian (or visceral) and neomammalian. (MacLean, 1970). According to this theory, the functional significance of this collection of various neural circuits is to provide the basis for social-related behaviors, such as nurturance, distress, and playfulness. (The reptilian brain controls most basic behavioral routines, such as locomotion, eating, drinking, and aggression. The neomammalian brain is seen as the neural substrate of higher psychological processes such as cognition). Discussion has however continued to this day regarding the exact brain structures that should be included under the label of limbic system (for discussion on this topic, see LeDoux, 1996, pp. 99-128, Panksepp, 1998, pp. 69-80, and Witter et al., 1989, pp. 163-164). For instance, in
MacLean's definition, the limbic system included the limbic cortex (subcallosal, cingulate and parahippocampal gyri), the mammillary bodies, the anterior thalamus, and the amygdaloid complex. To this were later added the septal region, the preoptic area, the hypothalamus, and the paramedian area of the mesencephalon. Most recently however, the limbic system is considered as including the hippocampal formation, the amygdaloid complex, the hypothalamus, and the associated cortical and subcortical formations (Witter et al., 1989).

Regardless of the debate on anatomical boundaries of the limbic system, the PHR and the hippocampal formation are important core structures within this larger functional unit. As the name indicates, the PHR surrounds the hippocampal formation and represents a major source of input and output for its circuits. Witter et al. (1989) divided it into a core formed by the entorhinal cortex (EC) and the adjacent Brodmann area 35, and a belt constituted of presubiculum (PreS), parasubiculum (ParaS), the postrhinal cortex (PostR), and Brodmann area 36. It should be mentioned that areas 35 and 36 together constitute the perirhinal (PER) cortex. These authors consider the belt a relay station through which the rest of the cortex interacts with the core, while the core itself is an area strongly connected with the hippocampal formation. The hippocampal formation encompasses the dentate gyrus (DG), the fields CA3-CA1 or hippocampus proper, and the subiculum (S).

Neural connections within the PHR are topographically organized. I will discuss in some detail the circuits within EC and hippocampal formation and their relationship with amygdala (AMG) and nucleus accumbens (ACC). The focus on AMG and ACC among the
other connections is justified because AMG is the center of a different memory system (e.g. Kapp et al. 1979; LeDoux, 1993, 2000) which interacts with the hippocampally-based one in certain conditions, while ACC acts as a motor gate for both AMG and hippocampal formations (Mogenson, 1984).
1.1.1  **Entorhinal cortex.**

Based on cytoarchitectural descriptions, the EC has been divided into medial (MEA) and lateral (LEA) components. Independent of this classification, there is a second one based on rostro-caudal organization of intrinsic fibers. This cuts perpendicularly onto the MEA/LEA division. I will refer to the three areas included in this category as lateral, intermediate, and respectively medial longitudinal strip. A third dimension of EC anatomy is constituted by lamination: superficial vs. deep layers.

1.1.1.1  **MEA/ LEA division.**

LEA is located anterior and ventral to the MEA and is in close proximity to the PER. MEA is bordered dorsally by the PostR and ParaS. The LEA shows a clearer distinction between layer II and layer III neurons. Second, the cells of this area are clustered in islands. Third, the MEA layers IV – VI are more distinctive and show clearer columnar arrangement (Witter et al., 1989). Additionally, layer II of LEA is thinner that its MEA counterpart (Dolorfo and Amaral, 1998b). Axons of MEA and LEA neurons constitute the perforant path (PP) which provides input from EC to the hippocampal formation (Hjorth-Simonsen and Jeune, 1972). Following the MEA/LEA origin, the PP also has two components, medial (MPP) and lateral (LPP). These have different anatomical distributions, as well electrophysiological and pharmacological properties (see next sections). Compared to the MPP, the LPP is distributed more widely along the longitudinal axis of the hippocampal formation (Ruth et al., 1982, 1988). Associational fibers originating in the lateral portion of
LEA target the LEA itself, while fibers originating in the medial LEA project to the MEA; MEA projects heavily to LEA (Dolorfo and Amaral, 1998).

The PP synapses onto DG granule cells and onto pyramidal neurons of CA3-CA1 and S. These projections are topographically organized. In the DG and CA3 the connections are arranged in 2 horizontal planes, with LPP synapsing on the outer 1/3, and MPP on the middle 1/3 of the dendrites. In the CA1 and S, the projections are organized vertically. LPP projects to the distal CA1 and proximal S, while MPP projects to the proximal CA1 and distal S (proximal and distal are defined relative to DG; for an illustration see figure 1.1 reproduced with permission from Amaral and Witter, 1995, figure 19). Projections back to EC are also topographically organized, such that each EC area receives fibers from the hippocampal regions to which it preferentially projects.
Figure 1.1 Topographical organization of projections between EC and hippocampal formation. Reproduced with permission from Amaral and Witter, 1995.
1. Entorhinal Cortex
2. Perirhinal Cortex
3. Infrahilimic Cortex
4. Mammillary Nucleus (rostral)
5. Nucleus Accumbens
6. Lateral Septal Region

1. Midline Thalamus
2. Entorhinal Cortex

1. Retrosplenial Cortex
2. Presubiculum
3. Anterior Thalamic Complex
4. Mammillary Nucleus (caudal)
5. Entorhinal Cortex
1.1.1.1 Inputs/outputs to the MEA and LEA.

*Cortical inputs.* Burwell and Amaral (1998b) have recently provided a summary of the cortical afferents to the EC. MEA receives a larger proportion of projections from cingulate (mainly dorsal retrosplenial cortex, but also anterior cingulate), posterior parietal and occipital (primarily visual associational, but also primary visual) cortices. Both MEA and LEA receive input from the piriform cortex. LEA receives a heavier projection from PER and MEA a heavier projection from PostR. PER receives a large input from temporal cortex, piriform cortex, insula, and EC. Thus, PER cortex receives uni- and polymodal information, which is transmitted preferentially to LEA. The PostR cortex receives visual and visuospatial input from the posterior parietal and retrosplenial cortices, which is relayed mainly to the MEA.

*Subcortical inputs.* Although ParaS projects to both MEA and LEA, PreS and ParaS outputs are directed mainly to the MEA. So are the projections from the medial septum, which terminate in layer II and reach the EC after crossing the AMG (Witter et al., 1989). Projections from the hypothalamus or brain stem do not seem to segregate between MEA and LEA with the exception of lateral hypothalamic area which projects preferentially in the rostrolateral LEA (Witter et al., 1989).

*Cortical outputs.* The issue of EC projections back to the neocortex is still subject of dispute (Amaral and Witter, 1995), although Swanson and Köhler (1986) had previously reported
that EC, particularly the LEA sends output to the infralimbic, prelimbic, somatosensory, motor, and auditory cortices.

**Subcortical outputs.** Projections to the hippocampal formation constitute a large component of EC output. Aside from these, EC sends a strong projection to the lateral septum which has not been clearly dissociated based on the MEA/LEA distinction.

1.1.1.2 Longitudinal strips (lateral, intermediate, and medial).

There has been a relatively large amount of research outlining the topographical organization of the projections from the longitudinal strips to the hippocampal formation (Ruth et al., 1982, 1988; Witter and Groenewegen, 1984; Van Groen and Witter, 1985; Witter et al., 1989; Dolorfo and Amaral, 1998a; for reviews see Witter et al., 1989 and Amaral and Witter, 1995). Data obtained with both anterograde and retrograde labeling are highly coherent and indicate that for both MEA and LEA, cells located in the lateral strip project predominantly to the dorsal areas of the hippocampal formation, cells in the intermediate strip project to the intermediate, splenial region, and cells in the medial strip project mainly to the most ventral tip (see figure 1.2, reproduced with permission from Amaral and Witter, 1995, figure 14). In general, there is little overlap between the target areas within the hippocampal formation (Dolorfo and Amaral, 1998a) and their return projections to the EC tend to be confined to the areas that originated them (Köhler, 1985a; Van Groen and Wyss, 1990).
Figure 1.2 Topographical organization of EC projections along the longitudinal hippocampal axis. Reproduced with permission from Amaral and Witter, 1995.
In a study of EC organization, Dolorfo and Amaral (1998b) recently confirmed earlier findings that within strips, connections are organized such that projections tend to be confined to the area which also contains the cell bodies. Thus, neurons that project to the more dorsal levels of the hippocampal formation do not communicate directly with neurons which project to the more ventral levels.

1.1.1.2.1 Input/output connections of the longitudinal strips.

Cortical inputs. The three longitudinal strips receive different types of information (for comprehensive reviews see Burwell and Amaral, 1998a; Amaral and Witter, 1995; Witter et al., 1989). The lateral strip receives mostly projections from unimodal and polymodal associational cortical areas through the PER cortex. The intermediate strip also receives mostly sensory information, but PostR cortex additionally sends input here.

Subcortical inputs. The most medial strip receives mostly information from the AMG, as well as from ventral S, anterior thalamus, medial septum and ventral parts of PreS and ParaS. Dorsal S and PreS project to the lateral longitudinal strip (Witter et al., 1989).

Cortical outputs. The lateral strip sends output to the PER and the medial, but not intermediate EC strip (however see Dolorfo and Amaral, 1998b). It is still unclear whether the EC projects directly to the neocortex (for review of literature, see Amaral and Witter, 1995, p. 482-483). The intermediate zone projects to the other two strips (Witter et al.,
1989). Projections from the most medial zone are confined within this zone itself (Dolorfo and Amaral, 1998b).

**Subcortical outputs.**

While septal input to the EC originates mainly in the medial part of the structure, the output back from the EC is directed mainly to the lateral septum. The lateral strip is connected with the dorsomedial lateral septal nucleus and the medial strip with the ventromedial lateral septal nucleus (Witter et al., 1989). These connections are in register with projections from the hippocampal formation. Dorsal CA1 and S, which receive input from the most lateral EC strip, also project to the dorsomedial portion of the lateral septal nucleus, while the ventral CA1 and S, which receive input from the most medial EC strip, project to the ventromedial lateral septal nucleus. The lateral septum in turn projects to the medial septum (which is the source of input for the EC), but the significance of this topography relative to the hippocampal formation is still not known (Witter et al., 1989, pp. 199-201).

There is a weak connection from the most medial EC strip to the mammillary nuclei (Witter and Groenewegen, 1986). Connections to the AMG and ACC will be discussed separately.

1.1.1.3 Lamination.

The EC has six cell layers. Of these, layers II and III contain the neurons whose axons form the PP, while projections back from the hippocampal formation are received by neurons
from the deep layers IV-VI (Steward, 1976; Steward and Scoville, 1976; Ruth et al., 1982, 1988; Witter and Groenewegen, 1984; Dolorfo and Amaral, 1998b). Layer II projects to DG and CA3, while projections to CA1 and S originate in layer III. Layers IV-VI send output to layers II and III (Dolorfo and Amaral, 1998b) and possibly to the cortical areas that provide the sensory input to the PHR (Swanson and Köhler, 1986; but see Amaral and Witter, 1995).

1.1.1.3.1 Input/output connections of EC cell layers.

Generally, superficial EC layers receive neocortical input while deep layers originate the output. Additionally, there are projections from the deep layers of EC towards the superficial layers and horizontal projections within the superficial layers. These connections are organized such that the longitudinal strip separation is preserved (Dolorfo and Amaral, 1998b).

*Cortical inputs.* Cortical areas that project to the EC have been summarized above. This input contains sensory information that has passed through several stages of processing and is directed to layers I – III.

*Subcortical inputs.* Fibers originating in the septum are directed to layers II-IV of the EC. Projections from the thalamus are directed to the superficial layers, while projections from the hypothalamus and brain stem terminate difusely across all divisions and layers of EC.
**Cortical outputs.** Projections back to the areas that originated input to the EC arise almost exclusively from deep layers of LEA (Witter et al., 1989).

**Subcortical outputs.** Projections from the deep layers of the EC are directed to the claustrum, striatum, and septum.

1.1.2. **Hippocampal formation.**

The overall shape of the structure resembles a letter C with the superior pole located next to the septal nuclei and with the inferior pole terminating more caudally in the depth of the medial temporal lobe. The longitudinal dimension of the HPC is known as either dorso-ventral or septo-temporal axis. Perpendicular on the longitudinal axis is the transversal axis.

1.1.2.1 **Organization of connections on the transversal axis.**

The arrangement of the MPP and LPP projections to the DG, CA3-CA1 and S have been described above. Additionally, there are also intrinsic connections among the fields of the hippocampal formation. The topographical organization of these connections has been synthesized by Amaral et al. (1991). Granule cells of the DG generate the mossy fibers which synapse *en passant* onto CA3 pyramidal neurons located throughout the transversal extent of the field. The axons of pyramidal cells send collaterals to the contralateral hippocampus (commissural fibers) and to the other neurons in CA3 (recurrent collaterals). Thus, the CA3 has an autoassociative network. The CA3 projections to CA1 (Schaffer collaterals) are topographically organized (see figure 1.1): proximal CA3 is connected to
proximal CA1 and distal CA3 to distal CA1. CA1 relays input to S in a similar, but not identical topographical fashion: proximal CA1 to distal S and distal CA1 to proximal S.

1.1.2.2 Organization of connections on the longitudinal axis.

These have been summarized by Amaral et al. (1991; figure 16 reproduced with permission as figure 1.3). Neurons located proximally in CA3 tend to project to more septal levels of CA1, while neurons in the distal CA3 project to more temporal levels of CA1 (Ishizuka et al., 1990; Amaral et al., 1991; Amaral and Witter, 1995). However the proximal CA3 axons tend to shift their terminations from the distal to the proximal CA1 as they travel septally. In contrast, axons originating in distal CA3 shift from proximal to distal CA1 neurons as they travel temporally. Within CA1, there are very few longitudinal associational fibers (Amaral et al, 1991). Thus, information may be channeled from proximal CA3 to distal CA1 and proximal S with a branching in CA1 towards more septal and more proximal areas, and from distal CA3 to proximal CA1 and distal S with a branching in CA1 towards more ventral and more distal areas.
Figure 1.3 Organization of connections on the longitudinal axis within the hippocampal formation. Reproduced with modification from Amaral et al., 1991.
1. Entorhinal Cortex LEA  
2. Perirhinal Cortex  
3. Infralimbic Cortex  
4. Mammillary nucleus (rostral)  
5. Nucleus Accumbens  
6. Lateral Septal Region

1. Midline Thalamus  
2. Presubiculum  
3. Anterior Thalamic Complex  
4. Mammillary Nucleus (caudal)  
5. MEA

Subicular afferent data from:  
Witter et al. (1990), European J. Neurosci. 2:718-725  
Witter (personal communication)
1.1.2.3 Inputs/outputs of the hippocampal formation.

Cortical projections to the hippocampal formation constitute the PP. Clarification of hippocampal output organization has been an important step in understanding the functional significance of this structure (Swanson and Cowan, 1977). Connections back to the EC and other cortical areas originate in CA1 and S. Projections to and from subcortical areas are grouped in four main pathways. Three of these take a dorsal course: the fimbria, the dorsal fornix, and the supracallosal stria. They contain axons connecting bidirectionally the dorsal and splenial areas of hippocampal formation with medial and lateral septum, the vertical limb of the nucleus of the diagonal band, hypothalamus, mammillary bodies, and the brain stem. The fourth pathway, called the ventral angular bundle, contains fibers connecting the most ventral parts of the hippocampal formation with the AMG and the horizontal limb of the nucleus of the diagonal band as well as with brain stem structures such as the locus coeruleus and the mesencephalic raphe (Amaral and Witter, 1995; Van Groen and Wyss, 1990, Cassel et al., 1997; Maren and Fanselow, 1995, Swanson and Cowan, 1977). The existence of this fourth pathway, usually overlooked, may be of particular importance because it remains intact following fimbria-fornix lesions.

Cortical inputs. These are provided by the EC and have already been discussed.

Subcortical inputs. Medial areas of the medial septum project mainly to the dorsal HPC pole, while cells located more laterally in the medial septum project mainly to the ventral HPC pole. The projections reach all hippocampal fields and are in register with the ones
from the medial septum to the longitudinal strips of the EC. Other important sources of input to the hippocampal formation are the hypothalamus and the brain stem.

**Cortical and subcortical outputs.**

**MPP/LPP division.** DG sends outputs only to the CA3 field (the mossy fibers). Similar to DG, the CA3 field does not send other outputs except to the next hippocampal relay station, which is the CA1 field. Thus the cortical output of the hippocampal formation originates in the CA1 and S. Distal CA1 and proximal S, which are projected upon mainly by LEA, send output back to the LEA, as well as to PER, infralimbic cortex, ACC, and lateral septum. Proximal CA1 and distal S, projected upon by the MEA, send output back to this area, as well as to retrosplenial cortex, presubiculum, anterior thalamus, and mammillary nucleus (see figures 1.2 and 1.3; Amaral and Witter, 1995; Amaral et al., 1991; Van Groen and Wyss, 1990; Swanson and Cowan, 1997).

**Dorsal/ventral division.** Projections to prefrontal cortex originate in the splenial and temporal, but not septal areas of CA1 and S (Verwer et al., 1997). Splenial hippocampus sends output to the lateral prefrontal cortex, but there are collaterals also to the medial prefrontal cortex. Temporal hippocampus projects exclusively to the medial prefrontal cortex. Swanson and Cowan (1977) found that dorsal S projects to mammillary bodies and ventral S to hypothalamus. Septal CA1 and S project mainly to the lateral EC strip while temporal hippocampal areas are connected mainly with the medial strip (Amaral and Witter, 1995).
One important point of anatomical organization of subcortical projections from the hippocampal formation is that they are not entirely accomplished through the fimbria-fornix-supracallosal stria complex. Van Groen and Wyss (1990; fig. 10) indicate that fibers leaving the temporal CA1 reach AMG by coursing directly rostrally.

1.1.3 Connections with AMG and ACC.

1.1.3.1 AMG

Like PHR and hippocampal formation, AMG is part of the temporal lobe structures. It is presently considered as the neural basis of affective memory system (Rolls, 1999 pp. 94-112; LeDoux, 1992, 1993, 1996; Selden et al., 1991; Gaffan, 1992; Davis, 1992; Aggleton, 1993; White and McDonald, 1993; Davis et al., 1994). It is located anterior of the temporal hippocampal pole with which it has close connections, as it has with PHR. AMG is subdivided in a series of nuclei (for a comprehensive review see McDonald, 1998), but a good comprehension of their functional importance is presently lacking. Swanson and Petrovich (1998) differentiated between an accessory olfactory system, a main olfactory system, an autonomic system, and a frontotemporal system. Of importance for the interactions with the hippocampal formation is mainly the latter. Encompassing the lateral (L) and basolateral (BL) AMG nuclei, it interacts bidirectionally with frontal and insular cortices. Additionally, L AMG nucleus has close connections with temporal cortex and hippocampal formation. Both L and BL AMG project to the ACC.
1.1.3.2 EC-AMG projections.

1.1.3.2.1 Amydalofugal connections.

**MEA/LEA.** The exact topography of AMG projections to the EC has not been mapped as well as in the case of EC to HPC connections. However, a series of studies (Krettek and Price, 1977; Ottersen, 1982; Witter et al., 1989; Pikkarainen et al., 1999) indicate that the L AMG projects mainly to the LEA (layer III), while MEA receives input from the BL AMG (layers III and V).

**Longitudinal strips.** As already discussed, AMG projections to the EC target specifically the medial longitudinal strip.

**Lamination.** AMG projections are directed to the superficial EC layers. (McDonald, 1998).

1.1.3.2.2 Amygdalopetal connections.

As a general principle, amygdalopetal projections from the EC originate in the deep layers, which receive input from the hippocampal formation (but see above). Russchen (1982) and Room and Groenewegen (1986) found retrograde labeling in LEA (deep layers) following injections in the L AMG. Witter et al. (1989) consider that the strongest projections from the
EC are directed to the B AMG. In the literature there are however descriptions of projections to both L and BL AMG nuclei (Price et al., 1987; Amaral et al., 1992).

1.1.3.3 AMG – HPC projections.

1.1.3.3.1 Amygdalofugal connections.

AMG projects to the temporal areas of the CA3, CA1 and S, while no projections have been described to the DG (Krettek and Price, 1977; Ottersen, 1982; Pkkarainen et al., 1999).

1.1.3.3.2 Amygdalopetal connections.

The temporal thirds of CA1 and S project to a series of AMG nuclei (mainly the accessory basal) but not to the L AMG (Canteras and Swanson, 1992; Van Groen and Wyss, 1990; Amaral and Witter, 1995; Price et al., 1987). As already emphasized above, connections from the ventral hippocampal formation to BL AMG do not reach their destination after coursing through the Fx, but directly, through the ventral angular bundle.

1.1.3.4 ACC

ACC is the ventral component of the basal ganglia (Joel and Weiner, 1994; McGeorge and Fauill, 1989) and is also known as the ventral striatum. It is seen as an output gate for both AMG and hippocampal formation to the motor system (Mogenson et al, 1980; Mogenson 1984, Lopes Da Silva et al., 1985).
Anatomical connections between ACC, HPC and AMG have been summarized by Groenewegen et al. (1987), Pennartz et al. (1994), and McDonald (1991). The lateral longitudinal EC strip, which projects mainly to the dorsal hippocampal areas, projects mainly to the rostrolateral ACC, while the medial longitudinal EC strip, connected mainly with ventral hippocampal region, projects to the medial ACC. Projections from the hippocampal formation to ACC course through the Fx and respect a similar topography: dorsal hippocampal area projects to lateral ACC, while ventral HPC area is connected to the medial ACC. BL AMG projects selectively to caudomedial ACC. Thus, the medial ACC is the site of convergence for fibers originating in BL AMG and ventral HPC. This anatomical organization seems to have electrophysiological significance (see Mulder et al., 1998; for more details, see section 3.4).
1.1.4 Behavioral relevance of anatomical connections.

Speculations on the functional relevance of these anatomical connections have been discussed in detail by Witter et al. (1989), Amaral and Witter (1995), Pennartz et al. (1994).

Empirical evidence discussed in this section suggest that EC is organized along three directions. First, there is a MEA/LEA division based on cytoarchitectural criteria. Second is the organization of intrinsic EC along longitudinal strips. These strips project to selective areas of the hippocampal formation. Third is the laminar EC division into superficial and deep neuronal layers.

The pattern of connections on the transversal hippocampal axis creates the possibility of information channeling through this structure. The MEA/LEA segregation of input, the output from CA1 and S to the deep EC layers and the intrinsic EC connections from deep to superficial layers may create closed loops within which information is processed repeatedly. It is important to notice that the separation between these loops would be relative rather than absolute. Cross-referencing does occur in DG-CA3 fields, which receive input from both MEA and LEA. A second convergence could occur in the longitudinal-strip system.

MEA receives mainly information from S, PreS, ParaS, and PostR and sends a strong input to LEA. Thus, its function may be related more to information processing within the HPC formation itself rather than to processing of sensory information (however, see Burwell and Amaral, 1998b). In contrast, LEA receives input from, and sends output to PER. Thus, it is
connected bidirectionally with a multitude of cortical association areas and therefore its function may be more as a gate through which sensory input converges from cortical areas onto the hippocampal formation.

Perpendicular to the MEA/LEA division there is a second system of parallel circuits which separates dorsal, splenial, and ventral hippocampal areas. The dorsal HPC circuit encompasses the lateral longitudinal EC strip, the lateral ACC, and the anterior prelimbic cortex. It receives mainly sensory information and thus it may be involved mainly in situations which demand orientation in an environment. The ventral HPC circuit includes the most medial longitudinal EC strip, the medial ACC, the BL AMG, and the posterior prelimbic cortex. Due to its connections with AMG, its function may be more relevant in situations which demand association of cues with an affective response, as well as modulating autonomous reflexes triggered by contextual stimuli. Dorsal hippocampal areas project predominantly to lateral ACC and ventral hippocampal areas to medial ACC. Medial ACC is the zone receiving dual hippocampal-AMG input. Thus, there are separate input and output pathways for dorsal vs. ventral areas of the hippocampal formation.

Subcortical connections of the hippocampal formation course mainly, but not exclusively, through the fimbria-fornix (Fx). Septal input originates in the medial nucleus and reaches the dorsal hippocampus through the Fx, while connections with ventral hippocampal area are not contained in this pathway. Output from the entire dorso-ventral CA1-S axis is sent to the lateral septum through the Fx. These connections are important, as the septum has been seen as a pacemaker for hippocampal theta oscillation, a form of slow-wave activity.
associated with REM sleep and locomotion (e.g. Petsche et al., 1962, 1965; Rawlins et al., 1979; Lynch et al. 1978) and related to learning processes (Wilson and McNaughton, 1994). The Fx also contains fibers to and from the hypothalamus, mammillary bodies, and brain stem. These projections terminate diffusely within the hippocampal formation and probably are more related to overall modulation of hippocampal activity. A second pathway (sometimes called the ventral bundle) connects the ventral pole of the hippocampal formation with the AMG and other brain stem nuclei which generate cholinergic, noradrenergic, and serotonergic input. The existence of this pathway is particularly relevant because it remains intact following Fx lesions. These connections are illustrated in figure 1.4.

It should be emphasized that separation of neural circuits within PHR is relative, rather than absolute. As described, both MPP and LPP input is directed to the whole transversal extent of DG and CA3. Thus, EC projections to CA1 and S preserving the MEA/LEA division are combined with projections to DG and CA3 which relay MEA and LEA information throughout the entire horizontal extent of these fields. Similarly, segregation of projections based on longitudinal EC strips and dorsal/ventral areas of hippocampal formation will be complemented by longitudinal associational fibers.
Figure 1.4. Cortical and subcortical connections of the hippocampal formation. The Fx subserves mainly dorsal hippocampus, while ventral hippocampus receives subcortical input and output through the ventral angular bundle. Connections with AMG are also illustrated. Evidence suggests that AMG and ventral hippocampal output converge in the medial ACC, while output from the dorsal hippocampal formation seems is directed to lateral ACC.
While in some previous experiments I investigated the behavioral relevance of MEA/LEA division (Ferbinteanu et al., 1999), the work described in this thesis concerns the role of dorsal/ventral HPC circuits in spatial learning and two forms of conditioning. In the rest of this chapter I will review the evidence for differences between the electrophysiology and pharmacology of the dorsal/ventral HPC circuits, I will summarize the behavioral literature on this topic, and I will finally provide a rationale for the choice of behavioral paradigms that I have employed.
1.2 Neurochemical aspects of PHR organization.

No systematic investigation has been carried out regarding the issue of whether the topographical anatomy of PHR is associated with a parallel neuropharmacological organization. Empirical data are rather scarce and their behavioral significance is presently obscure.

1.2.1 Differences between MPP and LPP.

Fredens et al. (1984) found that enkephalin is specific to the terminations of LPP onto the dendrites of DG granule cells, while cholecystokinin is specific to the MPP projections. Interestingly, the enkephalin immunostaining was weaker for septal hippocampal slices. Presently there are no investigations of the behavioral implications of this finding, although there have been some studies of the neurophysiological role of opioid receptors (see next section). Morris and Johnston (1995) summarized evidence indicating that the enkephalins increase, while dynorphins suppress excitability and plasticity of the PP synapses onto the granule cells and hypothesized that these neurochemicals play a role in modulation of hippocampal plasticity.

1.2.2 Differences between dorsal and ventral areas of the hippocampal formation.

Nomura et al. (1997) found that the densities of nonprincipal neurons immunoreactive for chemically distinct subpopulations of cells was higher in the ventral than in the dorsal hippocampal areas. The differences were not identical for all classes of neurons. Thus, immunoreactivity to calretinin was higher in the ventral region of DG, CA3 and CA1;
immunoreactivity to nitric oxide synthase was higher in the ventral DG and CA3; and immunoreactivity to somatostatin was higher in the ventral DG and CA1. As in the case of the MPP/LPP, it is not known whether these differences have behavioral relevance.
1.3 Electrophysiological characteristics of anatomically distinct PHR structures

The hippocampal formation and EC have been the focus of a large number of electrophysiological studies. A comprehensive summary of the entirety of this work is beyond the scope of this section. I will instead concentrate on data arguing for the existence of separate functional systems a) along the transversal hippocampal axis and b) along the longitudinal (septo-temporal) hippocampal axis. This section will be organized by areas of research: 1) intracellular and field potential recording; 2) unit recording and 3) recording of network oscillatory activity.

1.3.1 Transversal axis: circuits based on the MEA/LEA systems.

1.3.1.1 Field potential recording.

Differences between transmission in the two components of the PP have been long known. Two types of recording are studied: excitatory postsynaptic potentials (EPSP), believed to reflect presynaptic processes, and population spikes (PS), related to active neuronal firing. EPSPs elicited with LPP stimulation have higher peak latencies, half widths, and rise times than the ones following MPP activation (McNaughton and Barnes, 1977), This reflects more remote activation sites (LPP synapses on the outer third of the granule cells dendrites) and slower depolarization of cell population. In contrast, MPP activation of the same cells results in shorter latencies and higher amplitude PSs. These differences were found to be due to active synaptic properties of the two pathways and not to differential passive decay following variation of distance between synaptic site and soma of granule cells.
McNaughton (1980; Abraham and McNaughton, 1983). McNaughton (1980) also reported that in contrast to LPP, responses elicited by MPP depressed fast after stimulation at a frequency of 2Hz. The ratio of EPSP to presynaptic fiber response was larger in MPP, suggesting that stimulation of these fibers releases a larger quantity of neurotransmitter per impulse. The work of Bramham et al. (1991a, 1991b, 1996) on LPP showed that in this pathway, LTP requires activation of δ and μ opioid receptors and that it is NMDA receptor-independent. The peptidergic transmission characteristic of LPP may be involved in hippocampal seizure development and may be involved in modulating spatial learning (for review, see Bramham, 1992).

LTP induction in vitro was found to be more successful with MPP stimulation, but LPP showed higher EPSP facilitation (Colino and Malenka, 1993). Wang and Wojtowicz (1997) confirmed these results and found additionally that simultaneous activation of the two pathways facilitated LTP only in the MPP. These authors proposed that MPP-LPP interaction results in boosting of associative LTP in MPP, but not LPP. Doyère et al. (1997) investigated depotentiation in the two pathways. These authors found that both newly established and days-old LTP could be depotentiated in MPP by tetanization of the LPP while the reverse was not true.

Taken together, this evidence indicates that the two components of EC input to the hippocampal formation are organized into two physiologically distinct systems. Data suggest that LEA input modulates synaptic plasticity in the MPP by either augmenting or depotentiating transmission in this pathway.
1.3.1.2 Unit recording.

There are no investigations of MEA/LEA differences using unit recording.

1.3.1.3 Oscillations.

Buzsáki et al. (1990) investigated spontaneous and evoked physiological activity at different septotemporal levels of the hippocampus. This work supports the idea that output of individual hippocampal areas is directed to the EC regions that originated the corresponding input. Other empirical evidence indicates that monosynaptic projections from the EC to CA1 are not only physiologically functional, but in fact they may represent a major means of activating the pyramidal CA1 neurons (Doler and Weight, 1982; Yeckel and Berger, 1990). Oscillations in parallel EC-CA1/S loops have been reported (Iijima et al., 1996). Thus, input provided through distinct fiber pathways by MEA and LEA may be processed separately in different areas of the hippocampal formation and returned to the same EC areas that generated it. There is some evidence for differences between the theta rhythmic activities in MEA and LEA (Alonso and Garcia-Austt, 1987), but systematic studies have not been performed.

Recently, it has been found that the EC acts as a frequency switch: low-frequency stimulation of layer III neurons (which generate input to CA1 and S) was found more likely to generate action potentials. In contrast, high–frequency stimulation activated layer II, but
not layer III cells (Gloveli et al., 1997). Stimulation at frequencies between 5 and 10 Hz, which is within the theta rhythm range, activated both layer II and layer III neurons. It thus seems that the DG-CA3 neurons activation occurs with high-frequency EC activity, while at low frequencies the parallel EC-CA1/S loops are preferentially active. According to Buzsáki (1989), activity of the CA1 and S neurons is characteristic to non-exploratory behaviors (awake immobility, consummatory behavior, and slow wave sleep) and may constitute a second stage of memory trace formation, when long-term modifications of synaptic efficacy take place. It has been speculated (Jones, 1993) that certain types of memory acquisition could be mediated by the monosynaptic projections from EC to CA1 and CA3.

1.3.2 Longitudinal axis: dorsal/ventral hippocampal areas.

1.3.2.1 Field potential recording.

Van Groen and Witter (1985) used anterograde labeling in order to demonstrate the topographical organization of input from lateral-to-medial EC longitudinal strips to dorso-ventral hippocampal areas. The authors then stimulated the EC along the latero-medial axis and recorded responses along the longitudinal axis. Stimulation in the most lateral EC strip resulted in maximum evoked field potentials in the dorsal hilus, while stimulation in the more medial strips resulted in larger evoked responses in progressively more ventral hippocampal areas. Interestingly, it was also found that the projections from lateral EC to septal hippocampus had higher conduction velocities, suggesting thus that in normal conditions, it is this area of the hippocampus which first receives input, after which activity
spreads towards more ventral levels. Other data (Buzsáki et al., 1990) indicate that the point of maximum efficacy of EC input to the CA1 field is situated more dorsal to the point of maximum DG activation. One possible reason for this result may be the particular topography of longitudinal association fibers.

1.3.2.2 Unit recording.

There is an abundance of data on the activity of cells in the dorsal hippocampal formation. In contrast, recording in ventral hippocampus is rather scarce, mainly because it is technically more difficult. Thus, there are only a few studies directly comparing the properties of dorsal and ventral cells. One experiment investigating specifically activity in the ventral hippocampus (Poucet et al., 1994) found that the properties of place cells in ventral CA3 and CA1 were very similar to the ones of place cells found in dorsal areas. The only direct comparison between units in the dorsal and ventral hippocampus (Jung et al., 1994) found fewer complex spike cells with place sensitivity in the ventral hippocampus. Additionally, these neurons had lower spatial resolution. The authors speculated that both dorsal and ventral hippocampus are probably involved in processing of space, but while the dorsal areas may deal with high-resolution spatial information characteristic to a small environment, the ventral areas may be involved in processing of low-resolution input for a large environment. McNaughton et al. (1996) incorporated the differences in properties of place fields between dorsal and ventral units in a path-integration model of hippocampal function. This supports the idea that the physiological differences between dorsal and
ventral hippocampal neurons may have important consequences regarding information processing.

1.3.2.3 Oscillations.

Despite differences found between dorsal and ventral areas with field potential and unit recording, Buzsáki et al. (1990) found that theta waves occurred in phase and were highly coherent along a network of electrodes implanted at different septo-temporal levels. It is important however to notice that no electrodes were implanted in ventral areas as defined by behavioral investigations using localized lesions (see next section; Buzsáki et al., 1990, figure 2). Interestingly however, the same study found the density of parvalbumin-immunoreactive cells, which are inhibitory interneurons, to be higher at the more septal levels. The significance of this result is not yet clear, but it has been proposed (Buzsáki and Chrobak, 1995) that the activity of inhibitory interneurons network provides a 'context' for the 'content' encoded by the network of principal neurons. The 'context' would be constituted by entraining fluctuation in membrane potential of principal cells as a response to the oscillatory activity of inhibitory interneuron network.

It is also known that the ventral hippocampus is more sensitive to epileptiform discharges (Czéh et al., 1998; Lee et al., 1990; Gilbert et al., 1985), but the implications of this characteristics for learning processes is not clear.
1.3.3 **AMG modulation of HPC activity.**

The functional aspects of anatomical projections from AMG to hippocampal formation are not well known. There are a few studies employing field potential recordings which demonstrate that AMG stimulation modulates synaptic plasticity in the EC-hippocampal system. Thomas et al. (1984) reported that stimulation of L AMG generated an evoked response in DG granule cells similar to the one obtained by PP activation. DG response to PP stimulation was enhanced if L AMG stimulation preceded it by 50 msec. Although it is not specified in the paper, the configuration of the evoked responses suggests that it is the MPP that was studied. Ikegaya et al. (1994) found that BL AMG lesion was associated with attenuation of LTP induced in the DG by MPP stimulation. The lesions did not affect already-induced LTP and did not depend on the frequency of the tetanic stimulation of MPP. Stimulation of medial AMG (Ikegaya et al., 1995) facilitated MPP – DG transmission (as measured by PS amplitude). These results were supported by a later study (Ikegaya et al., 1996) and it seems that this effect is specific to the β-noradrenergic neurotransmitter system (Ikegaya et al., 1997). Thus, the AMG seems to facilitate signal transmission and LTP induction in the MEA-DG synapses. In agreement with Thomas et al. (1984), Finch et al. (1986) demonstrated an excitatory projection from the L and BL AMG to layers II, III, and V of both LEA and MEA and to the S. AMG activity thus seems to facilitate synaptic plasticity of the projections from the EC to the hippocampal formation.
1.3.4 Modulation of AMG activity by the EC-hippocampus system.

Layers III and V of LEA stimulation elicited excitatory responses followed by prolonged inhibition throughout the AMG (Brothers and Finch, 1985). A much more complete study was performed by Maren and Fanselow (1995). These investigators recorded BL AMG responses following ventral angular bundle stimulation. Single pulse stimulation resulted in evoked field potentials with short latency. Additionally, the field potentials were correlated with action potentials and required glutamate-based synaptic transmission. Second, paired pulse stimulation resulted in short-term changes in synaptic plasticity. Third, train stimulation of the ventral angular bundle resulted in NMDA receptor-dependent LTP.

In parallel with electrophysiological characterization of these projections, Maren and Fanselow carried out a behavioral study. Electrolytic lesions of EC, ventral S, and ventral angular bundle impaired acquisition of conditioned fear to contextual cues, while lesion of BL AMG produced a non-selective deficit in fear conditioning (response to both context and acoustic signal were abolished). The authors concluded that the projections from the hippocampal formation to the AMG are involved in acquisition of conditioned fear to contextual cues. Although certainly suggested by the data, this statement will have to be cross-validated by future research. As the authors themselves acknowledge, the lesions were not sufficiently selective, encompassing areas whose function may have various behavioral relevance.
1.3.5 AMG and HPC input to ACC.

The ACC receives input from both hippocampal formation (through the Fx) and AMG, and projects to motor areas such as the pallidum, the midbrain extrapyramidal system, substantia nigra and ventral tegmental area. This structure is therefore in a position of acting as an output gate for two different memory systems: the one based on EC-hippocampal formation and the one based on AMG. It has been postulated that ventral striatum associates movements, motivation, and spatial representation (Redish, 1999, pp. 89-92). This statement should be further elaborated in order to allow formulation of clear predictions, but empirical data seem to indicate that the ACC is involved in reward processes in which the hippocampal formation is not (Redish 1999, ibid.). Topographical organization of anatomical connections suggests that AMG and hippocampal memory systems may be competing for motor output at this level.

Physiological data supporting this hypothesis were obtained by Mulder et al. (1998) who investigated the interaction between the outputs of BL AMG and hippocampal formation in the ACC neurons. These authors found that cells in the dorsomedial shell of the ACC and ventromedial caudate-putamen (dorsal striatum, DS) were responsive exclusively to Fx stimulation and cells in the ventrolateral ACC responded exclusively to BL AMG activation. In contrast, input from hippocampal formation and from AMG converged on cells in the medial shell and core of the ACC. Recordings of activity in neurons receiving dual input showed that stimulation of BL AMG previous to Fx activation resulted in enhanced transmission in the hippocampal-ACC projections. In contrast, preceding Fx stimulation
resulted in depressed transmission in the AMG-ACC pathway. Thus, AMG seems to facilitate hippocampal formation output, while hippocampal formation activity seems to inhibit AMG output. This result is particularly relevant for behavioral tasks such as conditioned place preference (CPP) which require synergistic activity from the two memory systems (for details see section 1.5 and chapter 4).
1.4 Behavioral evidence indicating dissociation of PHR circuits.

As in previous sections, evidence summarized in this section will follow first transversal (MEA vs. LEA) and then longitudinal (dorsal vs. ventral) hippocampal axes. Empirical data in the first case are scarce. More studies have been done on the second topic, but the behavioral paradigms employed in earlier experiments are vastly different and cannot be easily integrated within more modern theories of hippocampal function. A second problem with earlier research is lack of precision in lesion localization. The aspiration and electrolytic lesion techniques usually used in these studies resulted in damage encompassing not only cell bodies but also fibers of passage. Thus, relevant evidence comes mainly from most recent studies.

Third, synergistic activity between AMG and hippocampal formation will be considered. Finally, a section will be dedicated to evidence comparing Fx and hippocampal lesions. As described in section 1.1, anatomical evidence suggests that Fx lesions may leave the circuitry of the ventral hippocampus partially functional.

A note should be made on the interpretation of results obtained with PP vs. EC lesions. Physiological evidence (Quirk et al., 1992) indicates that MEA cells exhibit firing patterns similar to those of hippocampal neurons. This suggests that some processing of information may take place independently in the EC. EC lesions interfere with these processes and with EC input to the hippocampal formation, while only the latter is abolished when PP fibers are sectioned. Additionally, EC contains other cells aside from the neurons generating the
perforant path. Third, EC is source of input/output for other structures (AMG among others). Thus, lesions of the EC may be similar, but not identical to PP transections. Here I will consider data regarding the latter case only.

1.4.1 Transversal axis: MEA vs. LEA circuits.

Myhrer (1988a) investigated exploratory behavior and reaction to novelty in animals with MPP, LPP, or total PP lesions. Compared to controls, all lesion groups showed increased locomotion, but the LPP lesion group was most active. MPP lesions had no effect on rearing, while higher scores followed total PP and LPP lesions on this behavioral parameter. Third, animals with total PP did not respond to novelty at all, the LPP lesion group spent less time in contact with novel objects, and the MPP lesions group was only slightly impaired. Finally, both MPP and LPP lesion groups exhibited less exploration than either the total PP lesion group or the controls. A second study (Myhrer, 1998b) investigated more closely reaction to novelty and object identification. Compared to MPP lesion group, the LPP lesion group spent less time in contact with novel stimuli which were of three kinds: visual/tactile, olfactory, or visual. A third study (Myhrer, 1989) indicated that disruption of connections between temporal cortex and LEA reduced reaction to novelty and impaired acquisition and retention of a brightness discrimination task. The author concluded that LEA processing is related to object identification, but comparisons with MEA function are missing.
The rationale of using novelty tests is that the hippocampal formation is the neural substrate of recognition memory. The evidence for this hypothesis is mixed (Redish, 1999, p. 191; Steckler et al., 1998) partly due to the large array of behavioral paradigms employed. It is difficult to integrate these results with most recent theories of hippocampal function (Memory Systems 1994; Cohen and Eichenbaum, 1993; see following section for a more detailed discussion), which focus on spatial navigation and formation of associations among stimuli. In agreement with these theoretical considerations, a previous study (Ferbinteanu et al., 1999) tested animals with either MPP or LPP lesions on spatial learning and discriminative context conditioning. The results showed that MPP, but not LPP lesions were associated with impaired spatial acquisition. Neither MPP nor LPP lesions interfered with normal performance on a preference test of discriminative context conditioning, but animals with LPP lesions showed enhanced discriminative freezing conditioning. This is similar to a different finding indicating that LEA lesions facilitate conditioned odor aversion (Ferry et al., 1996). In agreement with previous data demonstrating impairment on the Morris water task after PP section (Skelton and McNamara, 1992), these results indicate that processing of information on the transversal hippocampal axis is necessary for spatial navigation, but neither MEA nor LEA input taken separately seem to be essential for comparisons among a multitude of cues with conflictual affective valence. The enhancement effect obtained with LPP lesions may be explained by a possible facilitation of AMG control on behavior, but there are other possibilities. A detailed discussion of this issue will be provided in chapter 5.
1.4.2 Longitudinal axis: lesions of dorsal vs. ventral hippocampal formation.

One of the earliest papers on this topic belongs to Nadel (1968). Rats with electrolytic lesions of either dorsal or ventral hippocampal formation were scored on exploratory activity (rearing-sniffing, walking-sniffing, and sitting-sniffing), inactivity (sitting and lying) and grooming. Both lesion groups showed more exploratory activity in the beginning, but became more inactive towards the end of the testing interval. Animals with ventral hippocampal lesions seemed to have become inactive at a faster rate. The novelty level (low vs. high) did not differentially affect lesion groups, but animals with ventral lesions habituated faster than controls if the level of novelty was low. Ability to suppress behavioral action was measured in a conditioned emotional response procedure in which a tone (CS) was paired with electrical shock (US) when animals drank water from a water-spout (instrumental response). Both lesion groups could suppress responses at a rate similar to the controls, but animals with dorsal hippocampal lesions suppressed the response more than controls when not habituated to the CS. Dorsal lesions, but not damage to the ventral hippocampus, resulted in deficits in an active avoidance task. There were no differences found on the extinction time curve measured in a test of retention of stimulus-shock association, but dorsal hippocampal lesion group displayed stronger conditioning than controls. Although these results indicated that placement of lesion within hippocampal formation influenced its behavioral effects, it is difficult to integrate these results within present theories of hippocampal function. One possibility is that avoidance learning could be interpreted as acquisition of information about places where punishment occurs (Black et al., 1977), or that context conditioning can be interpreted as spatial learning (Nadel and Willner,
This argument is valid as long as there is access to distal spatial cues. This is not true in every context conditioning paradigm (see chapter 3), which are run in a large number of variants. The heterogeneity of design is in fact likely to be part of the reason for which it is still disputed what aspects of behavior are controlled by which cues (Holland and Bouton, 1999; section 1.5). The former explanation is not consistent with more recent data (see below) clearly indicating that dorsal hippocampal lesions are more effective than ventral lesions in disrupting spatial learning. If avoidance learning as described above can be explained as spatial learning, animals with dorsal hippocampal lesions should show weaker, not stronger conditioning than the controls. The later position would predict that the effect of dorsal/ventral HPC lesions on spatial learning and context conditioning would be the same. Results presented in chapters 2 and 3 argue against this view. Thus, these two behavioral paradigms are likely to involve different memory processes.

Stevens and Cowey (1973) investigated the effects of dorsal vs. ventral hippocampal lesions on spontaneous alteration, probability learning, and lever alternation. On the first task, they found that ventral hippocampal lesions were associated with perseveration at short intertrial interval and normal alternation at long delays. Rats with dorsal hippocampal lesions chose randomly with short intervals and perseverated at long delays. Ventral, but not dorsal hippocampal lesions impaired probability learning. In contrast, dorsal hippocampal lesions accelerated acquisition on the lever alternation task, which in this case was designed as a form of delayed-non-matching-to-sample (DNMS) paradigm. It is difficult to interpret these results because the neural networks involved in alternation and DNMS tasks do not necessarily include the hippocampus (Aggleton et al., 1986; Rawlins et al., 1993; Cassaday
and Rawlins, 1995, 1997; Steckler et al., 1998). Even less is known about the neuroanatomical substrate of probability learning and this task has been little used in more modern investigations of hippocampal function.

Lanier and Isaacson (1975) found increased locomotion in animals with either dorsal or ventral hippocampal lesions. The hyperactivity however diminished in the dorsal lesion group shortly after the surgery. These results seem to be in agreement with Nadel's (1968), but it is worth mentioning that in this case the lesions seemed to be less well localized and more variable in size. Problems with localization of lesion were also prominent in a different report (Sinnamon et al., 1978), where dorsal hippocampal lesions extended to the dorsal thalamus and ventral hippocampal lesions encompassed large portions of both MEA and LEA as well as PER. Thus, although the results of this study did seem to indicate that dorsal hippocampal lesions are more disruptive of spatial learning, the data are not easily interpretable.

Recent studies using more localized lesions focused on spatial learning. Moser et al. (1993, 1995) demonstrated that dorsal hippocampus is more efficient in acquiring spatial information, but on the other hand normal animals employ a large extent of their hippocampal network (Moser and Moser, 1998a). Hock and Bunsey (1998) found impairment with dorsal, but not ventral hippocampal lesions on a spatial alternation task. In contrast, Stubley-Weatherley et al. (1996) reported that both dorsal CA1 and ventral CA3 played a role in spatial orientation, but the asymmetry and confinement of lesion areas to the CA fields make direct comparisons with other work difficult. The involvement of ventral
hippocampus in spatial learning is supported by data obtained with a task using the hole board circular platform (Poucet et al., 1991) where rats were motivated to localize a particular hole, and by data from an experiment in which the subjects' reaction to removal of a salient stimulus was measured (Thinus-Blanc et al., 1991). Additionally, Long and Kesner (1996) reported that both the dorsal and ventral hippocampus could process information about allocentric distance. Supporting the role of dorsal hippocampal formation in spatial learning were data reported by Bannerman et al. (1999) and Tuvnes et al. (1999). Interestingly, in a parallel paper Richmond et al. (1999) did not find impairment on the same spatial task with either dorsal or ventral hippocampal damage. Taken together, current data demonstrate fairly clearly that dorsal hippocampus has an important role in spatial learning, but are ambiguous on the role of ventral hippocampus. Elucidating this aspect of hippocampal function was the purpose of two separate experiments, as described in chapter 2.

A second task sensitive to dorsal/ventral hippocampal lesions is context conditioning, but data available on this topic are scarce. Hock and Bunsey (1998) found that both dorsal and ventral hippocampal lesions impaired formation of associations between an internal cue (e.g., hunger) and shock. Tuvnes et al. (1999) found that ventral hippocampal lesions reduced corticosterone levels in plasma. Richmond et al. (1999) reported that ventral, but not dorsal hippocampal lesions affected conditioned freezing in a signaled-shock procedure as described by Kim and Fanselow (1992) and Phillips and LeDoux (1992). Ventral hippocampal involvement was also demonstrated in a passive avoidance procedure, very similar to context conditioning (Ambrogi-Lorenzini et al. 1997) in which access to distal
spatial cues was blocked. Thus, some results support dissociation between the functions of the two hippocampal areas, while others do not. This issue was the topic of an experiment described in chapter 3.

1.4.3 AMG and hippocampal formation: together or separately?

While spatial navigation seems to involve the hippocampal formation, context conditioning involves additionally a second memory system, namely the one based on AMG function (however, see Holland and Bouton, 1999). There is a large number of studies employing this type of task and a complete review of this literature would cover many pages. A good synthesis is provided by Holland and Bouton (1999), who concluded that depending on the particular setting, context conditioning involve memory processes that may or may not require hippocampal function. Thus, while some studies seem to point to impairment in context conditioning following hippocampal lesions, other studies (Winocur, 1997) indicate enhanced responding. The general consensus on this issue seems to be that normal animals will tend to use strategies requiring learning of relationships among cues, while animals with hippocampal lesions, unable to form stimulus-stimulus associations, will tend to use behavioral strategies controlled by single cues. The latter would presumably be based on the function of the AMG memory system. I will therefore limit the present discussion to studies directly comparing AMG and hippocampal functions.

Phillips and LeDoux (1992) performed an experiment in which animals were presented with a tone (CS) paired with shock (US). Conditioning to the context and the CS were measured
by assessing freezing upon reintroducing the animals in the shock chamber and then presenting the CS. The results indicated that AMG lesions reduced freezing to both CS and context, while dorsal hippocampal lesions interfered only with freezing to context. The authors concluded that AMG but not hippocampal formation is required for conditioning to simple, modality-specific cues such as a tone, and that both structures were involved in context conditioning. A similar conclusion was drawn by Kim and Fanselow (1992), who found that dorsal hippocampal lesions abolished conditioned contextual fear if the lesions were performed 1 day after training. The same animals conditioned normally to the CS (tone). Dorsal hippocampal lesions were also found to have no effect on fear-potentiated startle (McNish et al., 1997). Transient inactivation of this area had a similar effect on conditioning to contextual cues, while AMG inactivation had an effect on both contextual and auditory cues. (Sacchetti et al., 1999). Phillips and LeDoux (1994) investigated the role of the CS in context conditioning. It was found that dorsal hippocampal lesions did not affect conditioning to context in the absence of the CS (tone), but when shock was paired with tone animals conditioned to the CS and not to the context. The authors interpreted these results as indicating that single cue associations (CS-US) are mediated by the AMG (and therefore are not affected by hippocampal lesions), while hippocampal function is involved in forming associations between the context (which is a collection of cues) and the US.

The studies mentioned above used freezing as a fear measure. In contrast, Selden et al. (1991) assessed conditioning by measuring suppression of lick response and site preference in an apparatus formed of two chambers, one of which was associated with shock.
difference was that hippocampal lesions were produced neurochemically (as opposed to electrolytically) and encompassed the whole formation rather than only its dorsal area. AMG lesions did not interfere with preference for the ‘safe’ chamber, but affected CS conditioning as measured by suppression of licking response (which occurs in normal animals upon CS presentation). Animals with hippocampal damage conditioned just as well as the controls, but spent less time in the ‘safe’ compartment of the two-chamber apparatus. Thus, the literature seems to support the idea that hippocampal formation is involved in context conditioning, where relationships among a multitude of cues have to be acquired, while AMG is involved in simple cue-fear response associations.

There is some discrepancy in empirical data, as AMG lesions impaired conditioning to context in some, but not all cases. One important issue is the particular behavioral parameter used to assess conditioning. Freezing, defecation, chamber preference, and avoidance are all behaviors that have been measured in conditioning paradigms. In the studies cited above, AMG lesions interfered with freezing but not with chamber preference. Phillips and LeDoux (1992) briefly discussed the possibility that the two behaviors may be controlled by different neural structures. Thus freezing, a classical conditioned fear response, may be more dependent on AMG, as this structure is the neural basis of a memory system modulating affective responses. In contrast, the hippocampal function may be more related to performance in the preference test, where a choice has to be made between two sets of cue combinations. Supporting this conclusion are data reported by Frankland et al. (1998) showing that lesions of the dorsal hippocampal formation do not interfere with context
recognition (as measured by freezing), but impairs context discrimination (measured in a chamber preference test).

This may be related to the fact that context-induced freezing and context discrimination (chamber preference) are assessed in different conditions. In discriminative paradigms, freezing is measured upon confinement in a particular part of the apparatus. In contrast, context discrimination is measured (by definition) while the animal moves freely between chambers. The passive condition may favor control of behavior by AMG vs. hippocampal memory systems. Data obtained in conditioned place preference (CPP) tests point to this direction. CPP is a modification of the classical radial maze task in which animals are presented with a pair of arms, one of which is reinforced (food) and one of which is not. At the end of the training interval, subjects are allowed free access to both arms and preference is assessed for the location previously paired with food. A series of experiments (White and McDonald, 1993; McDonald and White, 1995a,b) demonstrated that AMG lesions abolish conditioning in this task if the cue presentation is passive (i.e., pairing between food and location takes place by confining the animal at the end of the maze arm. If the pairing is active (rats are allowed to move between two arms, one containing food and one empty), AMG function is not necessary. Instead, the hippocampal and dorsal striatum memory systems are involved. This evidence and its implication for the dorsal/ventral hippocampal function will be discussed in detail in chapter 5. For now, it is important to note that these data support the idea that the particular procedure used for measuring freezing may favor AMG control over behavior, while the procedure used to measure chamber discrimination may tip the balance in favor of hippocampal formation. Second, these data show that AMG
and hippocampal formation act synergistically at least in some types of conditioning paradigms.

Synergistic interaction is however not the complete story. AMG and hippocampal formation can act antagonistically as well, and most interestingly, in the same type of conditioning paradigms. In the report cited above, Frankland et al. also reported that post-, but not pre-training hippocampal lesions interfered with context recognition. Thus, when the hippocampal formation is active during training (post-training lesions), other memory systems cannot support acquisition of context identity. This implies that the hippocampal formation engages in antagonistic interaction with other neural structures, in this case most likely AMG. In agreement with this hypothesis is the finding that in the CPP task AMG lesions are followed by impaired conditioning, but disruption of hippocampal function through Fx lesions results in enhanced conditioning (McDonald and White 1995a, b; White and McDonald, 1993). One way to explain this result is that activity in the hippocampal network may have an inhibitory effect on AMG function. Such a hypothesis is strongly supported by Mulder et al. (1998) report discussed above.

To summarize, data provided by conditioning paradigms suggest that both synergistic and antagonistic interaction between the hippocampal formation and AMG are possible. Topographical organization of anatomical connections along the longitudinal axis shows that ventral, but not dorsal hippocampus is related to the AMG. Thus, one question that should be answered regards the role each of these hippocampal areas plays in conditioning. Evidence regarding this issue will be presented in chapters 4 and 5.
1.4.4 **Fornix and hippocampal lesions: is there a difference?**

Related to the issue of hippocampal – AMG interaction is the one of Fx lesions. I have discussed anatomical evidence suggesting that Fx lesions may affect more the function of dorsal, rather than ventral hippocampal pole. Behavioral evidence indicating that Fx and whole hippocampal lesions do not have identical effects on behavior is also accumulating. Rawlins et al. (1993) found transient differences between rats with Fx vs. hippocampal lesions on a nonspatial matching-to-sample task. Whishaw and Jarrard (1995) reported that hippocampal lesions were less disruptive than Fx lesions on a spatial task and on circadian activity. A more systematic comparison between the effects of the same two lesions on different configural learning tasks (McDonald et al. 1997) showed that unlike hippocampal damage, Fx transection is not associated with acquisition deficits. Sziklas et al. (1998) found that although both Fx and hippocampal lesions impaired acquisition of a spatial working memory task, the deficit was transient in the first group of animals. Cassel et al. (1998) found a higher increase in activity and more impairment in a spatial radial maze paradigm with hippocampal than with Fx lesions. Therefore, although there is strong evidence that impairment in spatial learning occurs following either method of disrupting hippocampal function (Morris, 1981; Morris et al., 1982; Sutherland and Rodriguez, 1989; Morris et al., 1990; Sutherland and Rudy, 1988; Eichenbaum et al., 1990; Whishaw et al., 1995), there are reasons to believe that the effects are not entirely equivalent (see also Redish, 1999, pp. 235-236). Therefore, a comparison was performed between the effects of whole hippocampal,
dorsal hippocampal, ventral hippocampal, AMG, and Fx lesions on CPP. These data are presented in chapter 4.
1.5 Behavioral paradigms

The choice of behavioral paradigms employed in this work has been directed by the present debate on the topic of hippocampal function. Because the focus of this thesis was not the role of the hippocampal memory system *per se*, but the existence of subsystems within it, only a brief review of the theories regarding the role of the hippocampus in the rodent will be provided here.

Theoretical perspectives and design of empirical studies investigating hippocampal function have been shaped by the understanding of learning and memory processes in general. Early theories were influenced on one hand by the (then current) view of cognitive psychology that memory is a unitary type of phenomenon which can be either short- or long-term, and on the other hand by the triune theory of MacLean (see section 1.1). One additional factor that hampered progress for some time was the inability to produce sufficiently well localized lesions. Thus, research focused on changes following hippocampal lesions on fear responses (which was later demonstrated to be more related to AMG function), levels of locomotor activity (later connected to ACC and changes in dopamine levels), exploration (issue still controversial today), spontaneous alteration, or hormonal levels (for more extensive reviews see Redish, pp. 177-180 and Isaacson, pp.169-190). There were problems with all of these conceptualizations because they explained only a small part of the data, usually the ones that generated the theory to begin with. Publication of O'Keefe and Nadel's *The Hippocampus as a Cognitive Map* (1978) fundamentally changed this situation. These authors' approach was rooted in Tolman's idea that learning means formation of stimuli-stimulus associations
organized in a cognitive map. These authors distinguished between a taxon and a locale system. The former, hippocampally independent, is related to stimulus-response associations. The latter, hippocampally based, involves the organization of environmental stimuli in a map utilized by the animal for spatial navigation. Due to its predictive and explanatory powers, this theory has proved an excellent theoretical framework which has been extremely influential in directing later research. Presently, views on hippocampal function are generally divided into two major categories: the ones that agree with the spatial map theory, and the ones that view spatial learning as one aspect of the more general ability of learning about relationships among cues (see below).

Parallel to O'Keefe and Nadel's account, Hirsh (1974) formulated the contextual retrieval theory. According to this proposal, the function of the hippocampus is to acquire information about the context within which stimulus-response associations are embedded. A key point of this perspective was that the hippocampus was viewed as controlling behavior in conditional situations, such as learning in a food-deprivation situation vs. learning in a water-deprivation situation (reviewed in Hirsh, 1980). Hirsh's theory was more general than O'Keefe and Nadel in that it postulated a map-type of organization for spatial and non-spatial events (Hirsh, 1980).

Hirsh's theory provided the basis for the more recent formulation of relational (Eichenbaum et al., 1992) and configural (Sutherland and Rudy, 1989; Rudy and Sutherland, 1995) theories. With slight differences (see section 1.5.2), both these approaches emphasise the
role of the hippocampus in forming relationships among cues of various nature (visual, spatial, olfactory, sensory, etc.).

A yet different view was proposed by Olton, who distinguished between working and reference memory (Olton et al., 1979). The former, hippocampally dependent, is defined as acquisition of representations for a limited time interval of time, until a behavioral response is performed. After this the representation is discarded. Reference memory, which is hippocampally independent, concerns information needed for an undetermined period and it deals with issues such as rules and procedures. Although this account on hippocampal function has been influential, it is easily disproved, as it is well known that hippocampal lesions impair navigation to a submerged platform maintained in the same position across a number of days (the classical Morris water task). This theory was also disproved by work demonstrating that hippocampal lesions disrupt spatial but not cued 'working memory' in the radial maze (O'Keefe et al., 1975; Aggleton et al., 1986; McDonald and White, 1993; Jarrard, 1993).

More recent theoretical developments on the topic of hippocampal function have been rather refinements influenced by accumulating electrophysiological data of either the spatial or relational-configurational views. Thus, Shapiro and Olton (1994) proposed that the result of hippocampal activity is a reduction of interference (negative transfer) defined as the slowing down of information acquisition following prior learning. It is interesting to note that Rudy and Sutherland (1995) revised version of configural theory also viewed the hippocampal function as enhancing the salience of a complex (as opposed to elemental) stimulus. In their
account, Shapiro and Olton emphasized that units in CA1 and DG respond to various types of stimuli (visual, auditory, and olfactory). This argues against the idea that hippocampus is involved exclusively in acquisition of spatial information. The authors viewed spatial and configural representations as particular cases of relational representations (as defined by Eichenbaum). The difference between this account and Eichenbaum's seems to be that relational representations are understood as organized hierarchically. Eventually, these two positions were 'collapsed' (through the stage of hippocampus as associator of discontiguous events; Wallenstein et al., 1998) in the proposal that the hippocampus generates not a map, but a memory space (Eichenbaum et al., 1999; Eichenbaum, 1999; Shapiro and Eichenbaum, 1999). According to the proponents of this approach, activity in the hippocampal neural network does not encode distances and angles, but relationships between spatial cues specific to one event on one hand and on the other hand common elemental cues that span across distinct behavioral episodes and are non-spatial in nature. Thus, the resulting representation is not a map in the sense that it does not enable the animal to perform computations regarding positions, but a 'memory space' functioning through transitive inference from one pair of cues to another (from AB to BC to CD and so on).

Parallel to this theoretical development is a second one, similarly influenced by electrophysiological data, but with a computational bend. Example of this account on hippocampal function are represented by Whishaw (1991), McNaughton et al. (1996), and Redish's book Beyond the Cognitive Map (1999). This work focuses almost exclusively on spatial navigation and it is based on O'Keefe and Nadel distinction between taxon and locale systems. One way of understanding the function of the locale system is as a path integrator,
i.e., as a network whose input is direction and speed at moment $t_1$ and output is position at moment $t_2$. According to Redish, what the hippocampus does is self localization at the entrance in a familiar environment and replay of recently travelled routes (p. 195). A similar argument has been formulated by Whishaw and collaborators (e.g. Whishaw and Jarrard, 1996; Whishaw et al., 1995; Whishaw and Gorny, 1999) who argue that impairment in spatial navigation following hippocampal lesions is due to inability to integrate self-movement. One problem with this account is that it focuses exclusively on explaining spatial navigation data and it does not fit with results reported by White and Ouellet (1997), which demonstrated that not self-generated movement, but alternative view of cues is necessary for spatial learning. Against this view is also the argument that residual learning abilities of animals with hippocampal lesions can be explained by activity of alternative memory systems (McDonald and White, 1995; McDonald and Hong, 2000; Devan et al., 1999; Devan and White, 1999; Muller et al., 1996).

Finally, an approach still in embryonic stage is generated by the study of complex systems dynamics. This area of science investigates how functional patterns emerges from basic organizational laws of a system composed of a large number of units. Obviously, the brain is one such system and this approach has been already utilized by Freeman in his study of odor representation (Freeman and Schneider, 1982; Freeman et al., 1985; Freeman, 1991); there are examples in other areas of neuroscience as well (Gray, 1997). In the area of hippocampal function research this approach is best represented by electrophysiological recording of large network oscillations, among which Buzsáki's work is probably the best and most articulated example. Development of this type of approach would be instrumental
in explaining not only how particularities of the anatomy and physiology of hippocampus influence processes such as learning and memory, but also how different memory systems interact with each other in directing behavior.

Data discussed in this thesis are relevant mostly to the dispute between spatial and configural-relational theories. Animals with either dorsal or ventral hippocampal lesions were tested on spatial learning, context conditioning (illustrative for configural-relational learning), and conditioned place preference (task requiring formation of associations between distal cues usually presented in spatial tests, and emotional response, usually involved in conditioning paradigms). A further discussion regarding the rationale of choosing these tasks is provided below.
1.5.1 **Spatial learning.**

Evidence that the hippocampal formation is involved in spatial learning in the rodent is very strong. The behavioral paradigm most frequently employed is the one introduced by Morris (1981) which I will refer to as the classical version. This consists of submerging a platform in a pool filled with opaque water. Thus the platform is invisible and animals can find it only by spatial navigation. After a number of trials, normal animals perform quite well. Animals with either Fx or hippocampal formation lesions are notoriously poor at this task, while lesions of either DS or AMG (sites of different memory systems) do not interfere with performance (Sutherland and McDonald, 1990; McDonald and White, 1994).

In section 4.2 I have summarized evidence indicating that disruption of the the dorsal hippocampal function is particularly important for spatial learning. The role of ventral hippocampal area is less clear. Moser and Moser (1998b) claimed an absolute dissociation between the two hippocampal areas, but data seem to indicate otherwise. The studies mentioned above used the classical version of the Morris task. In the present experiments, variations of this paradigm were used. The first test has been previously used by McDonald and White (1994). It requires swimming to a visible platform for three days and to an invisible platform during the fourth. The sequence is then repeated three times (thus, the total time of training is 12 days). During this interval, the platform position remains the same. At the end, the visible platform is moved to a new, diametrically opposed position (competition test). Animals with disruption to hippocampal function respond normally to the
visible cue and are impaired in spatial navigation, while the reverse is true for animals with DS lesions. On the competition test, DS lesioned animals respond only to location and hippocampally lesioned animals only to visible cue. (McDonald and White, 1994; Devan, McDonald and White, 1999; Devan and White, 1999). Within a group of normal animals, half respond to the visible cue (the platform in the new position) and half respond to location (the position to which they have been trained for 12 days). In these experiments, this paradigm was employed for demonstrating a double dissociation between hippocampal and DS memory systems. In the present case, the use of this test had a different purpose. First, by training animals to a visible platform, we attempted to reduce demand on hippocampal function (Sutherland and Rudy, 1988) and overcome possible procedural differences between groups (Whishaw et al., 1995), as it is known that animals with hippocampal lesions tend to swim in circles at a relatively constant distance from the pool wall (thigmotaxic behavior). Second, we also attempted to control for any possible motor and motivational differences between lesion groups, as connections with 'visceral' areas (AMG and hypothalamus) are directed selectively towards the ventral hippocampal area.

In the second experiment we used a paradigm known as one-trial place learning. This is similar to the classical Morris task in that it requires navigation to an invisible platform from a number of distinct starting positions. The difference is that the position of the platform is modified daily. Previous data (Morris, 1983; Whishaw, 1985, 1991; Sutherland et al., 1997, 2000, Auer et al., 1989, Steele and Morris, 1999; Hoh et al., 1999) demonstrated that the task is particularly sensitive even to subtle hippocampal dysfunction. For example, in one experiment (Auer et. al., 1989), even ischemic damage restricted to unilateral CA1 resulted
in impaired spatial learning. In a more recent study, Sutherland et al. (1997) showed that kindled rats, which acquire normally the classical version, were impaired on the one-trial place task. Other data show that, unlike the classical version, this task requires NMDA-dependent LTP (Hoh et al., 1999; Steele and Morris, 1999). Thus, the purpose of this paradigm was to test animals with lesions restricted to only dorsal or ventral hippocampal areas in conditions which are particularly demanding on hippocampal computation.

The two modified versions of Morris water task provided a range for computational demand on hippocampal function. If the distinction between the roles of dorsal vs. ventral hippocampal areas is all-or-none, as Moser and Moser (1998b) have argued, then modifying the difficulty of the task should not affect the effect of dorsal hippocampal lesions. These animals should show as large a deficit with the 'easy' as with the 'difficult' task. On the other hand, animals with ventral hippocampal lesions should be at no point different than controls. If both dorsal and ventral hippocampus are involved in spatial learning, then this should be reflected in the results: an 'easier' task would show dissociation of lesion effects while a more 'difficult' one would expose deficits after both types of lesion. Second, dissociations would be transient, disappearing as learning occurs based on the remaining hippocampal network.

1.5.2 Context conditioning.

The involvement of hippocampal formation in spatial learning is well documented in the rodent literature. This promoted the formulation of the so-called spatial cognitive map theory (O'Keefe and Nadel, 1978; Nadel, 1991) according to which hippocampus is
involved in learning relationships among a constellation of distal cues. However, accumulating empirical data showed that disruption of hippocampal function affects performance in other types of task. This has led to an alternative view of hippocampal role. According to this perspective, the hippocampus is involved in acquiring general relationships among a multitude of cues, when the nature of these cues is by no means spatial only (Eichenbaum et al., 1992; Sutherland and Rudy, 1989; Rudy and Sutherland, 1995). Space is seen as only one particular aspect of the kind of information processed by the hippocampus. There are in fact two different theories adopting this general outlook. Eichenbaum and his collaborators formulated the so-called relational theory, which in its most recent version, states that hippocampal function is to generate a memory map that encodes any regularity in the environment and links it to behavioral episodes (Eichenbaum et al., 1999; Shapiro and Eichenbaum, 1999). Rudy and Sutherland (1995) proposed the configural theory according to which the role of the hippocampus is to increase the salience of a unique representation formed for a conjunction of cues. The difference between the relational and configural theories is rather small and has to do with whether the stimuli participating in the ‘compound’ still have an individual representation or they are encoded as one unit. Because the evidence discussed in this thesis is not conclusive for one or the other of these positions, I will refer to both of these views as the relational-configural theory.

According to the position described above, the hippocampus encodes not just spatial relationships among cues, but relationships in general. Conditioning paradigms are tasks in which a choice between discrete cues has to be made based on information provided by other, more general cues, such as spatial location or experimental chamber (Cohen and
Eichenbaum, 1993, pp.165-166). Discriminative context conditioning was used to test the effect of dorsal vs. ventral hippocampal lesions. Unlike water task paradigms, performance in this test involves both AMG and hippocampal function. Connections between the ventral hippocampal area and AMG suggest that location of lesion within hippocampal formation may have different effects on freezing and chamber preference. Data relevant to this issue are presented in chapter 3.

1.5.3 **Between space and conditioning: conditioned place preference.**

The version of context conditioning used in the present studies eliminated distal cues. However it has been argued that conditioning paradigms may also have a spatial component (Nadel and Willner, 1980). An example of a task that involves acquisition of spatial cues - affective response compounds is CPP, where the animal learns to associate a particular location with reward. Thus, this test blends the spatial component of the water task with the conditioning aspect of paradigms used to demonstrate the relational-configural function of the hippocampal formation.
1.6 Summary.

Anatomical, neurochemical, and physiological evidence indicates that the EC–hippocampal formation is a heterogeneous functional system. The EC is organized along three main axes. The first is the classical division into MEA and LEA. Perpendicular to this is the division into longitudinal strips (lateral, intermediate, and medial). Third is the lamination of the EC: deep vs. superficial neuronal layers. MEA and LEA projections throughout DG and CA3 are segregated horizontally, while connections to CA1 and S are organized in columns. Anatomical tracing and physiological recording indicates that EC–hippocampal system is constituted of parallel loops EC (superficial layers) – hippocampal formation – EC (deep layers) – EC (superficial layers). EC division into longitudinal strips is maintained within the hippocampal formation. The lateral strip is connected to the septal hippocampal pole and receives mostly specific sensory information. The medial strip is connected to the temporal hippocampal pole and receives mostly visceral input from subcortical areas such as AMG. The ventral tip of the hippocampus seems also to have higher concentration of various neurochemicals. Output to the ACC originating in the septal pole is directed to lateral areas, while the temporal pole projects mainly to medial areas. Dual input hippocampal formation–AMG is found in the medial ACC. The Fx connects mainly the dorsal and splenial hippocampal areas with other subcortical structures. The ventral angular bundle fulfills the same role for the ventral hippocampus.

The focus of this thesis is the behavioral relevance of topographical organization of the hippocampal formation along its longitudinal axis. Three different types of tasks have been
employed: water task, discriminative context conditioning, and CPP. Moser and Moser (1998b) proposed that spatial navigation has as neural substrate the dorsal and splenial, but not ventral hippocampus. The purpose of the water task experiments (chapter 2) was to test this conclusion by modifying demand on computations within the hippocampal network.

The context conditioning experiment (chapter 3) investigated the role of dorsal vs. ventral hippocampus in acquiring information about combinations of non-spatial cues. This paradigm was designed based on relational-configural theories of hippocampal function. As opposed to water tests, normal performance in this case requires synergistic interaction between AMG- and hippocampus-based memory systems. The behavioral parameters used to assess learning were freezing and chamber preference. Empirical data suggest that the former may be controlled preferentially by AMG, while the latter is most likely hippocampal dependent.

Evidence presented in chapter 4 used a different type of conditioning paradigm, the CPP. This task is similar to context conditioning in that it trains animals to associate sensory stimuli with an affective response. However it is also similar to water tasks in that the sensory stimuli are spatial in nature. Thus, the demands that this paradigm sets are at once spatial and configural-relational. The first experiment investigated the performance of animals with Fx, AMG or whole hippocampal lesions. It has been previously shown that AMG lesions impair, while Fx lesions enhance CPP performance. The issue was whether
hippocampal lesions have a similar effect as Fx lesions. The second experiment looked specifically at the effects of dorsal vs. ventral hippocampal lesions.
Note. Data presented in experiment 1, chapter 2 together with data presented in chapter 3 have been accepted for publication as J. Ferbinteau and RJ McDonald – Dorsal and ventral hippocampus: same or different? Psychobiol, in press. Data presented in chapter 4 have been accepted for publication in Hippocampus. Data presented in experiment 2, chapter 2 are presently in review with Hippocampus.

Lesions involving the hippocampal formation were produced using NMDA and destroyed cells in DG and fields CA3-CA1, but not S. I will refer to this type of lesion as HPC lesion.
2. DORSOVENTRAL HIPPOCAMPAL AXIS AND ACQUISITION OF SPATIAL INFORMATION.

The view that hippocampal formation is involved in spatial learning has been formulated for some time (O’Keefe and Nadel, 1978) and data supporting it have been reported repeatedly (Morris et al., 1982, 1990; Sutherland et al., 1982; Sutherland and Rudy, 1988). Topographical organization of connections with EC suggests that sensory information reaches mainly the septal pole of the hippocampus, while the ventral pole receives predominantly visceral and affective input (Amaral and Witter, 1995). This view is also supported by physiological and neuropharmacological data. Previous work (Moser et al., 1993, 1995) demonstrated that lesions of dorsal hippocampus interfere with acquisition of spatial information, while damage to ventral areas is not as disruptive. It has been proposed (Moser and Moser, 1998a) that dorsal hippocampus is exclusively involved in spatial navigation.

To test this theory, two experiments were performed. The first used a paradigm that diminished demand on hippocampal function by training animals to visible and invisible platforms in a water task. If spatial learning depends exclusively on dorsal hippocampus, it is expected that animals with dorsal HPC lesions would show consistent impairment in the
invisible platform trials, as opposed to animals with ventral HPC lesions, which would be no different than controls.

The second task increased the demand on hippocampal function by modifying the position of invisible platform in a water task. In this case, exclusive dependence of spatial learning on dorsal HPC network should result in the same pattern of results: permanent impairment with dorsal HPC lesions and no impairment with ventral HPC damage.

An alternative view is that spatial learning employs circuits extended along the whole longitudinal hippocampal axis, but dorsal areas are more specialized for processing this type of information than ventral areas. This view is suggested by the relative, rather than absolute separation of neural circuits (see section 1.1.4 and Amaral and Witter, 1995). In support of this perspective is the observation that in the Moser et al. (1995) study, progressively larger ventral HPC lesions incorporated larger portions of ventral HPC network, and not, as the authors argue, larger parts of dorsal HPC circuits (see fig. 1 in the cited paper). According to the alternative perspective, diminished demand on the hippocampal function should still result in impairment following dorsal HPC lesions, but this would evolve from highly visible to subtle, as ventral HPC network acquires information. Impairment following ventral HPC lesions may or may not be present, but increased demand on hippocampal function should uncover this subtler deficit. The effect of dorsal HPC lesions should of course still be highly visible. However, because both types of lesion leave part of the
hippocampal circuitry intact, extended training should reduce differences in performance between lesion groups and differences between lesion groups and controls.
2.1 MATERIALS AND METHODS

2.1.1 Experiment 1: visible/invisible platform

2.1.1.1 Subjects.

Thirty-nine Long-Evans male rats (Charles River Colonies) were used in this experiment. The animals were individually housed in clear plastic cages, were given food and water *ad libitum*, and were kept on a 12/12 hr light/dark cycle. All behavioral testing occurred during the dark cycle. Animals were randomly assigned to three groups: control (n = 13); dorsal HPC lesion (n = 13) and ventral HPC lesion (n = 13). No surgical procedures were performed on the control group.

2.1.1.2 Surgical procedures.

All animals weighed between 275 and 300 g at the time of the surgery. Rats were anesthetized with sodium pentobarbital 65mg/kg (Penlong, MTC Pharmaceuticals) and placed in a stereotaxic apparatus. The skin of the scalp was cut on the midline and deflected. Each rat received a total of 10 injections (5 per side) of N-methyl-D-aspartate (NMDA) in phosphate buffer (5 mg/ml), pH 7.4, at locations as indicated in Table 1, using a 10 μl Hamilton syringe connected to a cannula (30 Ga). An interval of 3 min was allowed at the end of each injection before the cannula was retracted. Rats were given an interval of 5 to 7 days for recovery.
Table 2.1. Injection coordinates and volumes

<table>
<thead>
<tr>
<th>Dorsal</th>
<th>Ventral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. AP -3.1; L +/-1.0; V -3.6 0.25μl</td>
<td>1. AP -5.0; L +/-5.2; V -5.0 0.25μl</td>
</tr>
<tr>
<td>2. AP -3.1; L +/-2.0; V -3.6 0.25μl</td>
<td>2. AP -5.0; L +/-5.2; V -7.3 0.25μl</td>
</tr>
<tr>
<td>3. AP -4.1; L +/-2.0; V -4.0 0.25μl</td>
<td>3. AP -5.8; L +/-4.4; V -4.4 0.25μl</td>
</tr>
<tr>
<td>4. AP -4.1; L +/-3.5; V -4.0 0.25μl</td>
<td>4. AP -5.8; L +/-5.1; V -6.2 0.40μl</td>
</tr>
<tr>
<td>5. AP -5.0; L +/-3.0; V -4.1 0.25μl</td>
<td>5. AP -5.8; L +/-5.1; V -7.5 0.40μl</td>
</tr>
</tbody>
</table>

AP = antero-posterior from bregma; L= lateral from bregma; V = ventral from bregma

All coordinates are given in mm.

2.1.1.3 Behavioral testing.

The animals were tested in three tasks: conditioned place preference, spatial navigation in the water task, and discriminative context conditioning. The results of the conditioned place preference task were inconclusive due to a procedural error and will not be reported here. The results of the discriminative context conditioning task will be reported in chapter 3. The experiment was run in stages, so that the rats whose data were included for final analysis (following the lesion assessment, see below) were assigned to counterbalanced groups in the context discrimination task.

2.1.1.3.1 Task.

This paradigm has been previously used by McDonald and White (1994) and is constituted by an alternation of visible and invisible platform training. In this experiment, this protocol served two purposes: a) to control for motivational, motor, or other impairments that might have occurred in the lesion groups, and b) to provide an alternative to the classical Morris
water task which places less computational demand on the HPC. Testing took place in a windowless, well-lit room.

2.1.1.3.2 Apparatus.

A white plastic pool 180cm in diameter was filled with water (temperature 21°C) mixed with nontoxic white paint. A Plexiglas platform with a 12 X 12 cm rectangular top was submerged in the SW quadrant of the pool. To this, a second, wooden platform painted black on the sides and white on the top could be attached. All testing was conducted using a tracking system (VP118, HVS Image) controlled by an air-operated device used to start and end each trial. Extramaze cues were provided by three posters (of different sizes and orientations) mounted on three different walls, by the computer rack, and by the experimenter. Lighting was provided by ceiling fixtures that could not provide any specific information about platform position.

2.1.1.3.3 Procedure.

The platform was maintained in the same position during the first 12 days of the procedure. During days 1-3, the rats were trained to the visible platform, while during day 4, the visible top was removed and the animals were required to find the invisible platform. This cycle was repeated 3 times. During day 13, the platform with the visible top attached was placed in the NE quadrant in a position diametrically opposed to the one used during days 1-12. Thus, during days 4, 8, and 12 the task required spatial navigation, during days 1-3, 5-7, and
9-12 animals swam to a visible platform, and during day 13, an option between a response to the visible cue and a response to location was available.

For days 1-12 (see fig. 2.1 for an illustration), training consisted of 4 individual trials, each starting at a different cardinal compass point of the room. The sequence of starting points was pseudorandom and was the same for all subjects. All subjects were kept in the testing room in individual wire cages mounted in a cage rack. Each animal was removed from the cage and placed in the pool facing the wall. The recording of the swim started when the rat was released and ended either 30 sec later, or when the rat found the platform, whichever came first. Regardless of whether it found the platform or not, the rat was placed on the top of the platform (visible or invisible) for 10 sec before being returned to his assigned cage. Animals were all given their first trial, then all given their second trial, etc.; therefore, the intertrial interval varied with type of task (visible vs. invisible platform) and with level of training. During day 13, only two trials were run, starting equidistantly between the old and new platform positions.
Figure 2.1. Illustration of the behavioral procedure during days 1-12 in the visible/invisible platform paradigm. The platform position was maintained constant throughout the testing interval, but it was visible during days 1-3, 5-7, and 9-11 and invisible during days 4, 8, and 12.
1. Visible platform - days 1-3; 5-7; 9-11

2. Invisible platform - days 4; 8; 12

3. Competition day 13
On each trial, four parameters were calculated: a) latency to find the platform; b) length of trajectory traversed in the water; c) time spent in the platform quadrant (as a % of latency); and d) heading angle deviation. The latter was defined as the angle between a straight line connecting starting and platform positions on one hand, and a second line connecting starting position with a point located 36 cm on the trajectory on the other hand. For each day, an average for the four trials was calculated for latency, path length, and platform quadrant and reported as one data point. For the heading angle, data resulting from the starting position closest to the platform were excluded because the distance between these two points was shorter than 36 cm and thus a large bias was introduced, particularly in the visible platform data.

2.1.1.3.4 Histology.

At the end of behavioral testing, the lesioned animals were deeply anesthetized and perfused intracardially first with 0.9% saline and then with a 10% formalin solution. The brains were removed and placed in 10% formalin for a minimum of 24 hours, then moved in a 15% sucrose solution. Following crioprotection, the brains were sectioned in the coronal plane (40 µ) and stained with a metachromatic thionin stain.

Each lesion was assessed by marking the damaged tissue onto histological plates showing the hippocampus in coronal sections. Animals whose lesions were unilateral, or showed partial sparing of neurons in the corresponding dorsal or ventral HPC areas were classified as incomplete and data from these animals were discarded from the study. The area of the
lesion was measured in each case (Scion software) and the total volume of the lesion was calculated using the formula:

\[ \text{Volume lesion} = \sum \left[ \frac{\text{area}_i + \text{area}_j}{2} \times \text{distance(\text{area}_i, \text{area}_j)} \right], \]

where \( \text{area}_i \) and \( \text{area}_j \) were the areas of the lesion as measured on two successive histological plates. The total volume of the hippocampus was calculated in a similar way. Finally, the volume of the lesion was expressed as a percentage of the total volume of the hippocampus.
2.1.2 Experiment 2: one-trial spatial learning.

2.1.2.1 Subjects.

40 male Long Evans rats (Charles River Colonies) were involved in total in this experiment. All animals were individually housed in clear plastic cages with food and water available *ad libitum* and maintained on a 10 a.m. – 10 p.m. dark/light cycle. In all cases, testing took place during the dark period. Subjects were randomly assigned to one of three groups: dorsal HPC lesion (n = 14), ventral HPC lesion (n = 14), and sham (n = 12).

2.1.2.2 Surgical procedures.

These were identical to the ones described in section 2.1.1.2.

2.1.2.3 Behavioral testing.

Animals involved in this experiment performed only this task. At the end of the 15 days of testing, they were perfused and the brain sectioned as described in *Histology*.

2.1.2.3.1 Task.

This task has been used before (Morris, 1983; Whishaw 1985, 1991; Sutherland et al., 1997, 2000; Steele and Morris, 1999) in order to increase the computational demand onto the hippocampal function (for a more detailed discussion, see pp. 67-68). In this experiment, this paradigm was used to the same purpose.

2.1.2.3.2 Apparatus.

The same pool was used as in experiment 1.
2.1.2.3.3 Procedure.

Testing lasted a total of 15 days. During each day, each subject performed 8 swimming trials. The sequence of starting positions was randomized within each day and was the same for all animals, but differed from one training session to the other. The position of the platform was changed daily, but remained the same within one given session. Thus, results of the first trial reflected search in the absence of information about platform position, results of the second trial reflected ability to locate a given position within the room, while results of the last trial reflected spatial learning within the particular training session.

During testing, the rats were held in wire cages mounted on a rack. Each animal was in turn removed from the holding cage and placed in the pool facing the wall. Using the air-controlled device, the experimenter started the trial when the rat was released in the water and stopped it either when the animal reached platform or after the elapse of a 60 sec interval, whichever came first. A 60, rather than 30 trial duration has been chose in order to avoid ceiling effects at the beginning of testing, when even normal animals regularly spend more than 30 sec to locate the platform. The animal was placed for a 10 sec interval on the top of the platform at the end of each trial regardless of whether it had found the platform or not, after which it was returned to its holding cage. During each day, the subjects were all given the first trial, then the second trial, and so on. Therefore, the intertrial interval depended on the amount of training.

2.1.2.3.4 Histology.

The histological assessment was performed as described in section 2.1.1.3.4.
2.2 RESULTS

2.2.1 Experiment 1: visible/invisible platform.

2.2.1.1 Histology.

Figure 2.2 shows the extent of the smallest (black) and largest (hatched) lesions for both dorsal and ventral HPC lesion groups. In this study, we attempted to produce lesion of medium size (40-60% of total hippocampal volume, as described by Moser et al., 1995). Our measurements indicated that the dorsal lesions encompassed an average of 38.838% (+/- 2.73) of the total hippocampal volume, while the ventral lesions were slightly larger, enclosing 48.122% (+/- 3.64). The size of the lesion was not correlated with the results of the behavioral tests for either lesion group, but this is not surprising given the consistency of the lesion sizes and the restricted number of data points obtained from experimental groups of 8 subjects.

_Dorsal HPC lesions_. Of the 13 rats that underwent this type of surgery, 5 were eliminated because the lesions were incomplete. Thus, 8 animals were included in this group. All but one of these rats presented some cortical damage at the cannula insertion site. The dentate gyrus was completely destroyed bilaterally in all cases. Some small bilateral sparing in the CA3 field at the anterior pole was present in 3 cases. CA2 sparing, found in 5 animals, was restricted only to most posterior areas (close to splenial region) of the dorsal HPC. Partial bilateral CA1 sparing was observed in one rat at posterior levels. Small unilateral damage to the dorsal thalamus occurred in two cases, one of which was selected as the smallest lesion.
Bilateral damage of the same structure was found in one rat; this animal was selected as showing the largest lesion.

_Ventral HPC lesions._ As in the case of the dorsal lesion group, of the total 13 rats assigned to this group, 5 were eliminated because the lesions were incomplete. Thus, data from 8 animals were included in the final analysis. In all cases, some cortical damage was present at the cannula insertion point. Sparing of most ventral tip of the dentate gyrus, similar to the one shown by Moser et al. (1993), Moser et al. (1995), and Hock and Bunsey (1998), occurred in all cases. Fields CA3 and CA2 were completely damaged bilaterally in all 8 animals. Unilateral sparing in field CA1 was found in 2 instances, while two other animals showed very limited bilateral sparing of this field in the most posterior regions. Some subicular damage occurred only at the most anterior levels of the field (at the border with CA1). Partial damage to pre- and parasubiculum was found in 3 cases. Of these, the one encompassing the most extensive portions of these two structures was selected to represent the largest lesion.

The technical means available did not allow a computerized volumetric analysis of lesion size similar to the one presented by Moser et al., (1995). However, based on a comparison between our plates and the plates presented previously (Moser et al., 1993), as well as on the volumetric lesion reconstruction and on the position of the sections presented by Moser et al. (1995), we estimated that both our dorsal and our ventral lesions would most likely encompass 40-60% of total hippocampal structure. Thus, there is some limited overlap between the dorsal and the ventral lesions, as it can be seen in figure 2.2.
Figure 2.2. Reconstruction of dorsal (left) and ventral (right) hippocampal lesions. The largest lesion is showed in hatches and the smallest in solid black. Results of quantification procedure indicated that dorsal hippocampal lesions were close to 40%, and ventral hippocampal lesions close to 50% of total hippocampal volume.
2.2.1.2 Water task.

Results from both visible and invisible platform testing are shown in figure 2.3.

Visible platform test. Overall lesion X day ANOVAs on the latency, trajectory, quadrant preference, and heading angle indicated in all cases a significant effect of day ($F_{8, 208} = 57.84$, $p < 0.0001$; $F_{8, 208} = 48.68$, $p < 0.0001$; $F_{8, 208} = 10.79$, $p < 0.0001$, and $F_{8, 208} = 16.63$, $p < 0.0001$, respectively), but no significant effects of lesion. The analysis also revealed a significant interaction day X lesion ($F_{16, 208} = 1.95$, $p = 0.0181$) for latency only. Because the data indicated no differences for days 5-7 or 9-11 for latency, and no significant interactions for the other measurements were found, no further investigations were performed. The results were interpreted as indicating no differences between the groups in motivation, motor ability, or ability to associate a visible cue with a motor response (dorsal striatum type of task).

Invisible platform test. The dorsal HPC group showed longer latencies in finding the platform, swam longer distances, and spent less time in the platform quadrant when compared to the controls and the ventral HPC group. These differences diminished towards the end of the testing. Thus, overall lesion X day ANOVAs on latency, trajectory, and quadrant preference showed in all cases a significant effect of day ($F_{2, 52} = 4.65$, $p = 0.0139$; $F_{2, 52} = 8.09$, $p = 0.0009$, $F_{2, 52} = 9.67$, $p = 0.0003$) and lesion ($F_{2, 26} = 9.66$, $p = 0.0007$; $F_{2, 26} = 7.77$, $p = 0.0023$; $F_{2, 26} = 6.14$, $p = 0.0065$). Comparisons using the Student-Newman-Keuls post-hoc test indicated that for latency, there were significant differences between the dorsal HPC lesion group and the other two groups during both first and last day of spatial learning.
The same tests run on trajectory showed dorsal HPC vs. control and ventral HPC differences during day 4, but no differences during day 12. Quadrant preference was significantly higher for controls compared to both lesion groups during day 4, but there were no differences during day 12. Heading angle measurement showed an overall day effect which approached significance ($F_{2, 26} = 3.10, p = 0.0533$), but no significant lesion lesion X day effect. Data for this last parameter did not suggest any differences between groups during any of the invisible platform tests.

**Probe trial – day 13** Previous results (McDonald and White, 1994; Devan et al., 1999; Devan and White, 1999) indicated that in a group of normal animals, half responded to the cue (visible platform) and half responded to location (position trained to during days 1-12). None of the animals with complete HPC lesions responded to location. Thus, while some normal animals show strong association between cue and motor response, some others respond better to location. HPC lesion abolishes response to location. Figure 2.4 shows latency data obtained with all three groups in this experiment. 8 out of 13 control animals had latencies longer than 6 sec (a typical latency to reach the visible platform is about 3 sec). Of these eight, 7 animals actually crossed into the quadrant where the platform had been placed during the previous days. Animals with short latencies (2.4 to 4.3 sec) did not cross this quadrant at all. In the dorsal HPC group, 1 out of 8 animals had latencies longer than 6 sec. This rat did not enter the quadrant previously containing the platform, but two others, with latencies of 5.1 and respectively, 5.9 sec did. In the ventral HPC group, three animals had latencies longer than 6 sec and two of them entered the location quadrant. A third animal, which had a latency of 5.2 sec, also crossed the same quadrant. ANOVA on the
latency data revealed a significant lesion effect for the first trial ($F_{2, 26} = 5.90, p = 0.0077$) and comparisons using the Student-Newman-Keuls test showed significant differences between controls on one hand and both lesion groups on the other hand. No differences were found for the second trial, indicating that one trial was sufficient to introduce a modification in behavior (animals chose the new location).
Figure 2.3. Results of the modified version of Morris water task, as reflected by latency, path length, time spent in the platform quadrant, and heading angle deviation. The platform was submerged during days 4, 8, and 12, and visible for the rest of the testing.
VISIBLE AND INVISIBLE PLATFORM TRIALS

LATENCY (sec)

PATH (cm)

PLATFORM QUADRANT (% total time)

HEADING (degree deviation)

DAY

DAY

DAY

DAY

control

dorsal HPC

ventral HPC
Figure 2.4. Latency in the competition trial. Both lesion groups were faster than the controls in reaching the visible platform. This indicates that both types of lesion altered the equal split between spatial and visual cue responders present in the normal population.
COMPETITION TRIAL
2.2.2 Experiment 2: one-trial spatial learning.

2.2.2.1 Histology.

Histological assessment indicated that 4 animals in the dorsal HPC lesion group and 3 animals in the ventral HPC lesion group had either incomplete damage or extended cortical lesions; data from these animals were discarded. Thus, final analysis included data from 10 animals with dorsal lesions, 11 animals with ventral lesions, and 12 shams. Figure 2.5 shows the lesions with maximum and minimum extent of dorsal (right) and ventral (left) HPC. Dorsal HPC lesions represented an average of 35.99 +/- 4.55 % of total HPC volume, while ventral HPC lesions were an average of 46.02 +/- 4.12 %. In the dorsal lesion group, 6 animals had no cortical damage and 4 had bilateral cortical damage at the cannula entrance points, the largest of which is shown in figure 1. 4 animals had damage to the inferior blade of dentate gyrus (DG) and to CA3 field similar to the one found with the largest lesion. The animal with the smallest lesion in this group presented sparing of the most anterior tip of the dorsal HPC.

In the ventral lesion group, there was no cortical damage in 3 cases, unilateral damage in 3 cases, and bilateral cortical damage in 5 cases. Maximum cortical damage occurred in the animal with the largest ventral HPC lesion (see figure 2.5). Sparing of most ventral HPC tip occurred in all cases. Subicular damage was present in one animal in areas immediately adjacent to the most posterior levels of the DG and otherwise it was very restricted and
present in the ventral HPC areas (7 cases). At most posterior levels, small unilateral sparing of CA1 (7 cases) and of DG (2 cases) was found. The largest CA1 sparing is shown in figure 2.5 as smallest ventral HPC lesion. Comparison with lesions produced by other researchers (Moser et al., 1993; Hock and Bunsey, 1998) did not help us in making a decision of whether to exclude this rat from the final analysis. Its data were not notably different from the ones of other animals in the same group. In the end we resolved to include it and perform statistical analyses with and without its data.
Figure 2.5. Reconstruction of dorsal (a) and ventral (b) HPC lesions. In both cases, the lesion with smallest volume is shown in black, and the lesion with largest volume is shown in gray. Extended sparing of CA1 was found in the animal with smallest ventral HPC lesions.
2.2.2.2 Water task.

The results of the task are shown in figure 2.6. As mentioned above, data from first, second and eighth trials were investigated. To control for starting position relative to platform location, each data point was obtained by averaging results of either days 1-3 (start) or days 13-15 (end). For each parameter, a separate lesion x trial 2-way ANOVA was performed for the start and respectively the end of training. In each case, simple effects of lesions were investigated by using the REGW test (based on the same idea as the Newman-Student-Keuls test, but adjusted for unequal means). A note will be made when differences were found between the analysis including data from all animals and the one excluding data from the animal with smallest ventral lesion (see above).
Figure 2.6. Results of one-trial spatial learning water task. Data from first trial reflect searching in absence of information about platform position. Second trial results indicate how efficiently the animals could locate the platform after spending a 10 sec interval on its top at the end of trial 1. Eighth trial results reflect spatial learning within one training session.
**Latency.** Main effects of lesion were significant at the start (F_{2,30} = 4.26, p < 0.05) and at the end (F_{2,30} = 5.20, p < 0.05). A similar situation was found for trial number (F_{2,60} = 80.21, p < 0.001 and F_{2,60} = 43.00, p < 0.001 respectively). No significant lesion\times trial interactions were found in either case. There were no differences between groups for trials 1 and 8 either at the beginning or at the end. Significant differences were found for trial 2. At the start, the results of the dorsal and ventral HPC lesion groups were statistically different, but neither of them was different from the results of the sham group. At the end, the results of the dorsal and ventral HPC lesion groups were not different from each other, but both groups needed significantly longer times than the controls to reach the platform. Exclusion of the data from the animal with the smallest lesion did not introduce any modification in these results.

**Trajectory length.** Main effect of lesion was found significant at the beginning (F_{2,30} = 5.10, p < 0.05) and at the end (F_{2,30} = 7.71, p < 0.01) of testing. A similar situation emerged for main effect of trial number (F_{2,60} = 76.28, p < 0.001 and F_{2,60} = 41.86, p < 0.001). Investigation of simple effects indicated differences between groups on trial 2. At the start, dorsal HPC lesion rats swam longer distances than ventral HPC lesion group but not the shams. There were no differences between shams and ventral HPC lesion group. At the end of testing, dorsal HPC lesion group swam longer distances than the ventral HPC lesion group, which in turn swam longer distances than the sham group. Additionally, on trial 1 at the end of training dorsal HPC lesion group swam longer distances than both ventral HPC and sham groups, which were not different from each other. These differences were not maintained when analysis was run on the smaller data set.
**Quadrant preference.** Overall ANOVAs showed no significant effect of lesion or lesion x trial interactions. A significant effect of trial was found only at the beginning of testing ($F_{2, 60} = 13.69, p < 0.001$), although trial effect at the end was very close to significance as well ($F_{2, 60} = 2.99, p = 0.0576$). There were no simple effects of lesion except during trial 8 at the end of the testing, when the shams spent more time in the platform quadrant than the animals with ventral, but not the ones with dorsal HPC lesions; the lesion groups were not different from each other. These differences disappeared when the analysis was repeated for the smaller data set.

**Heading angle.** No lesion or lesion x trial effects were found at either beginning or end of testing. In both cases there was a main effect of trial ($F_{2, 60} = 12.63, p < 0.001$ and $F_{2, 60} = 10.99, p < 0.001$ respectively). There were no simple effects of lesion at any level of the trial variable.

Because the pattern described by the data is rather complex, the results of the statistical analysis are summarized in table 2.2.
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<th>Trial</th>
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<th>END</th>
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<tr>
<td></td>
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<tr>
<td></td>
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<td>D V Sh</td>
</tr>
<tr>
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<td>8</td>
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</tr>
<tr>
<td></td>
<td>8</td>
<td>no differences</td>
<td>no differences</td>
</tr>
</tbody>
</table>

D = dorsal HPC lesion; V = ventral HPC lesion; Sh = sham

___ = data not statistically different

* = no differences when data obtained with smallest ventral HPC lesion excluded
2.3 DISCUSSION

2.3.1 Experiment 1: visible/invisible platform.

This experiment demonstrated that lesions of dorsal HPC produced a greater learning deficit in spatial navigation than lesions of ventral HPC. However, dorsal HPC lesion group exhibited some limited learning, as its impairment diminished across training when compared to the other two groups. During the last day of invisible platform testing, only the latency measure showed a significant discrepancy. A general sense of platform position was present in the dorsal HPC lesion group even at early stages of training, as demonstrated by the lack of heading angle differences between groups. During probe trial (day 13), both dorsal and ventral HPC lesion groups responded preferentially to visible cue than to spatial position. Thus, similar to dorsal HPC lesion, ventral HPC lesion altered the normal equal split (McDonald and White, 1994; Devan et al., 1999; Devan and White, 1999) between ‘place learners’ and ‘cue learners’. Together, these results demonstrate that ventral HPC can support some spatial learning in the water maze and its lesion does interfere with processing of spatial information. Therefore, although there is a clear quantitative difference between the dorsal and ventral HPC regarding efficiency in spatial navigation, our data do not argue for an absolute dissociation.

This interpretation is more in line with the earlier position of Moser et al. (1993), who accepted a limited role for the ventral hippocampus in spatial learning. This is also
compatible with data reported by this group that show involvement of the ventral hippocampus in retrieval of information (Moser and Moser, 1998a). This is against the latest position of Moser and Moser (1998b), which argues for strict dissociation between functional roles of dorsal vs. ventral hippocampus. The reasons for this position are multiple and I will discuss them below, but first I will make a remark on the issue of spatial strategies.

One obvious explanation for the impairment following the partial HPC lesions is based on the cognitive map theory formulated by O'Keefe and Nadel (1978). According to this view, animals with HPC lesions are deficient in spatial navigation because they are not able to acquire spatial relationships among cues. A first alternative explanation is that the animals learn to perform a motor response to one particular stimulus in the room. Against this view is the finding that this particular type of association is based on the DS memory system. Clear double dissociation has already been demonstrated between spatial navigation, sensitive to HPC disruption, and stimulus-response association, sensitive to DS damage (McDonald and White, 1993, 1994). A second possibility is that animals perform a 'vector heading' response: they learn to approach one particular cue and then swim in a particular direction from that cue to the platform (Pearce et al., 1998; Kubie et al., 1999). This strategy however would require the presence of a salient, visible cue involved in a consistent spatial relationship with the invisible platform. Data presented by Pearce et al. (1998) argue against this. In that particular experiment, animals were trained to an invisible platform always located in a certain relation to a visible cue. The position of the cue-platform compound was modified with every training session. Normal animals showed longer latencies in the first
trial than animals with HPC damage, but by the fourth trial their performance was much better. Similar to that study, the absence of differences in heading angle data in the present experiment do indeed suggest that animals with HPC damage can use a heading vector. But if a heading vector strategy (rather than cognitive mapping) was the basis of spatial navigation, one would expect that latencies would be similar in visible and invisible platform trials, because when the location of the visible cue and the location of the platform are the same it is clear that vector heading cannot be performed relative to the visible platform (the vector calculated would be a null vector). A third possible strategy is path integration (as argued for instance by Whishaw; see section 1.5). Again, the discrepancies between visible and invisible trials data do not support this theory. If animals navigate by integrating idiothetic or allothetic cues with movement, they should be able to perform equally well in visible vs. invisible trials. Thus, it appears that a cognitive map theory is more suitable for explaining the empirical data.

I shall now consider the differences between this interpretation of the data and the ones presented by Moser and collaborators. First, four different measurements of spatial navigation were investigated. It is important to notice that as far as latency goes, our data replicate the results of Moser et al. (1995) (these were also obtained following a neurotoxic lesion procedure). However, when other parameters are included in the analysis, the lesion effects prove to be more complicated. Information provided by these multiple measurements has to be considered in an integrative fashion. Thus, an animal that has some, but not very precise representation of the platform position within the room may swim in the right direction (small heading angle deviation) and circle around the right position (high
preference for the platform quadrant), but latency for reaching the platform may be high. The path length may vary, depending on whether the animal is searching for platform slowly or not. Alternatively, a rat that has a very precise representation of platform position will probably show short latency and path length, but preference for the platform quadrant may be low because the animal will not spend a long time searching.

In this experiment latency, likely to be most sensitive to efficiency of behavior, showed most modifications. Heading angle, likely to concern mainly a general sense of platform position, was not much affected. This pattern of results suggests that control animals had a precise representation of platform location, while rats with dorsal HPC lesions started searching in the right area (small heading angle deviation, quadrant preference over chance level) but lacked the precision necessary to make this search efficient. The ventral lesion group acquired quickly a general sense of platform position but needed more training to make their navigation efficient as well.

Second, the paradigm used in this study is an alternative to the classical, submerged platform version. During visible platform training, normal animals acquire spatial information. Swimming to the visible platform also promotes procedural learning and does not favor thigmotaxic behavior, possibly present in the lesion groups. Third, in this experiment the rats were allowed an interval of 24 hours (as opposed to 4 hours, Moser et al., 1993 and Moser et al., 1995) between blocks of testing, time during which memory consolidation processes are likely to have occurred (Wilson and McNaughton, 1994).
Fourth, there are discrepancies between the results of previously published studies. Thus, Moser et al. (1993) found learning deficits with dorsal HPC lesions larger than 20%, and ventral HPC lesions larger than 39% of the total HPC tissue. The more recent study (Moser et al., 1995), employing a similar task, indicated that dorsal HPC lesions of 20-40%, and ventral HPC lesions larger than 40% (and up to 80%) of the total HPC had no effect. It is difficult to speculate what the cause of this discrepancy may be, but possibilities are the lesion technique (aspiration vs. neurotoxin) and inconsistencies in lesion size assessment.

In conclusion, these data support the view that HPC is heterogeneous in its efficiency of supporting spatial navigation: the gradient is maximal at the dorsal tip and decreases progressively towards the ventral tip.

2.3.2 Experiment 2: one-trial spatial learning.

This experiment investigated spatial learning in animals with either dorsal or ventral HPC lesions by assessing daily acquisition of location. The task was chosen to further investigate the previous finding that dorsal HPC lesions animals show some forms of spatial learning and that ventral HPC lesions interfere with this ability. These results did not support the idea of an absolute separation of function between dorsal and ventral HPC regarding spatial learning. Instead, there seems to be a gradient of efficiency, with septal pole being at the upper and temporal pole at the lower end of the scale.

The particular task used in experiment 1 was previously used to demonstrate dissociation between HPC and dorsal striatum memory systems (McDonald and White, 1994; Devan et
al., 1999; Devan and White, 1999). It differs from the classical Morris water task because it alternates training to a visible platform with training to an invisible platform located in the same position. Swimming to a visible platform requires forming an association between a visible cue and a motor response. This form of learning is dependent on the function of the dorsal striatum. In contrast, swimming to an invisible platform requires spatial navigation, a process dependent on HPC function. In our previous experiment, we used the visible trials for different reasons. First, we intended to control for possible differences in motivation or motor ability levels. Second, we facilitated overcoming of thigmotaxic behavior in animals with HPC damage. Thus, we attempted to separate spatial from procedural learning. In the present experiment, we controlled for procedural learning by extending the testing interval to 15 days. Simultaneously, we increased demand on HPC network function by daily modifying the platform position. Based on previous results, we expected to find more impairment in animals with dorsal than with ventral HPC lesions. Additionally, we predicted that higher processing requirements in the HPC network should reveal a more obvious deficit in the ventral HPC group. Third, if both dorsal and ventral HPC can support spatial learning, then extended training should reduce differences in performance between animals with dorsal vs. ventral HPC lesions.

The results confirmed these predictions. First, we replicated the differences in spatial navigation following dorsal vs. ventral HPC lesions. Results of trial 2 at the beginning of training show that dorsal HPC rats swam longer distances for longer intervals of time. As in our previous experiment, we found no differences between lesion groups on quadrant preference and heading angle, suggesting thus that neither kind of damage abolished a
general sense of direction. This demonstrated that dorsal HPC lesions produce a more severe learning deficit, although the impairment is reflected in some, but not all aspects of this behavior.

Second, we found acquisition deficits in the ventral HPC group similar to the ones obtained with dorsal HPC lesions. These were revealed at the end of training, when any differences on procedural strategies were likely overcome. During trial 2, there were significant differences on latency and path length between both lesion groups and sham group. This demonstrated that both dorsal and ventral HPC lesions have an effect on spatial learning.

Finally, our hypothesis regarding attenuation differences between lesions groups with extended training was also confirmed. This is demonstrated by modifications in performance with amount of training (start vs. end; trial 2 vs. trial 8). Thus, at the beginning of training, trial 2 results indicated significant differences between animals with dorsal and ventral HPC lesions on latency and path length. The differences on latency disappeared at the end of training. Additionally, both lesion groups performed similarly on quadrant preference and heading angle, suggesting thus that some aspects of spatial processing were not affected by lesion type. Second, comparison of results across trials shows that differences present during trial 2 disappeared on trial 8. An exception was constituted by quadrant preference data at the end of testing. However, this particular difference was not reliable because it was not preserved when data from one animal were excluded (see Results for details). Thus, given enough time and experience, it seems that spatial learning can be supported by either dorsal or ventral HPC networks.
The absence of differences between groups during trial 1 supports our previous conclusion that there are no procedural differences between groups. The trajectory data were an exception, showing that dorsal HPC lesion groups swam significantly longer distances on trial 1 at the end of training. This difference however was not reliable, because it disappeared when the analysis was repeated with the smaller data set.

Differences between groups obtained when comparing trial 1 to trial 2 data could be used to argue that the impairment following HPC lesions is due to interference with working memory. As mentioned in section 1.5, this theory of HPC function has been disproved several times. Additionally, comparison of trial 2 to trial 8 data does not support it. If animals cannot form a temporary representation for platform position between trial 1 and trial 2, it is difficult how they could form one between trial 7 and trial 8. A counterargument is that at this point, platform position is represented in reference memory. Again, the dissimilarity between performance during trial 1, 2 and 8 is against this idea. Olton (Olton et al., 1979) makes it clear in his comparison of working and reference memory that the latter refers to information about rules and procedures. If animals with HPC lesions have intact reference memory, but are impaired in the working component of the task, they would be expected to perform at similar levels on first, second, and eighth trials, because in all cases they would engage in search while unable to remember the exact platform position from one trial to the next.
Trial 2 data obtained at the end of training show a significant difference between lesion groups on path length but not on latency. This suggests differences between lesion groups regarding swim speed. This is particularly relevant because Bannerman et al. (1999) recently reported that lesions of ventral, but not dorsal HPC were associated with faster swimming. Latency, swim speed and path length are directly related (path length = latency * swim speed). Therefore we did not perform a separate analysis for swim speed. However, trial 1 results argued against this hypothesis. If the ventral HPC animals were indeed swimming faster, it would be expected that they would find the platform in a shorter time on this trial, which involves search in the absence of information about platform location. We performed an informal analysis by dividing average path lengths by average latencies. The results suggested no differences between groups at the beginning (all results between 28.39 cm/sec and 28.46 cm/sec) or at the end of testing. If anything, ventral HPC lesion group tended to have lower averages (25.63 cm/sec, 22.54 cm/sec, and 22.41 cm/sec on trials 1, 2 and 8 respectively) than the rest of the rats (28.4 cm/sec, 25.75 cm/sec, 29.136 cm/sec for dorsal HPC lesion group and 26.63 cm/sec, 21.875 cm/sec, and 24.6 cm/sec for shams). Our results therefore do not support the conclusion drawn by Bannerman et al. (1999). One factor we thought may account for this discrepancy is lesion size. These authors estimated the size of their ventral HPC lesions as 25% to 45% of total HPC, while we estimated ours as an average of 46.03 +/- 4.12%. Our group seems therefore to have had larger lesions. This rules out the possibility that lack of swimming speed modifications is due to smaller lesion size in our case. It is difficult to estimate differences between lesioned and spared areas, because Bannerman et al. sectioned the brains horizontally. Thus, at this point there is no
obvious explanation for the discrepancy in results. Bannerman et al.’s use of the Duncan test may be part of the problem, as this test does not control familywise error rate.

To summarize, the results of this experiment are consistent with the view that although dorsal HPC is more efficient in acquiring spatial information, the process occurs along the whole dorso-ventral HPC axis. Thus, our conclusion is in contrast with the one drawn by Moser and Moser (1998) that ventral and dorsal HPC areas perform qualitatively different functions. These authors’ opinion was based on data obtained in two different studies (Moser et al., 1993; Moser et al., 1995), the first using electrolytic, the second neurotoxic lesions. Dorsal HPC lesions were more effective in disrupting spatial learning in both cases, but there are discrepancies. Electrolytic lesions of ventral HPC encompassing 39-52 % of total HPC volume resulted in impairment. In contrast, neurotoxic lesions encompassing 40-60 % of total HPC did not. Electrolytic dorsal HPC lesions of 20-30% resulted in impairment, but neurotoxic lesions of 20-40 % of the same area did not. One reason for these differences may be electrolytic damage of passage fibers connecting HPC with other brain areas involved in spatial learning, such as the septum. Although there is some evidence in support of this hypothesis (Whishaw and Jarrard, 1996), systematic studies of this issue are lacking.

A second problem may lay in the interpretation of the lesion effects. A 3-dimensional reconstruction of neurotoxic lesions (Moser et al. 1995, figure 1) indicates that damage in ventral HPC lesion group advances from splenial to ventral HPC area and not vice-versa, as it is indicated on the diagrams (figure 2). The reconstruction of electrolytic lesions (Moser et
al., 1993, figure 1) seems to corroborate this observation. If this is the case, then impairment obtained with larger ventral HPC lesions in both studies is due to encompassing progressively more ventral rather than more dorsal HPC areas. Consequently, these data cannot be used as support for the argument that ‘...the circuit required for spatial learning is not graded. It seems to be distributed throughout the dorsal two-thirds of the hippocampus, with septal and splenial parts being equally important.’ (Moser and Moser, 1998).

Third, in the experiments cited above training to the invisible platform was performed twice a day, 4 trials/session, for a total of 6 days. In the present experiment, we performed one training a day, 8 trials/session, for a total of 15 days. Thus, our paradigm allowed more than double the time for task acquisition. Similarly, in our previous experiment training lasted 12 days. It is thus possible that longer acquisition intervals minimized differences between lesion groups by the end of training. This hypothesis is supported by a similar finding (De Hoz and Morris, 1999) that training spaced in time resulted in attenuation of differences found on the Morris water task between animals with dorsal vs. ventral HPC lesions.

Finally, there is the issue of multiple parameters assessment. Moser et al. (1993) and Moser et al. (1995) reported only latency data. Results indicate that latency is the measurement most affected by HPC lesions, probably because it requires both good orientation towards the proper location and efficient navigation to that position. Were other parameters recorded in the studies cited above, a situation similar to ours might have emerged, in which differences would be found mainly on latency and less on other measurements.
The conclusion that processing of spatial information occurs along the whole septo-temporal HPC axis is in agreement with other results. Both dorsal and ventral HPC were found to be involved in working memory for allocentric distance (Long and Kesner, 1996). Lidocaine inactivation of the ventral HPC impaired performance in a hole-board task (Poucet et al., 1991). It is interesting that in a parallel study, using identical lesions and procedures as Bannerman et al. (1999), Richmond et al. (1999) did not obtain overall impairment with dorsal HPC lesions. Unfortunately, these authors analyzed only overall lesion effects and did not perform comparisons between groups at different stages across training. Analysis of time spent per pool quadrant indicated however that animals with both dorsal and ventral HPC lesions spent significantly more time in the platform quadrant, as opposed to animals with whole HPC lesions. This result thus supports the idea that spatial learning can occur in either dorsal or ventral HPC.

Systematic investigations of electrophysiological activity in dorsal vs. ventral HPC is scarce. Jung et al., (1994) indicates that there are place cells in both these HPC areas, although ventral HPC has fewer complex spike cells responsive to space. The fields of these cells had lower resolution of spatial selectivity. Presence of place cells in ventral HPC has also been reported by Poucet et al. (1994), who found that the properties of these cells were similar to the ones of dorsal HPC neurons. Thus, electrophysiological data suggest that exclusive processing of spatial information in the dorsal HPC is an unlikely case. Presently, the exact significance of the differences in physiological characteristics is not known. Jung et al. (1994) speculated that dorsal HPC might be more involved in dealing with high-resolution spatial information found in a small environment, while ventral HPC may process low-
resolution spatial information found in a large environment, but further evidence is necessary to support this hypothesis.
2.4 CONCLUSION: SPATIAL LEARNING AND THE LONGITUDINAL HIPPOCAMPAL AXIS

In conclusion, our investigation of the role of dorsal vs. ventral HPC in a water maze task indicated that both areas are involved in spatial learning. Present results and other data (De Hoz and Morris, 1999) suggest that within HPC network, the septal pole is at the upper and temporal pole at the lower extremities of a graded efficiency in spatial information processing.
3. DORSOVENTRAL HIPPOCAMPAL AXIS AND ACQUISITION OF DISCRIMINATIVE CONTEXT CONDITIONING

Context conditioning is a behavioral task that requires learning the affective significance of a multitude of cues, presumably organized in a configural-relational representation. This task has been developed as a means for testing memory processes related to other types of information but spatial. As evidence presented in section 1.4 indicates, this paradigm involves both AMG and HPC function. Empirical data suggest that the roles of the two neural structures are different. AMG is viewed as the anatomical substrate of forming an association between a simple stimulus and an emotional response. As discussed, its function may modulate predominantly freezing. The hippocampal formation is seen as anatomical substrate of learning about relationships among cues. Its function may modulate predominantly performance in a preference test where a choice is given between (usually) two contexts (discriminative context conditioning).

The paradigm used in this experiment was designed to test discrimination among contextual cues while minimizing the role of spatial, distal cues. It used an apparatus consisting of two separated compartments, one of which was paired with shock. Chamber identity was defined by visual (color and shape) and olfactory cues. Both freezing and chamber preference were evaluated.
3.1 MATERIALS AND METHODS

3.1.1 Subjects.

As mentioned in chapter 2, the animals involved in this experiment were first tested in the visible/invisible platform water task.

3.1.2 Apparatus.

A diagram is presented in figure 3.1. The apparatus used for this task has been described in detail elsewhere (Ferbinteanu et al., 1999). Briefly, two large chambers connected by a middle alley (16.5 cm X 11 cm X 11 cm) were used. One of the chambers was a black triangular prism (61 cm edge, 30 cm height) associated with one particular odor (amyloacetate). The other chamber was a white rectangular prism (41 cm edge, 30 cm height) associated with a second, different odor (eucalyptus). Both chambers and the middle alley were covered with translucent plastic, which blocked access to the cues in the testing room. The apparatus was mounted on a transparent tabletop underneath which a mirror was mounted at a 45° angle. This set up allowed monitoring of the animal’s movements within the apparatus.
**Figure 3.1** Diagram showing the setup used for context conditioning. The two compartments of the apparatus were of equal area. One was a black cube while the other was a white triangular prism. Additionally, each chamber was associated with a different odor. A middle alley with a pivoting lid allowed communication between the two sides of the apparatus. Both chambers and the middle alley were covered with translucent plastic that blocked access to distal cues. Thus, the animals had to use exclusively local, contextual cues in guiding their behavior. Behavioral monitoring was performed through the angled mirror placed underneath the table.
1. Habituation
2. Training 1 (shock). Testing 1 (freezing)
3. Preference 1
4. Training 2 (shock). Testing 2 (freezing)
5. Training 3 (shock). Testing 3 (freezing)
6. Preference 2
3.1.3 Procedure.

During the whole procedure, the rats were kept on a cart in their home cages and were not allowed visual access to the testing room except when introduced and removed from the apparatus. During day 1, each rat was placed in the middle alley and allowed to freely explore the two chambers for 10 min (habituation). On days 2 and 3 (training days), the openings of the middle alley were obstructed. Each rat was assigned a particular combination of room identity (black vs. white), order of pairing (shock on day 2 or day 3), and order of testing (paired chamber on day 4 or day 5; see below). The animal was introduced in its assigned box and confined there for 5 min. On shock days, 0.6 mA of current (scrambled shock) were delivered for 2 sec at minutes 2, 3, and 4. In each case, signs of distress (flinch, vocalization, defecation) were recorded. During days 4 and 5 (testing days), freezing (absence of any movement except breathing) was measured upon confinement for 5 min in one of the chambers, according to the schedule particular to each rat. Response to both paired and unpaired compartments was measured, but on separate days. During day 6, the middle alley openings were unblocked and the animals were allowed to explore the apparatus for 20 min (preference). Following this test, there were two more training sessions (days 7 and 8 - second shock; days 11 and 12 - third shock), two more testing sessions (days 9 and 10 - second shock; days 13 and 14 - third shock) and one more final preference test (day 15). To summarize, the sequence of events was the following: day 1 - habituation; days 2 and 3 - training shock 1; days 4 and 5 - testing shock 1; day 6 - preference after first shock; days 7 and 8 - training shock 2; days 9 and 10 - testing shock 2;
days 11 and 12 – training shock 3; days 13 and 14 – testing shock 3; day 15 – final preference. This is illustrated in the following flow chart:

DAY 1: habituation ⇒
DAYS 2 & 3: training I ⇒ DAYS 4 & 5: freezing I ⇒ DAY 6: preference I ⇒
DAYS 7 & 8: training II ⇒ DAYS 9 & 10: freezing II ⇒
DAYS 11 & 12: training III ⇒ DAYS 13 & 14: freezing III ⇒ DAY 15: preference II

3.1.4 Histology.

At the end of the context conditioning procedure, the histological procedures were performed as described in chapter 2.
3.2 RESULTS

3.2.1 Freezing.

Figure 3.2 shows the freezing data collected after the first, second, and third shock pairing. Separate lesion X chamber ANOVAs were performed in each case and investigation of simple effects of chamber were run using an adjusted $\alpha$ of $0.05 / 3 = 0.0166$ as a criterion. After the first shock, there was an overall chamber effect ($F_{1, 26} = 6.55, p = 0.0167$), but no individual group showed significantly higher freezing in the paired condition. After the second shock, there was an overall chamber effect ($F_{1, 26} = 28.62, p < 0.0001$) and all groups froze significantly higher in the paired chamber ($F_{1, 12} = 9.23, p = 0.0103$ for controls, $F_{1, 7} = 4.57, p = 0.0156$ for dorsal HPC lesion group, and $F_{1, 7} = 12.37, p = 0.0098$ for ventral HPC lesion group). After the third shock, there was similarly an overall chamber effect ($F_{1, 26} = 37.82, p < 0.0001$), and significant differences for all groups ($F_{1, 12} = 18.15, p = 0.0011$ for controls, $F_{1, 7} = 12.86, p = 0.0089$ for dorsal HPC lesion group; $F_{1, 7} = 9.97, p = 0.0160$ for ventral HPC lesion group).
FIGURE 3.2. Acquisition of discriminative freezing following successive pairings with shock. Neither lesion group was different from the controls.
3.2.2 Preference.

The preference data (figure 3.3) were analyzed in a similar way as the freezing data. There was no overall significant preference for any of the groups during habituation. After the first shock, a significant overall chamber effect ($F_{1,26} = 5.41, p = 0.0289$) was found, but only the control group showed a significant preference for the unpaired (‘safe’) chamber ($F_{1,12} = 12.69, p = 0.0039$). The overall analysis for the second test also indicated a significant chamber effect ($F_{1,26} = 13.30, p = 0.0012$), but again only the control group spent significantly more time in the unpaired box ($F_{1,12} = 10.28, p = 0.0075$). Although data do indicate the development of a trend in the same direction for the lesion groups, the preference was not statistically significant even after two more shocks.
Figure 3.3. Chamber preference during habituation and after one and respectively three pairings with shock. Neither lesion group succeeded in acquiring a significant preference for the unpaired chamber.
PREFERENCE

HABITUATION

![Bar chart showing time percentages for dorsal, ventral, and control conditions under habituation.]

AFTER SHOCK 1

![Bar chart showing time percentages for dorsal, ventral, and control conditions after shock 1.]

AFTER SHOCK 3

![Bar chart showing time percentages for dorsal, ventral, and control conditions after shock 3.]

Legend:
- **paired**
- **unpaired**
3.3 DISCUSSION

The results of the discriminative context conditioning task showed that animals with both dorsal and ventral HPC lesions could acquire discriminative freezing at a rate similar to the control group. In contrast, neither group developed a preference for the ‘safe’ environment even after three training sessions. This discrepancy could be explained by particularities of neurobiological substrate that supports performance in this task. Context conditioning involves both AMG and HPC memory systems (Phillips and LeDoux, 1992; Kim et al., 1993; McDonald et al., 1995; Frankland et al., 1998). Training is accomplished by pairing a combination of sensory cues with an affective response. This is a situation similar to training in the passive (CPP) task as described by McDonald and White (1995a). In that task, rats are confined at the end of one arm in the presence of food and at the end of a different arm in the absence of reinforcement. Investigation of lesion type (Fx, AMG, DS), of arm position (opposite vs. adjacent), and of training procedure (passive presentation vs. free movement) demonstrated that AMG is involved in passive learning, while active place learning (rat moved between the paired and unpaired arms) required a functional HPC (McDonald and White, 1993; see also White and Ouellet, 1997). HPC acquires information during habituation to the maze, when alternative perspectives on the cues are available, but both AMG and HPC are involved in discriminating among cues, depending on the situation (McDonald and White, 1995a,b). In the present experiment, passive pairing of shock with a particular chamber may favor AMG control over behavior, especially as freezing is measured while the rat is confined in the given chamber. HPC may gain precedence over
AMG in directing behavior during the preference test, when exploration is possible and discrimination among cues is required.

The results of the preference test do not support the idea that rats use a spatial solution for chamber discrimination because animals with ventral HPC lesions, who can perform spatial tasks, were impaired on this test. Second, dorsal and ventral HPC areas seem to be equally important for adequate behavior in choosing the ‘safe’ environment, suggesting that discrimination between two sets of cues with conflicting emotional valence requires extensive integration along the dorso-ventral axis of the HPC. This is different than the case of spatial navigation which although using a large subset of hippocampal network in normal conditions (Moser and Moser, 1998a), requires only limited portions of the HPC (Moser et al., 1993, Moser et al., 1995; present report). The implications of this finding are discussed in chapter 5.

There are only two studies in the literature directly comparable with the present one. The first is by Hock and Bunsey (1998) who tested animals with either dorsal or ventral HPC lesions on a conditioning task that used internal state as a cue. The rationale behind this design was that ventral HPC, which is connected mostly to subcortical areas such as AMG and hypothalamus, may be preferentially involved in this type of learning. Freezing during the last 2 days of training and last 2 days of extinction were compared. It was found that both dorsal and ventral HPC lesions were associated with reduced freezing. The second study is by Richmond et al. (2000), who compared rats with dorsal, ventral, or complete HPC lesions to controls. These investigators found no differences between dorsal HPC and
control group, or between complete and ventral HPC lesion groups. Freezing had a significantly lower level in the latter than in the former case.

As opposed to the present case, results of both experiments cited indicate effects of dorsal/ventral HPC lesions on freezing. There are however important aspects that should be considered. The work discussed above used testing paradigms that lacked the discriminative aspect. In both cases, training and testing were performed in the same chamber. Additionally, Richmond et al. (2000) used tone as CS. A recent review (Holland and Bouton, 1999) summarizing research on HPC involvement in context conditioning has pointed out that one important reason behind the heterogeneity of experimental results is precisely the diversity of conditioning protocols. Phillips and LeDoux (1994) have demonstrated that there are important differences between conditioning in the presence vs. absence of distinct CS. These authors reported that electrolytic lesions of dorsal HPC abolished freezing when shock was paired with a tone. In contrast, when shock was presented alone there were no differences between controls and lesioned animals.

A second factor that should be considered is the type of lesion employed. Phillips and LeDoux (1994) and Maren and Fanselow (1997) used electrolytic lesions of dorsal HPC. Similar to this study, Richmond et al.'s experiment was based on neurotoxic lesions. Electrolytic lesions destroy both cell bodies and fibers and passage. Thus, electrolytic damage of dorsal HPC may be more similar in effects to Fx transections than to neurotoxic lesion of the area. To make matters even more complicated, a recent paper (Laurent-Demir and Jaffard, 2000) reported that Fx lesions enhance context conditioning in a paradigm using
pairing of a tone with shock while Winocur (1997) reported enhanced conditioning after electrolytic lesions of dorsal HPC. The importance of this issue is demonstrated by data presented in next chapter which indicate a clear dissociation of behavioral effects between neurotoxic dorsal HPC and Fx lesions.
3.4 CONCLUSION

In conclusion, systematically collected information on the issue of dorsal/ventral HPC lesions and context conditioning is lacking. Thus, it is difficult to make a strong statement about the precise role of these HPC areas in this type of task. Data from this experiment however demonstrate two things. First, spatial learning in the water task and acquisition in the discriminative context conditioning paradigm are not identical. This is because ventral HPC lesions produce only a modest deficit in the water task but as severe of an impairment as dorsal HPC lesions in the context task. This issue will be discussed in more detail in chapter 5. Second, behavioral parameters are affected differently. This is not the first report of similar dissociations. Phillips and LeDoux (1995) investigated freezing and passive avoidance conditioning and found that EC lesions disrupted the latter but not the former. One factor making the results of this study difficult to interpret is that the lesions included in fact ventral HPC and PER (see fig, 1). The important point is however that freezing and passive avoidance were affected differentially. The conclusion is that future investigations on the role of dorsal vs. ventral HPC areas should take into consideration the nature of the behavioral parameters used to assess learning.
4. DORSOVENTRAL HIPPOCAMPAL AXIS AND CPP. WHAT ABOUT THE FORNIX?

CPP is an example of conditioning paradigm that requires dual AMG and hippocampal involvement (White and McDonald, 1993; McDonald and White, 1995a,b). In this case, location rather than context is employed as CS. The task is a modification of the classical radial maze procedure requiring alternative confinement of the animal in the paired and unpaired arms. When given a choice, normal rats spend more time at the location previously paired with food.

Previous work showed that AMG lesions disrupt learning in this task while surprisingly, Fx lesions have the opposite effect. Thus the first goal of the present experiment was to directly compare the effects of AMG, Fx and whole HPC lesions. The second objective was to investigate whether dorsal and ventral HPC networks have distinct involvement in this type of learning.
4.1 MATERIALS AND METHODS

4.1.1 Experiment 1: Effect of Fx, AMG and HPC lesions on CPP.

In this experiment, sham animals and animals with AMG and HPC lesions underwent training in the CPP task (4 training trials; for details, see below). To demonstrate the lack of effect on spatial learning following AMG lesions we also tested these animals in a classical Morris water task. Sham animals and animals with Fx lesions were run in a similar CPP task that involved only 3 training trials.

4.1.1.1 Subjects.

CPP - 4 training trials. 36 male Long Evans rats (Charles River Colonies) were involved in total in this experiment. Animals were individually housed in clear plastic cages with water ad libitum and were maintained on a 10 A.M. – 10 P. M. dark/light cycle. Testing occurred during the dark period. All animals were food restricted during the behavioral procedures. The subjects were randomly assigned to one of three groups: sham (n = 12), HPC lesion (n=12) and AMG lesion (n=12). Due to incomplete lesions, data from 2 animals from each lesion group were discarded from the final analysis.

CPP - 3 training trials. 21 male Long Evans rats (Charles River Colonies) were involved in this experiment. Housing, testing and feeding conditions were identical to the ones described above. The subjects were randomly assigned to one of two groups: sham (n=11) and Fx lesion (n = 10). Following histological assessment, data from 7 animals with Fx lesions were included in the final analysis.
4.1.1.2 Surgical procedures.

CPP – 4 training trials. All animals weighed 275-300 g at the time of surgery. The rats were anesthetized using sodium pentobarbital (65 mg/kg body weight) administered i.p. Atropine (5 mg/kg body weight) was also administered in order to avoid fluid accumulation in the respiratory tract. Stereotaxic lesions were produced by using a 5mg/ml solution of NMDA in phosphate buffer (pH = 7.4) injected through a 30-Ga cannula attached to a Harvard minipump. HPC lesion rats received twenty injections (ten per side) while AMG lesion rats received four injections (two each side); the coordinates of each injection and the volumes injected are presented in Table 4.1. In order to prevent seizure development, valium was administered i.p. (10 mg/kg) and animals were monitored until completely awake and active in their home cages. Rats in the sham group were anesthetized and the scalp cut on the midline after which the skin was sutured with no further interventions.

CPP – 3 training trials. All rats were 275-300g at the time of the surgery. The anesthesia procedure was similar to the one described above. The Fx was damaged by passing 2.0 mA of direct current for 20 msec through the tip of a stainless steel electrode insulated except for 1.0 mm at the tip. Each rat received four lesions, 2 for each side; the coordinates are shown in Table 4.1. The electrode was left in place for 60 sec after the lesion in order to minimize damage during withdrawal from the tissue.
Table 4.1. Lesion coordinates and injection volumes

<table>
<thead>
<tr>
<th>Lesion</th>
<th>AP</th>
<th>L</th>
<th>V</th>
<th>Volume</th>
<th>AP</th>
<th>L</th>
<th>V</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPC lesion</td>
<td>1. AP -3.1; L +/-1.0; V -3.6</td>
<td>0.25μl</td>
<td>6. AP -5.0; L +/-5.2; V -5.0</td>
<td>0.25μl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. AP -3.1; L +/-2.0; V -3.6</td>
<td>0.25μl</td>
<td>7. AP -5.0; L +/-5.2; V -7.3</td>
<td>0.25μl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. AP -4.1; L +/-2.0; V -4.0</td>
<td>0.25μl</td>
<td>8. AP -5.8; L +/-4.4; V -4.4</td>
<td>0.25μl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. AP -4.1; L +/-3.5; V -4.0</td>
<td>0.25μl</td>
<td>9. AP -5.8; L +/-5.1; V -6.2</td>
<td>0.40μl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. AP -5.0; L +/-3.0; V -4.1</td>
<td>0.25μl</td>
<td>10. AP -5.8; L +/-5.1; V -7.5</td>
<td>0.40μl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMG lesion</td>
<td>1. AP -2.3; L +/-4.8; V -9.4</td>
<td>0.60μl</td>
<td>2. AP -3.3; L +/-4.6; V -9.4</td>
<td>0.60μl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fx lesion</td>
<td>1. AP -1.5; L +/-0.8; V -4.6</td>
<td>2mA/20sec</td>
<td>2. AP -1.5; L +/-2.2; V -4.6</td>
<td>2mA/20sec</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AP = antero-posterior from bregma; L = lateral from bregma; V = ventral from bregma
All coordinates are given in mm.

At the end of the behavioral procedures, each animal was deeply anesthetized with sodium pentobarbital and perfused intracardially with 0.9 % saline solution, followed by 10 % formalin solution. The brains were frozen and sectioned (40 μ / section). The tissue was stained with acid thionin stain.
4.1.1.3 Behavioral testing.

All animals were tested in the CPP paradigm. Additionally, animals in the HPC and AMG lesion groups and the corresponding controls were also tested on the classical version of Morris water task.

4.1.1.3.1 Apparatus.

An 8 arm radial maze elevated 60 cm from ground and painted white was used for the CPP task. The maze had an octagonal central platform (18 cm side). Each arm was 11 cm wide and 55 cm long. There was no separation between central platform and arms, but six wooden blocks were used to obstruct as many arms during pre-exposure and testing sessions (see fig. 4.1). Two similar blocks had panels attached to the end facing the animal in order to successfully confine the rat in the arm during training trials (see Procedure). The maze was situated in the middle of a windowless, well-lit room which contained various visual cues: a chair, a filing cabinet, a garbage box, a pail, and various posters.

For the Morris water task, a white plastic pool 180 cm in diameter was employed. The pool was filled with water mixed with non-toxic paint covering (2-3 cm) a platform made of transparent plastic with a surface of 12 X 12 cm. The room contained various visual cues: posters, the computer and computer rack, the door, the animal cages rack, and the experimenter. (for a more detailed description, see chapter 2).
FIGURE 4.1. The CPP paradigm. Animals are pre-exposed to the apparatus, after which a specific location is rewarded in a series of training trials. During the test session, normal animals spend more time at the location previously paired with food.
PRE-EXPOSURE

TRAINING

DAY 1
food

DAY 2
no food

TEST
4.1.1.3.2 Procedure.

CPP paradigm. The CPP procedure (see fig. 4.1) was identical to the one described by McDonald and White (1993; 1995a). Animals were maintained at 85% of their ad libitum body weight. Each animal was assigned one of four pairs of opposite arms (ie, 1-5, 2-6, 3-7, or 4-8). Of the pair, one arm was designated ‘paired’ and represented the location where food was presented; the other, where food was never found, was designated ‘unpaired’.

During the first day (pre-exposure) the rat was placed on the central platform and allowed to freely explore the assigned pair of arms for 10 min. Access to the rest of the apparatus was blocked by the six wooden blocks. Next, 4 training sessions for AMG and HPC groups, and 3 training sessions for the Fx group took place. Each training session required two days. During one day the rat was confined for 30 min at the end of the paired arm, where 50 Fruit Loops were placed. During the other day the rat was confined for the same interval but with no food at the end of the unpaired arm. Thus, the animal was exposed to distinct sets of visual cues in the paired vs. unpaired arms. Paired/unpaired identity of the arms and order of reinforcement (day 1 vs. day 2) were counterbalanced within each group.

For testing, the arms were detached from the central platform, their position shifted one slot, and then reattached. Thus information provided by local cues became irrelevant. No food was presented on the maze during this day and each rat was allowed to freely move between the designated paired/unpaired locations as during pre-exposure, but for an interval of 20
min. Records were kept of the time of entry and exit for each arm. A rat was considered in or out of the arm if both of its front feet had crossed the arm threshold.

Water task. Only animals with full HPC and AMG lesions and their controls participated. The Morris water task was run across 6 days. All recordings were performed using a tracking system (VP118, HVS Image). Dedicated software computed for each trial latency to find the platform, path length, time spent in the platform quadrant, and heading angle. During the first 5 days, each animal was placed in water facing the pool wall and allowed to swim until either finding the platform or until 60 sec elapsed, whichever came first. At the end of the trial the rat was allowed to stand on the platform for 10 sec. Each rat performed 8 running trials per day. The sequence of starting positions was the same for all animals and varied from day to day. At the end of the training session, the results of the 8 trials were averaged for each rat and considered as one data point. During the last day, the platform was removed and the rats swam freely for 30 sec.
4.1.2 Experiment 2: dorsal/ventral HPC and CPP

4.1.2.1 Subjects.

CPP - 3 pairings. A total of 60 male Long Evans rats (Charles River Colonies) were used for this experiment. Housing, feeding, and testing conditions were identical to the ones described in experiment 1. The animals were randomly assigned to one of three groups: dorsal HPC lesion, ventral HPC lesion, and sham. After histological evaluation, data from 11 rats with dorsal lesion, 14 rats with ventral lesion, and 16 shams were included in the final analysis.

CPP - 4 pairings. A total of 24 male Long Evans rats (Charles River Colonies) were used for this experiment. Housing, feeding and testing conditions were as described above. Animals were randomly assigned to either dorsal HPC lesion group or sham group. After histological assessment of the lesions, 8 rats with dorsal HPC lesions and 10 sham animals were considered for final analysis.

4.1.2.2 Surgical procedures.

All animals weighed 275-350 g at the time of the surgery. Surgical procedures were identical to the ones for neurotoxic lesions described in experiment 1. Dorsal HPC lesions were produced by performing injections 1-5 as described in Table 4.1. Ventral HPC lesions were produced by performing injections 6-10. We thus attempted to create lesions that would be in the middle range according to Moser et al. (1995) classification.
4.1.2.3 Apparatus.

The same 8 arm radial maze was used as described in experiment 1.

4.1.2.4 Procedure.

The CPP paradigm was the same as described for experiment 1. Animals with either dorsal or ventral HPC lesions, as well as shams, underwent 3 training trials. Animals with only dorsal HPC lesions and shams underwent 4 training trials.

4.1.2.5 Histology.

At the end of behavioral procedures, histological procedures were performed as described in chapter 2. An identical computerized lesion assessment was performed for the dorsal and ventral HPC groups.
4.2 RESULTS

4.2.1 Experiment 1: Effect of Fx, AMG and HPC lesions on CPP.

Figure 4.2a shows a reconstruction of AMG and HPC lesions. There was no damage to the Fx in either lesion group. Cortical damage, although present at the cannula insertion points, was minimal. HPC lesions encompassed both dorsal and ventral areas. Damage to S was minimal and restricted only to its most anterior part, immediately adjacent to CA1. Partial sparing of CA3's most anterior tip well as minimal parts of dorsal HPC occurred in two cases. None of the AMG lesions extended to the ventral HPC tip.

Figure 4.2b shows a reconstruction of Fx lesions. In 3 cases damage extended to the posterior parts of the septum; one such case has been selected as the largest lesion. In 2 other rats damage extended to the most anterior tip of dorsal HPC, but it disappeared at the level of 2.12 mm posterior to bregma (coordinates according to Paxinos and Watson, 1986). In all cases, fibers constituting the Fx were thoroughly disrupted.
FIGURE 4.2. Reconstruction of histological damage in animals undergoing experiment 1. Largest lesions are shown in gray, smallest lesions are shown in black. a. Diagrams showing the extent of HPC and AMG lesions. Fx was not affected in either case. b. Diagrams indicating the extent of Fx lesions.
Figure 4.3a shows the results of AMG, HPC and sham groups following 4 training trials. A 2-way lesion x location ANOVA showed no main effects of either lesion or arm, but a significant lesion x arm interaction ($F_{2, 29} = 3.42, p < 0.05$). Planned comparisons between time spent in the paired vs. unpaired location for each group indicated that neither AMG nor HPC groups showed a significant preference for either arm. In contrast, the control group preferred the paired arm ($F_{1, 11} = 8.78, p < 0.013$). Figure 4.3b shows the results of Fx and sham groups following 3 training sessions. To attain homogeneity of variance, a logarithmic transformation was applied to this data set, after which a similar statistical analysis as described above was performed. The 2 way ANOVA indicated a main effect of arm ($F_{1, 16} = 5.32, p < 0.05$) but no significant main effect of lesion or lesion x arm interaction. This lack of effect was not surprising because both sham and Fx lesion groups spent on average more time in the paired arm. However, comparisons within groups demonstrated that only animals with Fx lesions significantly preferred the paired location ($F_{1, 6} = 31.17, p < 0.01$).
Figure 4.3. Mean amounts of time spent in the paired vs. unpaired location. a. Results of AMG, HPC, and corresponding sham groups following 4 training sessions. The sham group only showed significant preference for the paired arm. b. Results of Fx and corresponding sham groups following 3 training sessions. The Fx but not the sham groups showed significant preference for the paired location.
CPP - 4 PAIRINGS

AMG
HPC
sham

LESION GROUPS

TIME (sec)

paired
unpaired

*
CPP - 3 PAIRINGS

LESION GROUPS

TIME (sec)

Fx sham

paired unpaired

*
Figure 4.4 shows latency, path length, platform quadrant preference, and deviation of heading angle for AMG, HPC, and the corresponding sham groups during the first 5 days of testing. Two-way ANOVA analyses for latency, path, and quadrant preference indicated main effects of day in all cases ($F_{4, 116} = 116.65, p < 0.001$; $F_{4, 116} = 112.19, p < 0.001$; $F_{4, 116} = 30.83, p < 0.001$) and main effects of lesion group ($F_{2, 29} = 10.81, p < 0.001$; $F_{2, 29} = 12.90, p < 0.001$; $F_{2, 29} = 5.85, p < 0.001$). Analysis on the heading angle data restricted to the last two days indicated a close to significance effect of lesion ($F_{2, 29} = 3.31, p = 0.0507$). Multiple comparisons using the Student–Newman–Keuls test showed that in all cases the HPC group was different from the other two, but there were no differences between AMG and sham groups. During the sixth day, heading angle was measured for each group (data not shown). One-way ANOVA showed a main effect of lesion ($F_{2, 29} = 3.99, p < 0.05$) due to poorer performance of HPC group relative to the other two groups.
FIGURE 4.4. Results of the Morris water task. Animals with HPC, but not AMG lesions showed impaired learning.
4.2.2 Experiment 2: Dorsal/ventral HPC and CPP.

Figure 4.5a shows a reconstruction of HPC damage in dorsal and respectively ventral lesion groups that underwent 3 training trials. Lesion size assessment indicated an average of 37.94 +/- 3.46 % in dorsal lesion group, and 42.89 +/- 5.63 % in ventral lesion group (% with respect to total HPC volume). In the dorsal lesion group, 10 animals presented no cortical damage and one of them showed unilateral damage at the site of cannula entrance. 5 animals had damage of the inferior blade of the DG that extended to the splenial region, as showed in Fig. 4.5a. One rat also had unilateral partial damage of CA3 field in the splenial HPC. In the ventral HPC lesion group, sparing of the most anterior tip of the structure was found, similar to the one reported by Moser et al. (1995). S was intact in all cases. Partial sparing of most posterior CA1 was found in 8 animals unilaterally and in 3 animals bilaterally. Partial sparing of the most posterior DG was found in 4 animals unilaterally and in 2 animals bilaterally. 3 rats had unilateral cortical damage at the cannula entrance point. 2 rats had bilateral cortical damage, the largest of which is showed in fig. 4.5a as the largest ventral lesion.

Figure 4.5b shows reconstruction of damage to dorsal HPC in the lesion group that underwent 4 training trials. Lesion size assessment indicated an average of 36.25 +/- 3.84 % of total HPC volume for this group. CA3 sparing was limited to posterior sections. The rat with the smallest damage had a 29.01 % lesion volume due mostly to unilateral sparing of fields CA3 and CA1, as shown in the histological plate. Unilateral cortical damage was present in 2 rats at the cannula entrance point. Bilateral damage was found in 2 animals; of
these, the one with the largest damaged cortical area was also the one with the largest dorsal HPC lesion.
FIGURE 4.5. Reconstruction of histological damage in dorsal and ventral HPC lesion groups. Largest lesions are shown in gray and smallest lesions are shown in black. a. Dorsal and ventral HPC lesions. Animals in these groups underwent 3 training sessions. b. Dorsal HPC lesions. Animals in this group underwent 4 training sessions.
Figure 4.6a shows CPP learning after 3 training trials. The dependent variable was time spent in a particular arm. The two independent variables were lesion type and location (paired vs. unpaired). To achieve homogeneity of variance among groups a logarithmic transformation was performed on the data. A 2-way lesion x location ANOVA showed no main effect of either lesion or location, but a significant lesion x location interaction ($F_{2, 38} = 4.76, p < 0.05$). Planned comparisons between time spent in the paired vs. unpaired arm showed that ventral HPC lesion group preferred the paired arm ($F_{1, 13} = 6.66, p < 0.05$). In contrast, neither dorsal HPC lesion nor sham groups showed significant preference for either location. Taken together, these data indicated enhanced learning in CPP following ventral, but not dorsal HPC lesions.

Figure 4.6b shows CPP learning following 4 training trials. A similar 2-way lesion x location ANOVA revealed a close to significance lesion effect ($F_{1, 16} = 4.38, p = 0.0525$) and no significant location effect or lesion x location interaction. To increase power, the error term used for planned comparisons was pooled across the whole data set. Normally, such a pooled error term is not recommended for repeated measures variable because of concerns regarding the sphericity assumption (Howell, p. 468). However, because in this case there are only two levels of the location variable the sphericity assumption does not apply (SAS/STAT User’s Guide, p. 954). The sham group spent significantly more time in the paired arm ($F_{1, 32} = 5.34, p < 0.05$), while dorsal HPC lesion group did not show location preference. These results indicated that dorsal HPC lesions are associated with impairment in acquisition of this version of CPP.
FIGURE 4.6. Mean amounts of time spent in paired vs. unpaired location in experiment 2. a. Results of dorsal /ventral HPC lesion groups and corresponding shams after 3 pairings. The ventral HPC group but not the other two showed significant preference for the location previously associated with food. b. Results of dorsal HPC and corresponding sham groups. Only shams showed a significant preference for the paired arm.
CPP - 3 PAIRINGS

![Bar graph showing time in seconds for different lesion groups: dorsal HPC, ventral HPC, and sham. The graph compares paired and unpaired conditions.](image)

**Lesion Groups**
- Dorsal HPC
- Ventral HPC
- Sham
CPP - 4 PAIRINGS

![bar chart showing time (sec) comparison between paired and unpaired groups for dorsal HPC and sham lesion groups.](chart.png)
4.3 DISCUSSION

4.3.1 Experiment 1: Effect of Fx, AMG and HPC lesions on CPP.

The main conclusion of this experiment is that complete HPC lesions (DG and fields CA3-CA1) impair, while Fx lesions enhance performance in this version of CPP. The results replicate effects of AMG and Fx lesions reported by White and McDonald (1993) and McDonald and White (1995a) using identical behavioral procedures. Taken together, the data demonstrate a clear dissociation between Fx and HPC lesions, indicating that there are important differences in the functional consequences following damage to these brain structures.

Second, the results of this experiment showed that CPP acquisition requires not only AMG but also HPC function. Because both AMG and HPC lesions interfered with normal behavior it follows that in normal animals performance requires a synergistic interaction of the memory systems based on these anatomical units. It is generally accepted that AMG is involved in conditioning paradigms and is not required for spatial learning. In agreement with this idea, it is likely that in CPP AMG participates in formation of cue-reward associations. The spatial component of the task is most likely solved through HPC function.

Because dorsal HPC areas have been found to be more important for spatial navigation, a question that followed directly from these results was whether normal performance in CPP requires integrity of the whole HPC network, or only of parts of it. This issue was investigated in experiment 2.
4.3.2 Experiment 2: Dorsal/ventral HPC and CPP.

Results obtained in this experiment demonstrated that given an intact AMG, dorsal HPC is necessary and sufficient for CPP learning. Ventral HPC function seemed to have a suppressive effect on this process because its disruption resulted in enhanced conditioning. Normal performance in CPP requires acquisition of spatial information. This is markedly affected by dorsal HPC lesions and only mildly impaired by ventral HPC lesions. Considered separately, the results of dorsal HPC lesions in CPP can be explained as due to impairment in spatial learning. This interpretation is however contradicted by the results of experiment 1. Fx lesions, known to produce severe spatial learning deficits, resulted in enhanced CPP learning. Additionally, no good explanation can be offered for the effect of ventral HPC lesions. Thus a different interpretation has to be found.
4.3.3 Which structure is doing what in CPP? A hypothesis.

The results raise two major problems. The first is explaining how impairment of spatial navigation follows both dorsal HPC and Fx lesions and yet performance in CPP, presumably requiring spatial learning, is impaired in the former but enhanced in the latter case. The second issue regards the enhancement effect found with Fx and ventral HPC lesions. Presently formulating conclusive statements on either of these topics is not possible due to lack of sufficient information. Some anatomical and physiological results do suggest a hypothesis, but this should be taken as tentative and will have to be cross-validated by future research.

Figure 4.7 shows a diagram based on anatomical data (Amaral and Witter, 1995; Cassel et al., 1997; Van Groen and Wyss, 1990; Pennartz et al., 1994; Gloor, 1997; McDonald, 1991; McGeorge and Faull, 1989). Projections from medial septum, nucleus of diagonal band, and brain stem reach dorsal HPC through the Fx. The Fx also contains fibers from dorsal HPC to lateral septum and lateral ACC, and from ventral HPC to lateral septum (not shown) and medial ACC. Input from nucleus of diagonal band and brain stem to ventral HPC is conveyed through ventral angular bundle. Bi-directional connections of AMG with ventral HPC and medial strip of EC are also located outside of the Fx. Basolateral AMG projections are restricted to medial ACC (McDonald, 1991). Of importance are also the topographically organized cortical-HPC projections. Dorsal HPC is connected to lateral longitudinal EC strip and ventral HPC to medial longitudinal EC strip.
FIGURE 4.7. Diagram indicating anatomical connections between septum, hippocampal formation, AMG, and ACC. For clarity purposes, projections from ventral HPC to lateral septum are not shown. The figure also demonstrates connections compromised following dorsal HPC lesions (top), ventral HPC lesions (middle) and Fx transections. Abbreviations as in figure 1.4.
The diagram in figure 4.7 also shows the particular anatomical projections affected by dorsal HPC lesions (top), ventral HPC lesions (middle), and Fx sections (bottom). Dorsal HPC damage compromises communication with lateral EC strip, input from medial septum, nucleus of diagonal band and brain stem, and output to lateral ACC. Lesions of ventral HPC compromise functionality of circuits related to medial EC strip and AMG and abolish output to medial ACC. Fx transections destroy subcortical input and output to dorsal HPC while leaving input to ventral HPC and retrohippocampal connections intact. Notably, the output of ventral HPC to medial ACC is also affected in this case.

The results presently reported can be explained if different functions are attributed to these circuits. Comparison between effects of dorsal HPC and Fx lesions indicates that in both cases the subcortical input and output of dorsal HPC is compromised. Thus, the septum-dorsal HPC-lateral ACC circuit may be essential for spatial navigation as done in the water maze tasks. A lesion to this area produces spatial learning deficits regardless of whether or not other circuits process spatial information.

Second, comparison between effects of dorsal and ventral HPC lesions indicates that input to medial ACC is affected only with the second type of damage. That AMG and ventral HPC outputs to ACC are restricted to medial ACC may be of particular significance. Mulder et al. (1998) have shown an interesting effect in medial ACC neurons that receive dual AMG and Fx input. Stimulation of Fx fibers prior to AMG activation results in inhibition of AMG effect on ACC neurons. In contrast, Fx-ACC transmission is facilitated if AMG is stimulated first. This finding suggests that AMG and ventral HPC compete for behavioral
output at the level of medial ACC. Although direct evidence for this hypothesis is missing, extrapolation from anatomical and physiological data suggests that the consequence of activating projections from ventral HPC to medial ACC may be to inhibit AMG control of behavior. Ventral HPC lesions would translate at the behavioral level as enhanced CPP learning. In this case, the spatial component of CPP can easily be solved within dorsal HPC lateral septum network. In contrast, dorsal HPC lesions render spatial learning slow and maintain inhibition on the AMG. Consequently CPP performance is impaired.

Comparison of Fx and ventral HPC lesion effects supports this explanation. In both cases AMG inhibition is abolished, creating thus the premise for enhanced CPP learning. In the case of Fx lesions, the spatial component of CPP may be solved through ventral HPC network activity, which has been shown to support this type of learning (De Hoz and Morris, 1999; chapter 2 of this thesis). It is likely that spatial learning in this version of CPP is not as demanding HPC function as spatial navigation in the water task. Previous data (McDonald and White, 1995b) demonstrated that an opposite-arm version of CPP, which lowers cue ambiguity, creates less demand on HPC processing. This may conceivably be within the functional abilities of ventral HPC network.

The view of medial ACC as site of AMG – ventral HPC interaction should not be interpreted simplistically. Mulder et al. (1998) found that medial ACC contained also cells that responded preferentially to either AMG or Fx stimulation, but not to both. Thus, single and dual input cells were interspersed in medial shell and medial core. In contrast, dorsal shell of ACC and ventromedial caudate contained only Fx-driven units. Ventrolateral core
and shell contained only AMG-driven units. It seems therefore that the functional organization of ACC is complex and may or may not overlap with conventional anatomical divisions. Additionally, there is some empirical evidence (Parkinson et al., 1999) that the shell/core division in ACC may have some behavioral relevance as well. Lesions of the core, but not the shell impaired Pavlovian conditioning. Lesions of the shell, but not the core abolished the stimulant effects of D-amphetamine.

Existence of closely intermeshed ACC circuits with distinct behavioral roles is compatible with available empirical data. Sutherland and Rodrigues (1989) found deficits in spatial navigation associated with lesions of medial ACC. At first sight this contradicts the view that it is lateral ACC that is essential for spatial navigation. There are however a number of factors that qualify this effect. First, the lesion reconstruction (see figure 1 D) is not very detailed and thus it is not clear how much of lateral ACC was actually affected. Second, direct comparison with anatomical data presented by Mulder et al. (1998, see figure 2) is difficult because of the differences in localization of sections. Third, these lesions were produced electrolytically rather than neurotoxically. Thus this evidence does not conclusively falsify the present hypothesis. Somewhat supportive are the results reported by Annett et al. (1989). These experimenters obtained impairment, but not abolishment of spatial learning following ACC lesions that spared medial and lateral extremes of the structure. The one study that found impaired CPP retention following complete ACC lesions (Everitt et al., 1991) is unfortunately irrelevant for the present hypothesis because it encompassed both medial and lateral ACC.
4.3.3.1 Fx vs. HPC lesion effects in the literature.

It is well known that Fx lesions disrupt spatial learning (see O'Keefe et al., 1975; Olton et al., 1978; Sutherland and Rodriguez, 1989), a behavior dependent on HPC-centered memory system (O'Keefe and Nadel, 1978). The possibility of differences in behavioral modifications following Fx vs. HPC damage has been briefly discussed by Olton et al. (1979), but extensive and systematic comparisons between the effects of the two procedures are missing from the literature. Rawlins et al. (1993) reported transient differences between rats with Fx lesions and rats with HPC lesions on a nonspatial matching-to-sample task, but it is worth noting that these investigators used aspiration as a lesion method. Additionally there was extended sparing of ventral HPC areas. It is true that aspiration destroys fibers of passage as well as cell bodies, and thus it is likely that ventral HPC was not fully functional in this case. Nonetheless, the effects of aspiration vs. neurotoxic lesions are not well documented and introduce additional difficulties in interpreting these data.

Whishaw and Jarrard (1995) found larger spatial learning impairment and disruption in circadian activity with Fx transections than with HPC damage induced by ibotenic acid. The authors attributed differences between groups to lesion of projection fibers possibly originating in the subicular complex, EC, or other subcortical structures and coursing through the Fx. It remains a puzzle why Fx lesions would cause more marked spatial learning deficits because in this case HPC cells are not destroyed, HPC-EC connections are not disrupted, and subcortical input to ventral HPC network is spared. Data from a different experiment (Sziklas et al., 1998) showed that acquisition of associations between visual
stimuli and their location was impaired in animals with dorsal HPC damage while animals with Fx lesions eventually learned the task, presumably by using the ventral HPC network. Not surprisingly, both lesion groups were impaired on a spatial working memory task. Cassel et al. (1998) compared the effects of Fx lesions and ibotenic acid HPC lesions on locomotor activity and spatial learning. They found that HPC lesions resulted in higher hyperactivity and greater disruption in spatial radial maze paradigms where the rats had to remember position of one arm across a number of days (reference memory task), or to retrieve food placed in each maze arm (working memory task). Again, differences may be explained by activity in the ventral HPC circuit in the Fx lesion group. Most evidence thus seems to tip the balance in favor of greater spatial learning deficits following whole HPC lesions, but more empirical data are needed before formulating definitive conclusions.

McDonald et al. (1997) used negative patterning, conditional contextual and biconditional discriminations as three different instances of non-spatial, configural-relational learning to test the effects of Fx and HPC lesions. Fx lesions were found to have no effect in all three cases. HPC lesions impaired acquisition of first task, had no effect on the second, and retarded learning on the third. According to the hypothesis formulated above, learning in the Fx lesioned animals would have to take place based on ventral HPC activity. This is entirely possible, especially as this is the HPC area best connected to AMG. Effects of whole HPC lesions seem to indicate that the only task requiring integrity of HPC network is negative patterning. Conditional contextual discrimination and biconditional discrimination may be accomplished based on a combination of EC and AMG activity, especially as connections
between these areas are left intact. Additionally, damaging the HPC implies that AMG control of behavior is facilitated.

To summarize, evidence provided by comparisons of Fx and HPC lesions effects seems to be compatible with the hypothesis we formulated. However, paucity of empirical data renders any conclusions only tentative.

4.3.4 CPP — what is new for the debate on hippocampal function?
The CPP task we used involves passive conditioning: animals are confined at the end of the arm and no free movement is allowed during training. This paradigm is identical to the one previously used by White and McDonald (1993), who showed that after 4 training trials control animals spend more time in the arm previously paired with food. AMG lesions, alone or in combination with Fx lesions, interfered with normal learning. Fx lesions resulted in preference for the paired arm after only 1 training trial. In a different experiment (McDonald and White; 1995a), control animals were presented with 2 training trials, but while one group underwent pre-exposure as described in this report, a second group was not pre-exposed to the apparatus at all, and a third was pre-exposed in a different room. Animals pre-exposed to the same room did not develop a significant preference at the end of training. However the other two groups did, suggesting thus that the inhibitory effect on AMG-based conditioning to location is due to information acquisition during pre-exposure. Fx lesions performed before pre-exposure resulted in enhanced learning. Fx lesions performed after pre-exposure or after 2 training trials were not associated with enhancement. Thus, the
authors hypothesized that a functional Fx is required for acquisition but not expression of the inhibitory effect on AMG function.

A third study (McDonald and White, 1995b) investigated the effects of varying ambiguity of spatial cues and of conditioning type (active vs. passive). Ambiguity of spatial cues was increased by training the rats in adjacent (45° angle) rather than separated (135° angle) arms. Active cue presentation was accomplished by allowing free choice between designated arms (one reinforced and one not) during training. With adjacent arms/passive presentation, control animals did not acquire CPP after 2, 4, or 8 training trials, unlike Fx lesioned animals that did so after 8 pairings. With adjacent arms/active cue presentation, control, AMG-lesioned and DS-lesioned, but not Fx-lesioned animals learned the task. With opposite arms/active cue presentation neither AMG nor Fx or DS lesions were followed by a learning impairment. Finally, with opposite arms/active cue presentation combined DS+Fx lesions resulted in impairment, AMG+DS lesions resulted in enhancement, and AMG+Fx lesions did not have any effect. The authors concluded that in the passive condition AMG function is necessary for normal learning regardless of ambiguity level. In active CPP high cue ambiguity requires HPC function, while either HPC or DS can direct behavior in the low cue ambiguity condition.

White and Ouellet (1997) found that unlike in the passive presentation condition, normal animals discriminated among highly ambiguous cues if passively switched from one arm to the other during training. This is presumably because in the second case they had access to alternative views of spatial cues during a short interval of time. Fx but not AMG lesions
impaired learning in this condition, demonstrating that cue discrimination requires HPC function. The same lesions did not interfere with learning in an opposite arms/passive switching condition.

The results of this complex series of experiments indicate that information necessary to normal performance in the radial maze version of CPP is acquired in parallel by 3 memory systems: HPC-based, AMG-based, and DS-based. The involvement of one vs. other memory system is contingent upon situation. Thus, passive presentation of widely separated, non-ambiguous cues requires AMG function. Active presentation of cues requires HPC function, which seems to be also sufficient for normal performance. DS activity is however involved in this condition as well, most likely because its role in associating cues and motor responses (McDonald and White, 1993; McDonald and White, 1994).

It thus seemed that normal performance in the low cue ambiguity/passive presentation condition is based exclusively on the AMG memory system. The present experiments indicate that this is not the case. Some HPC function, however limited, seems to be required for solving the spatial component of CPP. A second implication is that although different memory systems specialize for acquisition and processing of separate types of information, their synergistic activity may be necessary in solving some (but not all) learning tasks.

4.3.5 Interactions among memory systems.

The hypothesis formulated above implies that ACC is divided into two functional parts. The medial ACC is seen as site of competitive AMG-HPC interaction. HPC input to this area
diminishes AMG's ability to control behavior. In contrast, HPC output to lateral ACC could be related exclusively to spatial learning. This circuit can be conceived as permissive of cooperative AMG-HPC interaction. This type of relationship is necessary in solving tasks such as passive CPP. Active CPP performance seems to be dependent on both HPC and DS memory systems (McDonald and White, 1995b). In that experiment, AMG+DS lesions resulted in enhanced conditioning, suggesting that in certain conditions, HPC function can be suppressed by the other two memory systems.

Interestingly, Devan and White (1999) found that information processing in the DS occurs in two parallel circuits. These authors used the modified spatial learning paradigm in which training to visible (DS-dependent task) and invisible (HPC-dependent task) platform alternate. At the end of training, the visible platform is moved to a new position. This provides a choice between responding to location vs. responding to visible cue. Lesions of lateral DS did not prevent acquisition of either cue or spatial information, but caused increased preference for spatial response. Lesions of medial DS retarded both spatial and cue response learning and produced preference for cue response. Fx lesions resulted in spatial learning deficit, no impairment in cue learning, and preference for cue response. Combined HPC-medial DS lesions on contralateral sides resulted in slow spatial and cue learning and preference for cue response. The authors concluded that a) medial and lateral DS areas have different functional relevance; b) HPC may interact competitively with lateral DS and cooperatively with medial DS and c) the cooperative interaction may take place in a circuit including both HPC and medial DS. These conclusions were supportive of an earlier study (Devan et al., 1999) which found that lesion of medial DS impaired cue and place
acquisition in the water task, was followed by a preference for local rather than spatial cue response, and resulted in increased thigmotaxis. In contrast, lateral DS lesions were not associated with any of these effects. These results suggest that medial, but not lateral, DS is involved in integration of cognitive information and stimulus-response type of behavior.

Thus a medial-to-lateral topographical organization of striatum functionality may exist. Lateral DS seems to be important for formation of stimulus-motor response associations. In contrast, medial DS may act cooperatively with HPC by modulating spatial behavior. Lateral ACC may be the preferential output gate for spatial navigation. Finally, medial ACC may be the site of AMG-HPC competitive interaction and particularly relevant to conditioning-type paradigms. That medial DS – lateral ACC may constitute a functional unit is somewhat supported by the finding that both ACC and medial DS lesions impaired CPP retention (Everitt et al., 1991).
4.4 SUMMARY

The implication of these data for research on HPC function is that multiple factors have to be weighted when interpreting experimental results. First, the structures lesioned and the size of the lesions themselves should be carefully considered. It becomes increasingly clear that Fx and HPC lesions are not interchangeable. The same is true for partial vs. complete lesions of the HPC itself. Second, requirements posed by behavioral paradigms should be differentiated. Some tasks may involve spatial learning only, while others may additionally involve formation of associations between stimuli and motor or affective response. More demand on HPC function seems to be generated by tasks involving discriminations among multiple cues presented simultaneously (McDonald et al., 1997, chapter 3 of present thesis). Third and related is the interaction between memory systems. Depending on the particular setup, distinct memory systems may cooperate or compete for behavioral output. Thus, spatial learning in the water task may involve a different pattern of neural activity than spatial learning in the radial maze. Assessment of this factor may be particularly difficult because the interaction among memory systems may occur at various levels, cortical and/or subcortical. Future systematic comparisons between different types of tasks and different types of lesions should bring more light on these issues.
5. GENERAL DISCUSSION

The broader implication of this work for the function of the hippocampus is that it provides yet another argument for the position that hippocampal formation is involved in acquisition processes beyond the ones regarding spatial information. However, data suggest that spatial learning is not a particular kind of configural-relational learning, i.e., these two types of acquisition processes are not in the hierarchical relationship of set-subset. Instead, it seems to be the case that they are more like conjunctive sets: they share some, but not all of the neuroanatomical substrate and implicitly, processes. More detailed comments follow below.

5.1 Spatial navigation and context conditioning.

Based on their study of anatomical connections, Amaral and Witter (1995) proposed that the divergence of information input to HPC (exteroceptive to dorsal pole, interoceptive/emotional to the ventral pole) may have behavioral relevance. Spatial information, by definition, is related to exteroceptive input. Thus the conclusion that dorsal HPC is more efficient in supporting spatial learning agrees with Amaral and Witter’s hypothesis. That ventral HPC can support some limited acquisition of spatial information is also within this view because dissociation in anatomical connections is relative, rather than absolute. However, the anatomy also predicts a predominant involvement of ventral HPC in tasks that require processing of interoceptive information. Presently, there is no evidence to support this view. Hock and Bunsey (1998) found that both dorsal and ventral HPC lesion groups were impaired in associating an internal state (hunger/satiety) with shock. In fact, the p values indicate that the difference between the dorsal lesion group and controls was more
reliable. Data from earlier reports (e.g., Sinnamon et al., 1978; Lanier and Isaacson, 1975; Nadel, 1968) are difficult to interpret because, partly due to technological reasons, the lesions are not sufficiently inclusive or restrictive. In other cases (e.g., Stevens and Cowey, 1973) the paradigms employed are difficult to integrate within more modern views of HPC function. Thus, the functional specialization of ventral hippocampus, if any, remains presently unknown.

Different patterns of results emerged from spatial navigation and context conditioning tasks. As opposed to dorsal HPC lesions, ventral HPC damage produced small impairment in spatial navigation. In contrast, both types of lesion were associated with impairment in the context discrimination test. There are two possible explanations for this. First, formation of a representation in discriminative context conditioning may require a more extended neural network than in the case of spatial navigation. That both lesion groups could learn discriminative freezing at the same rate as control animals argues against this explanation because it implies that both lesioned groups formed some functional representation of the apparatus which they were able to express in certain conditions.

An alternative is that although the process of forming representations may not occur differently in the two lesion groups, the utilization of these representations in the larger context of behavior might differ. It is possible that performance in (at least some) configural/relational, non-spatial tasks require extended integration along the septo-temporal hippocampal axis, while spatial navigation tasks could be supported by restricted HPC segments. Anatomical (Amaral et al., 1991) and physiological (Buzsáki et al., 1991; Jung et
al., 1994; Poucet et al., 1994) data suggest that activity along the longitudinal hippocampal axis may be of a particular nature, necessary for some behaviors, but not others. If true, this view implies that a spatial navigation task is not simply a subset of the general category of configural tasks. This is in the sense that spatial navigation would not be based on configural-relational processing applied to spatial input. Instead, it would reflect spatial-specific processing applied to spatial information. The converse would be true of configural-relational learning: input regarding a constellation of cues would undergo configural-specific type of processing. Because performance in these two types of paradigms is supported by partially shared network, spatial-specific and configural-specific memory processes would probably not be completely independent. On the other hand, they would not be identical either.

The idea that spatial and configural learning are not the same is, of course, not new. O'Keefe and Nadel (1978) and Nadel (1991, 1994) have long argued that there are two memory systems: the locale, which is hippocampally based, and the taxon, which is hippocampally independent. One difference between them is that the locale system has exploration as motivational drive, while the taxon system is based on ‘standard motivations’ such as hunger (Nadel, 1994). Indeed, configural-relational tasks currently employed are based on associations of a stimulus with an affective response (either positive, generated by reward, or negative, typically generated by shock administration) and thus likely to involve AMG memory system, or of a stimulus with a motor response and thus likely to involve the DS memory system. Second, dorsal and ventral HPC send outputs to distinct areas of the ACC (Pennartz et al, 1994 for review). It is thus possible that configural-relational tasks involve a
neural network that straddles on HPC as well as other memory-related structures, while learning in spatial navigational tasks requires only the hippocampal memory system, particularly its dorsal area. In agreement with the position advocated by Nadel, spatial and configural-relational learning may be indeed different. The two would probably share some processes because they are supported by common anatomical substrate to some extent. However each case would also involve specific operations, namely the ones taking place in dedicated networks. Spatial and configural-relational leaning would therefore be neither disjunctive, nor identical, or one subset of the other, but rather conjunctive sets: some common and some specific elements. It is thus conceivable that HPC could be the anatomical site of more than one type of memory process.

5.2 Spatial learning: water task and CPP.

As discussed above, both paradigms require acquisition of spatial information. In addition CPP also requires associating spatial cues with reward. Data presented in chapters 2 and 4 indicate that spatial learning in the water task utilizes exclusively HPC network, particularly the dorsal areas, while conditioning paradigms require synergistic interaction between AMG and HPC. This pattern of results has already been explained in section 4.3 and the discussion will not be repeated here. One conclusion should be emphasized, namely that spatial learning in water tasks may not be identical to spatial learning in CPP. This supports the idea formulated in section 5.1 that spatial and relational-configural learning are not in a relation of set-subset, but rather of conjunctive sets.
5.3 Two types of conditioning? Context and place preference.

The two conditioning tasks employed in these experiments are based on the same general procedure: habituation is followed by training and then by measuring the animals’ ability to identify specific parts of the apparatus. However there were also important differences. Context conditioning employed an aversive US, while CPP used a positive one. Second, spatial elements were screened out in context conditioning while they were essential in CPP. Results indicated that dorsal HPC lesions impair performance in both CPP and context conditioning. In contrast, ventral HPC lesions produced enhancement in CPP and impairment in context conditioning.

It is difficult to specify which one of these two factors (nature of US and nature of information) was responsible for the differences in results. One possibility is that comparison of a multitude of non-spatial cues specifically requires activity along the septo-temporal HPC axis and involves predominantly EC-HPC communication. This hypothesis is supported by the finding that HPC damage abolished learning in negative patterning, slowed acquisition of biconditional discrimination, and had no effect on conditional contextual discrimination, while Fx lesions had no effect (McDonald et al., 1997). In contrast, behavior directed by a constellation of distal, spatial cues, and involving locomotion within the environment may depend mostly on dorsal HPC – lateral ACC network.
5.4 Longitudinal and transversal hippocampal axes – what is their relationship to general hippocampal function?

Previous work (Ferbinteanu et al., 1999) showed that selective MPP lesions impair spatial learning and do not affect context conditioning, while LPP lesions have no effect on spatial navigation but accelerate discriminative freezing in the context conditioning task. In this section, the implications of these results will be discussed in conjunction with work presented in this thesis.

5.4.1 Spatial learning in water tasks.

Experimental data indicate that disruption of activity on either the longitudinal or transversal axes of hippocampal formation results in deficient spatial navigation. Thus, it seems that this type of task requires normal activity in an extended hippocampal network. This idea is in agreement with results reported by Moser and Moser (1998b) showing that acquisition of spatial information normally involves widespread hippocampal areas. In contrast with their conclusion, this work indicates that spatial navigation is not the exclusive function of dorsal HPC. However, not all components of EC-hippocampal system are equally important. The reasons behind this functional heterogeneity are not clear. One factor is topographical organization of anatomical connections. The lateral EC strip receives mostly polymodal sensory information (Amaral and Witter, 1995). MEA receives strong visual input from
POR (Burwell and Amaral, 1998b). It is known that MEA neurons show place cells characteristics (Quirk et al., 1992) although it is not known how this compared to LEA neurons. A second factor could be particularities of physiological activity. Buzsáki (1989) proposed a two-stage information processing process in the HPC. According to this model, rapid firing of DG cells results in heterosynaptic potentiation of CA3 pyramidal neurons creating thus a memory trace. This would be reinforced during the second stage, when DG granule cells are silent and CA3 engage in repeated and synchronous activity. As argued elsewhere (Ferbinteanu et al., 1999), MPP lesions may be more disruptive for this process than LPP because the former pathway is more efficient in triggering an action potential in the granule cells.

Whichever one of these views is true, performance in a spatial learning task seems to require extensive integration along the whole hippocampal formation and is sensitive at disruption on either longitudinal or transversal axes. In contrast, context conditioning seems to impose different computational demands.

5.4.2. Discriminative context conditioning.

Discrimination between compartments of an apparatus seems to require activity along the longitudinal axis. Transversal axis activity appears to be less important, as neither MPP nor LPP lesions impair normal chamber preference in this task (Ferbinteanu et al., 1999). Thus comparison among cues seems to be dependent mostly on activity along the longitudinal HPC axis.
Freezing, the second behavioral parameter measured in the conditioning task, was affected differently. LPP lesions accelerated conditioning, MPP lesions seemed to have an overall suppressive effect on this behavior, while neither dorsal or ventral HPC lesions seemed to introduce modifications. Part of the explanation for these results may be that different AMG nuclei project to MEA vs. LEA (Pikkarainen et al., 1999). Little is known about the specific role of AMG nuclei in behavioral modulation, but one study (Kill cross et al., 1997) demonstrated that central AMG nucleus, which projects mainly to MEA, is related more to suppression of behavior elicited by a CS. BL AMG, which projects mainly to LEA, controls mainly active CS avoidance. It is thus possible that MEA and LEA interaction with AMG may play different roles in guiding behavior, but it is difficult to integrate these results with the ones of present experiments because data suggest that context conditioning with and without CS may be paradigms posing different demands on hippocampal networks (Phillips and LeDoux, 1994).

The important conclusion is that discriminative context conditioning data demonstrate a double dissociation between behavioral relevance of information processing along the longitudinal vs. transversal hippocampal axes. The former is important in preference types of tests, which may be more HPC-dependent, while the other is related to freezing, a parameter possibly more AMG-dependent.

5.4.2 CPP.

In view of evidence discussed above, it is interesting to determine the MEA/LEA roles in CPP. Presently this is under investigation. MPP lesions interfere with acquisition of spatial
information. It is possible that MPP lesions impair performance in this task as well. However, Fx lesions also impair spatial learning, but produce enhancement in CPP. The effect of LPP lesions is also difficult to predict. Discriminative freezing was accelerated in the context conditioning task, while no effect was obtained on chamber preference. It is possible that these lesions would accelerate place conditioning as well, but on the other hand freezing and preference seem to be different types of responses.

5.5 Is the EC-hippocampal formation divided into memory subsystems?

First, I will make the preliminary remark that although the data presently discussed are in agreement with evidence obtained from human subjects (e.g., Maguire et al., 2000), I will make no attempt to extrapolate these results to human literature. This is because the object of this study was the existence of hippocampal memory subsystems in the rodent, as a particular model of mammalian brain. Second, the differences between humans and rodents are quite large, and the amount of information we presently have does not allow easy crossing of barriers between bodies of empirical data without involving a large amount of speculation.

According to Schacter and Tulving (1994), the distinguishing properties of memory subsystems are:

kind of information processed (but using identical rules of operation)

and

neuroanatomical substrate.
Anatomical, physiological, and pharmacological data suggest that the memory system centered EC-hippocampal formation may not be homogenous, but composed of a series of subsystems organized along the longitudinal and transversal hippocampal axes. However, the neural circuits are not completely independent. Within HPC, points of convergence are represented by longitudinal associational fibers and by the dendrites of granule cells in DG and pyramidal cells in CA3 that receive input from both MEA and LEA. Projections to and from EC are not absolutely, but relatively segregated (Amaral and Witter, 1995). Therefore, if the criterion of neuroanatomical substrate mentioned above cannot be satisfied in the strong sense.

Similarly, behavioral data do not seem to support the strong version of the first criterion. Spatial information processing is dependent mostly on dorsal HPC, but ventral HPC is also involved. Conditioning data indicate that comparisons among several cues with different affective valence involve activity along the longitudinal hippocampal axis if the behavioral parameter measured is chamber preference, but activity along the transversal axis if the behavioral parameter is freezing. Additionally, conditioning tasks involve activity of an altogether different structure, the AMG, which is substrate of a different memory system. CPP and water tasks may both be based on processing of spatial information, but as the effects of ventral HPC lesions show, the kind of processing involved in each of these cases may not be the same.
The latter remark brings up an important point. If the same kind of information is processed differently, depending on interaction with other memory systems, then maybe the first criterion formulated by Schacter and Tulving should be characterized more specifically. Thus, instead of *kinds of information*, *behavioral relevance of information processing* may be a better formulation. This way, important dissociations could be made. Figure 5.1 is designed to illustrate this point. Both dorsal and ventral HPC networks can process spatial information. However, data presented in chapter 4 suggest that essential for spatial navigation are only connections between septum, lateral ACC, and dorsal HPC. Second, activity in the longitudinal associational network and LEA is important in situations requiring comparisons among cues. Because sensory information reaches HPC through EC it is likely that EC-HPC connections are also essential. Third, projections from ventral HPC to ACC may be important for AMG-HPC competition for behavioral output.
FIGURE 5.1. Three hypothetical memory subsystems within EC-hippocampal formation. Connections between septum, dorsal HPC, and lateral ACC, as well as MEA input may be essential for spatial navigation. LEA input and the longitudinal associational network may be required for comparisons among cues. Third, connections between ventral HPC and ACC may be part of a network within which HPC and AMG compete for behavioral output.
In conclusion, data discussed in this thesis do not support the hypothesis that EC-HPC formation system is organized in subsystems if the criteria formulated by Schacter and Tulving are taken in the strong sense. A modified version of this idea is that subsystems are distinguished based on

- neuroanatomical substrate – in relative, rather than absolute terms
- and
- behavioral relevance of information processing.

In this case, EC-hippocampal formation system may contain three distinct subsystems with different functional importance: a) spatial navigation; b) comparison among cues; and c) competition with AMG. Obviously, at this stage this hypothesis will have to be validated by future empirical data. Although it is clear that different hippocampal circuits are involved in different tasks, a conclusive demonstration of the distinctiveness of memory subsystems would involve demonstration of double dissociations between their behavioral relevance. In the absence of such dissociations, an alternative interpretation of the data is that the reason different types of lesions produce different types of impairment is because the tasks are all testing a general HPC function and they vary only in difficulty level. Thus, the present series of experiments does not allow formulation of a conclusive statement on the issue of hippocampal memory subsystems, but it provided information is useful in guiding future research directed towards this goal.
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7. APPENDIX