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**THE EFFECTS OF FIMBRIA-FORNIX TRANSECTION,
BILATERAL KNIFE CUT OF THE TEMPORAL-HIPPOCAMPAL
PATHWAY, AND ADRENALECTOMY-INDUCED HIPPOCAMPAL
DENTATE GRANULE CELL LOSS ON SPATIAL LEARNING IN
THE RAT.**

by

JOHN NORMAN ARMSTRONG, B.A., M.Sc.

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfilment of the requirements for the degree of
Doctor of Philosophy

Department of Psychology,
Carleton University,
Ottawa, Ontario,
Canada

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THE RAT.**

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Doctor of Philosophy

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ABSTRACT

The present study investigated the effects of fimbria-fornix transection, bilateral knife cut of the temporal-hippocampal pathway, and adrenalectomy-induced dentate granule cell degeneration on spatial learning in the rat. Adrenalectomy (ADX) of adult rats causes selective hippocampal dentate granule cell degeneration that is prevented by corticosterone. In the present study, 45 rats displayed behavior in the Morris water maze, 3-4 months after ADX, that was similar to 16 sham-operated control rats and 16 ADX rats maintained on corticosterone throughout the study. Fourteen of these 45 rats displayed a loss of granule cells 5 months after ADX that varied from minimal to extensive. ADX rats with moderate to extensive granule cell loss displayed latencies in the acquisition, retention and reversal of place learning that were remarkably similar to controls or ADX rats with minimal or no cell loss. Even the animal that displayed 95% loss of dentate granule cells performed like controls in the Morris water maze task. However, unlike controls or animals with bilateral knife cuts of the temporal-hippocampal pathway, or spatially impaired fimbria-fornix transected rats, ADX rats with moderate to extensive granule cell loss were severely but temporarily impaired immediately following alteration of either intramaze or extramaze cues even though they remembered an old place and learned a new place as quickly as controls. These results suggest that although a complete complement of dentate granule cells is not necessary for the acquisition of locations in a spatial learning task the granule cells might play an important role in the recognition of a familiar but altered spatial environment. These results also illustrate the serious limitations of the traditional 'trisynaptic' view of the functional organization of the hippocampus.

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I dedicate this thesis to my parents, Clyde and Binnie Armstrong.

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GENERAL INTRODUCTION

Understanding how the brain acquires and stores information requires both the identification of neural circuits essential for memory formation and a description of how these circuits function. While several circuits that appear critical for the acquisition and retention of information have now been identified in mammals, substantially less progress has been made in understanding how these circuits function. Over the past few decades a vast amount of evidence has accumulated that suggests that the hippocampus is critical for the acquisition and storage of information about spatial locations (O'Keefe and Nadel, 1978; Squire, 1987). In the paragraphs below I review some of this evidence and illustrate the rationale for determining the effects of fimbria-fornix transection, temporal-hippocampal pathway transection and adrenalectomy-induced dentate granule cell degeneration on the acquisition and storage of spatial information in the rat.

ANATOMY OF THE HIPPOCAMPUS

The hippocampus is a relatively simple cortical structure that occupies most of the ventroposterior and ventromedial walls of the cerebral cortex in the rat (Bayer, 1985). Observations made by Cajal (1911) and Lorente de No (1934) using the Golgi silver-impregnation technique, latter substantiated using the degeneration and silver

impregnation techniques of Blackstaad (1956) and Raisman (1966), revealed that the hippocampus is organized into a series of sequentially arranged processing modules known collectively as the "trisynaptic loop" . Anatomically, this loop is made up of the primarily unidirectional connections between four relatively simple cortical regions that include the entorhinal cortical projection to the granule cells of the fascia dentata (dentate gyrus), the mossy fiber output of the granule cells to the pyramidal cells of CA3, and the Schaffer collateral output of CA3 to the pyramidal cells of CA1 (Fig. 1.1).

In 1971, the results of a number of physiological studies were used to develop a hypothesis about the functional organization of the hippocampus (Anderson, Bliss and Skrede, 1971). The major feature of this hypothesis (known as the lamellar hypothesis) was that the hippocampus was presumed to act in a lamellar fashion, such that a single source of entorhinal activity would project its impulses through the trisynaptic pathway along a slice or lamella of hippocampal tissue oriented perpendicular to the long axis of the hippocampus (Anderson et al., 1971). As a result of the widespread acceptance of this hypothesis the hippocampus was presumed to consist of a number of cytoarchitectonically and connectionally stereotyped slices that were stacked up along its long axis (extending from its dorsorostral or septal pole to its ventrocaudal or temporal pole) such that the major flow of neural activity was within each slice rather than between adjacent slices and information entering at any particular level would not diverge to other septotemporal levels (Amaral and Witter, 1989).

Fig. 1.1 Schematic representation of a horizontal section through the hippocampal region of the rat brain. The various subdivisions and their interconnecting pathways are indicated. Abbreviations: ab, angular bundle; alv, alveus; CA1-3, subdivisions of Ammon's horn; DG, dentate gyrus; EC, entorhinal cortex; fi, fimbria; HDG, hilus of the dentate gyrus; PAR, parasubiculum; PC, perirhinal cortex; PRE, presubiculum; rs, rhinal sulcus; S, subiculum; sm, stratum moleculare; so, stratum oriens; sr, stratum radiatum; ve, lateral ventricle.

HIPPOCAMPUS AND MEMORY

Both clinical observations and animal experimentation indicate that the hippocampus plays a critical role in the establishment of long-term associative memories (Squire, 1987; Skaggs and McNaughton, 1992). In 1957, Scoville and Milner discovered that removal of the anterior two-thirds of the hippocampus and surrounding medial temporal lobe structures in a patient who had undergone successful treatment for intractable epilepsy also resulted in a severe and permanent inability of this patient to acquire new information, although the patient's general intellectual ability and short-term memory were preserved. Since this time, there has been, and remains to be considerable debate over the contribution of individual medial temporal lobe structures to this syndrome (for example: Mishkin, 1978; Zola-Morgan, Squire, Amaral and Suzuki, 1989; Zola-Morgan, Squire, Clower and Rempel, 1993).

However, in 1986, Zola-Morgan, Squire and Amaral reported the case of a patient (R.B.) who developed amnesia as the result of a bilateral ischemic episode that destroyed the entire CA1 field of the hippocampus. This was the first reported case of amnesia where the histological findings demonstrated damage limited to only a small portion of the hippocampus with no damage to other surrounding medial temporal lobe structures. Minor pathology, however, was found in other structures including: the left globus pallidus, right postcentral gyrus and left internal capsule. Extensive neuropsychological tests revealed that R.B. had a profound anterograde amnesia. He

performed poorly on tests of paired associative learning, story recall, and diagram recall, and displayed a deficit in verbal recall that was present up to at least 5 years after the ischemic episode (Zola-Morgan et al., 1986). Thus, this case provided the first compelling evidence that damage limited to the hippocampus would be sufficient to cause a severe and clinically significant memory impairment.

Clinical studies of amnesia have also demonstrated that the hippocampus is not the critical storage site for permanent memories because patients with damage to the hippocampus display deficits in new learning with very little or no retrograde amnesia (Zola-Morgan et al, 1986). This issue is often overlooked when studying the hippocampus. However, Zola-Morgan and Squire (1990) have demonstrated that hippocampal lesions made eight weeks or more after training on a delayed nonmatching to sample task failed to produce an impairment in retention when the animals were tested 4 months later. This evidence suggests that researchers should be looking for temporary rather than permanent changes in the functioning of the hippocampus.

Nonetheless, Nadel (1991) has argued that demonstrations of the time-limited role of hippocampus in memory illustrate little about the permanence of memory storage in the hippocampus; they address only the question of whether there is adequate information outside the hippocampus to support recall. Perhaps this is why no evidence exists as to where permanent memories are stored. Although it is commonly assumed that permanent memories are stored in the cerebral cortex in a widely distributed manner, Thompson (1992) has argued that this is only a reformulation of

the original question, and only serves to postpone the solution to the problem of where permanent memories are stored.

Furthermore, clinical studies of amnesia have demonstrated that damage limited to the hippocampus result in a comprehensive learning deficit where most new learning is impaired and only certain types of learning are spared (for detailed discussion of this point see Eichenbaum, Otto and Cohen, 1992). Thus, it is not known whether the hippocampus plays a specific role in the storage of a particular type of information, the type that subserves a critical learning strategy, such as information that can be consciously declared, or whether it is involved in a particular kind of memory process, irrespective of information content, such as consolidation or retrieval.

This debate concerning the nature of the deficit in a hippocampal lesioned animal still remains unresolved. For example, the cognitive mapping theory of O'Keefe and Nadel (1978), the declarative memory theory of Squire (1987) and the configural association theory of Rudy and Sutherland (1989) all speculate that the hippocampus is involved in the storage of a particular type of information; while the temporary memory buffer theory of Rawlins (1985), and the working memory theory of Olton, Becker and Handelman (1979) speculate that the hippocampus functions as a particular type of memory processor. However, Sutherland and McDonald (1990) have pointed out that while each one of these theories makes correct predictions within a particular domain of behavioural tasks, each one fails when applied beyond its original domain.

The inability of any one of these theories to account for a large share of the available data, however, arises from the lesion-based model of studying brain function (Nadel and O'Keefe, 1974). Using this model researchers have ascribed to the hippocampus those functions that are lacking in the animal without a hippocampus; however, the functions which are chosen often reflect the initial bias of the experimenter. While this results in the rapid accumulation of data on the behavioural effects of hippocampal lesions, each individual theory actually accounts for a smaller and smaller fraction of all the data. Perhaps a more effective method of understanding the role of the hippocampus in learning and memory would be to ascertain how the different components of its circuitry contribute to its overall functioning. In order to do this one must choose a particular behavioural task that has consistently demonstrated sensitivity to hippocampal lesions.

HIPPOCAMPUS FUNCTIONS AS A COGNITIVE MAP

One particularly prominent view about the selective role of the hippocampus in memory suggests that the hippocampus functions as a cognitive map (O'Keefe and Nadel, 1978). This theory, which was derived entirely from behavioural studies with rodents, was based on the discovery that the hippocampus contains cells (complex-spike units called place cells) that discharge selectively in relation to an animal's location (place field: O'Keefe and Dostrovsky, 1971; Ranck, 1973; McNaughton, Barnes and O'Keefe, 1983). Furthermore, this theory accounts for why hippocampal

lesioned animals are consistently and severely impaired when required to navigate to specific spatial locations (i.e., spatial learning, Morris, Garrud, Rawlins and O'Keefe, 1982; Sutherland, Whishaw and Kolb, 1983; Aggleton, Hunt and Rawlins, 1986, Morris, Schenk, Tweedie and Jarrard, 1990; Gallagher and Holland, 1992). For example, Morris et al. (1982; 1990) have demonstrated that rats with hippocampal lesions fail to learn the location of a hidden platform in a large circular pool of opaque water. Normally, rats learn to escape by swimming directly to and climbing on the small platform hidden beneath the surface of the water as long as the platform has remained in a fixed location over a series of trials (Morris, 1981). However, hippocampal lesioned animals take 5-10 seconds longer to escape to the platform because they must search for it rather than swim directly to it (Morris et al., 1982; 1990).

However, electrophysiologists who have investigated the unit activity of hippocampal cells using non-spatial paradigms, such as sensory discrimination learning, have reported that complex-spike cells fire primarily in relation to the critical discriminative cues of the task, or that the location of the animal was only one of many variables necessary to predict the temporal firing of these cells. For example, Wible, Findling, Shapiro, Jang, Crane and Olton (1986) found that some cells were specifically active in relation to conjunctions of goal box color and position in a delayed response task, or to conjunctions of odors and their presentation position in the case of rats performing an odor discrimination task.

Wiener, Paul and Eichenbaum (1989) have also shown that while hippocampal complex-spike cells have distinct single or multiple place fields, the firing rate of these cells also varies systematically with behavioural variables, including speed, direction, and turning angle of the rat as it moves through its place field. It was also demonstrated that during an odor task, most cells fired as the rat sampled discriminative cues or when the rat executed specific task-relevant behaviours. Wiener et al. argued that while the complex stimuli that compose spatial cues are reflected in hippocampal neuronal activity, this activity is not limited to the representation of spatial location. Furthermore, Eichenbaum et al. (1989) have argued that while hippocampal unit activity can be reliably related to both behavioural events and spatial location, spatial location provides a set of continuous variables that can be more easily mapped onto the anatomy of the hippocampus than other behavioural events. And thus, one could argue that the spatial properties of complex-spike cells are the consequence of some underlying behavioural or discriminative process and not the result of selective encoding of spatial information *per se*.

Furthermore, complex-spike cells in the hippocampus do not appear to have a lamellar organization (Barnes et al., 1983; Eichenbaum et al., 1989). Representation of perceptual features is highly organized in primary sensory cortical areas and is often characterized by a spatial topography where neighboring neurons have partially overlapping sensory receptive fields (Hubel and Wiesel, 1977). However, the results

from simultaneous recordings of multiple distinct neurons in freely-behaving rats suggests that there is only a very limited organization to complex-spike cells in the hippocampus (Eichenbaum et al., 1992). Analyses of the overlapping distributions of place fields of neighbouring hippocampal neurons suggest that although clusters of cells with overlapping place fields can be found, many cells with well defined place fields do not have neighbouring cells with overlapping place fields. These complex-spike cell clusters fail to show the organization that one would predict exists based on the lamellar hypothesis and on the organization that is known to exist in primary sensory areas.

It also is not known whether spatial information is linearly transmitted and refined along the known pathways. Recently, Barnes, McNaughton, Mizumori, Leonard and Lin (1990) compared the spatial and temporal discharge characteristics of area CA1 and CA3 to their target areas in the entorhinal cortex and the subiculum. They discovered that neither the entorhinal cortex nor subicular cells exhibited the patterns of location specific firing that they observed in CA1 and CA3. The lack of spatial selectivity found by Barnes et al. is inconsistent with the hypothesis that spatial information is progressively refined along the known pathways.

One of the most striking features reported by Barnes et al. (1983; 1990), however, was how rarely most of CA1 and CA3 cells were active. Skaggs and McNaughton (1992) have reported that in an alert moving rat a typical complex-spike cell fires less than 0.5 spikes per second on average. They argued that this 'sparsity' of firing makes

the hippocampus unique as it maximizes the capacity for the storage of associative information. The observation that firing increases (i.e., sparsity decreases) when an animal is quiescent or sleeping, and decreases when the animal is performing an exploration task supports the suggestion that sparse coding is important for the acquisition of spatial information.

HIPPOCAMPAL ELECTROGRAPHIC (EEG) ACTIVITY

Like most parts of the central nervous system, the hippocampus displays several rhythmic EEG oscillation patterns that can be classified according to specific characteristics (Mody and Soltesz, 1993). Power spectral analysis of this activity in the hippocampus has revealed at least three different patterns of activity (Vanderwolf, 1969): (1) rhythmic slow wave activity or theta rhythm (4-10 Hz), (2) fast wave activity (25-70 Hz), and (3) high- or low-amplitude irregular slow wave activity (0-25 Hz). Of these three patterns of activity, only the so-called theta rhythm is generated during the performance of an exploration-dependent task (O'Keefe and Nadel, 1978; Eichenbaum et al., 1992). Furthermore, intracellular recordings of pyramidal cells have revealed that the hippocampal theta rhythm is associated with a remarkably low rate of non-phase-locked pyramidal cell activity (Mody and Soltesz, 1993). These data suggest that theta may reflect the synchronized inhibition of pyramidal cells that is necessary for the generation of a sparse code (outlined above) and thus capable of maximizing the storage capacity of the hippocampus during an exploration task (Skaggs and

McNaughton, 1992; Mody and Soltesz, 1993).

ROLE OF THE FIMBRIA-FORNIX

Recent evidence suggests that the hippocampal theta rhythm is critically dependent upon the cholinergic and γ -aminobutyric acid (GABA) projections to the hippocampus that arise from the medial septum and vertical limb of the diagonal band of Broca (Bland, 1986; Colom, Nassif-Caudarella, Dickson, Smythe and Bland, 1991; Smythe, Colom and Bland, 1992). These studies have demonstrated that reversible blockade of the medial septum and diagonal band of Broca by microinjection of procaine hydrochloride (Smythe et al., 1992) or lesions to this area (Bland, 1986) result in the complete abolishment of the hippocampal theta rhythm.

The majority of fibers forming this septohippocampal pathway project to the hippocampus by way of the fimbria-fornix (Storm-Mathieson, 1978; Lopes da Silva, Witter, Boeijing and Lohman, 1990). Thus, the fimbria-fornix represents one of the primary input/output pathways of the hippocampus (Bayer, 1985). Estimates based on histochemical localization of acetylcholinesterase (AChE), the enzyme that degrades acetylcholine, and immunohistochemical localization of choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD), the enzymes responsible for the synthesis of acetylcholine and GABA respectively, have revealed that approximately 50% of the fimbria-fornix fibers are cholinergic (Storm-Mathieson, 1978; Amaral and

Kurz, 1985) and approximately 30% are GABAergic (Alonso and Kohler, 1984; Amaral and Kurz, 1985). Furthermore, transection of all the fibers in this pathway, which results in the bilateral depletion of AChE in the hippocampus and the subsequent abolishment of the theta rhythm, also results in a severe and permanent inability of these animals to perform normally in a spatial learning task (Sutherland and Rodriguez, 1989; Packard and McGaugh, 1992).

ROLE OF THE TEMPORAL-HIPPOCAMPAL PATHWAY

The hippocampus also distributes and receives highly integrated neocortical information from the entorhinal cortex (Hjorth-Simonsen and Jeune, 1972; Steward, 1976; Swanson and Cowan, 1977; Van Hoesen, 1982) and perirhinal cortex (Kosel, Van Hoesen and Rosene, 1983; Zola-Morgan et al., 1989; Zola-Morgan et al., 1991). In the rat, the fibers from the perirhinal cortex that run horizontally along the rhinal fissure before joining the entorhinal perforant path input to the hippocampus, has been referred to as the temporal-hippocampal pathway (Myhrer, 1991). In comparison to the fimbria-fornix, however, very little is known about the role of the temporal-hippocampal pathway in the normal functioning of the hippocampus including its role in the generation of the hippocampal theta rhythm, its role in place specific firing of cells in CA1 and CA3, and its role in the exploration-dependent encoding of spatial information during a spatial learning task.

ROLE OF THE GRANULE CELLS

Arguably, one of the most difficult problems for the hippocampus must be the initiation of the exploration-dependent encoding of spatial information. In fact, Skaggs and McNaughton (1992) have argued that much of the early processing of sensory events in an exploration task should be devoted to transforming highly correlated patterns of activity into much sparser and less correlated patterns of activity. Treves and Rolls (1992) have suggested using computational modelling that cells in the fascia dentata may serve this purpose. However, the role of granule cells in the firing of complex-spike cells in CA1 and CA3 has been extremely difficult to determine. Initially, Rose (1983) reported that cells in and near the granule cell layer have high average discharge rates that can be correlated with certain kinds of motor activity. However, Mizumori, McNaughton and Barnes (1989) later suggested that these cells were not granule cells but inhibitory interneurons called basket cells. Mizumori et al. reported that they were able to distinguish granule cells from basket cells using a number of physiological parameters and suggested that basket cells had higher spontaneous discharge rates, fired at a low stimulus threshold and were not suppressed during paired-pulse stimulation of the perforant path at intervals generating population spike inhibition. In contrast, granule cells had low spontaneous rates, fired at a higher stimulus threshold and were suppressed during the paired-pulse interval where the population spike was reduced. They argued that the granule cells reported by Rose

were actually basket cells and pointed out that that the spatial processing characteristics of granule cells still remain a puzzle.

There is reason to suppose, however, that granule cells might not contribute to place-specific firing and the encoding of spatial information in CA1 and CA3 pyramidal cells. McNaughton, Barnes, Meltzer and Sutherland (1989) have demonstrated that damage to the granule cells has no effect on the spatially selective firing of complex-spike cells in CA1 or CA3, while such damage had a severely detrimental effect on spatial learning. Bilateral microinjections of the neurotoxin colchicine at a number of levels along the septotemporal axis of the dentate gyrus resulted in the destruction of up to 75% of the granule cells. Animals that displayed as little as 50% loss in granule cells made significantly more errors during the acquisition and reversal of place learning on the circular platform test and took much longer to escape on the last four trials of acquisition in the Morris water maze than did controls. Yet despite this apparent spatial deficit, lesioning the granule cells had remarkably little effect on the spatial selectivity of complex-spike cells in CA1 and CA3. Single unit recordings from CA1 and CA3 subfields while rats performed a forced choice behavioural task on a 8-arm radial maze revealed that neither the spatial selectivity measures nor the mean discharge rates differed between control and colchicine injected animals (McNaughton et al., 1989). Although McNaughton et al. (1989) did observe a difference between groups in the temporal firing characteristics of these cells, these differences were attributed to the increase in the burst-discharge activity observed in the cells recorded in the colchicine-

injected animals rather than alterations in the principle input to complex-spike cells in CA1 and CA3.

ARE GRANULE CELLS NECESSARY FOR SPATIAL LEARNING?

These observations, however, raise an important question about the role of the granule cells in the functioning of the hippocampus. If the hippocampus functions to encode spatial information in CA1 and CA3, and this information is not conveyed directly to CA3 via the granule cells, why are the granule cells so critical for spatial learning?

Several groups have reported that spatial learning in the Morris water maze is severely impaired in rats that display dentate granule cell loss as a result of multiple local injections of colchicine (Sutherland et al., 1983; Whishaw, 1987; McNaughton et al., 1989). Impairment in these animals has been reported to be as severe as that observed in animals that have had their hippocampus removed (Sutherland et al., 1983). Thus, these findings demonstrate that an intact trisynaptic loop is necessary for normal hippocampal functioning (i.e., spatial learning). However, these studies cannot rule out the possibility that the observed impairment was the result of nonselective damage to surrounding pyramidal cells (Whishaw, 1987).

OUTLINE

The following 3 experiments were designed to examine the effects of fimbria-fornix transection, transection of the temporal-hippocampal pathway and adrenalectomy-induced dentate granule cell degeneration on spatial learning in the rat. The first two experiments demonstrate both the selective and sensitive nature of our Morris water maze procedure for detecting hippocampal-dependent spatial learning impairments in the rat.

Transection of the fimbria-fornix results in the disruption of one of the principal input/output pathways of the hippocampus, the abolishment of the cholinergic- and GABAergic-dependent hippocampal theta rhythm, and the pathological (i.e., epilepsy-prone) reorganization of the intrinsic circuits of the hippocampus including the loss of parvalbumin-immunoreactive GABAergic interneurons (Buzaki, Ponomareff, Bayardo, Ruiz and Gage, 1989; Chen, Freund, Buzaki, Baimbridge and Gage, 1989; Lahtinen, Miettinen, Ylinen, Halonen and Riekkinen, 1993). Therefore it is not surprising that fimbria-fornix (FFx) transection has become one of the most frequently used methods of producing hippocampal-dependent spatial learning impairments in the rat. In Experiment 1 of this thesis, spatial learning impairments associated with FFx transection were characterized in a detailed protocol using the Morris water maze. Unlike previous experiments, experiment 1 demonstrated that spatially-impaired FFx transected rats do not rely abnormally on intra- or extramaze cues to locate the hidden platform during

spatial learning in the Morris water maze.

The second major input/output pathway of the hippocampus is made up of the fibers that run horizontally along the rhinal fissure that arise from the surrounding parahippocampal regions that include the entorhinal and perirhinal cortices (Kose et al., 1983; Deacon, Eichenbaum, Rosenberg and Eckman, 1983). These areas have been referred to collectively as the temporal cortex (Myhrer, 1991). The hippocampus distributes and receives highly integrated neocortical information from the entorhinal cortex (Hjorth-Simonsen and Jeune, 1972; Steward, 1976; Swanson and Cowan, 1977; Van Hoesen, 1982) and perirhinal cortex (Kosel et al., 1983; Zola-Morgan et al., 1989; Zola-Morgan et al., 1991) through this temporal-hippocampal fiber pathway in the rat (Myhrer, 1991). Myhrer (1991) has previously demonstrated that lesions to the posterior temporal-hippocampal pathway result in profound deficit in the retention of a visual discrimination task. Experiment 2 examined whether this temporal-hippocampal pathway is also necessary for hippocampal-dependent spatial learning.

Experiment 3 utilized the discovery that adrenalectomy selectively destroys granule cells (Sloviter, Valiquette, Abrams, Ronk, Sollas, Paul and Neubort, 1989) to determine whether granule cells are necessary for spatial learning. The granule cells represent the first relay station in the conventional model of hippocampal functioning. According to this model, eliminating the granule cells should prevent spatial information from reaching the CA1 and CA3 and therefore, should result in a severe and permanent spatial learning impairment. However, unlike multisite injection of

colchicine, adrenalectomy results in the selective loss of dentate granule cells while leaving the pyramidal cells completely intact.

Experiment 1: Fimbria-fornix transection impairs spatial learning but has no effect on performance after cue alteration in the Morris water maze.

One reason why the hippocampus plays a critical role in spatial learning (Morris et al., 1982; Sutherland et al., 1983; Aggleton et al., 1986; Morris et al., 1990; Gallagher and Holland, 1992) might be that the hippocampus is critical for the storage of a certain type of information that is necessary to perform a spatial learning task (Ross, Orr, Holland and Berger, 1984; Sutherland and Rudy, 1989). However, an alternative theory has recently been proposed which suggests that the hippocampus is not critical for the storage of spatial information but is critical for its flexible use (Eichenbaum, Stewart and Morris, 1990). This theory was based on the observation that fimbria-fornix (FFx) transection did not interfere with the acquisition of spatial learning in the Morris water maze using specialized training and a *fixed* swim start location but did impair performance of FFx rats when extramaze cues were rotated (Eichenbaum et al., 1990).

However, in the Eichenbaum et al. experiment FFx rats were trained extensively in the Morris water maze to escape to a *visible* platform from a *fixed* start location. After many trials, the visibility of the platform was reduced until finally it was submerged. Subsequently, when extramaze cues were rotated and animals were released from a

novel start position, FFX rats displayed latencies that were three times as long as sham-lesioned rats. The authors argued that these data provide support to their view that the hippocampus is critical for the flexible use of spatial information.

However, I wondered if the acquisition of spatial learning by FFX transected rats was really normal or only apparently so with specialized *fixed* swim start training. To answer this question and to set the tone for the next two experiments, we trained FFX transected rats in our Morris water maze using a *novel* swim start procedure and then exposed these rats to manipulation of both intra- and extramaze cues.

MATERIALS AND METHOD

Animal treatment

Forty-eight Long-Evans hooded rats (220-249 g at surgery) were purchased from Charles River Laboratories (Quebec) and treated in accordance with the guidelines set by the Canadian Council on Animal Care. Accordingly, animals were tail-marked, placed into large (800 cm² x 18 cm deep) polypropylene cages (2 per cage) and housed in one room on a reverse light cycle (8:00 off, 20:00 on). Food and water were available *ad libitum*.

Surgery

Under pentobarbital anesthesia (50 mg/kg, i.p.), 34 rats underwent FFX transection. At a position immediately anterior to bregma, an elongated burr hole was drilled

through the skull to the dura mater extending 2 mm over the right hemisphere from the midline. Through this hole a modified #22 surgical blade was inserted 5mm from the medial aspect of the burr hole, at an angle of 45° from the surface of the skull. This insertion angle allowed the blade tip to pass beneath the midsagittal sinus into the left hemisphere above the fimbria-fornix. The blade tip was then drawn downward using a free-hand motion that went from left to right until it reached the lateral aspect of the burr hole. The blade was then withdrawn and the scalp incision was closed using wound clips. The rats were given 28 days to recover from surgery.

Apparatus and Procedures

The water maze consisted of a white circular pool (200 cm diameter x 61 cm height) which was filled with 25°C water, rendered opaque with powdered milk, and placed in the center of a room (4.3 x 3.5 m) with white walls and several movable posters. Immediately above the center of the maze was a light assembly to which a video camera was attached. Videotaping was used to record the behaviour of all the animals during specific portions of the testing.

Spatial probe: Spatial probe testing required each rat to swim in the pool for 60 s without the escape platform present. The number of times the rat swam in the quadrant over the exact site (annulus crossing) which would normally contain the platform was

compared to the number of times it swam over that same area in the other 3 quadrants. Each animal received 3 spatial probe trials: one on the first day in the water maze, and a second and third trial on the day before place learning day 3 and 11 respectively.

Cued learning: On the second day in the maze a black circular platform (10 cm diameter) was placed in the center of quadrant 4 and raised to the surface of the water so that it was easily visible to the swimming rat. Each rat received 4 consecutive trials on this task and the latency to reach the platform was recorded with a maximum swim-time of 120 s/trial. Each trial began from 1 of each of the 4 start locations (order determined randomly on each day).

Place learning: Place learning began the day following cued learning and required a rat to swim from the maze wall to a submerged transparent platform (10 cm diameter) hidden in quadrant 3. After mounting the platform, the rat was given 30 s to rest before starting the next trial. If the rat was unable to locate the platform within 120 s, it was lifted out of the water and placed on the platform for the rest interval. At the end of this time, the rat was placed again in the water at the next start location. Each animal received 4 trials of place learning per day for 11 days. The latency to reach the platform was recorded for each animal on each trial.

Rotation of intramaze cues: The day following the 11th day of place learning the pool

was rotated 90°. The hidden platform, however, was not rotated with the maze but was kept in the same position relative to the extramaze cues as in the previous place learning. Each animal received 4 trials from 4 different start locations and the latency to escape to the hidden platform was recorded.

Rotation of extramaze cues: On the day following intramaze cue rotation all moveable extramaze cues (2 posters and a animals rack) were rotated by 90°. The submerged platform, however, remained in quadrant 3, the same intramaze position as during original place learning. Each animal received 4 consecutive trials from 4 different start locations and the latency to escape to the hidden platform was recorded.

Random variation in location of the hidden platform: On the day following extramaze cue rotation, the search strategy of each animal was evaluated by randomly varying the location of the submerged platform between each of 4 trials. The latency to reach the platform was recorded.

Old place retention/reacquisition: After completion of the random platform experiment, the animals were given 7 days without testing. Immediately following this period, they were retested on 4 trials for their retention of the platform's original location (quadrant 3). The latency to reach the platform was recorded.

New place learning: New learning was evaluated after the retention experiment by moving the submerged platform to a new location (quadrant 2). Again, all rats were given 4 trials, each from a different start location, and their latencies to escape were recorded.

Histology

At the completion of behavioural testing, animals were transcardially perfused with 60 mL of 0.1M phosphate-buffered saline (pH=7.2-7.4) followed by 120 mL of 10% formalin in 0.09% saline. Coronal sections 50 µm-thick were cut on a Vibratome, mounted directly onto chrom alum- and gelatin-coated glass slides and air dried. Slides were dehydrated in graded alcohols and xylene, rehydrated, stained with 1.0% cresyl violet or processed for acetylcholinesterase (AChE) histochemistry (as described by Paxinos and Watson, 1986), dehydrated and coverslipped with Permount.

Statistical analyses

All statistical analyses involved univariate analysis of variance and, when necessary, multiple comparisons using Scheffé's procedure.

RESULTS

Histology

Of the 34 transected animals, 10 displayed bilateral loss of AChE staining (bilateral group), 8 displayed only unilateral AChE loss (unilateral group), and 16 displayed no obvious AChE loss at all (partial group). The remaining 14 animals served as unoperated controls (Fig. 2.1).

Behavioural testing

Cued learning: There were no group differences in the acquisition of cued learning.

All 4 groups performed at the same level and their performance improved across trials 1-4 [$F(3, 132)=11.4$, $p<0.0001$; Fig.2.2]. Thus, the perceptual, motivational and motoric demands of the task were apparently similar for all groups.

Place learning: During the 11 days of place learning, however, there was a significant *GROUP by DAY* interaction [$F(30,440)=2.85$, $p<0.001$]. This difference was initially the result of the longer latencies of the bilateral and unilateral group on the first place learning day compared to both the partial group and unoperated controls (Fig 2.3). However, only the bilateral group displayed consistently longer escape latencies [$F(3,44)=29.3$, $p<0.0001$] on all 10 remaining days of place learning (Scheffe's $p<0.01$). The unilateral and partial group performed like controls.

Spatial probe: On the first spatial probe trial, which occurred the day before cued learning, there was no quadrant bias shown by any group during their swim.

Fig. 2.1 Selective loss of hippocampal AChE-positive fibers 2 months after fimbria-fonix transection. (A) unoperated control (JNA117). (B) bilateral FFx rat (JNA121). Note the loss of AChE-positive fibers in the hippocampus of the bilateral FFx rat. AChE loss was not apparent in the hippocampus of animals that made up the partial FFx group.

A

Fig. 2.2 Mean ($\pm$ s.e.m.) latency to escape to the black platform during 4 trials of cued learning. No differences were apparent between any of the groups during the performance of this task.

Fig. 2.3 Mean ($\pm$ s.e.m.) latency to escape to the hidden platform during 11 days of place learning (4 trials per day). Note that the Bilateral group took significantly longer to escape to the hidden platform on all 11 days when compared to the control group. See text for further details.

However, on the second spatial probe trial, which occurred the day following the second day of place learning, all groups except the bilateral group showed a bias for the annulus in quadrant 3 (Fig. 2.4A). On the other hand, by the third spatial probe trial, which occurred after place learning day 10, a spatial bias was now apparent in the bilateral group. All animals crossed over the annulus in quadrant 3 significantly more times than in the annulus of any other quadrant [$F(3,176)=34.1$, $p<0.001$; Fig. 2.4B]. In addition, during this third probe trial, the unilateral group showed significantly more annulus crossings than all other groups.

Analysis of the amount of time spent in each of the 4 quadrants during the 3rd spatial probe trial revealed that, like controls, the rats in the bilateral group spent more time searching quadrant 3 than any other quadrant [$F(3,176)=24.1$, $p<0.001$]. These rats failed to differ from controls in the amount of time spent in any one of the quadrants (Fig. 2.4C). However, the unilateral group spent more time than controls in quadrant 3 and less time in quadrant 4.

Schenk and Morris (1985) have questioned whether these 2 measures of localized search strategy are related (annulus crossings versus amount of time). In the present experiment, we observed a nearly significant correlation between annulus crossings and time spent in quadrant 3 for the normal rats ($r = 0.48$) but not the partial, unilateral or bilateral rats (Table 2.1).

Fig. 2.4 Mean ($\pm$ s.e.m.) number of annulus crossings in the four quadrants during the (A) 2nd and (B) 3rd spatial probe trials. (C) Mean ($\pm$ s.e.m.) amount of time spent in quadrants 1-4 during the 3 spatial probe trial. Note the strong bias for quadrant 3 for all groups during the 3rd probe trial. Asterisk (*) indicates a significant difference from control.

A. Spatial Probe 2

B. Spatial Probe 3

C. Amount of Time

Table 2.1. Correlations between dependent measures on the 3rd spatial probe trial.

Group	Control (n=14)	Partial (n=16)	Unilateral (n=8)	Bilateral (n=10)
r_{c_1}	-0.57*	-0.17	0.57*	-0.24
r_{c_2}	-0.16	0.36	0.73*	0.40
r_{c_3}	0.48**	-0.23	0.01	0.16

where. r_{c_1} = correlation between # *annulus crossings* and *latency* to reach the annulus,
 r_{c_2} = correlation between *amount of time spent in the quadrant* and *latency*
 r_{c_3} = correlation between # *annulus crossings* and *amount of time spent in the quadrant*,
and * indicates significance ($p < 0.05$, two-tailed; ** nearly significant).

Rotation of intramaze cues: The bilateral group displayed significantly longer latencies across the 4 trials when compared to all other groups [$F(3,44)=12.6$, $p<0.0001$; Fig. 2.5A] when the intramaze cues were rotated by 90° . However, there was no difference between the performance of the bilateral group on place day 11 and any one of the 4 trials following intramaze cue rotation.

Rotation of extramaze cues: The latency to reach the hidden platform was also significantly longer for the bilateral group when compared to all other groups [$F(3,44)=8.31$, $p<0.001$; Fig. 2.5B] when the extramaze cues were rotated 90° . There was no difference in the performance of the bilateral group on this day and on place day 11.

Random variation in location of the hidden platform: There were no differences between groups in the latency to reach the platform when it was randomly placed in the annulus area of the 4 different quadrants (Fig. 2.5C). The mean latency on this day did not differ from the mean latency on intramaze and extramaze cue rotation days for the animals in the bilateral group. However, the mean latency on this day was significantly longer when compared to the mean on the previous 3 days for those animals of the other three groups (Fig. 2.6). This observation suggests that the search strategy used by the bilateral group in the random platform location day was similar to that used during the last day of place learning, as well as during trials on intramaze and

Fig. 2.5 Mean ($\pm$ s.e.m.) latency to escape to the hidden platform during 4 trials of (A) intramaze cue rotation, (B) extramaze cue rotation and (C) random placement of the platform to the middle of each quadrant between trials. The bilateral group took significantly longer (*) to escape to hidden platform on all trials in (A) and (B) but their performance was similar to controls during trials 1-4 in (C).

Fig. 2.6 Comparison of mean ($\pm$ s.e.m.) latency measures across different test days.

Compared to the previous place learning trials, intramaze and extramaze cue rotation trials, the latencies of the bilateral group during the 4 trials of random placement of the platform remained relatively unchanged ($p>0.05$), while those of the other three groups increased dramatically ($p<0.01^*$). Perhaps the search strategy used by the bilateral group in the random platform experiment was similar to that used during the last day of place learning, as well as during trials on intramaze and extramaze cue rotation days.

extramaze cue rotation days.

Old place retention/reacquisition: Seven days after the random platform test rats belonging to the bilateral group displayed longer escape latencies during reacquisition of the original place learning task when compared to all other groups [$F(3,44)=8.10$, $p<0.001$; Fig. 2.7A]. They performed similar to controls on the first and second trials of reacquisition but unlike controls failed to shorten their latencies across reacquisition trials 3 and 4. The bilateral group continued to perform at a level statistically similar to that which they displayed on place day 11.

New place learning: A nearly significant groups effect [$F(3,44)=2.56$, $p=0.06$] occurred after the platform was moved to a new location as the result of the consistently longer mean escape latency of the bilateral group compared to controls. Importantly, however, all groups displayed improved performance across the 4 trials [$F(3,132)=10.1$, $p<0.001$; Fig. 2.7B], suggesting that even the rats in the bilateral group were capable of learning some aspects of the new task. This result is quite different from the results of Eichenbaum et al. (1990) where under a novel starting procedure FFX rats were unable to improve their performance 18 trials after being presented with a new platform location.

Fig. 2.7 Mean ($n = 10$) latency to escape to the hidden platform during (A) trials 1-4 of retention of the original location of the hidden platform, and (B) new place learning. Compared to controls, the Bilateral group was significantly (* $p < 0.05$) slower escaping to the hidden platform on trials 2-4 in (A) and consistently slower to escape to the hidden platform on trials 1-4 in (B).

DISCUSSION

In this experiment, fimbria-fornix transected (FFx) rats with extensive bilateral loss of AChE in the hippocampus (bilateral group) were consistently slower to escape to the hidden platform during 11 days of place learning in the Morris water maze when compared to 3 control groups. The impairment observed in the bilateral group was not the result of obvious differences in activity, motoric capacity or motivation to escape because these rats failed to differ from controls during 4 trials of cued learning, when the escape platform was easily visible, and during 4 trials of random platform location, when the position of the hidden platform was randomly varied between trials. The spatial learning impairment which was observed in these rats despite preserved motoric capacity, was very similar to those impairments reported previously for FFx transected rats (Packard and McGaugh, 1992; Sutherland and Rodriguez, 1989) and hippocampal lesioned rats (Kesner, Bolland and Dakis, 1993; Gallagher and Holland, 1992; Morris et al., 1982).

Despite the longer escape latencies during the 11 days of place learning, the bilateral FFx group did display substantial improvement in their performance as they achieved asymptotic latency levels that were only 5-10 seconds longer than controls. Rats with unilateral hippocampal depletion of AChE (unilateral group) initially showed acquisition deficits nearly as great as the bilateral group, but after 3 days of training their performance improved considerably and became indistinguishable from the partial

AChE-depleted group and the unoperated control group. Clearly, bilateral transection of the fimbria-fornix was necessary to produce an enduring interference with the acquisition of spatial learning.

Analyses of the learning strategies during both place learning and the 3 spatial probe trials suggested that unlike controls the bilateral FFX rats located the platform by exploring near it, but without precise knowledge of its location. The difference between the bilateral group and controls was particularly evident during the second spatial probe trial where the bilateral group failed to show a spatial bias for the annulus in quadrant 3. However, they did display a spatial bias for the annulus in this quadrant by the third spatial probe trial suggesting that despite having taken longer to reach the annulus in quadrant 3, the animals of the bilateral group were capable of searching there as frequently as controls. It is possible that the bilateral group improved their performance on the third spatial probe by adopting a search strategy that was both quantitatively and qualitatively different from controls.

These results are supported by an additional measure of spatial bias during the third spatial probe; amount of time spent in each of the 4 quadrants (see Fig. 4c). Rats in the bilateral group spent more time in quadrant 3 than in any other quadrant but failed to differ from unoperated controls in the amount of time spent in any one of the quadrants. Schenk and Morris (1985) have questioned whether these 2 measures of localized search strategy are related (annulus crossings versus amount of time). In the present experiment, we observed a nearly significant correlation between annulus crossings and

time spent in quadrant 3 for the normal rats ($r = 0.48$) but not the partial, unilateral or bilateral rats (Table 2.1). These results suggest that the varied surgical disconnections represented by partial, unilateral and bilateral FFx may result in search strategies that share only some components with the normal unoperated control group.

Since latency to reach the platform was our primary measure of spatial learning, we also examined the latency to reach the annulus in the third spatial probe and its correlation with the number of annulus crossings and time spent in quadrant 3. As expected, the latency to reach the annulus was significantly longer in the bilateral group (12.20 ± 3.65 ; mean $\pm$ s.e.m.) than in all other groups (7.61 ± 1.79), which suggests that although the bilateral rats spent as much time as controls in quadrant 3 they took longer to reach the annulus there. However, we also observed that this latency was not significantly correlated with annulus crossings for any of the FFx groups. Therefore, we conclude that while all FFx groups adopted a search strategy that was different from unoperated controls, only the strategy adopted by the bilateral group resulted in consistently longer escape latencies during place learning.

Although it has proven difficult to precisely characterize the strategy used by “spatially impaired” animals (for discussion see Schenk and Morris, 1985; DiMattia and Kesner, 1988 or Sutherland and Rodrigues, 1989), impairments associated with hippocampal damage have been hypothesized to represent a deficit in the ability of a hippocampal lesioned animal to use spatial information (e. g. Morris et al., 1990).

However, very few of these experiments have documented the nature of these impairments. Therefore, we have found it critical to determine the characteristics of the impairment associated with bilateral FFX.

The bilateral group appeared to learn the maze in a manner that differed substantially from unoperated controls, therefore we examined the importance of several spatial cues in their place learning performance. After 11 days of place learning, the performance of the bilateral group and the 3 control groups during the intra- and extramaze cue rotation experiments was similar to their performance on the last day of place learning. As with the previous place learning, the bilateral rats took longer than controls to find the hidden platform on all trials of intra- and extramaze cue rotation. Thus, neither the bilateral group nor the 3 control groups were influenced by intramaze or extramaze cue rotation. This result was surprising because hippocampal lesioned animals have been reported to compensate for their inability to use an effective spatial strategy by using intramaze cues (M'Harzi and Jarrard, 1992). In fact, place neurons in FFX transected animals may respond preferentially to intramaze cues (Miller and Best, 1980).

The lack of influence of extramaze cue rotation upon the performance of our bilateral FFX rats was also unexpected because Eichenbaum et al. (1990) previously reported that FFX rats took longer than sham controls to find the hidden platform on one trial of extramaze cue rotation because they either followed the rotated cues or got lost. However, as indicated above, Eichenbaum et al. used a version of the task which required highly overtrained rats to swim repeatedly to the platform when released from

a *fixed* starting point. In that experiment, the rats were reported to have based their escape response on the invariant position of the extramaze cues proximal to the hidden platform. However, dependency upon these cues in the FFX rats was unclear because the rats were also switched at this time from a fixed start procedure to a *novel* start procedure. Close examination of these data suggest that it was more likely the novel start and not the extramaze cue rotation that produced the slow escape latencies in the FFX transected rats because manipulation of extramaze cues did not additionally alter the FFX rats' escape latencies or swim paths (notably the 'west novel start') beyond the switch to a novel start procedure alone. Thus, with an unaltered hidden platform location, the escape latencies of Eichenbaum et al.'s FFX rats during all of their *novel* start trials (with or without cue rotation or cue deletion) were remarkably similar to each other and to our novel start bilateral group. However, under our protocol the novel start procedure allowed bilateral FFX rats to easily acquire the procedural demands of the task and to adopt a reasonably successful search strategy that did not result in the abnormal sensitivity of these animals to alterations in extramaze cue information.

It has been assumed that spatial learning depends upon stable extramaze cues (Morris, 1982). However, we have reported that the latencies of control animals are unaffected by the rotation of extramaze cues both after (Armstrong, McIntyre, Sloviter and Neubort, 1991) and during (J. N. Armstrong and D. C. McIntyre, unpublished observations) the acquisition of spatial learning. There are a number of reasons to suppose why such a result might be obtained. First, it is difficult to rotate all of the

extramaze cues in the environment where spatial learning has occurred because not all of the extramaze cues are moveable and because we do not know what constitutes an extramaze cue for a rat. Second, Beigler and Morris (1993) have recently demonstrated that geometric stability is the only prerequisite for an extramaze cue to be used during spatial learning. Therefore, the defining boundaries for a given spatial environment (e.g. corners) might act as extramaze cues because they are inherently geometrically stable and therefore immovable. And finally, spatial learning in an acquired spatial environment may require only one salient extramaze cue. McNaughton, Barnes, Mizumori, Green and Sharp (1991) have argued that spatial learning requires the computation of a set of linearly independent vectors which combine current head direction and angular velocity with the known location of previous cues to allow for the computation of novel optimal trajectories between locations using only a single extramaze cue. The lack of effect on extramaze cue rotation trials in the present experiment observed in all groups demonstrates that the behaviour of spatially impaired FFX transected animals is not disrupted by alteration of intra- or extramaze cues.

Although our novel start protocol produced nearly identical results to most of the Eichenbaum et al. experiment, it might appear that the previous fixed start training did not affect Eichenbaum et al.'s FFX transected rats after being switched to novel starts. However, comparing our results from the new place learning experiment to those of Eichenbaum et al. (1990) indicates that this was not the case.

In the new learning experiment, we observed only slightly longer latencies in the bilateral group compared to the 3 control groups. On the first trial, all groups showed a latency increase that was approximately 2 times their normal value. However, normal latency values were reacquired by the fourth trial. By contrast, both the FFX transected and sham control rats in the Eichenbaum et al. experiment showed latencies that increased 10 times their normal value on the first trial of new learning. In addition, while the sham controls took 13-18 trials to finally reacquire their normal latency values, the FFX transected rats showed no improvement at all. Indeed, the latency data suggested that the FFX transected rats never reacquired even the procedural aspects of the new place learning task. These results indicate that overtraining with the fixed start procedure produces behavioural inflexibility in both the FFX transected *and* the sham control rats. Unlike the more flexible controls, however, this behavioural rigidity prevented any subsequent improvement in performance in the FFX transected rats. Such profound inflexibility was not apparent in our procedure using novel start training. It seems that the flexible use of environmental cue information in FFX transected rats may be more related to the original form of exposure to that information than a property of the lesion *per se*. More specifically, a fixed start procedure may simply train inflexible use of information in contrast to a novel start procedure.

It is not clear why FFX transection produces such a profound and reliable impairment in spatial learning. Certainly FFX transection disconnects the pathway through which substantial information must flow into and out of the hippocampus, but FFX transection

may also result in the pathological reorganization of intrinsic hippocampal circuits (e.g., Buzaki et al., 1989; Geddes et al., 1992; Lahtinen et al, 1993; Chen et al., 1989).

The present results confirm that the fornical afferents and/or efferents to and/or from the hippocampus are required for the normal hippocampal-dependent acquisition of spatial learning, but that rats lacking these afferents/efferents are insensitive to alteration of environmental cues if they have received extensive training in the Morris water maze task using a novel or variable start procedure.

Experiment 2: The effects of bilateral knife cuts of the temporal-hippocampal pathway on spatial learning in the rat.

It is clear based on the results of experiment 1 that transection of the fimbria-fornix does result in a spatial learning deficit that can be characterized by longer escape latencies in the acquisition, retention and reversal of place learning in the Morris water maze. It is not clear, however, why such profound spatial learning deficits are observed in the FFX transected animal. As pointed out earlier, FFX transection results in both the elimination of one of the principal input/output pathways of the hippocampus, elimination of the cholinergic- and GABAergic-dependent theta rhythm and the disruption of the hippocampus' intrinsic GABAergic interneurons. Any one of these phenomena could be responsible for the spatial learning impairment in a FFX transected animal. Therefore, it might be useful to compare the effects of FFX transection to transection of the second major input/output structure of the hippocampus, namely the temporal-hippocampal fiber pathway.

The hippocampus distributes and receives highly integrated neocortical information from the entorhinal cortex (Hjorth-Simonsen and Jeune, 1972; Steward, 1976; Swanson and Cowan, 1977; Van Hoesen, 1982) and perirhinal cortex (Kosel et al., 1983; Zola-Morgan et al., 1989; Zola-Morgan et al., 1991) through the temporal-hippocampal fiber pathway in the rat (Myhrer, 1991). Evidence suggests that at least

some of this information is critical for spatial learning because bilateral lesions of the entorhinal cortex result in a severe and lasting impairment on the 8-arm radial maze (Olton, Walker and Gage, 1978; Olton, 1983) and the Morris water maze (Schenk and Morris, 1985).

Bouffard and Jarrard (1988), however, have questioned the importance of the entorhinal cortex in spatial learning. They reported that ibotenic acid-induced lesions of the entorhinal cortex and subiculum failed to produce behavioural impairments in the acquisition of a complex spatial task even though aspiration-induced lesions did. They argued that aspiration lesions produce additional hippocampal and extrahippocampal damage that may be directly responsible for the observed impairments and that prevention of this damage results in normal spatial learning. Consistent with these observations, Rothblat, Vnek, Gleason and Kromer (1993) have reported that N-methyl-d-aspartic acid (NMDA)-induced lesions of the entorhinal cortex failed to impair performance on a spatial alteration task but produced aspiration-like impairments on a visual discrimination task. These results suggest that parahippocampal structures other than the entorhinal cortex may be critical for spatial learning.

There is substantial evidence that the amygdala (Mishkin, 1978), piriform cortex (Lynch, 1986) and perirhinal cortex (Zola-Morgan et al., 1989; Zola-Morgan et al., 1991) are critical for the processing of information during nonspatial learning and memory tasks. For example, Zola-Morgan et al. (1989) have demonstrated that monkeys with lesion-induced damage limited to perirhinal cortex display memory

impairments that are similar to, or greater than those produced by lesions restricted to the hippocampus. The perirhinal cortex lies in close proximity to the hippocampus and entorhinal cortex in both the monkey and the rat, and is likely damaged by large aspiration lesions to these areas. Anatomical data have demonstrated that the perirhinal (PRh) cortex projects directly to CA1 in the monkey (Suzuki and Amaral, 1990), and the subiculum in the rat (Kosel et al., 1983). Clearly, additional research is needed to clarify the role that this parahippocampal area might play in spatial and non-spatial learning.

Unfortunately, very little is known about the organization of the PRh cortex in the rat. Therefore, the following study addressed whether spatial learning was impaired following disruption of the temporal-hippocampal fiber pathway in the rat. Transection of this pathway rostral to the entorhinal cortex results in the elimination of fibers projecting to and/or from the hippocampus while sparing all other surrounding parahippocampal structures. Therefore, this technique is ideal for determining the general involvement of the PRh cortex in spatial learning.

MATERIALS AND METHOD

Animal treatment

Forty Long-Evans hooded rats (220-249 g at surgery), were purchased from Charles River Laboratories (Quebec) and treated in accordance with the guidelines set

by the Canadian Council on Animal Care. Accordingly, animals were tail-marked, placed into large (800 cm² x 18 cm deep) polypropylene cages (2 per cage) in one room on a reverse light cycle (8:00 off, 20:00 on). Food and water were available *ad libitum*.

Surgery

Twenty-nine bilateral knife cuts of the temporal-hippocampal pathway were made manually by intracranial coronal incision using a modified surgical blade. Rats were anesthetized with sodium pentobarbital (50 mg/kg) and held stationary in a Kopf stereotaxic apparatus such that bregma and lambda were in the same horizontal plane. A large incision was made in the scalp exposing the skull, following which the muscle adhering to the lateral ridge of the skull was detached and retracted to expose the zygomatic arch and the side of the skull overlying the perirhinal cortex. A small series of burr holes were drilled just above the zygomatic arch (and rhinal sulcus) 1 mm posterior to bregma. The surgical blade was then inserted 5 mm at an angle of 45° from the surface of the skull so that its tip passed underneath the rhinal sulcus and extended into the ventral PRh cortex. The blade was subsequently drawn medially and 3mm vertically from this lateral position. Then the blade was withdrawn from the tissue. Using wound clips, the scalp incision was closed and the animals allowed 14 days to recover.

Apparatus and Procedures

The apparatus, test room and procedures used in this experiment were identical to those used in experiment 1.

Histology

At the completion of behavioural testing all 39 animals were transcardially perfused with 60 mL of 0.1M phosphate-buffered saline (pH=7.2-7.4) followed by 120 mL of 4.0% paraformaldehyde/14% saturated picric acid in 0.1M phosphate buffer (pH 7.2). Horizontal sections 50 μ m-thick were cut on a Vibratome, mounted directly onto chrom alum- gelatin-coated glass slides, air-dried, dehydrated in graded alcohols and xylene, rehydrated, and stained with 1.0% cresyl violet or acetylthiocholine iodide for AChE histochemistry (as described by Paxinos and Watson, 1986).

RESULTS

Histology

Inspection of the bilateral knife cuts in the temporal-hippocampal pathway of 29 animals revealed that 22 of the animals had transections through the pathway (Bilateral group) and 7 had complete transection of pathway on only one side (Unilateral group). The Bilateral group was subsequently divided into 2 groups. The first group was made up of those animals that had bilateral knife cuts through the fiber pathway but displayed no damage to other surrounding tissue (Bilateral group, n=9). The second group was

made up of the those animals that had complete knife cuts that also displayed additional damage to the surrounding areas (Bilateral⁺ group, n=13; Fig. 3.1).

Behavioural testing

Cued learning: There were no differences between groups during the acquisition of cued learning. All groups improved their performance across trials 1-4 [$F(3,105)=12.8$ $p<0.001$; Fig. 3.2]. Thus, the perceptual, motivational and motoric demands of the task were similar for all groups.

Place learning: There were no differences between groups during the acquisition of place learning across 11 days (Fig. 3.3). All groups displayed escape latencies that were indistinguishable from controls and all groups significantly improved their performance across days 1-11 [$F(10,350)=34.1$, $p<0.001$].

Spatial probe: On the second spatial probe trial, the day following the second day of place learning, all groups showed a quadrant bias for the annulus in quadrant 3 (Fig. 3.4A). However, no differences were apparent between the groups in the mean number of annulus crossings in quadrant 3, or any of the remaining 3 quadrants.

By the third spatial probe trial, after place learning day 10, all groups crossed over the annulus in quadrant 3 significantly more times than any of the other quadrants

Fig. 3.1 AChE staining in horizontal (50 μm) sections 2 months after receiving knife cuts. (A) A complete knife cut that is representative of the Bilateral group (JNA245). (B) A knife cut that is representative of the Bialteral⁺ group (JNA244). Note the extensive damage to the caudate in (B).

A

B

Fig. 3.2 Mean ($\pm$ s.e.m.) latency to escape to the black platform during 4 trials of cued learning. No differences were apparent between any of the groups during the performance of this task.

Fig. 3.3 Mean ($\pm$ s.e.m.) latency to escape to the hidden platform during 11 days of place learning (4 trials per day). No differences were apparent between the groups.

Fig. 3.4 Mean ($\pm$ s.e.m.) number of annulus crossings in the four quadrants during the (A) 2nd and (B) 3rd spatial probe trials. No differences were apparent between the groups although all groups crossed over the annulus in quadrant 3 significantly more times than in any other quadrant.

A. Spatial Probe 2

B. Spatial Probe 3

[$F(3,105)=52.0$, $p<0.001$; Fig. 3.4B]. However, no differences were apparent between the groups in the mean number of annulus crossings in quadrant 3.

Rotation of intramaze cues: There were no differences apparent between the groups in latency to reach the platform (Fig. 3.5A) when the maze was rotated by 90° . Latencies on this day were indistinguishable from those recorded on the last day of place learning.

Rotation of extramaze cues: Performance after extramaze cues were rotated 90° was not different between groups. However, the mean latency on the first trial (9.97 ± 2.70) was slower than the mean latency on the last trial [6.10 ± 2.21 ; $F(3,105)=3.30$, $p<0.05$; Fig. 3.5B]. However, performance on all extramaze cue trials was not different than that recorded on the last day of place learning.

Random variation in location of the hidden platform: There were no differences between groups in the latency to reach the platform when it was randomly placed in the annulus area of the 4 different quadrants (Fig. 3.5C).

Old place retention/reacquisition: No differences were observed between groups when the animals were tested for their retention of the platform's original location (Fig 3.6A). All groups improved their performance across trials 1-4 [$F(3,105)=3.28$, $p<0.05$].

Fig. 3.5 Mean ($\pm$ s.e.m.) latency to escape to the hidden platform during 4 trials of (A) intramaze cue rotation, (B) extramaze cue rotation and (C) random placement of the platform to the middle of each quadrant between trials.

Fig. 3.6 Mean ($\pm$ s.e.m.) latency to escape to the hidden platform during (A) trials 1-4 of retention of the original location of the hidden platform, and (B) new place learning. No differences were apparent between the groups on the trials of these two tasks.

New place learning: A significant interaction was observed across trials 1-4 after the platform was moved to a new location [$F(9,105)=2.70$, $p<0.001$]. This interaction was the result of the longer mean escape latency of the Unilateral group when compared to the Bilateral⁺ group on the 1st trial of new place learning (Fig. 3.6B). However, no differences were apparent between groups across trials 2-4. All groups improved their performance across trials [$F(3,105)=3.70$, $p<0.01$].

DISCUSSION

In the present study, bilateral knife cuts of the temporal-hippocampal pathway failed to have any effect on spatial learning. Animals that received extensive cuts of this pathway performed like controls during acquisition, retention and reversal of place learning in the Morris water maze when compared to unoperated controls and animals that displayed one intact pathway. Although there are no direct comparisons that can be made between the results presented here and the results of any similar spatial learning investigation, these results do fail to support the more general findings that suggest the PRh cortex is important for retention of information in a number of tasks, including delayed nonmatching to sample task in the monkey (Zola-Morgan et al., 1989, 1991, 1993), conditioned fear (Rosen, Hitchcock, Miserendino, Falls, Campeau and Davis, 1992), odor-guided delayed nonmatching to sample (Otto and Eichenbaum, 1992) and object discrimination in the rat (Meyer, Meyer and Clound, 1986; Myhrer, 1991).

There are, however, a number of reasons why the knife cuts used in the present study may have failed to have any effect on spatial learning in the Morris water maze. First, the knife cuts used in the present study may have failed to include some critical aspect of this pathway that lies either dorsal or ventral to the rhinal sulcus. According to Paxinos and Watson (1986) fibers in this pathway can transverse between 1.5 mm dorsal and 0.5 mm ventral to the rhinal sulcus. This means that this pathway extends between 2 and 4 mm dorsal to stereotaxic-zero (STX0) at the rostrocaudal location of the knife cuts used in the present study. Rosen et al. (1992) have previously demonstrated that bilateral lesions to the PRh cortex that did not include damage dorsal and ventral to the rhinal sulcus in this area failed to block the potentiated acoustic startle reflex in a rat model of conditioned fear. Although lesions limited to the cortex just dorsal to the rhinal sulcus failed to block this potentiation, Rosen et al. were unable to determine whether the effects of lesions to the area ventral to the rhinal sulcus would produce the conditioned response. Deacon et al. (1983) have previously suggested that neocortical inputs to the PRh cortex, which arise from allocortical areas such as the piriform and entorhinal cortex, terminate in the area of the PRh cortex which is ventral to the rhinal sulcus. In the present study, the knife cuts in the Bilateral group (n=9) were located approximately 2.64 ± 0.22 mm (mean $\pm$ s.e.m.) dorsal to the horizontal plane of STX0. Although this measurement varied from 1.9 to 3.9 mm, no relationship was found between the ventral extent of the knife cut and latency on the last day of place learning ($r = 0.20$, $p > 0.05$ for $n = 9$). However, it is possible that the area of the

temporal-hippocampal pathway ventral to the rhinal sulcus is critical for spatial learning and that the knife cuts used in the present study failed to transect critical fibers of passage in this area.

The second reason why bilateral knife cuts used in the present study failed to have any effect on spatial learning is that the anterior and the posterior fibers in this pathway may play differentially distinct roles in different learning and memory tasks. Rosen et al. (1992) have demonstrated that lesions to the PRh cortex that included the areas dorsal and ventral to the rhinal sulcus completely eliminated the potentiated startle reflex only when these lesions were placed in the area extending 1.8 to 2.3 mm posterior to Bregma. Similar lesions placed slightly more posterior had no effect. In the present study, the most anterior placements of the bilateral knife cuts in the bilateral group (n=9) were found approximately 3.09 ± 0.29 mm posterior to Bregma. This measurement varied from 1.5 mm posterior to Bregma to 4.0 mm posterior to Bregma. However, we were surprised to find a significant correlation between the distance the knife cuts were placed posterior to bregma and latency on the last day of place learning ($r = 0.53$, $p < 0.05$) in the Bilateral group (n=9). Therefore, it is possible that the knife cuts used in the present study failed to have any effect on spatial learning because they were placed in a rostrocaudal area of the temporal-hippocampal pathway where they did not disrupt critical connections to and/or from the hippocampus. These results suggest that the posterior and not the anterior fibers are important for learning in a spatial

learning task. Future research should address whether different rostrocaudal areas of this pathway are critically involved in spatial learning.

The third reason why the knife cuts used in the present study failed to have any significant group effect on spatial learning is that this pathway may not be involved in spatial learning in either the rat or monkey. In the monkey there is substantial evidence that lesions to the parahippocampal areas cause severe and permanent impairments in a number of object retention tasks including concurrent discriminations (Zola-Morgan et al., 1989), paired associate learning (Murray, Gaffan and Mishkin, 1993) and delayed nonmatching to sample in both visual and tactile modalities (Horel, Pytko-Joiner, Voytko and Salsbury, 1987; Zola-Morgan et al., 1989; Suzuki et al., 1993). However, performance of animals on these tasks does not necessarily reflect how animals will perform on a spatial learning task. For example, recent evidence suggests that the PRh cortex and hippocampus may play different roles in learning and memory. Evidence in the monkey suggests that the PRh cortex plays an important role in object-recognition memory (Suzuki, Zola-Morgan, Squire and Amaral, 1993) whereas the hippocampus and fimbria-fornix seem critical for the acquisition and retention of spatial information (Gaffan and Harrison, 1989; Murray et al., 1989). Recently, Murray et al. (1993) have argued that there are three distinct associative memory mechanisms within the medial temporal lobe of the monkey. The first mechanism seems to play a critical role in retention of stimulus-reward memories and its circuits encompass the amygdala. The second mechanism seems critically important for spatial memory and its circuitry

encompasses the hippocampus and fimbria-fornix. The third mechanism seems critical only for the retention of object-recognition memories, and its circuits encompass the PRh cortex. The results presented in this study support Murray et al.'s speculation that there are distinct and separate memory circuits within the medial temporal lobe.

Compared to the monkey, however, very little is known about the function of the PRh cortex in the rat. Previously, Meyer et al. (1986) have demonstrated that bilateral aspiration lesions to both the entorhinal and posterior perirhinal cortex result in a severe impairment on a spatially discontinuous component of a black-white visual discrimination task. The PRh cortex, however, did not seem to be involved in visual perception because animals with bilateral PRh and entorhinal cortex lesions failed to differ from controls on the contiguous component of the task and on a test of form perception that required discrimination between two sets of oblique stripes. These results suggest that the PRh and/or entorhinal cortices are important for the attentional processes in the acquisition and/or retention of visual associative memory. However, Meyer et al. were unable to determine whether lesions made separately to either the entorhinal cortex or PRh cortex would result in the impairment.

In the rat, there is also evidence that the PRh and/or entorhinal cortices are important for odor discrimination. For example, Otto and Eichenbaum (1992) have demonstrated that combined lesions of the entorhinal and PRh cortex produce severe deficits in retention, but not acquisition, of an odor-guided delayed nonmatching to sample task. During the performance of this task rats were required to make a choice

response to an odor presented 3, 30 or 60 seconds after presentation of a sample odor. Otto and Eichenbaum found that PRh/entorhinal cortex lesioned animals displayed fewer correct responses than controls during the 30 and 60 second delay interval, but not during the 3 second delay. These results suggest that these parahippocampal areas play an important role in the maintenance of memories for odors and support recent findings in the monkey that suggest parahippocampal areas are particularly important for object-recognition memory across a number of stimulus presentation modalities (Suzuki et al., 1993). However, like the results of Meyer et al., these results cannot rule out the possibility that the observed impairment in odor memory was the result of damage to the entorhinal cortex and not the PRh cortex per se, because these authors failed to include a group of additional rats that had lesions restricted to either cortex alone.

Furthermore, there is now substantial evidence that suggests performance of a rat on a object discrimination task is not indicative of performance on a spatial learning task (Aggleton et al., 1986; Rothblat and Kromer, 1991; Kesner, 1993). This evidence suggests that the PRh cortex and hippocampus may play different roles in learning and memory depending on the behavioural task employed. A number of explanations have been provided to explain this observation. For example, Eichenbaum et al. (1992; Otto, Eichenbaum and Cohen, 1993) have argued that spatial tasks differ from recognition tasks in both the nature of the information encoded and the how long that

information is retained. According to Eichenbaum et al., hippocampal lesions should only impair performance on a task that requires the animal to make a permanent association between the relationships among objects of a perceptually independent nature. In contrast, parahippocampal lesions should impair performance on a task that requires the animal to make only a temporary association between individual objects. This argument, however, simply demonstrates the need for more detailed comparisons between lesion types and performance on a number of different behavioural tasks.

Experiment 3: The effects of adrenalectomy-induced hippocampal dentate granule cell degeneration on spatial learning in the rat.

Based on the results of both experiment 1 and 2, it is clear that the Morris water maze procedure used in this thesis is selectively sensitive to hippocampal-dependent spatial learning impairments. Experiment 1 demonstrated that transection of the fimbria-fornix in the rat produces a spatial learning deficit that can be characterized by longer escape latency in the acquisition, retention and reversal of place learning. Although it is not clear, however, why such profound spatial learning deficits are observed in the FFX transected animal, a similar transection of the other major input/output pathway of the hippocampus, namely the temporal-hippocampal fiber pathway, in experiment 2 of this thesis failed to produce a spatial learning impairment in this Morris water maze task. These data suggest that one of the primary reasons such profound spatial learning impairments are observed in a FFX transected animal might be the direct alteration of the intrinsic circuitry of the hippocampus. Therefore it is important to investigate the effects of altering the primary circuitry of the hippocampus on spatial learning in the rat.

The ability to assign particular functions to specific brain regions depends on the ability to produce selective and focal brain lesions. Previous studies investigating the selective role of the dentate granule cells in spatial learning have shown that

intrahippocampal injections of colchicine produce both hippocampal and extrahippocampal damage that is associated with slower spatial learning in the Morris water maze (Sutherland et al., 1983; Whishaw, 1987; McNaughton et al., 1989). The discovery that adrenalectomy (ADX) selectively destroys granule cells (Sloviter et al., 1989; Sloviter, Dean and Neubort, 1993; Sloviter, Sollas, Dean and Neubort, 1993) has enabled us to readdress the role that the dentate granule cells play in spatial learning in the rat.

MATERIALS AND METHODS

Animal treatment

Sixteen sham-operated and 102 adrenalectomized rats (ADX; 153-210 g at surgery) were purchased from Harlan-Sprague-Dawley, Inc. and treated in accordance with the guidelines set by the Canadian Council on Animal Care. Animals were tail-marked and placed into large (800 cm² x 18 cm deep) polypropylene cages (2 per cage). All animals were housed in one room with a reverse light cycle (8:00 off, 20:00 on). During the course of the 5 month experiment, food and saline or water were available *ad libitum* and the weight of each rat was determined weekly.

Throughout the study, the drinking water for all ADX rats contained 0.9% NaCl. However, corticosterone (Sigma; 20 mg/ml 100% ethanol) was added to the saline drinking solution to a final concentration of 20 µg/ml to prevent the death that occurs in many animals during the month after adrenalectomy. After one week on hormone, the

corticosterone concentration was halved and, after another week, halved again. After 3 weeks, corticosterone was removed from the saline drinking solution.

To determine the effects of corticosterone replacement on behaviour or granule cell loss, a special group of 16 ADX rats were maintained on 20 µg/ml corticosterone replacement (CR group) throughout the experiment. The sham-operated control rats were maintained on tap water without the NaCl supplement.

Recent studies have shown that a minority of ADX animals exhibit no granule cell loss (Sloviter et al., 1989; 1993b) because of the survival of ectopic adrenal tissue (Richter, 1941; McNeill, Master and Finch, 1991). The animals that exhibit obvious granule cell loss are always among the rats with the lowest weight gain (Sloviter et al., 1989; 1993b). Therefore, the heaviest 57 ADX animals were eliminated from this study 3 months after adrenalectomy. This left 45 of the lightest ADX rats to be compared to 16 corticosterone replacement (CR) ADX rats and 16 sham-operated rats.

Apparatus and Procedures

The apparatus and procedure used in the present study were identical to those used in experiment 1 and experiment 2 of this thesis.

Histology

Five months after surgery, the 45 ADX rats, 16 CR rats and 10 of the 16 Sham rats

were decapitated. Each brain was removed from the skull, bisected midsagittally and immersed in 1.0% paraformaldehyde/ 1.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4). Sections 25 μ m-thick were cut on a Vibratome, mounted directly onto chrom alum- and gelatin-coated glass slides and air-dried. Slides were dehydrated in graded alcohols and xylene, rehydrated, stained with 1.0% cresyl violet, dehydrated and coverslipped with Permount. One half of each brain was cut coronally and the other horizontally.

Granule cell loss was quantified in a subset of ADX rats (see below) by measuring the area of the entire granule cell layer with a histo- morphometric program (VIDS system; Analytical Measuring Systems, Cambridge, U.K.). With this method the outline of the granule cell layer was traced on the computer screen and the area bounded by the perimeter was calculated. To be conservative, the perimeter outline was expanded to include *any* neuron in this layer (e.g. basket cells). Thus, a perimeter score was obtained for the granule cell layer even if no granule cells were evident. For quantitative analysis, a section from each rat similar to plates 38-40 of the atlas of Paxinos and Watson (1986) was used. Sections from five sham rats were used for counts to generate a mean granule cell area at this representative rostrocaudal level. For comparisons between individual ADX rats and the control mean, the granule cell layer from each rat was measured three times to generate a mean value and variability estimate.

Statistical analyses

All statistical analyses involved univariate analysis of variance and, when necessary, multiple comparisons using Scheffe's procedure.

RESULTS

Histology

Nissl-stained sections from all surviving ADX rats were evaluated blindly by two observers. Of the 45 ADX rats completing the experiment, 14 exhibited "obvious" granule cell loss. This loss consisted of a thinning of the granule cell layer with the greatest loss in the lateral portion of the suprapyramidal blade. This qualitative assessment in the ADX rats was considered a conservative judgement because the animals that did not appear obviously different from controls were scored as not exhibiting obvious loss.

Within the group of 14 "obviously" affected animals, the loss of granule cells ranged from minimal (7 rats) but with a clear thinning of the superior blade, to moderate (n=3) or extensive (n=4) loss. Table 4.1 presents quantitative data on the degree of cell loss in one coronal plane through the posterior dorsal hippocampus. All three ADX groups showed substantial granule cell area loss compared to the sham rats; the minimal, moderate and extensive groups displayed 60%, 56% and 38% of the granule cell area of controls, respectively.

It is important to note that ADX never causes a complete loss of all the granule

2 of/de 2

PM-1 3½" x 4" PHOTOGRAPHIC MICROCOPY TARGET
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PRECISIONSM RESOLUTION TARGETS

Table 4.1. Area of Dentate Granule Cell Layer 5 Months after Adrenalectomy

<u>Group</u>	<u>Rat#</u>	<u>Area (mm²) ± s.e.m. ^a</u>	<u>% of Sham ± s.e.m.</u>	<u>Qualitative Score ^b</u>
Sham	23	0.321 ± 0.007		0
	24	0.396 ± 0.012		0
	25	0.324 ± 0.004		0
	26	0.333 ± 0.003		0
	28	0.331 ± 0.005		0
		0.341 ± 0.034 ^c		
Minimal	96	0.248 ± 0.001	72.8 ± 7.3	1
	112	0.221 ± 0.007	64.7 ± 6.1	1
	17	0.217 ± 0.007	63.5 ± 6.7	1
	70	0.201 ± 0.005	59.1 ± 6.1	1
	113	0.194 ± 0.002	56.8 ± 5.7	1
	76	0.187 ± 0.005	54.9 ± 5.7	1
	94	0.157 ± 0.005	46.1 ± 4.9	1
		0.204 ± 0.004	59.8 ± 6.1	
Moderate & Extensive	66	0.196 ± 0.004	57.4 ± 5.9	2
	9	0.196 ± 0.009	57.5 ± 6.4	2
	114	0.186 ± 0.006	54.6 ± 5.7	2
	75	0.178 ± 0.003	52.2 ± 5.3	3
	80	0.153 ± 0.011	44.7 ± 5.6	3
	44	0.131 ± 0.006	38.4 ± 4.2	3
	102	0.056 ± 0.003 ^d	16.3 ± 1.8	3
		0.156 ± 0.002	45.8 ± 5.0	

(a) Mean area and standard error of 3 measurements taken from the same section.

(b) Qualitative measurement of cell loss; 0=Sham, 1=Minimal, 2=Moderate, 3=Extensive (see text).

(c) Group mean ± s.e.m.

(d) In the granule cell area measurement for rat # 102, there were no granule cells evident.

cells. Some granule cell in the ventral or temporal dentate gyrus always survive (Sloviter et al., 1989; 1993a, b). However, in the most severely affected rats, for example rat 102, there is complete loss of the granule cells throughout approximately 80% of the dentate gyrus and reduced numbers in the remaining area. Therefore, rat # 102 is missing about 90-95% of its granule cells. Figure 4.1 shows a representative spectrum of the degree of granule cell loss characterized by the three different cell loss groups. A more detailed anatomical analysis of the phenomenon has been published (Sloviter et al., 1993b).

For all statistical comparisons, the seven rats with moderate to extensive granule cell loss (GCL group) were compared to the other ADX and control groups. Since there were no differences found between the minimal granule cell loss group and the other ADX rats not showing granule cell loss, their data were combined.

Weight

As shown previously (Sloviter et al., 1989; 1993b), ADX induces clinical signs that include reduced weight gain. Adrenalectomized rats were significantly ($p < 0.0001$) lighter than controls ($448.0 \text{ g} \pm 7.0$ vs $523.0 \text{ g} \pm 11.0$, respectively; mean $\pm$ SEM) 5 months after adrenalectomy. Although the 14 ADX rats that exhibited obvious granule cell loss tended to be lighter than the other ADX rats, they could not be distinguished from them statistically.

Fig. 4.1 Selective dentate granule cell degeneration after adrenalectomy. (A) Sham-operated control (#28). (B-D) Minimum, moderate and extensive granule cell loss in 3 different animals 5 months after adrenalectomy (ADX). B: #113; C: #80; D: #102; the latter rat exhibited the most extensive damage in the granule cell loss group. Note that cell loss is most apparent in the lateral tip of the inner blade of the granule cell layer (arrows). Also note survival of the pyramidal cells (P). (E and F). Sham-operated control (E) and ADX 102 (F) showing at high magnification the extensive granule cell loss after adrenalectomy. Abbreviations: G-granule cell layer; P-pyramidal cell layer. Cresyl violet-stained 25 μ -thick Vibratome sections. Magnification: A-D: 19.5X; E and F: 39X.

Behavioural Testing

Cued learning: There were no differences between the groups in acquisition of cued learning. Analysis across the 4 trials revealed that all groups improved their performance from trial 1-4 [$F(3,219)=22.8$, $p<0.001$; Fig. 4.2].

Place learning: There were no differences between groups during the acquisition of place learning across 11 days (Fig. 4.3). All 4 groups displayed escape latencies that were indistinguishable from sham operated controls and all groups significantly improved their performance across days 1-11 [$F(10, 730)=105$, $p<0.001$]. However, visual inspection and post hoc analysis of the escape latencies across the 11 days revealed that the rats with moderate or extensive granule cell loss (GCL group) displayed statistically longer latencies on place learning days 6, 7 and 8 ($p<0.01$, Scheffe's). On days 9, 10 and 11, however, the mean latency of the GCL group was indistinguishable from controls.

Spatial probe: No differences were observed between groups in the number of annulus crossings in each quadrant or the total number of annulus crossings during the each of the three separate spatial probe trials. However, on the second and third spatial probe trial all groups displayed a quadrant bias for the annulus in quadrant 3.

Fig. 4.2 Mean ($\pm$ s.e.m.) latency to escape to the black platform during 4 trials of cued learning. No differences were apparent between the groups on any aspect of this task.

Fig. 4.3 Mean ($\pm$ s.e.m.) latency to escape to the hidden platform on 11 days (4 trials per day) of place learning. Although the GCL animals performed like controls throughout the majority of this task, they were slightly slower than controls on day 6, 7 and 8.

By the third spatial probe trial (Fig. 4.4) the mean number of annulus crossings in quadrant 3 was substantially greater (4.2 ± 0.2) than the mean number of crossings in any of the other quadrants [1.0 ± 0.1 ; $F(3,219)=96.7$, $p<0.001$].

Rotation of intramaze cues: With the platform in the same position relative to the extramaze cues as during place learning and the maze rotated by 90° , the GCL group required a longer time to reach the platform than any other group [$F(3,73)=5.57$, $p<0.01$]. This difference, however, was the result of the longer mean latency of the GCL group compared to all other groups on the first trial of intramaze cue rotation [$F(9,219)= 3.08$, $p<0.01$; Fig. 4.5]. The performance of the GCL group was similar to that of the controls on the remaining trials.

Rotation of extramaze cues: After all moveable extramaze cues were rotated 90° in the maze room, the mean latency to reach the hidden platform was significantly longer in the GCL group than any other group on trials 1 and 3 [$F(9,219)=1.92$, $p=0.05$; Fig. 4.6]. Further analysis revealed that there was no correlation between the degree of cell loss and the level of performance in the GCL group. For example, the rat with 95% loss of granule cells performed very close to the average score of the GCL group (or better) on all tests. Thus, rats with moderate granule cells loss were as behaviourally

Fig. 4.4 Mean ($\pm$ s.e.m.) number of annulus crossings in each of the 4 quadrants for all groups during the 3rd spatial probe. No difference were apparent between the groups and all groups crossed over the annulus in quadrant 3 significantly more times than in any other quadrant.

Fig. 4.5 Mean ($\pm$ s.e.m.) latency to escape to the hidden platform on trials 1-4 after 90° rotation of the intramaze cues. Note that the GCL animals performed significantly different from all other groups during the performance of this task. Asterisk indicates a significant difference ($p < 0.01$) from controls.

Fig. 4.6 Mean ($\pm$ s.e.m.) latency to escape to the hidden platform on trials 1-4 after 90° rotation of all the moveable extramaze cues. Note that the GCL animals performed significantly different from all other groups during the performance of this task. Asterisk indicates a significant difference ($p < 0.01$) from controls.

disrupted (or more) as the most severely affected rat.

Random variation in location of the hidden platform: The latency to reach the hidden platform when it was placed randomly in the 4 different annuli (without quadrant repetition) for 4 consecutive trials revealed that all groups performed similarly. Not surprisingly, however, the latency to reach the platform was shorter (28.3 ± 3.2) for all groups on the final trial when it was located in quadrant 3 (original place learning) compared to any of the other quadrants (48.3 ± 3.9 ; Fig. 4.7).

Old Place Retention/reacquisition: After 7 days without exposure to the maze, all groups reacquired the original place learning task. No differences were apparent between groups, although the performance of all groups improved across trials [$F(3,219)=32.7$, $p<0.001$]. As can be seen in Figure 4.8, retention of the original place learning over this period was modest. This may have been the result of interference from the numerous maze cue perturbations each rat encountered shortly before the retention interval.

New Place Learning: No differences were apparent between groups when the animals acquired a new location (Fig. 4.9). All groups significantly improved performance across the 4 trials [$F(3,219)=14.4$, $p<0.001$].

Fig. 4.7 Mean ($\pm$ s.e.m.) latency to escape to the hidden platform when the platform was randomly moved between each trial. No differences were apparent between groups during the performance of this task.

Fig. 4.8 Mean ($\pm$ s.e.m.) latency to escape to the hidden platform during retention (or reacquisition) of the original place learning. No differences were apparent between groups.

Fig. 4.9 Mean ($\pm$ s.e.m.) latency to escape to the hidden platform during acquisition of a new place (reversal learning). No differences were apparent between the groups during the performance of this task.

DISCUSSION

In this experiment, adrenalectomized (ADX) rats that exhibited specific, extensive, bilateral dentate granule cell loss displayed escape latencies on 11 place learning days in the Morris water maze that were statistically similar to the latencies displayed by controls. Although the 7 rats with moderate to extensive granule cell loss (GCL group) performed like controls over the first 5 days and last 3 days of acquisition, GCL rats were slightly slower than controls on days 6, 7 and 8. Importantly, however, the rat with the most extensive loss of dentate granule cells was indistinguishable from controls throughout all 11 days of place learning.

This relatively small impairment in the GCL group differs somewhat from those "severe" spatial learning impairments that were reported when granule cell destruction was produced by multi-site, intrahippocampal injections of colchicine (Sutherland et al., 1983; Whishaw, 1987; McNaughton et al., 1989). For example, animals displaying substantial loss of dentate granule cells after adrenalectomy did acquire the location of the hidden platform in a way that was quantitatively similar to controls; whereas colchicine injected animals never reached a control-like level of performance. The discrepancy between the severe impairment reported previously and the control-like latencies observed here is somewhat surprising because both procedures produced a loss of dentate granule cells which was remarkably similar in both numbers and distribution. There are several possible reasons for this discrepancy. First, in our

study, adrenalectomy was performed in juvenile rats. Thus, unlike the adult rats used in the colchicine studies, the younger rats may have developed compensatory mechanisms to overcome granule cell loss. Second, although ADX results in considerable granule cell loss within 1-2 weeks (Sloviter et al., 1993b), colchicine kills granule cells more rapidly. Therefore, the ability to adapt to granule cell loss following ADX might be greater than that which occurs following intrahippocampal injection of colchicine. And third, both Sutherland et al. (1983) and Whishaw (1987) have reported that in addition to the destruction of granule cells, injection of colchicine resulted in the loss of CA1 pyramidal cells, damage to the neocortex and laterodorsal thalamic nuclei, and the induction of reflex epilepsy. By contrast, ADX produces highly selective dentate granule cell degeneration (Jaarsma, Postema and Korf, 1992; Sloviter et al., 1993b). Thus, the severe spatial learning impairment in the Morris water maze following intrahippocampal colchicine injections may not have resulted from granule cell loss *per se*, but from colchicine's effect on CA1 or damage due to invasive surgery.

Although the GCL rats in the present experiment acquired the place learning task marginally slower than controls, this was not due to obvious differences in activity, motoric capacity, motivation or specific search strategies. That is, the rats in all groups easily escaped to the raised black platform during the 4 trials of cued learning and therefore appeared equally motivated and physically capable of escape. In addition, on each of the 3 spatial probe days, the number of annulus crossings did not differ between the various groups.

If the rats with granule cell loss were not different motivationally and motorically from controls, and their search strategy appeared like controls, why were they slower than controls in three of the final six days of acquisition of the place learning task? The relative slowness of the GCL rats could mean that these animals learned the place task in a manner qualitatively different than controls. If GCL rats judged the location of the annulus by its approximate relationship to the many associated cues in both the proximal and distal environment, they may not have developed the same sense of location that was characteristic of control rats. If the representation of the platform's location in the GCL rats was highly dependent upon the integrity of the cue complex (i.e., relationship between intra- and extramaze cues) acquisition might be more difficult or slower for these rats. Our data suggest that this might be the case.

The first manipulation in our study to reveal a strong impairment in performance of GCL rats compared to controls was intramaze cue rotation. Although the intramaze cues were undoubtedly quite subtle (perhaps slight variations in the pool wall surface or the top-cut edge of the pool), the GCL rats took 3-4 times longer to reach the hidden platform on the first intramaze cue rotation trial when compared to all other groups. Similarly, the following day, the GCL rats showed longer latencies on the first trial of extramaze cue rotation. Therefore, any change in the cue complex that altered the intramaze-extramaze cue relationship, was problematic for the GCL rats.

The performance of the GCL rats on the first trial of the two cue rotation tasks was

similar to that reported previously for rats with loss of the fimbria-fornix (FFx transected). Eichenbaum et al. (1990) previously reported that on a single trial of extramaze cue rotation, five of seven FFx transected rats exhibited longer latencies than controls in finding the hidden platform because they followed the rotated extramaze cues, while the remaining two FFx transected rats simply appeared "lost". In the present study, the four rats with the greatest loss of granule cells showed latencies in excess of 30 seconds in the first trial of intramaze cue rotation and were likely "lost" temporarily. Similarly, on trial 1 of extramaze cue rotation, longer latencies occurred in two of three rats with moderate cell loss. Thus, one trial of cue rotation (intra- or extramaze) was problematic for the rats with granule cell loss.

The disruption of GCL rats during cue rotation could also be interpreted as a consequence of their not attaining the same level of performance as controls prior to cue rotation. The controls reached near asymptotic performance by training day 6, while the GCL rats required 3 more days to achieve those same short latencies. One could argue that the slower GCL group may not have developed a complete sense of location like the controls and, as a result, were more easily confused by the cue rotation procedures. However, we have also observed that control rats, exposed to either intramaze or extramaze cue rotation on day 4 (which is well before reaching asymptotic performance), exhibited no change in their swim latencies compared to rats not experiencing cue rotation (J. N. Armstrong and D. C. McIntyre, unpublished observation). Thus, the difference in latencies between groups with and without

granule cell loss, during the cue rotation trials, was not likely to be the result of the differences between groups in their level of performance prior to cue rotation.

The performance deficit of the GCL group also was not a product of reduced corticosterone *per se*, since the other ADX rats with reduced corticosterone, but with minimal or no granule cell loss, were similar to sham rats on all behavioural tasks. In future experiments, corticosterone replacement at the time of behavioural testing would be an important control to ensure that the behavioural deficits in the GCL group were not the product of an interaction between granule cell loss and corticosterone insufficiency.

As a result of the similarity in performance among all groups of rats during the four trials of retention, we conclude that rats with or without obvious granule cell loss were capable of retaining information and/or readjusting to familiar surroundings. In addition, in the final 4 trials the hidden platform was moved to a new location and the GCL group acquired the new response in a manner that was indistinguishable from controls.

This latter result is completely different from FFX transected rats which were unable to learn a new platform location in the presence of the familiar cues (Eichenbaum et al., 1990). Eichenbaum et al. (1990; 1992) argued that disruption of the hippocampal system by FFX transected ablation results in "behavioural inflexibility", whereby an animal is unable to use previously learned information in a new context. Since our GCL rats learned the new location of the hidden platform with an ease indistinguishable

from controls, we can conclude that, to maintain 'behavioural flexibility', the fornical afferents and/or efferents to and/or from the hippocampus may be required, but a full complement of dentate granule cells is not.

Whether the difference between FFX transected and GCL rats is qualitative or quantitative cannot be determined from these data. Obviously the loss of dentate granule cells is different than the loss of fimbria-fornix fibers. Therefore it is not surprising that the behavioural consequence of these two lesions is also different. Using a variable start location, FFX transected rats were very seriously impaired (Nilsson, Shapiro, Gage, Olton and Bjorkland, 1987) compared to our GCL rats. Perhaps the FFX transected rats are simply more compromised by their lesion than our GCL rats.

The traditional concept of hippocampal circuitry and function, however, emphasizes the importance of the "trisynaptic loop" (Andersen et al., 1971), a circuit running from the entorhinal cortex to the dentate granule cells and from the granule cells to CA3 and CA1. Since the neurons comprising this loop are completely spared in FFX transected rats, but are not in the GCL rats, one might have expected the FFX transected rats to be less impaired spatially than the GCL rats. Clearly, this was not the case.

If the pyramidal cells of CA3 and CA1 are the cells in the trisynaptic loop most sensitive to and necessary for spatial learning (O'Keefe and Nadel, 1978), and CA3 and CA1 cells are innervated by the dentate granule cells (Andersen et al., 1971), how

did our rats with granule cell loss acquire the place learning task? As hippocampal participation is believed necessary for spatial learning (e.g., Morris et al., 1982), there are at least two ways that the GCL rats could have acquired the task. First, place learning might require only a minimum number of granule cells to provide CA3 with all the innervation it needs for a rat to learn the task (Amaral and Witter, 1989). Or second, the critical information for place learning might access CA3 and CA1 not from the entorhinal cortex through fascia dentata, but from the entorhinal cortex to CA3 and CA1 directly. There are anatomical data to support both these possibilities. In the first case, recent detailed studies of the intrinsic organization of the connections between CA3 and CA1 have revealed that projections from any particular septotemporal level of CA3 diverge to involve much of the septotemporal extent of CA1 (Ishizuka, Weber and Amaral, 1990). Furthermore, the associational projection of the dentate gyrus has extensive divergence in the septotemporal axis of the hippocampus (Amaral, 1991; Amaral and Witter, 1989). Thus, these two divergent connections would allow relatively few granule cells, such as those in the temporal pole, to distribute information throughout much of the hippocampus. In the second case, it has been shown that the entorhinal cortex (Kohler, 1988; Witter, Griffioen, Jorritsma-Byham and Krijnen, 1988) and other parahippocampal structures (Suzuki and Amaral, 1990) make direct connections to pyramidal cells in CA1 and CA3. In fact, Tamamaki (personal communication) has shown that a single entorhinal cell innervates *both* the dentate molecular layer and the apical dendrites of CA1 and CA3.

These observations are particularly interesting because McNaughton et al. (1989) have shown that complex-spike cells in CA3 and CA1 respond to spatial stimuli even if the granule cells have been destroyed by colchicine. As a result, they suggested that the main circuit for elaboration of spatial representations might involve the direct projections to CA3 from the entorhinal cortex. Stimulated by the suggestion of Kohonen (1988), McNaughton, Barnes, Mizumori, Green and Sharp (1991) subsequently proposed that the granule cells may act as a "novelty filter" during spatial learning. They suggested that the filter normally allows input to CA3 when the animal is exposed to a novel environment. This input provides CA3 with enough tetanic stimulation to produce synaptic enhancement. Of course, without novel input, the direct connections to CA3 from the entorhinal cortex would still allow the CA3-1 cells to respond normally to spatial stimuli. From their proposal, one would predict that granule cells are activated *only* by the cues of a *new* environment, and only at the time when that environment is being spatially encoded. Subsequently, as the novelty of the new environment wears off, a decay in granule cell activity must occur. This, of course, is what McNaughton et al. (1991) have observed. Furthermore, if the granule cells normally promote synaptic enhancement only in the presence of novelty, then their threshold for activation must be quite high. Indeed, Mizumori et al. (1989a, b) have shown that granule cells exhibit a high threshold for activation and show little spontaneous activity.

Therefore, if the granule cells act as a high threshold novelty filter that initiates

synaptic enhancement only when that threshold is reached, one would predict that deletion or alteration of some cues in an familiar spatial environment must not provide sufficient novelty to activate the granule cells. Indeed, one might assume that it is the insensitivity of hippocampal complex-spike cells to altered environmental cues (O'Keefe and Conway, 1978; O'Keefe and Speakman, 1987) that allows the experienced rat to perform very well with many cues deleted or altered (Eichenbaum et al., 1990; J. N. Armstrong and D. C. McIntyre, unpublished observation).

Consistent with this theoretical framework, one would predict that a loss of granule cells should result in reduced synaptic enhancement and abnormal encoding of information in CA3 and therefore slower spatial learning. However, if the granule cells are activated by novelty and they signal the complex-spike cells in CA3 to enhance spatial learning in a novel environment, and cue alterations are not sufficiently novel to activate the granule cells in a familiar spatial environment why were the GCL rats in the present study not insensitive to cue alterations like controls? Presumably, if the spatial memories of the GCL and control rats develop in the same way, then the lack of granule cell input to CA3 after spatial learning -- either because of reduced granule cell *activity* in controls or reduced number of granule cells in GCL rats -- should not have resulted in spatial disorientation during cue rotation in the GCL rats. This observation suggests, therefore, that there is either a problem with the model of McNaughton et al. (1991) or, more likely, that our prediction from that model was inappropriate because

our rats with granule cell loss learned the place task in a manner *qualitatively* different from controls.

The results of this experiment demonstrate that a full complement of granule cells is not necessary for spatial learning in the Morris water maze and suggest that granule cells may play a role in the recognition of a familiar but altered spatial environment. Future research should address the questions raised in this experiment by comparing the response of complex-spike cells in CA1 and CA3 in normal and GCL animals after intramaze and extramaze cues have been rotated in a familiar spatial environment.

GENERAL DISCUSSION

The results of both experiment 1 and 2 demonstrated that the Morris water maze procedure used in this thesis was sensitive to hippocampal-dependent spatial learning impairments. In experiment 1 bilateral transection of the fimbria-fornix in the rat produced longer escape latency of these animals during the acquisition, retention and reversal of place learning in the Morris water maze. Although it is not clear, however, why such profound spatial learning deficits are observed in these FFX transected animal, a similar transection of the other major input/output pathway of the hippocampus, namely the temporal-hippocampal fiber pathway, in experiment 2 of this thesis failed to produce a spatial learning impairment in this task. The results of experiment 3 demonstrated that a full complement of hippocampal dentate granule cells are *not* necessary for the acquisition and retention of spatial information in the Morris water maze. However, the results of this experiment also suggest that dentate granule cells may play a role in the recognition of a familiar but altered spatial environment. In the paragraphs below I will discuss additional evidence that supports the findings reported in experiment 3 of this thesis. But first, it is important to note that a number of observations made in the first two experiments of this thesis support the results of experiment 3.

First, in experiment 1 of this thesis bilateral FFX transection resulted in a consistent

and marked spatial learning impairment in the Morris water maze. This impairment was characterized by the consistently longer escape latencies of bilateral FFx rats during 11 days of acquisition, 4 trials of retention, and 4 trials of reversal or new place learning, and during 4 trials of extramaze and intramaze cue rotation. The impairment observed in the bilateral FFx group in experiment 1 of this thesis is consistent with what has been reported previously for FFx animals who were trained using a novel start procedure (Sutherland and Rodriguez, 1989; Nilsson et al., 1987).

These results, however, do not agree with one previous report where FFx animals were trained using a fixed starting procedure (Eichenbaum et al., 1990). Contrary to the results of Eichenbaum et al., experiment 1 of this thesis demonstrated that bilateral-FFx transected animals did not rely abnormally on extramaze cues even though these animals displayed consistently slower escape latencies in spatial learning when trained on the Morris water maze using a novel start procedure. Bilateral FFx transected animals failed to alter their behaviour when either intramaze or extramaze cues were rotated by 90°. Although it was not clear why bilateral-FFx transected animals display consistently longer escape latencies, longer latencies have previously been reported to be the hallmark of a hippocampal lesioned animal (Morris, 1982). Importantly, however, the results of experiment 1 demonstrate that both the apparatus and procedure used in experiment 2 and 3 of this thesis are sensitive to spatial learning and memory impairments associated with hippocampal alteration. Furthermore, the behavioural consequences of hippocampal alteration associated with FFx transection in experiment

1 highlight the unique role that dentate granule cells must play in controlling the sensitivity of an animal to a familiar but altered spatial environment because a similar sensitivity was not seen in either partial, unilateral or bilateral FFX transected rats.

Experiment 2 in this thesis demonstrated that bilateral knife cuts of the temporal-hippocampal pathway do not result in spatial learning and memory impairments in the Morris water maze. However, correlational data within this experiment suggested that the knife cuts may have been anterior to the portion of the temporal-hippocampal pathway that plays a role in spatial learning. Animals that displayed unilateral or bilateral knife cuts to the temporal-hippocampal pathway, or animals that displayed extensive damage to the areas surrounding this pathway failed to alter their behaviour when intramaze cues or extramaze cues were rotated by 90° on two consecutive days of testing. These results further demonstrate the uniqueness of the impairment observed in animals that displayed moderated to extensive adrenalectomy-induced granule cell loss in experiment 3. The results of experiment 2 are the first to investigate the role of the temporal-hippocampal pathway in spatial learning in the rat.

In addition, these results demonstrate the special nature of spatial learning as a learning and memory task and suggest that both the Morris water maze apparatus and procedure used throughout this thesis are selectively sensitive to the functioning of circuits that are critical for spatial learning.

REPLICATION OF EXPERIMENT 3

Since the first presentation of the data in experiment 3 (Armstrong et al., 1991) there have been a number of reports that support our conclusions. First, in a replication of experiment 3 of this thesis, Conrad and Roy (1993) demonstrated that adrenalectomized rats were able to learn the spatial components of a task in both the Morris water maze and eight-arm radial maze despite showing an 80% reduction in the volume of the dentate gyrus 7 months after adrenalectomy. Although Conrad and Roy did report that ADX rats displayed slightly longer escape latencies during the initial trials of spatial learning in the Morris water maze compared to controls, performance of ADX animals in latter trials was indistinguishable from controls and no correlation was found between Morris water maze performance and volume of the dentate gyrus. The two rats that displayed the most extensive damage to the dentate gyrus were among the best performers in the acquisition of the task. Furthermore, Conrad and Roy failed to find any differences between ADX rats with granule cell loss and controls during 8 trials of reversal learning in the Morris water maze. These results support our conclusion that a full complement of dentate granule cells is not necessary for the acquisition and reversal of spatial learning in the Morris water maze.

In addition, Conrad and Roy (1993) demonstrated that ADX rats with extensive loss of the dentate granule cells were indistinguishable from controls during the acquisition of spatial learning in an eight-arm radial maze when four of the eight arms were baited. ADX rats failed to differ from controls on all aspects of the task including: latency to

retrieve the bait in all four arms, number of reentries into baited arms and the number of entries into nonbaited arms. These results suggest that spatial learning in the eight-arm radial maze is completely unimpaired by adrenalectomy-induced loss of the dentate granule cells.

The results presented in this thesis (Armstrong et al., 1991) and those of Conrad and Roy (1993) provide substantial evidence that a full complement of dentate granule cells is not necessary for spatial learning.

HIPPOCAMPAL ANATOMY REVISITED

Contrary to the lamellar hypothesis, dentate granule cells may not be necessary for spatial learning because according to the most recent anatomical studies, the pyramidal cells in CA3 and CA1 do not rely exclusively on input from the granule cells. There has been a number of changes in our understanding of the organization of the hippocampus within the last few years (Amaral and Witter, 1989; Van Hoesen, 1990; Schwartzkroin, Scharfman and Sloviter, 1990). For example, Amaral and Witter (1989) have demonstrated that three of the four major hippocampal projections are as extensive and highly organized in the septotemporal axis of the hippocampus as they are in the traverse axis. Contrary to the lamellar hypothesis (Anderson et al., 1971) these data suggest that there is substantial dispersion of information along much of the long axis of the hippocampus.

The classic Golgi studies of Ramon y Cajal (1911) and Lorente de No (1934)

demonstrated that the entorhinal cortex is the origin of a strong projection to the dentate gyrus and hippocampus. This projection consists of fibers that emanate from the entorhinal cortex and extend into the underlying white matter where they perforate the subiculum and invade the outer two-thirds of the dentate molecular layer, stratum lacunosum-moleculare of CA3 and the molecular layer of the subiculum (Bayer, 1985; Witter et al., 1988). This so called "perforant path" is organized such that the lateral entorhinal cortex terminates in the superficial third of the lacunosum molecular layer of CA3 and in the superficial third of the dentate molecular layer while the medial entorhinal cortex terminates in the deep third of lacunosum molecular layer of CA3 and in the middle third of the dentate molecular layer (Bayer, 1985).

Although Anderson et al. (1971) have argued that the perforant path is organized in a lamellar fashion, the detailed organization of this projection was not determined until the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) was recently introduced (Gerfen & Sawchenko, 1984). Small iontophoretic injections of PHA-L into several regions of the rat lateral entorhinal cortex (layer II) resulted in the labeling of terminals in the dentate gyrus along a substantial portion of the septotemporal axis of the hippocampus. While the density of the terminal field varied at different septotemporal levels of the dentate gyrus, it extended for approximately 4.5 mm along this septotemporal axis (Witter & Jorfitsma-Byham, unpublished observation; cited in Amaral & Witter, 1989) such that 10% of the entorhinal cortex projected to at least 25%

of the dentate gyrus. Furthermore, injection of two fluorescent retrograde tracers into different levels of the septotemporal axis of the dentate gyrus resulted in the double labeling of cells in layer II of the lateral entorhinal cortex providing additional evidence that these individual entorhinal cells give rise to collaterals that innervate distant septotemporal levels of the dentate gyrus (Witter, Van Hoesen and Amaral, 1989). These data suggest that the entorhinal cortical projection to the dentate gyrus is highly divergent in the septotemporal axis of the hippocampus and that information relayed from the entorhinal cortex diverges over a large extent of the entire dentate gyrus.

Furthermore, there is substantial evidence that cells in the entorhinal cortex send collaterals that terminate in the molecular layer of CA3 and CA1. Tamamaki (personal communication) has demonstrated using intracellular injection of biocytin, that in addition to the projection to the molecular layer of the dentate gyrus, neurons in layer II of the entorhinal cortex also send axon collaterals to the subiculum, CA2 and CA3, while neurons of the deeper layers (III-VI) send axons to CA3 and CA1. These data suggest that information from the entorhinal cortex can access CA1 and CA3 directly -- bypassing the granule cell layer altogether.

The pyramidal cells of CA3 have highly collateralized axons that terminate within CA1 (Blackstaad, 1956; Raisman, 1966). These axon branches are known as the Schaffer collaterals. Recent detailed studies of the intrinsic organization of the Schaffer collaterals has revealed that projections from any particular septotemporal level of CA3 diverge to involve much of the septotemporal extent of CA1 (Ishizuka et al.,

1990). Injection of the anterograde tracer PHA-L into various transverse and septotemporal levels of CA3 resulted in the labeling of projections in CA1 such that CA3 cells close to the dentate gyrus labeled projections in CA1 above the injection site, and CA3 cells located closer to CA1 labeled projections in CA1 located below the injection site (Ishizuka et al., 1990). The greatest density of fiber and terminal labeling was located at a distance of 1 mm or more away from the injection site. Ishizuka et al. demonstrated that CA3 cells located close to the dentate gyrus project further and more heavily in a septal direction whereas cells located near the CA1 border project further and more heavily in a temporal direction. Thus, the Schaffer collateral projection to CA1 arising from one small injection in CA3 can project to as much as three-quarters of the entire septotemporal extent of CA1. These data suggest that information processed at any single septotemporal level of CA3 will be distributed throughout almost the entire extent of CA1.

The overwhelming conclusion of these anatomical studies is that aside from the mossy fibers, none of the intrinsic connections of the hippocampus are organized in a lamellar fashion (Amaral & Witter, 1989). The lamellar hypothesis would suggest that the hippocampus is organized primarily in the transverse and not in the septotemporal axis. In contrast to this hypothesis, however, the projections of the entorhinal cortex and CA3 appear to be specifically organized in such a way as to integrate different septotemporal levels of the hippocampus rather than to isolate them.

The recent anatomical data that emphasizes the septotemporal organization of the

hippocampus suggests two possible ways animals with 95% loss of granule cells could function normally in a spatial learning task. First, these anatomical data provide substantial evidence that information from the entorhinal cortex is dispersed throughout CA1 and CA3 without passing through the granule cells and therefore the trisynaptic loop. Thus, animals without granule cells could function normally during the acquisition of a spatial learning task because all the necessary information is distributed to CA1 and CA3 directly from the entorhinal cortex. However, another explanation supported by these data suggest that spatial learning would be normal in animals that display a 95% loss of granule cells because the remaining granule cells may provide CA3 with all the information needed for CA1 and CA3 to function normally in a spatial learning task. Previously it was noted that any septotemporal level of CA3 sends projections to almost the entire septotemporal extent of CA1 (Ishizuka et al., 1990). This would allow the remaining granule cells in the temporal pole of the hippocampus to provide CA3 and therefore CA1 with all the information necessary to perform the task. Unfortunately however, both these explanations fail to explain why granule cell lesioned animals performed abnormally in a familiar but altered spatial environment. Below I will review some new electrophysiological evidence that suggests why animals that displayed a significant loss of dentate granule were temporarily impaired in spatial learning when exposed to a familiar but altered spatial environment.

ENTORHINAL-HIPPOCAMPAL CONNECTIONS

Recent electrophysiological evidence suggests that the perforant path projections from the entorhinal cortex to the hippocampus, which are now known to include direct projections from the entorhinal cortex to CA1 and CA3 (Tamamki, personal communication), activate different subareas of the hippocampus depending on the frequency of activity generated in the entorhinal cortex (Jones, 1993).

The principle component of the perforant path is the projection from the spiny stellate neurons of layer II of the entorhinal cortex to the dendrites of the dentate granule cells. In fact, this projection makes up the majority of fibres in the perforant path. However, Tamamaki (personal communication) has demonstrated that neurons in layer II of the entorhinal cortex send axons to the dentate granule cells and collaterals to the subiculum, CA2 and CA3; while neurons of the deeper layers (III-VI) send axons to CA3 and CA1. Electrophysiological evidence suggests that layer II neurons are under the rigid controls of local interneurons and therefore have a very high activation threshold; while the thresholds in the layers III-VI are much lower. These data suggest that the dentate granule cells would be activated only when sufficient activity is generated in the entorhinal cortex to overcome the inhibitory postsynaptic potentials (IPSP's) in the layer II stellate cells.

In vivo recordings from electrodes placed in the different layers of the entorhinal cortex in the rat suggest that the synaptic response of layer II cells is dominated by fast and slow inhibitory potentials at low frequency stimulation of input structures such as

the subiculum or adjacent neocortex; while the response in the deeper layers is excitatory (Jones, 1993). These IPSPs are most likely the result of inhibitory basket cells in layer II of the entorhinal cortex. Jones (1993) has demonstrated that as the frequency of the stimulation increases the activity of layer II neurons becomes more and more excitatory allowing greater transfer of synaptic information to the dentate gyrus. This evidence is supported by the observation that low frequency stimulation (0.2 Hz) of the perforant path produced monosynaptic suprathreshold activation of CA1 and CA3 but was subthreshold for the dentate granule cells; while perforant path stimulation at higher frequencies (5-10 Hz) produced suprathreshold polysynaptic excitation in CA1 and CA3 (Yeckel and Berger, 1990).

Yeckel and Berger (1990) have further demonstrated that neurons within the three major subfields of the hippocampus discharge simultaneously in response to input from a given subpopulation of entorhinal cortical neurons and that the initial monosynaptic excitation of pyramidal cells in CA1 and CA3 is often followed by weaker excitatory volleys transmitted through the granule cell layer. Activity in CA1 and CA3 could be evoked by entorhinal cortex stimulation when dentate granule cell activity was blocked by either local pressure injection of the GABA type A agonist muscimol, or when dentate granule cell activity was greatly inhibited by pairing entorhinal cortex stimulation with single pulse stimulation of the contralateral dentate hilus. Even when the granule cells were uninhibited, Yeckel and Berger found that the response of CA3

pyramidal cells to perforant path stimulation often preceded the response of dentate granule cells suggesting that input from the entorhinal cortex initiates two feed forward phases of excitation of pyramidal cells. First, the cells in the entorhinal cortex provide feedforward excitation of CA1 and CA3, and second, the granule cells and CA3 provide feedforward excitation of CA1.

These electrophysiological data provide substantial evidence that low frequency activity in the entorhinal cortex and perforant path may be preferentially oriented towards CA1 and CA3, while high frequency activity may be necessary to activate the granule cells. This evidence further supports our conclusion that a full complement of dentate granule cells are not necessary for spatial learning, as they suggest that granule cell activity may only be necessary to modify ongoing activity in CA1 and CA3 under special circumstances, circumstances that generate enough high frequency activity in the entorhinal cortex to overcome the tonic inhibition of the layer II neurons. Therefore, animals without granule cells would function normally during the acquisition of a spatial learning task because all the necessary information is distributed to CA1 and CA3 directly from the entorhinal cortex, and thus, the granule cells are not activated because the activity in the entorhinal cortex is low. However, once the activity in the entorhinal cortex becomes sufficiently high enough to overcome tonic inhibition of layer II entorhinal cortical neurons, granule cells would become active and would act to modify the processing of information that is already taking place in CA1 and CA3 as a result of activity in all other layers of the entorhinal cortex. Therefore, animals without

granule cells would perform abnormally during behavioural tasks that generate enough high frequency activity in the entorhinal cortex to overcome inhibition in layer II neurons. This explanation would suggest that alteration to a familiar environment might be sufficient to produce enough activity in the entorhinal cortex to activate the dentate granule cells. In fact there are data that demonstrate that there is an increase in synaptic strength between the axons in the perforant path and dendrites in the molecular layer of the dentate gyrus after an animal has been placed in a novel or altered spatial environment (see below).

SHORT-TERM EXPLORATORY MODULATION

Almost all modern theoretical accounts of the biological bases of learning and memory incorporate the notion that synaptic connections between neurons are strengthened when simultaneously active (Hebb, 1949). While there is substantial evidence that this notion is true, most of this evidence is based on the observation that there is an increase in synaptic strength following the generation of short pulses of high frequency activity in certain pathways of the hippocampus in the *in vitro* slice preparation (i.e., long-term potentiation; LTP). Little evidence exists that supports the notion that synaptic strength can be increased as a result of behavioural stimulation. However, Sharp, McNaughton and Barnes (1985) have demonstrated that exposure of an animal to a spatially complex environment for 20 days after 30 days of exposure to a spatially impoverished environment results in an increase in the evoked field excitatory

postsynaptic potential (EPSP) of the dentate gyrus following perforant path stimulation. These data suggest that initial exposure of an animals to a spatially complex environment is enough to induce the type of change that has been proposed to underly learning and memory.

Further analyses of this phenomenon has revealed that exploration of a unfamiliar environment is sufficient to cause this increase in the evoked field-EPSP in the dentate gyrus (Sharp, McNaughton and Barnes, 1989; Green, McNaughton and Barnes, 1990; Moser, Mathiesen and Anderson, 1993). This phenomenon, which has been named short-term exploratory modulation (STEM), persists for 30-60 minutes following cessation of exploration and has been specifically hypothesized to reflect an increase in the synaptic strength because it is associated with a decrease in the amplitude and onset latency of the population spike (Sharp et al., 1989). These results are supported by the findings of Green et al., (1990) who demonstrated that neither the field-EPSP growth nor the reduction in the population spike onset latency could be attributed to a change in perforant path or granule cell excitability. Exploration-dependent electrographic activity in the hippocampus also could not account for the field-EPSP growth or the reduction in the latency onset of the population spike because injection of the local anesthetic tetracaine into the medial septum failed to affect the persistent alterations in the evoked response even though it completely abolished the exploration-dependent hippocampal theta rhythm (Green et al., 1990). Increased locomotor activity also failed to account

for the persistent changes in the evoked response because there was no consistent change in the population spike parameters as a result of stationary locomotor activity on a treadmill (Green et al., 1990). When animals were repeatedly exposed to the same test apparatus over 5 test sessions Green et al. observed a reduction in both exploratory behaviour and field-EPSP growth in all rats. However, when this apparatus was altered by the addition of a red light on the 6th test session Green et al. subsequently observed an increase in both exploration and the evoked field-EPSP. These results demonstrate that exploration of a novel spatial environment results in a substantial increase in the response of dentate granule cells to perforant path stimulation. Furthermore, the results of Green et al. suggest that the granule cells of the dentate gyrus might be specifically attuned to any change in the characteristics of a familiar spatial environment.

However, in contrast to the findings of Green et al. (1990), Moser et al. (1993) demonstrated that the increase in evoked field potentials recorded after exploration of a novel environment is linearly related to an increase in brain temperature caused by the physical demands of exploration. When Moser et al. simultaneously recorded brain temperature and the evoked dentate field-EPSPs while rats explored novel items on a raised platform the increase and subsequent decrease in the dentate field-EPSP 30-60 minutes after cessation of exploration was identical to the corresponding change in brain temperature. Furthermore, warming the brain by radiant heating gave a similar increase in brain temperature and field-EPSP slope in all nineteen animals tested.

Increasing the animals body temperature by forced running on a treadmill also produced

similar changes in brain temperature and field-EPSP in the dentate gyrus. However, when Moser et al. forced rats to run on treadmill to which they had not fully habituated they found identical results to those of Green et al. (1990) reported above. Finally, when the brain temperature of an animal was kept above the maximum value recorded during exploration on the previous day, additional exploration failed to increase the f-EPSP any further despite tests demonstrating that the field-EPSP was not yet at its maximum. Moser et al. concluded that these temperature-related effects on the field-EPSP of dentate granule cells may mask any learning induced changes in these cells. Erickson, McNaughton and Barnes (1993) have stated that regardless of the physiological mechanism underlying STEM, it would be very surprising if this phenomenon did not have important consequences for information processing in the hippocampus.

Recent investigations in both these laboratories have suggested that the enhancement and subsequent decay of the evoked dentate field potential that is observed for 30 to 60 minutes following exploration of a novel testing environment can also occur when brain temperature is held constant (Moser, Moser and Anderson, 1993; Erickson, McNaughton and Barnes, 1993). These results suggest that the increase in the evoked response of dentate granule cells may reflect an increase in synaptic strength that is the result of some specific aspect of spatial learning rather than a consequence of a more general aspect of behaviour such as an increase in physical exertion and brain temperature.

Both the results of Green et al. (1990) and Moser et al. (1993) indirectly support the conclusion that dentate granule cells play a role in the recognition of a familiar but altered spatial environment. Both groups demonstrated that there is an a persistent increase in the evoked field-EPSP in dentate granule cells after exposure of an animal to a novel test environment. Furthermore, Green et al. demonstrated that while there was a general reduction in both exploratory behaviour and EPSP growth over 5 testing sessions, there was a substantial increase in both these measures after the environment was altered. These results suggest that granule cells might only be necessary for the processing of spatial information when an animal is exposed to a environment that it has never experienced before, or when a familiar environment has been altered.

The suggestion that granule cells only play a role in the processing of spatial information upon initial exposure of an animal to a new or altered environment might explain why Conrad and Roy (1993) observed longer latencies in ADX rats on the first 3-4 days of place learning in the Morris water maze when compared to controls. Conrad and Roy gave their animals only one trial to explore the apparatus before initiating the first place learning trial. Therefore, the animals in this study were exposed to a relatively novel environment; an environment that might have required granule cell activation for the normal processing of spatial information. On the other hand, we failed to find any difference between ADX animals that displayed granule cell loss and controls over the first 5 days of place learning perhaps because these animals had been

exposed to the test environment 5 trials before the first trial of place learning. Thus, by the time we actually started to measure place learning latencies, the test environment in which spatial learning was taking place was no longer novel enough to require granule cell activation. The observation that GCL animals in experiment 3 of this thesis were temporarily impaired following intramaze cue and extramaze cue rotation suggest that these conditions provided enough alteration to the familiar test environment to require the activation of granule cells for the normal processing of spatial information in the control group and the abnormal processing of spatial information in the animals without granule cells. However, additional investigations are needed to determine the effects of intramaze cue and extramaze cue rotation on the evoked dentate field-potential in the normal and adrenalectomized rat.

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