# The Effects of Neurotoxic Lesions of the Hippocampus and Subiculum on Memory

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Submitted for the degree of M.Sc.,
St Andrew's University.
March 1988.

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ACKNOWLEDGEMENT

I should like to thank Dr. R. Morris for supervising this thesis, Miss E. Forrest for supervising the histology and the animal technicians for looking after my rats.

ABSTRACT

Jarrard (1986) showed that ibotenic acid lesions of the hippocampus caused only a transitory impairment in the performance of a place task using the radial arm maze. In contrast, ibotenic acid lesions of the subiculum caused a sustained impairment on this task. Accordingly, rats were given identical lesions and tested on a spatial water-maze task (HIP, N = 14; SUB, N = 14; operated controls, N = 7; controls, N = 7). A strict histological criterion reduced the group sizes (HIP, N = 5; SUB, N = 6) and necessitated forming two more groups (HIP+SUB, N = 6 SUB+HIP, N = 4). The results on the place task were similar to Jarrard's (1986) result. However, a spatial working memory task revealed an impairment in all groups given lesions, in contrast to Jarrard's (1986) findings on the radial maze. In a further experiment, spatial working memory was also found to be impaired over each of 4 retention intervals (0 minutes, 3 minutes, 24 minutes, 3 hours) after a single training trial. A visual discrimination task revealed learning deficits in both the HIP and the HIP+SUB groups. Comparison of sub-groups trained 12 days or 100 days after surgery showed no time-dependant improvement in HIP rats but a significant improvement in SUB and SUB+HIP rats. The reasons for the different results obtained in the water-maze compared to the radial maze are discussed.

CONTENTS

# CHAPTER 1

# THEORY

## 1.1 Introduction

This thesis was designed to assess the extent to which the hippocampus mediates spatial information processing. The principle foundations on which this research is based are the spatial mapping theory (O'Keefe and Nadel, 1978) and the working memory theory (Olton, Becker and Handelmann, 1979).

O'Keefe and Nadel (1978) proposed that the hippocampus and adjacent structures (such as the medial septal nucleus, fimbria fornix, subiculum and entorhinal cortex) act as the neural substrate of place hypotheses, that is, they are involved in learning the spatial relations between objects in the environment and building a cognitive map. This map is updated during a trial to accommodate the sensory information arriving from the environment. This involves changing the whole map and not just the location of the rat (O'Keefe and Speakman, 1987). These changes only take a short period of time to complete but they can last for at least thirty minutes during which time they can produce large shifts in direction of movement within a trial. Distal cues play an important role in the cognitive mapping theory. O'Keefe and Nadel (1978) propose that rats learn the relative location of distal cues irrespective of the location of reward.

In the search for an alternative explanation, Olton et al. (1979) proposed the working memory theory. Although this is a more general theory than the spatial mapping theory, they are similar in that they both consider the hippocampus to be involved in memory and they are both cognitive theories.

## 1.2 Cognitive Mapping Theory

The cognitive mapping theory predicts that animals with hippocampal lesions will be impaired in storage and retrieval of allocentric spatial information, that is, they will be unable to perform either acquisition or retention tests on the radial arm maze or the watermaze. The cognitive mapping theory expects perseveration to appear whenever normal rats cannot use a cognitive mapping strategy and whenever rats with damage in the hippocampal system are unable to perform as well as normal rats.

O'Keefe and Nadel (1978) and O'Keefe (1983) hypothesised that there are several spatial systems. These include locale systems (learning about places) which are differentiated from taxon systems (learning to approach specific visible or audible cues), distinguished by guidance and orientation hypotheses, and involving different neuroanatomical systems which appear to act independently of each other. The locale-taxon distinction also includes differences in the way internal and external cues are used to solve problems during both acquisition and retention.

Several factors are important in influencing the choice of hypothesis. Place learning will only occur if there are a large number of cues spread out in the environment, rats will use a guidance hypothesis if the only cues are all behind the goal, and an orientation hypothesis if there are too few cues. However, other factors such as the reason for one hypothesis overshadowing another have not been specified. Rats vary in their choice of retrieval cues to locate the goal.

Properties of place strategies include rapid learning and behavioural flexibility (O'Keefe and Nadel, 1978). These have been confirmed by Morris (1981). Animals with hippocampal lesions lose the functions mediated by the locale system and so must depend upon

taxon mechanisms and in particular the behavioural orientation system which bias the rat towards persistent and stereotyped behaviour. This behavioural orientation system is involved in controlling behaviour in terms of the position of body axes within space (O'Keefe, 1983). Few of the properties of this orientation system have received experimental support. One property that has been demonstrated is that changes in the system require that the orientation vector rotates within an egocentric polar coordinate space as a result of a previous turn. Experiments have indicated that the orientation system incorporates changes as a function of experience. However, these changes may not be evident in certain behavioural paradigms which are only sensitive to large shifts in behaviour causing the rats behaviour to be misinterpretated as response perseveration. Other properties, if demonstrated, would help to explain the hippocampal deficit. These include the time dependent properties following turning and also the effects of reward on the change in orientation.

O'Keefe (1983) hypothesised that the three spatial systems can operate simultaneously. He proposed that in order to learn a task which required place (or guidance) hypotheses, the orientation hypothesis would need to be rejected. At the presolution stage the animals would use the two hypotheses.

Whishaw (1985) hypothesised that there are two kinds of place navigation. Normal rats initially solve the task using a locale strategy but eventually transfer navigation to taxon control. Whishaw (1985) suggested that atropine blocks both acquisition and retention of a place response when it is acquired rapidly because the animal is using a locale strategy. However, atropine does not block performance if an animal is given extensive training because it allows the rat to transfer to a taxon strategy. However these

results could be explained by the use of two locale systems with different storage processes. Alternatively, if there is only a locale or taxon system it may be less efficient under atropine.

O'Keefe rejects Restle's (1957) idea that place and cue learning only differ through the use of the distal cues in place learning. Even so, he has not determined the criteria to distinguish spatial and nonspatial learning. He has been criticised by Gray (1979) for not specifying the part of the brain responsible for taxon strategies.

O'Keefe and Nadel (1978) proposed three stages for the mapping system involving the three main subregions (dentate gyrus, CA3 and CA1). In the first stage the dentate gyrus organises sensory inputs for the map via the taxon systems. In the second stage, the CA3 forms the initial part of the map and represents places and the connections between these places in an environment. Finally, in CA1 the formation of the map is continued. Here also, is a misplace system to detect a mismatch between the environmental inputs and the animal's map. These mismatch signals cause exploration in order to acquire the information for initially building and subsequently updating cognitive maps. The sensory information for this system arrives via the perforant path and information about the rat's movements in space and also possibly arousal or attention travels in via the brainstem - medial septal pathway.

The strongest evidence for this theory comes from unit recordings. Results show single units, especially those in the CA1 cell field but also in CA3 and CA4 cell fields, discharging in relation to spatial position cues (O'Keefe, 1976; O'Keefe and Dostrovsky, 1971; Olton, Branch and Best, 1978; Kubie and Ranck, 1983). However, O'Keefe and Dostrovsky (1971) data show that only 8 out of 76 cells tested responded only when the rat was in a

particular place or was oriented in a particular direction but not where he intended to go to. Therefore, although this data is certainly suggestive, it does not prove that the hippocampus is capable of creating maps and does not explain how rats choose the appropriate direction to find the goal. Quantitative analyses have involved estimating field sizes from videotapes of behaviour (Hill, 1978) or calculating firing rates for an arm of a maze (Olton et al., 1978; Miller and Best, 1980). Precise quantitative analyses has been difficult because the firing in the place field is not homogeneous and the selection of the cut off point for the boundary of the field has been random.

O'Keefe (1983) has proposed four possible functions for the place cells in memory. First, synaptic change in the place cell may form place representations. Experiments have shown that a cell will fire when only a small percentage of synapses on to either the dentate granule cells (McNaughton, Barnes and Andersen, 1981) or the CA1 pyramidal cells (Andersen, Silvenius, Sundberg and Sveen, 1980) are activated. Simultaneous activation of some of these would increase the potency of each. This might account for O'Keefe and Conway's (1976) finding that any two of the four extramaze cues cause place cells to fire. Secondly, connections may be formed between the place cells themselves in order to form place representations. Thirdly, place cells may preferentially encode the location of salient features. However, evidence shows that food or water is not encoded differently from other cues. Finally, O'Keefe (1983) proposes that place cells encode the temporal order of visiting locations although he can find no evidence to support this.

Pharmacological work supports this theory. Atropine sulphate causes impaired spatial localisation in the watermaze (Sutherland, Whishaw and Regehr, 1982; Hagan, Tweedie and Morris, 1986).

## 1.3 Working Memory Theory

Olton et al. (1979) contrasted reference memory for retaining information that remains constant in the animal's environment with working memory for retaining information useful for only a single trial (see Honig, 1978). In the working memory theory, rats with lesions of the hippocampus should differentially have a performance deficit in acquisition or retention of a task requiring working memory; reference memory should be spared. This claim by Olton et al. (1979) that there is a category of allocentric spatial tasks which rats with damage to the hippocampus can solve is the basis for comparing the spatial mapping theory with the working memory theory in this thesis.

However, the distinction between working and reference memory can never be complete as working memory experiments always contain some reference memory components (Morris, 1983; Jarrard, 1986). The mechanism of action of these two systems has not been determined and the differing characteristics not described. However, Olton et al. (1979) suggest several possibilities. Working memory tasks require flexible stimulus-response associations while reference memory tasks are composed of fixed stimulus-response associations. Properties of working memory include limited capacity and thus the necessity for resetting at the end of a test (Olton, 1978a). The contents of working memory do not decay rapidly over time (Olton and Samuelson, 1976).

This theory is less satisfactory than the cognitive mapping theory as it is only based on operational criteria. It is incompatible with the cognitive mapping theory because it implies that brain areas outside the hippocampus are capable of allocentric spatial perception. Furthermore, in contrast to the spatial mapping

theory, the working memory system processes both spatial and non-spatial information. O'Keefe's view of the radial arm maze as a test for cognitive mapping with the extramaze cues being the components of the map conflicts with Olton's view of the maze as a test of spatial working memory and the extramaze cues as isolated stimuli by which the rats identify the arms of the maze.

Behavioural evidence for the working memory and cognitive mapping theory will be reviewed in the next chapter.

## 1.4 Interference Theory

Jarrard and Elmes (1982) dispute that the role of the hippocampus is to control a particular function such as working memory or cognitive mapping. Instead, they propose that the hippocampal formation is involved in retrieval processes. Evidence for this stance comes from the finding of retroactive interference in control animals when relearning a set of 4 arms in a 12-arm radial maze after training on a different second set of 4 arms. This is contrary to the predictions of the cognitive mapping theory. Jarrard and Elmes (1982) suggest that during relearning control animals choose incorrectly due to the increased processing involved. Animals with lesions of the hippocampus showed no effect of retroactive interference which Jarrard and Elmes (1982) suggest is due to their total inability to retrieve relevant information. Thus they hypothesise that the hippocampus is involved in the correct retrieval of responses.

## 1.5 Comparator Theories

Two theories consider areas of the hippocampal formation to act as comparators. Douglas (1967) hypothesised that the hippocampus proper was a comparison device which produced its output after

comparing inputs from the entorhinal area and the fimbria fornix. Conventional lesion studies involving the sectioning of the extrinsic connections of the hippocampus (Olton, Walker and Gage, 1978) are consistent with this theory. Gray (1982) considers the subiculum to be the central comparator which receives sensory information from the entorhinal cortex. This information also travels to the dentate gyrus where it is assessed and if important sent on to the subiculum via the CA3 and CA1 cells. These two inputs to the subiculum are compared in order to decide the next item of importance. Evidence exists for an input to the subiculum from layers II, III and IV of the medial entorhinal cortex (van Groen and Lopes da Silva, 1986; Van Groen, Van Haren, Witter, and Groenewegen, 1986), as well as a return pathway from the subiculum to the entorhinal cortex, suggesting that the entorhinal cortex may also be an area which could serve a comparator function.

## 1.6 Inhibitory Theories

Several theories propose an inhibitory role for the hippocampus. These vary from an inability to generate internal inhibition (Kimble, 1968; Douglas, 1972) to an inability to eliminate errors (Douglas and Pribram, 1966) an inability to inhibit attention (Hendrickson, Kimble and Kimble, 1969) or an inability to inhibit hypotheses (Isaacson and Kimble, 1972). However, these theories cannot adequately account for the results found on spatial tasks.

A more recent behavioural inhibition theory postulates that the hippocampus is involved in control of anxiety (Gray, 1982). However, unlike anti-anxiety drugs, hippocampal lesions do not lower the level of arousal elicited by conditioned aversive stimuli (Gray, 1982).

# CHAPTER 2

# ANATOMY

## 2.1 Definition

The hippocampus is defined as the hippocampus proper (Cornu Ammonis) and the dentate gyrus (fascia dentata) (Gottlieb and Cowan, 1973). The hippocampal formation is defined as the hippocampus and the subiculum.

## 2.2 Site

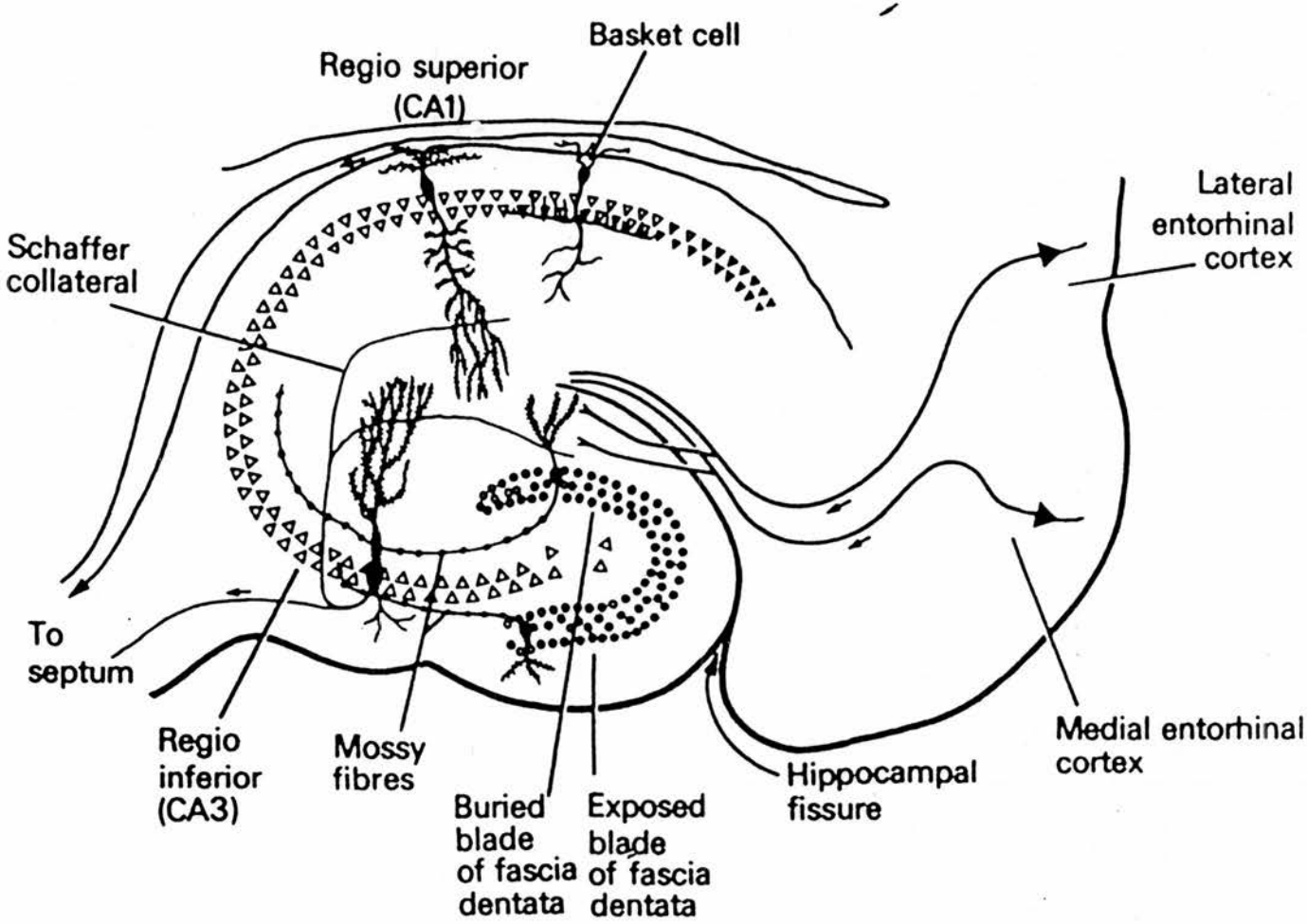
The hippocampal formation occupies a large part of the rat forebrain. O'Keefe and Nadel (1978) have described it as a large sausage shaped structure (see Fig. 1) The dorsal part lies posterior to the septum, the posterior part is where the sausage bends ventrally and laterally and the ventral part lies in the temporal area of the brain. The subiculum lies caudal to the hippocampus.

## 2.3 Hippocampal circuitry

Both anatomical and electrophysiological research has shown that the hippocampal formation has a neuronal circuit consisting of entorhinal cortex → perforant path → dentate gyrus → mossy fibres → CA3 cell field → Schaffer collaterals → CA1 cell field → alveus → subiculum → entorhinal cortex (Hjorth-Simonsen, 1973; Swanson, Wyss and Cowan, 1978; Andersen, Bland and Dudar, 1973). This circuitry divides the hippocampal formation into a series of lamellae that run at right angles to the septo-temporal axis. Each lamella consists of perforant path-dentate gyrus-CA3-CA1 connections that project primarily to the other components on that level (Andersen, Bliss and Skrede, 1971; Rawlins and Green, 1977; Ruth and

FIG. 1

Diagram of the hippocampus (from O'Keefe and Nadel, 1978).


Basket cell
Regio superior
(CA1)
Lateral
entorhinal
cortex
Schaffer
collateral
To
septum
Regio
inferior
(CA3)
Mossy
fibres
Buried
blade
of fascia
dentata
Exposed
blade
of fascia
dentata
Hippocampal
fissure
Medial entorhinal
cortex

Routtenberg, 1978).

## 2.4 Cytoarchitecture

Most knowledge of the fine structure of the hippocampus and subiculum is due to Cajal (1911) and Lorente de No (1934) using reduced silver and Golgi impregnation. This is discussed by O'Keefe and Nadel (1978); Gray (1982); Swanson (1978, 1983); Isaacson (1974) and Chronister and White (1975).

## 2.5 The Hippocampus

The hippocampus proper and dentate gyrus are two interlocking C-shaped structures. Three fundamental layers exist (the external plexiform, pyramidal and polymorphic cell layers), and secondary laminae are formed by the arrangement of axons and dendrites.

### a. Dentate Gyrus

The dentate gyrus has an outer blade adjacent to the diencephalon and rostral midbrain and an inner blade along the hippocampal fissure. Within the "C" of granule cell bodies is the hilar region (CA4).

The dentate gyrus is made up of three layers. The laminated molecular layer contains the apical dendrites of the granule cells. Their axons (mossy fibres) traverse from the polymorph layer to the interior of the "C" to cross through the stratum pyramidale and form the infrapyramidal bundle below the stratum pyramidale of the hippocampus proper and the suprapyramidal bundle above it. The suprapyramidal bundle extends across the entire mediolateral extent of field CA3. The infrapyramidal mossy fibres extend along the outer blade of the dentate gyrus (Haug, 1974). The granule cell layer consists of densely packed granule cells and basket cells which exert

an inhibitory influence on the granule cells. Horizontal cells and cells with horizontal axis cylinder are found in the polymorph layer. In the molecular layer there are two types of cell with short axis cylinders. Commissural and associational afferents from the hilar regions terminate in the molecular layer. The outer molecular layer receives projections from stellate cells of the ipsilateral lateral entorhinal cortex and a sparse contralateral projection. The middle molecular layer is innervated primarily by the ipsilateral medial entorhinal area. These entorhinal afferents travel via the perforant path (the main input to the dentate gyrus and hippocampus proper). The supragranular strip, adjacent to the cell bodies, receives septal and hypothalamic inputs and inputs from ipsilateral and contralateral CA4. Interneurons in the infragranular zone are innervated by granule cells, the septum, locus coeruleus and raphe nuclei (Mellgren and Srebro, 1973; Mesulam, Mufson, Wainer and Levey, 1983; Mosko, Lynch and Cotman, 1973).

b. Hippocampus Proper

The hippocampus proper consists of cell fields CA3 (or regio inferior), which is a semicircle close to the dentate gyrus, CA1 (or regio superior), which lies next to the prosubiculum, and CA4, lying inside the hilus of the dentate gyrus. These cell fields will now be discussed.

CA4 consists of scattered pyramidal cells. These are similar to the cells in CA1 and CA3 in that they are pyramid-shaped, receive mossy fibres and send their axons to the fimbria. All the pyramidal cells (CA1, CA3, CA4) receive inputs from entorhinal cortex (Hjorth-Simonsen, 1971; Swanson and Cowan, 1977; Steward, 1976). The hilar region (CA4) projects to the stratum moleculare of the dentate gyrus (Zimmer, 1971; Gottlieb and Cowan, 1973). In addition, the CA4

has efferents to the septum and to neurons in the vertical and horizontal limbs of the diagonal band (Segal and Landis, 1974). Also, CA4 (and CA3) innervates the contralateral hippocampus (Segal et al., 1974; Swanson and Cowan, 1977).

The CA3 cell field adjoins the dentate gyrus and consists of large pyramidal cells and pyramidal basket cells which form the stratum pyramidale. The proximal dendrites from these cells form the stratum lucidum and the stratum radiatum and the distal dendritic zone is called the stratum lacunosum/moleculare (Blackstad, 1956), where the mossy fibres terminate or form fibres en passage. The stratum radiatum is innervated by the ipsilateral and contralateral regio inferior while the stratum moleculare receives entorhinal afferents.

Basal dendrites form the stratum oriens (Blackstad, 1956). This layer receives commissural projections from contralateral pyramidal cells of area CA3 (Segal and Landis, 1974). This layer contains horizontal cells, basket cells and poligonal cells. The cells are influenced by the medial septal nucleus and adjacent interneurons (the medial aspect of medial septal nucleus projects to the dorsal hippocampus, while the lateral aspect projects to the ventral hippocampus to both CA3 and CA1) (Segal and Landis, 1974). The axons of the CA3 pyramidal cells bifurcate creating Schaffer collaterals projecting to the stratum radiatum of regio superior and branches that terminate in the septum and contralaterally in regio superior or inferior. Some pyramids send collaterals to form an association path. Like the Schaffer collaterals, the association path terminates in stratum radiatum and stratum oriens (Hjorth-Simonsen, 1973). The associational connections to the regio superior from field CA3 do not extend into the stratum lacunosum-moleculare (Swanson et al, 1978). A few efferents travel

to the septum and lamina IV of the medial entorhinal cortex (Hjorth-Simonsen, 1971). In addition, field CA3 gives rise to small projections to the presubiculum, parasubiculum, entorhinal cortex, perirhinal, retrosplenial and the cingulate areas (Swanson, 1979).

In the CA1 field there are a few rows of densely packed pyramids in close contact with basket cells to form the stratum pyramidale. The axons of these pyramidal cells give off several ascending collaterals that ramify in the stratum radiatum and several others which remain in the stratum oriens (Swanson et al., 1978). Apical dendritic zones include stratum moleculare and stratum lacunosum-moleculare. CA1 projects to the stratum moleculare of the subiculum and to a lesser extent to the pyramidal cell somata. The projection of field CA1 to the subiculum shows a topographic ordering (Swanson and Cowan, 1977).

The alveus becomes continuous with the fimbria-fornix along the medial border of the hippocampus carrying extrinsic hippocampal projections plus commissural projections and entorhinal fibres which pass caudally to the anterior commissure. The temporal part of field CA1 (or possibly the adjacent part of the ventral subiculum) gives rise to fibres which form part of the rostral extension of the postcommissural fornix and travel to the bed nucleus of the stria terminalis, the nucleus accumbens, the medial and posterior parts of the anterior olfactory nucleus, the taenia tecta, and the infralimbic area (Swanson and Cowan, 1977). Some of the axons of the pyramids in field CA1 have long recurrent collaterals which travel through the stratum oriens and the alveus, beyond the subiculum, to the presubiculum and parasubiculum and to the entorhinal, perirhinal, retrosplenial and cingulate areas (Swanson, 1979). The stratum moleculare is innervated by Schaffer collaterals and commissural fibres. The stratum lacunosum-moleculare receives bilateral

entorhinal input via the alvear pathway (tempero-ammonic tract). Medial entorhinal afferents terminate in CA1 farthest from the subiculum while lateral fibres terminate in CA1 adjacent to or in the subiculum. The stratum oriens receives commissural and septal afferents.

Autoradiography has revealed that every level of the diencephalon and brainstem projects directly to a part of the hippocampal formation (Wyss, Swanson and Cowan, 1979). This will now be discussed. Both CA1 and CA3 cell fields receive cholinergic input from the medial septal nucleus and the diagonal band of Broca (CA1 receives the least) (Mellgren et al., 1973; Mesulum et al, 1983; Mosko et al., 1973). The hippocampal cells receive noradrenergic and serotonergic input from cells in the locus coeruleus and the dorsal and median nuclei of raphe respectively. (Dahlstrom and Fuxe, 1964; Segal and Landis, 1974; Moore and Halaris, 1975). The nucleus locus coeruleus projects to the hippocampus ipsilaterally (Pickel, Segal and Bloom, 1974; Segal, Pickel and Bloom, 1973) and contralaterally (Segal and Landis, 1974). Neurons in the ventral tegmental area project to the hippocampus suggesting a dopaminergic input (Wyss et al., 1979). There is a projection from the nucleus reuniens and the adjoining paraventricular and parataenial nuclei of the thalamus to the hippocampus proper via the cingulum (Herkenham, 1978; Wyss et al., 1979; Swanson and Cowan, 1975). Some scattered cells in the preoptic region and in the adjacent parts of the substantia innominata and anterior amygdaloid area, and in the lateral hypothalamic area project to the hippocampus (Wyss et al., 1979). However, these cells are so diffusely arranged that the intrahippocampal distribution has not been determined. The supramammillary region projects to the hippocampus (Segal and Landis, 1974; Pasquier and Reinoso-Suarez, 1976). Adjacent cell groups such

as the tuberomammillary and the ventral premammillary nuclei and restricted parts of the lateral and medial mammillary nuclei (especially the pars posterior of the latter) are involved in the hypothalamo-hippocampal projection (Wyss et al., 1979). The supra-mamillary and adjacent regions project to the dendrites of the dentate granule cells (Wyss et al, 1979). Neurons in the reticular formation (from the midbrain to the medulla) and in the central gray project directly to the hippocampus (Wyss et al, 1979). The interpeduncular nuclear complex and part of the dorsal tegmental nucleus project to the hippocampus (Wyss et al, 1979): the interpeduncular nuclear complex indirectly receives hippocampal input via the septum (Swanson and Cowan, 1976) while the dorsal tegmental nucleus receives a projection from the subiculum via the mammillary nuclei (Guillery, 1956; Cruce, 1977).

There are pathways between the primary somatosensory cortices and the hippocampal formation in the monkey through the inferior parietal areas (Vogt and Pandya, 1978; Pandya, Van Hoesen and Mesulam, 1979; Seltzer and Van Hoesen, 1979; Van Hoesen, 1980; Seltzer and Pandya, 1980). In the case of visual input, the hippocampus receives an input from primary visual cortex which travels through the prestriate and inferotemporal areas (Kuypers, Szwarcbart, Mishkin and Rosvold, 1965; Pandya and Kuypers, 1969; Jones and Powell, 1970), on to the entorhinal and parahippocampal areas (Whitlock and Nauta, 1956; Jones and Powell, 1970; Moss, 1974; Seltzer and Pandya, 1976; Van Hoesen and Pandya, 1975a) and so to the hippocampus (Van Hoesen and Pandya, 1975b; Van Hoesen, 1980).

## 2.6 The Subicular Complex

The subiculum has been subdivided into the presubiculum, subiculum and parasubiculum. The subiculum lies immediately

adjacent to field CA1 of the hippocampus (Blackstad, 1956). The parasubiculum lies immediately adjacent to the entorhinal cortex. The presubiculum is continuous with parasubiculum. The transition from presubiculum to subiculum is characterised by the appearence of the laminar principalis externa of the presubiculum.

a. The subiculum

The morphology in the subiculum is transitional between the simple cortical structure of the hippocampus and the six-layered entorhinal neocortex. Here the cells are no longer in a row. The main cells are the pyramids lying in what is a continuation of the stratum pyramidale of the hippocampus. A plexiform layer contains the dendrites of these cells. Polygonal cells are found in the deep part of the pyramidal layer. Part of the perforant path travels in the wide plexiform layer en route to the dentate gyrus. Some of the axons from the pyramidal cells in the deep pyramidal layer go backwards and enter the presubiculum; others travel to the entorhinal and perirhinal cortices (Swanson et al., 1978). However the majority go in a rostral direction entering the fimbria.

The subiculum projects to the septum, nucleus accumbens and to the hypothalamus (Kelley and Domesick, 1982; Swanson, 1979), and to the entorhinal cortex (van Groen etal, 1986). Postcommissural fibres (primarily from dorsal subiculum and presubiculum travel to the hypothalamus, thalamus and particularly the mammillary bodies (Swanson and Cowan, 1975, 1977). The medial cortico-hypothalamic tract arises in the ventral subiculum and is known to terminate in the ventromedial nucleus of the hypothalamus from the level of the suprachiasmatic nucleus to the level of the medial mammillary nucleus (Swanson and Cowan, 1977). The subiculum is important as the area where the majority of both cortical and subcortical hippocampal

efferents originate (Swanson and Cowan, 1975, 1977; Rosene and Van Hoesen, 1977). The ventral part of the subiculum receives projections from the basolateral nucleus of the amygdala, the endopyriform nucleus and the periamygdaloid cortex (Krettek and Price, 1977). Further there are septo-subicular fibres (Swanson and Cowan, 1979).

b. Presubiculum

The presubiculum consists of two layers of cells called the lamina principalis externa and interna. These are separated by a cell-poor area called the lamina dissecans. The presubiculum contains a dense layer (II) consisting of small granular cells (Blackstad, 1956). Commissural fibres terminate in most of the presubiculum. There is a bilateral projection to layers I and III of medial entorhinal cortex, the crossed projection reaching the contralateral side by way of the dorsal hippocampal commissure (Shipley, 1975).

c. Parasubiculum

The parasubiculum consists of two parts "parasubiculum a" and "parasubiculum b" (Blackstad, 1956). There is a bilateral projection to the parasubiculum from the contralateral parasubiculum (Swanson and Cowan, 1977). The parasubiculum projects to layer II of the ipsilateral entorhinal cortex, with a crossed projection to the contralateral entorhinal area. Both pre- and para-subiculum project to the mammillary nuclei, the anterior thalamic and associated lateral dorsal nucleus (Swanson and Cowan, 1977). Parasubiculum a receives fibres from the basolateral nucleus of the amygdala (Krettek and Price, 1977). The subicular complex receives a projection from the nucleus reuniens of the thalamus and adjoining paraventricular

and parataenial nuclei of the thalamus (Wyss et al., 1979).

# CHAPTER 3

# BEHAVIOURAL REVIEW

## 3.1 Introduction - Two behavioural tasks

The results from two behavioural tasks have been the main contributing factors in the controversy between O'Keefe's spatial mapping and Olton's spatial memory theories. These tasks are the radial arm maze (Olton and Samuelson, 1976) and the open-field water-maze (Morris, 1981). Both of these behavioural paradigms are used to study allocentric spatial reference memory (O'Keefe and Nadel, 1978; Suzuki, Augerinos and Black, 1980; Sutherland and Dyck, 1984). Both apparati can be surrounded by many spatial cues. Other spatial tasks used previously, such as T-mazes, have been criticised for not requiring true allocentric processing and instead only requiring egocentric (location relative to the body, e.g. left, right), or guidance processing (true taxis, e.g. approach to a specific object).

The radial arm maze is now considered to be a test of spatial working memory (Olton, 1979; Olton and Collison, 1979; Olton, Collison and Werz, 1977; Olton and Papas, 1979; Roberts, 1979; Suzuki, et al 1980). It is a variation of the delayed conditional discrimination procedure because correct choices in the radial maze are conditional on previous choices made by a rat earlier in a trial (and not upon some overt stimulus such as an

olfactory trail). It consists of eight arms pointing outwards from a central platform like the spokes of a wheel. The spatial location of each arm is defined by extra-maze cues. One trial is given each day. Food is hidden at the end of each arm; then a rat is left in the centre to run out along the arms. The trial ends when the rat has eaten all eight pellets of food, ten minutes have elapsed since the start of the trial or two minutes have elapsed in which the rat has made no choice. Olton and Samuelson (1976) demonstrated that after approximately twenty tests (one test per day) and using the optimal strategy of choosing each arm once, rats could achieve the criterion of seven correct responses in the first eight choices for five consecutive days. Also, rats are able to perform accurately on a similar maze with 17 arms, but performance falls off on the later arm-choices (Olton et al. 1977). Solving this task is helped by the rats strong win-shift tendency (Olton et al. 1977).

Ability to perform this task requires that the animal acquire general information that applies to all trials (e.g. the behaviour of searching for food around the maze, the fact that food can be obtained only at the end of each arm etc.), which Olton refers to as "reference memory" components of the task following the terminology of Honig (1978). Also, the rat must remember information that is only applicable to a single trial and so is short lasting, which Olton refers to as "working memory" components of

the task following the terminology of Honig 1978. Olton's experiments suggest that rats have a specialised system for processing both spatial and non-spatial memory.

The second paradigm used is a "water-maze". This is a circular pool situated in a room surrounded by salient cues. Curtains can be pulled round the pool to exclude these cues. The water is made opaque and so prevents a rat from seeing a platform lying beneath the surface, by filling the pool with water mixed with dried milk. (Morris, 1981).

Several different tasks can be conducted in the water-maze (Morris, 1984). The basic hidden platform task is very simple, normal rats needing only about 4-10 trials to learn it. This is operationally, a spatial reference memory task which may require "spatial mapping" (O'Keefe and Nadel, 1978). Several measures of the rat's behaviour are consistent with this, including transfer tests (when the escape platform is absent) which show that a much larger proportion of their swimming time is spent traversing the training quadrant than any other quadrant.

The water-maze can not only be used to study long term memory in the hidden platform task but can also be used to study working memory by giving two trials to the same position and changing that position daily. The latency of the second trial is about half of the first trial after a 30 second delay (Morris 1983).

Both the radial arm maze and the water-maze indicate

that spatial working memory is long lasting. This varies from tens of minutes (Olton et al. 1979) to up to eight hours (Beatty and Shavalia, 1980) in the radial arm maze. The latter result suggests a ceiling effect where the memory can be recalled, however weak, using long term memory. However, in contrast to this result Buresova (1980) has found that rats were unable to perform on the radial maze after a 1 hour interruption period in the middle of a trial. Using the water-maze, Panakhova, Buresova and Bures (1984), studying retention after a 1 minute, 60 minutes, 4 hours or 24 hours interval between trial 1 and trial 2 in the working memory task, found that retention deteriorated as the interval increased. These observations agree with the trace decay hypothesis (Roberts and Grant, 1976) which considers that the memory trace decays at a constant rate from stimulus termination.

## 3.2 Lesions of Extrinsic Connections

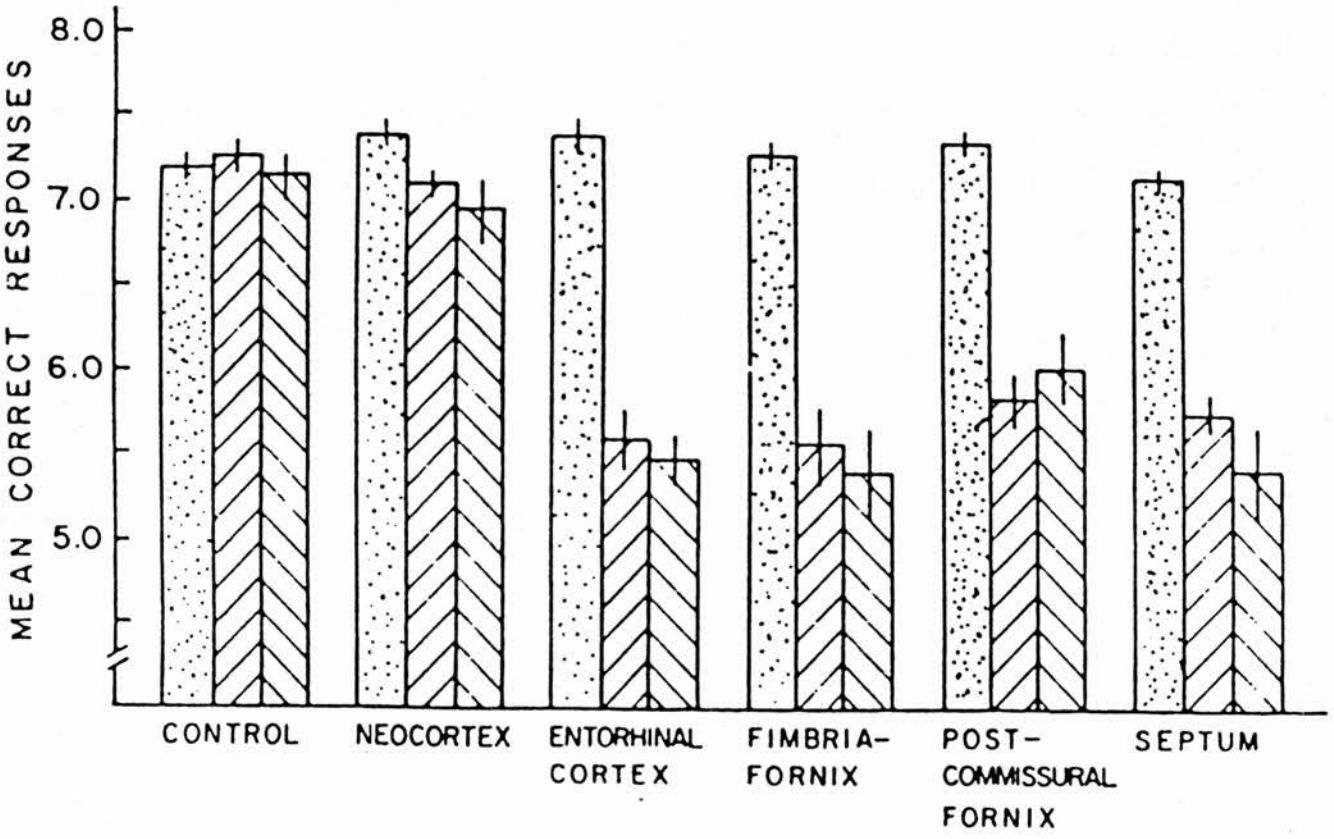
In early experiments it was assumed that the hippocampal formation had an integrated function. Hence experiments involved interrupting the fibres from the hippocampus to nearby anatomical structures. However, these lesions are not of the hippocampus proper which must be remembered when making inferences about hippocampal function. Despite the fact that the effects of fimbria-fornix lesions and entorhinal cortex lesions on learning tasks are equivocal, much of the support for both the

spatial mapping and especially the working memory theories is based on studies involving lesions of these connections. The working memory theory assumes that any lesion that produces complete bilateral destruction of an extrinsic fibre connection of the hippocampus will disrupt choice accuracy in a working memory procedure. Results from several experiments support this assumption.

Following preoperative training in an 8-arm radial arm maze, rats were given bilateral electrolytic lesions in either the entorhinal cortex, fimbria-fornix, precommissural fornix, or postcommissural fornix, (Olton, et al., 1978). They were then given 50 test sessions (5 times as many test sessions as in preoperative training). The lesioned animals were severely impaired in choice accuracy (working memory) compared to controls and the degree of impairment was similar for the four lesioned groups. (See Fig. 2). Further analysis of the choice patterns for the lesioned groups showed that after about two correct responses at the beginning of each test they began to repeat their previous choices. However, these animals could still remember the general rules of the experiment, such as running down the arms and collecting the food (i.e. reference memory). These results support the working memory theory (Olton et al. 1979). They can also be interpreted as support for the cognitive mapping theory, with the poor choice accuracy of the lesioned groups being due to an inability to identify correctly the

FIG. 2

This diagram shows the mean number of correct responses in the first 8 choices. For each group the first bar (light stipple) represents the preoperative criterion data, the second bar (lines slanted down to left) represents the first postoperative testing data, the last bar (lines slanted down to right) represents the 50-day postoperative testing data. (From Olton, et al. 1978).


MEAN CORRECT RESPONSES
8.0
7.0
6.0
5.0
CONTROL
NEOCORTEX
ENTORHINAL CORTEX
FIMBRIA-FORNIX
POST-COMMISSURAL FORNIX
SEPTUM

spatial location of each arm. The repeated sequences of choices is also consistent with the failure to inhibit responses in animals with hippocampal lesions (Douglas, 1967; Issacson, 1974; Gray 1982). Equivalent performance by the fimbria-fornix and entorhinal cortex animals suggests that the dentate gyrus acts as a throughput system for information.

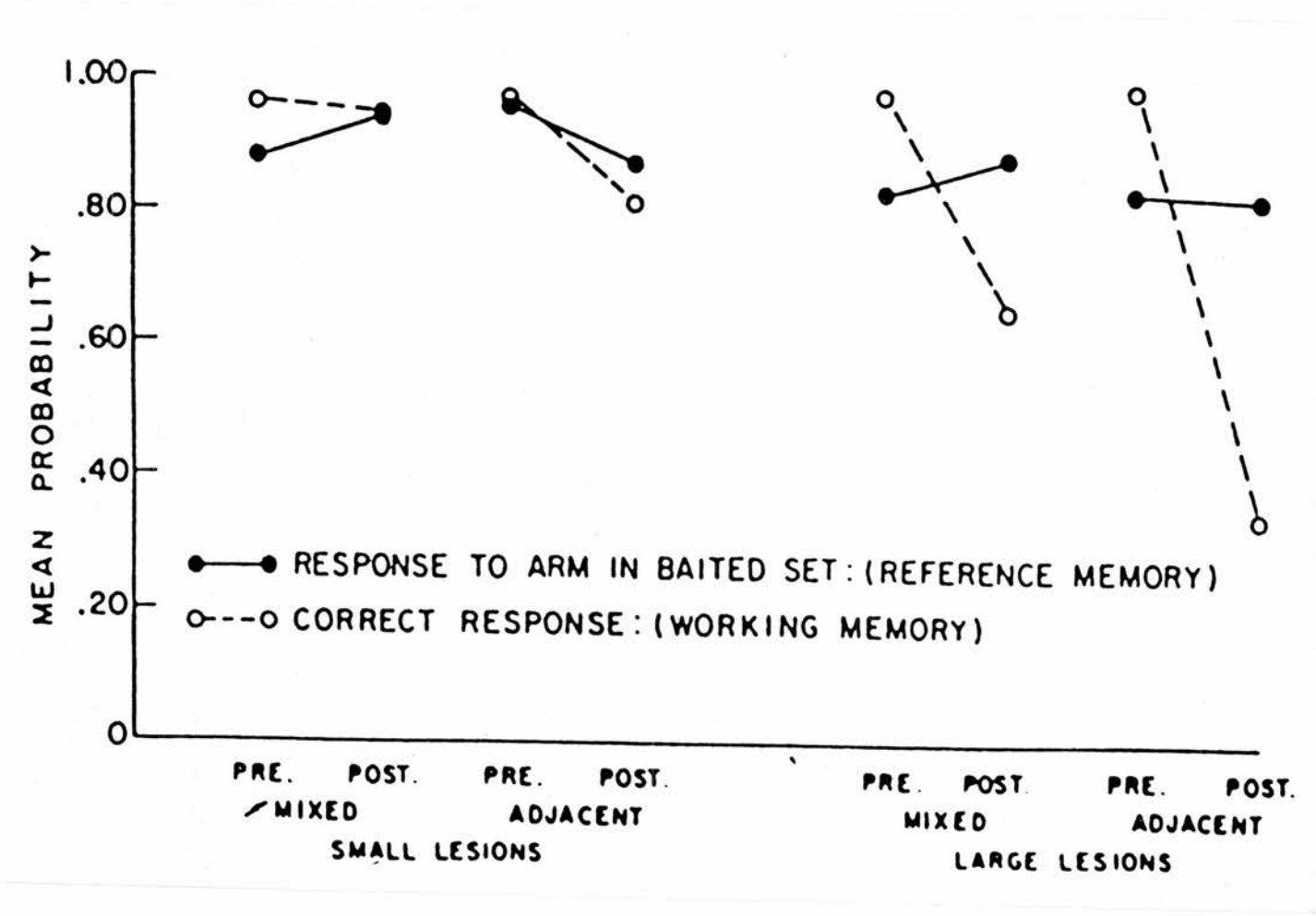
In a disconnection analysis of the hippocampal system, unilateral lesions of the fimbria-fornix and entorhinal area (on contralateral sides) were combined with a commissural section (Olton, 1978). The fornix and hippocampal commissures were cut with a scalpel blade while the entorhinal cortex was ablated using radio frequency current. The operations followed preoperative training on the 8-arm radial arm maze. Retention testing showed that the contralateral fimbria-fornix and entorhinal lesions combined with the commissural section, reduced choice accuracy to the levels found after bilateral entorhinal or bilateral fimbria-fornix lesions alone. These data demonstrate that an intact connection between the entorhinal cortex and the fornix through the hippocampus is necessary for correct performance on this task. Olton et al. (1979) considers that the function of the hippocampus would be either to transfer information from a cortical to a sub-cortical system (or vice versa) or to compare cortical and sub-cortical information and thus influence its output. In any event, this experiment

is important in acknowledging that the hippocampal connections to both cortical and sub-cortical structures, as well as the hippocampus, are necessary for normal choice accuracy in a working memory task.

Olton and Papas (1979) obtained more precise information about reference memory and working memory by incorporating the two procedures in the same test. This experimental design is ingenious in having the advantage of minimising variability. It is possible because Olton et al. (1979) consider that the two memory systems process information separately. However, Squire (1983) has criticised this design on logical grounds wondering how the animal would know what events are to be stored in which memory system. This behavioural dissociation procedure was tested on a 17-arm maze with 8 of the arms baited and 9 unbaited. Correct performance on the reference memory procedure required running down a baited arm, while correct performance on the working memory procedure required running down a baited arm that still had food. Rats were given preoperative training followed by fimbria-fornix lesions. Their results showed a normal ability in the reference memory procedure but very poor accuracy in the working memory procedure (See Fig. 3). This result was held to support the working memory theory (Olton et Papas, 1979). The cognitive map theory cannot account for the observed dissociation. If the rats were using a map strategy, then they would have been unable to

FIG. 3

The mean probability taken from the first 8 choices during the last 10 preoperative tests and the last 10 postoperative tests. (From Olton and Papas, 1979).


1.00
.80
.60
.40
.20
0
MEAN PROBABILITY
RESPONSE TO ARM IN BAITED SET: (REFERENCE MEMORY)
CORRECT RESPONSE: (WORKING MEMORY)
PRE.
POST.
PRE.
POST.
MIXED
ADJACENT
SMALL LESIONS
PRE.
POST.
PRE.
POST.
MIXED
ADJACENT
LARGE LESIONS

solve either the reference or working memory components of the task.

A major criticism of Olton's early experiments (e.g. Olton et al. 1978) was that the animals were not tested on a comparable non-spatial task, necessary because there is evidence that allocentric and guidance learning may interact (Diez-Chamizo, Sterio and Mackintosh, 1985).

Olton and Feustle (1981) tested rats with fimbria-fornix lesions (which had been given preoperative training) on an enclosed 4-arm non-spatial working memory task. Unlike previous tasks, where extra-maze cues were available only, intra-maze cues with a changing topological relationship were provided, to prevent learning by mapping strategies. The lesioned rats performed at chance levels. The result suggests a selective involvement of the hippocampus in working memory and contradicts the idea of the hippocampus being necessary only for behaviours requiring spatial maps. This study shows that fimbria-fornix lesions can produce a deficit on both spatial as well as non-spatial tasks indicating that the hippocampus is similarly involved. However, control rats were much slower learning this task than the spatial 4-arm maze, taking 50 trials to reach criterion. Therefore it must be taken into account that the task may be so difficult as to be disrupted by any brain damage.

Other studies with similar lesions have obtained

results which support the cognitive mapping theory (O'Keefe and Nadel, 1978). However, they used other experimental paradigms. O'Keefe, Nadel, Keightley and Kill, (1975) have shown that fimbria-fornix lesions prevented place learning in a circle maze. In a water finding task, thirsty rats with fimbria-fornix lesions were unable to learn the place component of the task but were able to learn a cue component (O'Keefe et al. 1975).

In an attempt to solve the controversy between the spatial mapping and working memory theories Jarrard (1983) developed a new procedure for the 8-arm radial maze that permits determining both place and cue learning and both working and reference memory. The place task was situated in a room with normal lighting and many extra-maze cues while the cue task had a reflector above it in order to emphasise intra-maze cues. In the place task the arms remained in the same spatial location over all trials while in the cue task the cues were moved randomly between trials (but not, unlike Olton and Feustle (1981), between choices). Only 4 out of the 8 arms (or cues) were baited. An important procedural difference is that Jarrard's rats could choose the next arm immediately, while in the Olton experiments the animals were confined to the central platform after each choice by guillotine doors. (A comparison between Jarrard and Elmes' (1982) study and Olton and Papas' (1979) study indicates that confinement interrupts response patterns and may reduce the number of

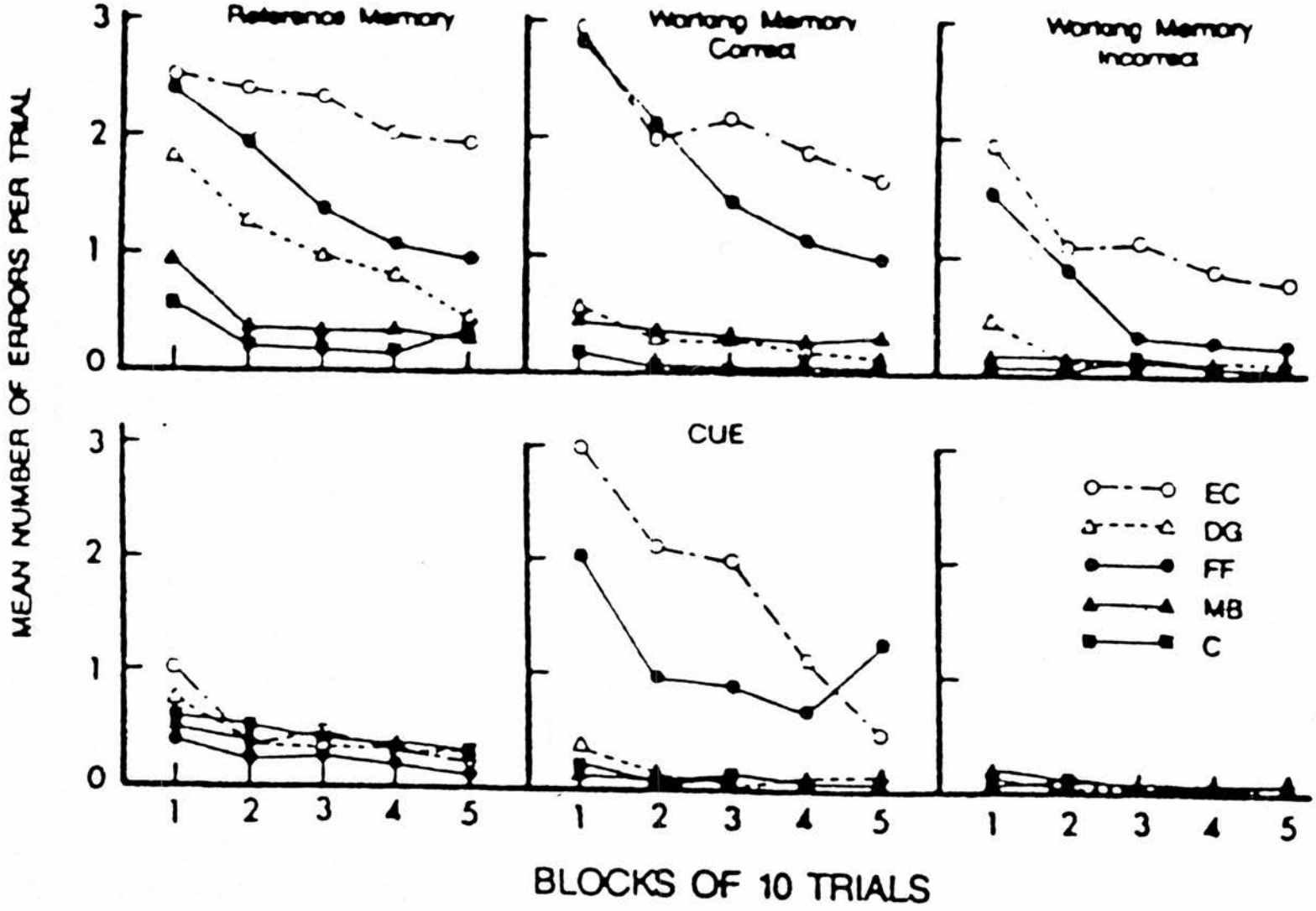
errors, especially on unbaited arms). A within subject design was used with a trial consisting of a rat being placed on the maze until all the food was eaten, until they had made 16 choices or until the time limit of 5 minutes had expired. The rats were tested on each task daily with the order of the tasks alternating between days. After preoperative training, electrolytic lesions were made to destroy the fimbria-fornix or entorhinal cortex, and the rats given 50 postoperative trials (Jarrard, Okaichi, Steward and Goldschmidt, 1984). The rats were then given a reversal test (to learn the opposite 4 arms or cues). The results showed that the rats were most impaired on the place task. The rats were capable of remembering which arms were baited in the cue task but not in the place task (reference memory). Normal performance on the cue task suggests that the lesions had not affected motivation or motor control. This supports the spatial mapping theory and conflicts with the interpretation of the results found by Olton and Papas (1979). However, the rats made a similar number of working memory correct errors on the cue and place tasks, i.e. they returned to already visited arms that had been baited that trial. They also reentered arms that were never baited on the place task (working memory incorrect) (See Fig. 4 ). The data from the reversal training showed that the fimbria-fornix and entorhinal cortex rats kept responding to the previously correct cues longer than

FIG. 4

Reference memory and working memory performance on place and cue tasks.

Abbreviations:

EC = entorhinal cortex; DG = dentate gyrus; FF = fimbria-fornix; MB = Mammillary bodies; C = operated and unoperated control. (From Jarrard, et al. 1984).


Reference Memory
Working Memory Correct
Working Memory Incorrect
CUE
EC
DG
FF
MB
C
MEAN NUMBER OF ERRORS PER TRIAL
BLOCKS OF 10 TRIALS
0
1
2
3
1
2
3
4
5

controls, but did eventually learn this reversal task. The result suggests that animals with lesions of the hippocampus are more influenced than controls by previous experience (Douglas, 1967; Jarrard, 1976). However, there is controversy over the criteria to distinguish reference and working memory (Jarrard, 1983; Morris, 1983).

This has been further studied with an experiment in the water-maze, Morris et al. (1986), which has indicated that spatial working memory is impaired by lesions of the hippocampus. Training involved two trials per day to find a hidden platform whose location changed daily. This ensures that only short-term memory can be used on this task and so it is therefore a working memory task. The controls showed a savings between trials 1 and 2 which was not evident in rats with aspiration lesions.

### 3.3 Lesions of the hippocampus

In order to determine the function of the hippocampus more accurately, lesions have to be made of the hippocampus proper rather than its extrinsic connections. This has enabled investigators to relate the functional evidence to the anatomical and electrophysiological data. In this way direct evidence would be available to confirm or refute the cognitive mapping theory. Furthermore, the relevance of the working memory theory based on fimbria-fornix lesions could be established. Swimming by rats with lesions of the hippocampus has been found to be

normal (Vanderwolf, Kolb and Cooley, 1978). Studies in the water-maze using conventional lesions of the hippocampus show impairment of place learning. Morris, Garrud, Rawlins and O'Keefe (1982) gave 10 rats aspiration lesions of the hippocampus. Using the hidden platform task, these rats' performance were impaired relative to controls. However, over the 28 trials of training the latency was reduced although never to a level better than controls searching for a platform moved randomly between trials. In contrast to the direct route the controls took to the platform, the paths of the rats with hippocampal lesions were both longer and more circuitous, with the rats setting off in a random direction when placed in the pool. When the platform was removed from the pool (transfer test) the rats with hippocampal lesions swam in a circular path round the pool, although not against the wall. Morris et al. (1982) demonstrated this by marking annuli on the video screen indicating the exact surface area and former positions of the platforms in each of the four cardinal quadrants. These rats spent as much time in each quadrant and passed over the former platform location no more frequently than the annuli in the other quadrants. In contrast, the controls spent most time in the training quadrant and crossed the training annulus frequently (see Fig. 5). A visible platform placed in the diagonally opposite quadrant for a further 11 trials (trials 30 - 41) and equated with the hidden platform for reinforcement

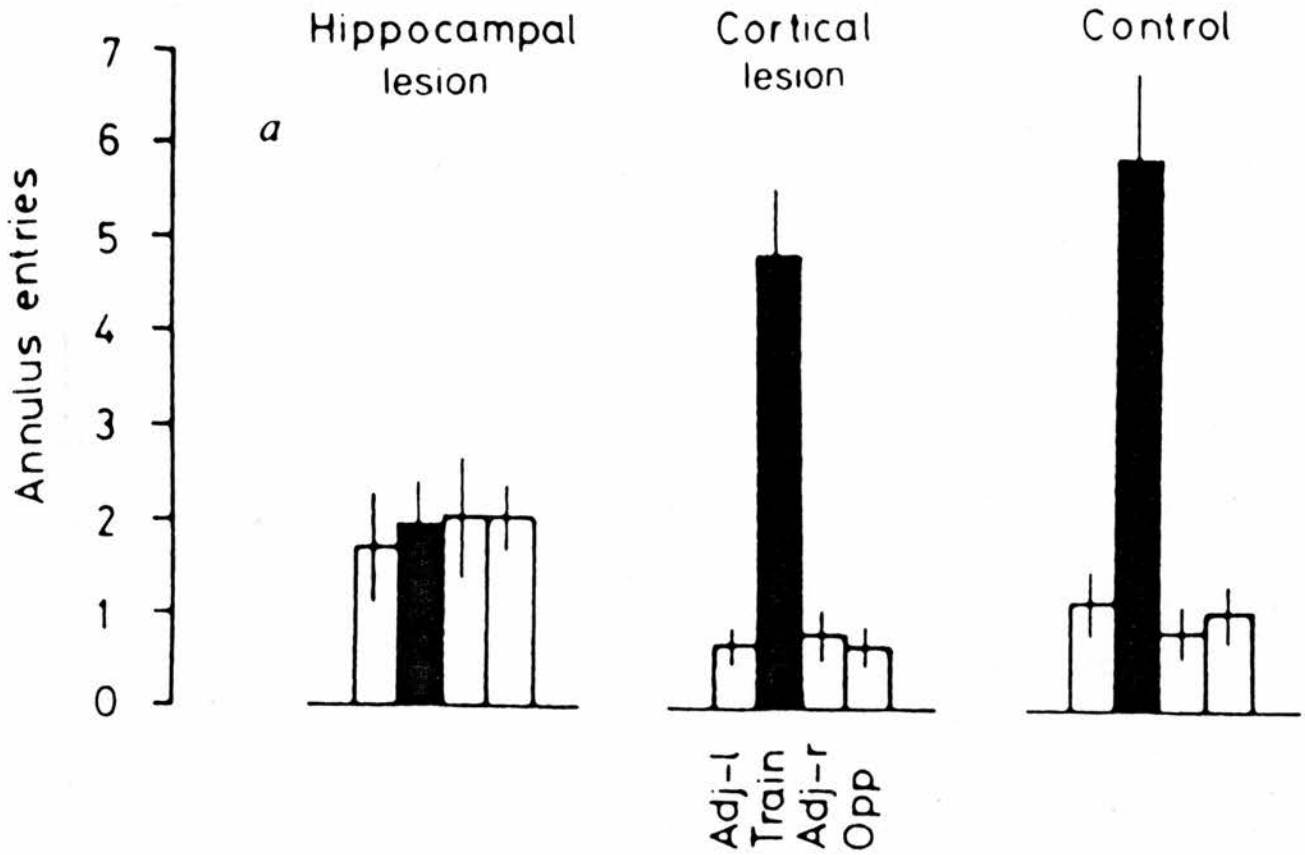
FIG. 5

Mean number of crossings of the annuli during a transfer test after place training .

Abbreviations:

train = former training location.
Opp = the annulus in the opposite quadrant.
Adj-l = the annulus in the adjacent left quadrant.
Adj-r = the annulus in the adjacent right quadrant.

(Adapted from Morris, 1984).

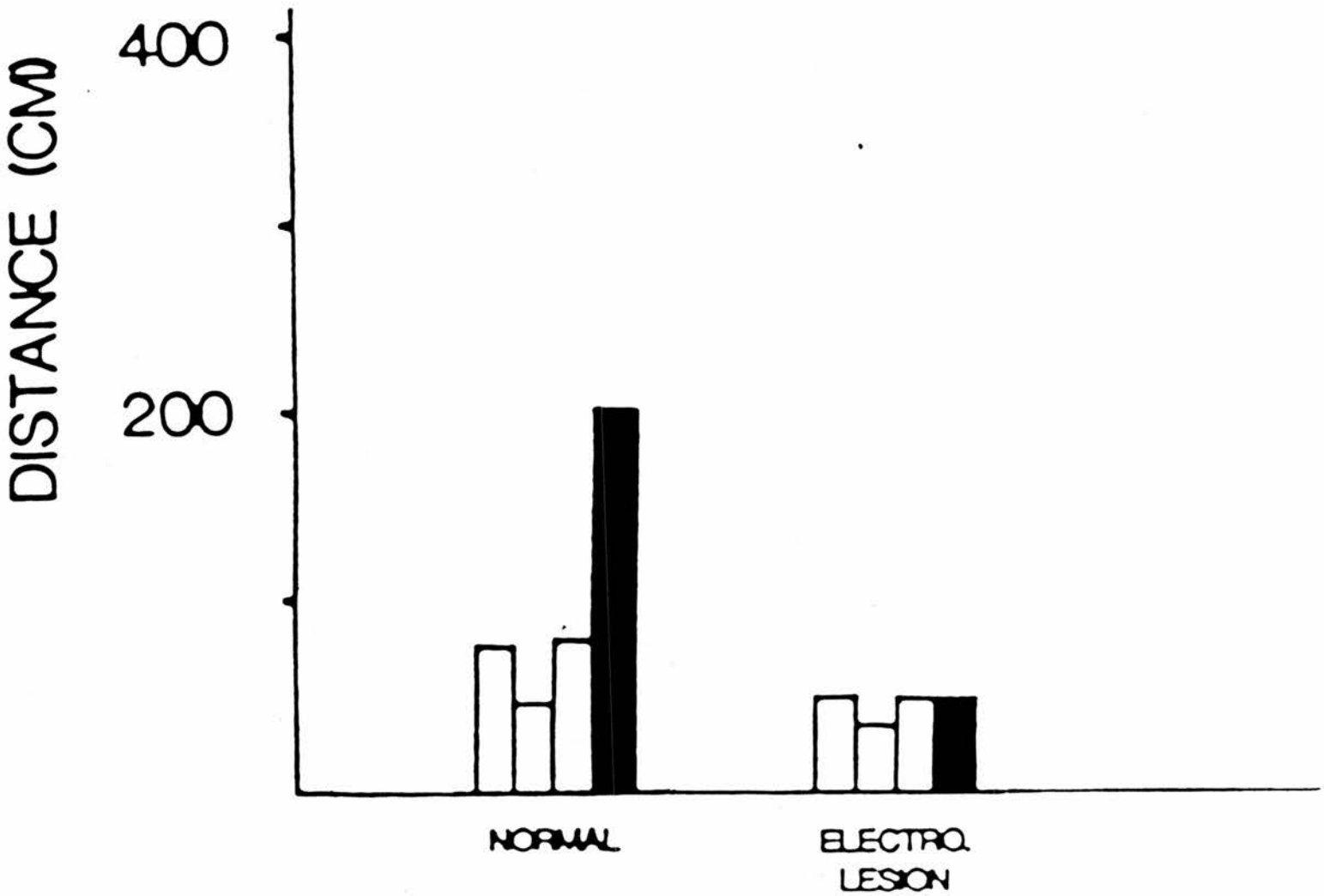

Annulus entries
7
6
5
4
3
2
1
0
a
Hippocampal lesion
Cortical lesion
Control
Adj-l
Train
Adj-r
Opp

(escape from water) gave similar escape latencies for all groups showing that motivation and sensori-motor factors were unaffected by the hippocampal lesion. The deficit in the group with hippocampal lesions reappeared when they had to find the hidden platform in the previous location of the visible platform over the next 8 trials. These results show that after aspiration lesions of the hippocampus, animals are not capable of spatial learning although they can learn an escape strategy. As the task is, operationally, a reference-memory procedure, these results support the spatial mapping theory but not the working memory theory.

A similar result was found by Sutherland, Whishaw and Kolb (1983) using electrolytic lesions (see Fig. 6). During trials 1-20 the hidden platform was located in the centre of the northwest quadrant after which the platform was moved to the southeast quadrant for trials 21-36. Analysis of trial 21 showed that swimming distance did not differ between the controls and the rats with hippocampal lesions although the controls swam a greater distance in the quadrant where the platform had been located while the rats with hippocampal lesions swam in all quadrants equally. Post hoc analysis of rearing showed that the incidence of rearing for controls declined over trials 1-20 but then increased immediately after changing the platform position and then again declined. In contrast, for the animals with lesions of the hippocampus, the

FIG. 6

The mean swimming distance in the four quadrants of the pool on the first trial after the change in platform location. The shaded bar represents the distance in the quadrant where the rat had been trained (adapted from Sutherland et al, 1983).


DISTANCE (CM)
400
200
NORMAL
ELECTRO.
LESION

rotation of the platform had no effect and so the incidence continued declining from trial 1-36. As in the Morris et al. (1982) experiment this shows an impairment in spatial learning in animals with hippocampal lesions in the water-maze. An issue which will be addressed in this thesis is whether ibotenic acid lesions of the hippocampus will similarly impair place learning in the water-maze.

Morris et al's. (1982) and Sutherland et al's. (1983) studies have been criticised for not using a comparable test for non-spatial learning. A different study achieved this aim (Morris, Hagan and Rawlins 1986). This used a two platform discrimination task for both the spatial and non-spatial test. Extra-maze cues were obscured from view in the non-spatial task. Ten trials were given per day in both tasks, with a trial ending when a rat reached the rigid platform. In the visual task the platforms were positioned quasirandomly around the pool, while in the spatial task the rigid platform remained in the same place over trials. In the visual task half the rats had to discriminate between a striped platform and a gray float and half vice versa. In the spatial task, the platform and float were a similar colour. The results showed that the eleven animals with lesions of the hippocampus were severely impaired in the spatial task (although by the end of training they did perform better than chance); they therefore offer ambiguous support for the spatial mapping theory, but no support for the working memory theory.

There was no significant difference between the groups in the visual discrimination task, indicating that hippocampal lesions do not affect the discriminability of stimuli in the water-maze.

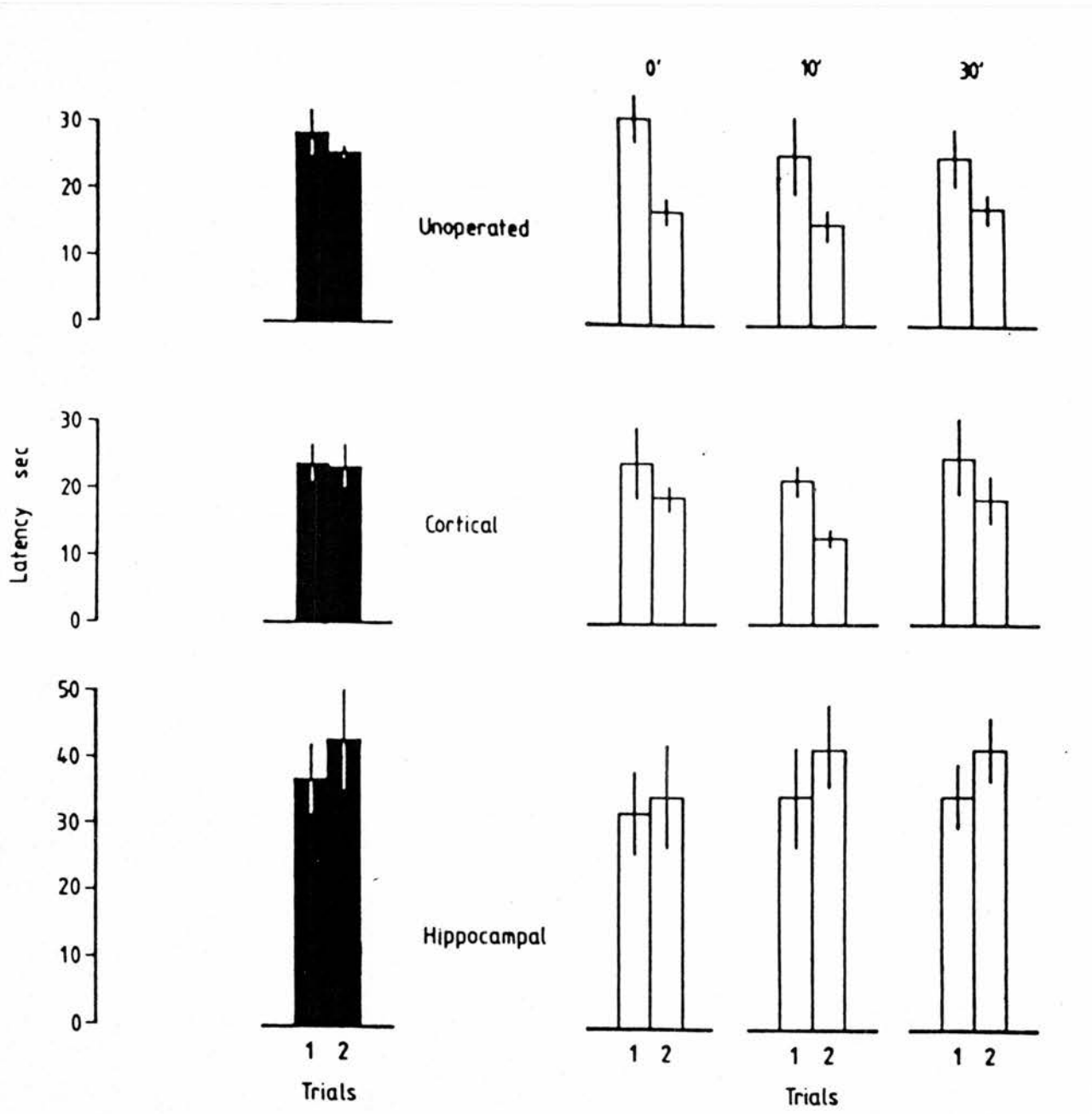
A spatial working memory task using the rigid and floating platforms involved changing the correct platform daily. This experiment gave a similar result to the reference memory task with the performance of the hippocampal group improving above chance. In addition, the hippocampal group was capable of one trial allocentric spatial learning. The equivalent visual task proved too difficult to learn. These results do not agree with either of the two main theories. A possible explanation for these results is that rats were using an alternative strategy (taxon strategy) to solve this task.

In a further working memory experiment, the hidden platform was moved daily in the pool, with the rats learning the position of the platform on trial 1 and then remembering it on trial 2. Animals with hippocampal lesions showed no sign of retention (see Fig. 7).

Results using conventional lesions of the hippocampus tested in the radial maze agree with Morris et al's (1982) place learning deficit. Following preoperative training on the complex place and cue task rats were given aspiration lesions of the hippocampus (Jarrard, 1983). Training then continued for 50 trials. The hippocampal group showed a significant impairment on

FIG. 7

Mean latency to escape in the working memory task (From Morris et al. 1986).


0'
10'
30'
30
20
10
0
Unoperated
Latency sec
Cortical
50
40
Hippocampal
1 2
Trials

the place task where they chose more incorrect arms that were not baited (reference memory errors) than correct arms that they had already entered within the trial (working memory errors). Performance on the cue task was unimpaired relative to controls (see Fig. 8). This impairment on the place but not the cue task is surprising taking into account that there is a place component to the cue task since the intra-maze cues remain in the same location within a trial thus confounding place with cue. However, Jarrard's (1983) result could have been due to a performance deficit. Analysis of running times showed that the animals with lesions of the hippocampus ran faster than controls. The reversal training showed the rats with hippocampal lesions were impaired on both the place and cue tasks. These results do not support either the spatial mapping or working memory theories.

Other results using the radial maze disagree with Jarrard's findings. Gage (1985) baited only three arms in two configurations in a reference/working memory task in the 8-arm radial maze. Choices to unbaited arms were punished with a brief period of confinement. After postoperative training the rats were trained on a different reference memory task. Postoperatively trained hippocampals were unable to learn the tasks. Preoperatively trained animals showed no signs of retrograde amnesia and they were able to learn the second tasks. These results are inconsistent with O'Keefe and

FIG. 8

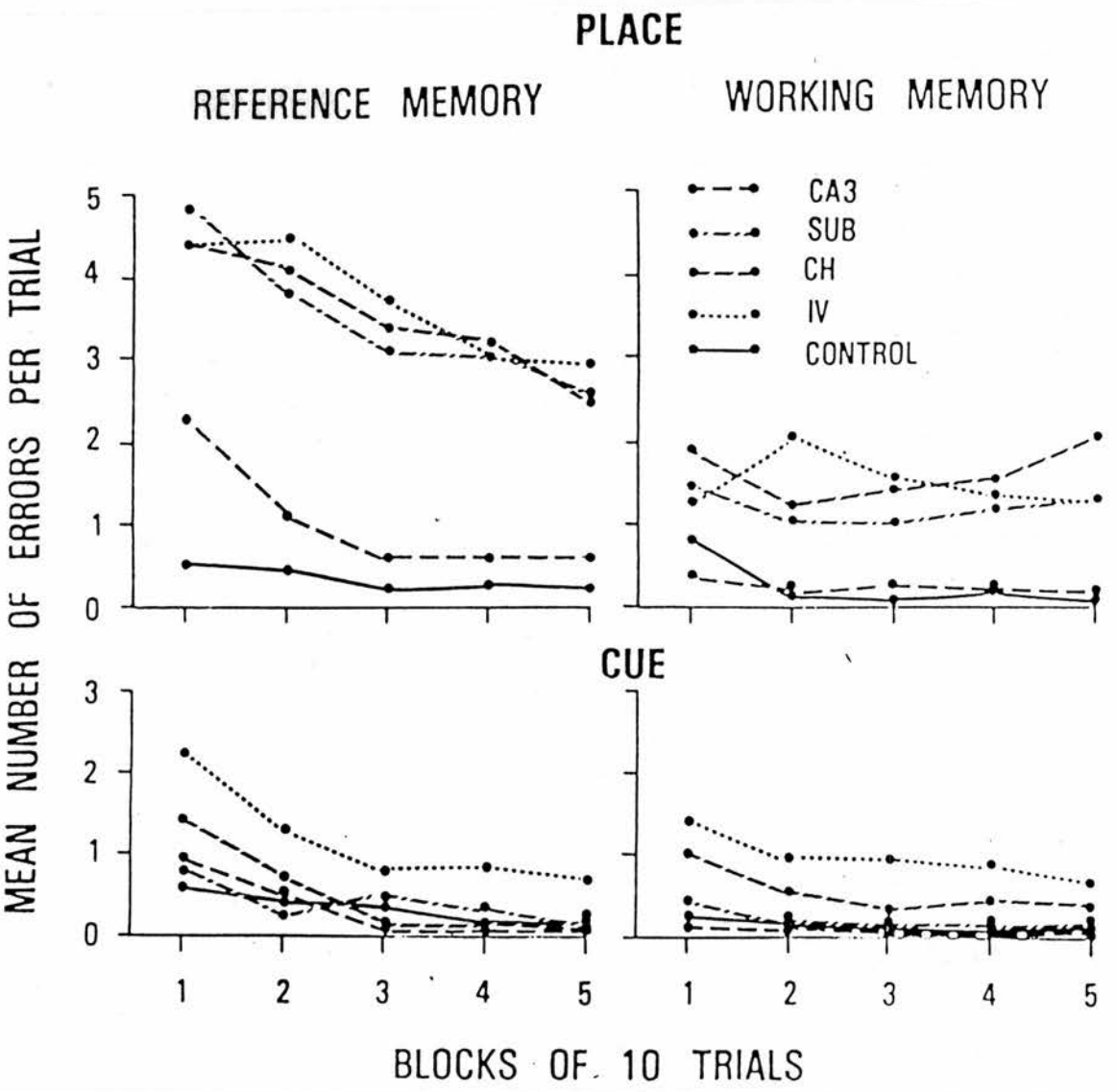
Reference memory and working memory performance on place and cue tasks.

Abbreviations:

SUB = Subiculum, IV = intraventricular CH = complete hippocampal

(From Jarrard, 1983)

Rats with intraventricular injections of KA were included since this procedure results in loss of hippocampal cells together with a pattern of brain damage similar to the distant damage found after KA lesions of CA3 cells or subiculum.


PLACE
REFERENCE MEMORY
WORKING MEMORY
CA3
SUB
CH
IV
CONTROL
MEAN NUMBER OF ERRORS PER TRIAL
CUE
BLOCKS OF 10 TRIALS

Nadel's (1978) view that the hippocampus is the storage site for cognitive maps. Instead the data suggest a role for the hippocampus in the processing of spatial information. The data is also inconsistent with Olton's working memory theory.

The use of neurotoxins has revealed more information about the function of the hippocampus. Jarrard (1986) injected ibotenic acid at 14 different sites resulting in 9 animals with cell loss in most of the hippocampus. Retention testing on his complex place and cue task (Jarrard, 1983) showed that these rats were impaired on the place task (only reference memory errors) for 20 trials after which performance was similar to controls (see fig. 9). This result is contrary to O'Keefe and Nadel's (1978) theory. There was no retention deficit on the cue task. As there was no working memory deficit Olton's theory is contradicted. Jarrard's (1986) results are evidence that the hippocampus is not necessary for spatial and non-spatial learning in the radial maze. Also, it is evidence that the ibotenic acid lesions are different from aspiration lesions. These results suggest that ibotenic acid lesions have less marked behavioural consequences (Jarrard, 1986).

### 3.4 A comparison of lesions of extrinsic connections and hippocampal lesions.

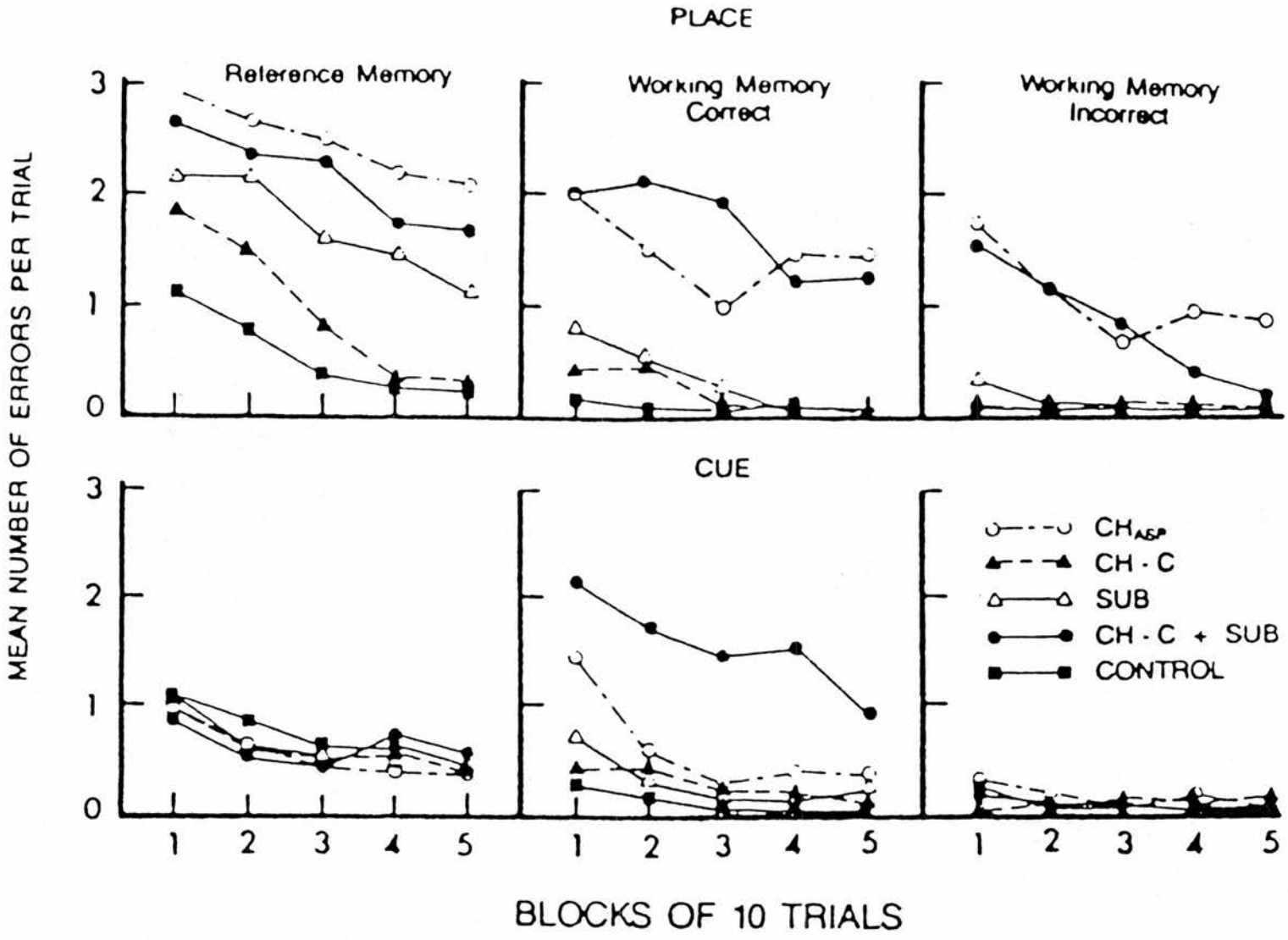
An important issue is whether lesions to the fimbria-fornix and entorhinal cortex give similar behavioural impairments as direct damage to the hippocampus. O'Keefe and Nadel assumed that these were similar and used

FIG. 9

Reference memory and working memory performance on place and cue tasks.

Abbreviations:

CH asp = complete hippocampal, aspiration lesion;
CH-C = complete hippocampal, ibotenic acid lesion;
SUB = subiculum, ibotenate lesion; CH-C + SUB = combined complete hippocampal and subiculum ibotenic acid lesions;
Control = operated and unoperated control.
(From Jarrard, 1986).


PLACE
Reference Memory
Working Memory Correct
Working Memory Incorrect
CUE
MEAN NUMBER OF ERRORS PER TRIAL
BLOCKS OF 10 TRIALS
CH-ASP
CH - C
SUB
CH - C + SUB
CONTROL

fimbria-fornix lesions as evidence for the function of the hippocampus and spatial mapping. Certainly, some experiments have shown a similarity. Early experiments with the radial maze show that both aspiration lesions of the hippocampus and lesions of the fimbria in animals similarily cause postoperative acquisition and retention deficits (Jarrard, 1978). However, in a later experiment using his complex place and cue task enabling him to analyse both reference and working memory errors, Jarrard (1986) showed that in contrast to animals with fimbria-fornix and entorhinal cortex lesions, animals with aspiration lesions of the hippocampus did not have a general working memory impairment. He also showed that ibotenate lesioned rats had neither a reference nor working memory impairment. This greater effect on behaviour caused by damaging the extrinsic connections demonstrates that the extra hippocampal structures may be more important for spatial learning than the hippocampus proper.

3.5 Lesions of the subfields of the hippocampus

(a) Conventional Lesions - In order to test the details of the cognitive mapping theory hypothesising certain functions for the subfields of the hippocampus, Jarrard (1978) selectively damaged these areas with aspiration lesions. He found that destruction of the CA1 and alveus caused both reference and working memory errors during

acquisition of an 8-arm radial maze task (similar to complete hippocampals) but no errors during retention (Jarrard 1978, 1980). Disruption of the CA3 field (by bisecting the fimbria) impaired both acquisition and retention of the task (a similar result to that of complete hippocampal lesions). This is evidence for the CA1 being necessary for forming new memories but not for the retrieval of old ones, while CA3 is necessary for both. This differential role for the subfields in behaviour showed that CA1 and CA3 are not involved together in making a spatial map. Also, these data are inconsistent with the working memory theory. Instead, Jarrard (1980) has proposed that the deficit reflects an increased susceptibility to interference.

(b) Neurotoxic Lesions - There is controversy over the effects of kainic acid lesions of the CA3 cell field. Handelmann and Olton (1981) found that all rats with CA3 lesions, given preoperative training, relearned the radial arm maze task to preoperative levels. Jarrard (1983) observed a temporary impairment (first 10 trials) in the place task on the radial arm maze but the rats performed normally on the cue task showing that the initial impairment was not due to problems with motivation or motor control. Without a naive control group it is impossible to know whether Jarrard's (1983) results with kainic acid are due to the facilitative effect of

preoperative experience or the diminution of extra-hippocampal degeneration with the use of valium. This thesis does not examine this issue. Also animals with CA3 lesions could not learn the radial arm maze task above chance if they were not preoperatively trained (Handelmann and Olton, 1981). A similar result was found by Sutherland et al. (1983), with unilateral lesions of CA3, using the hidden platform task in the water-maze, although no preoperative training had been given. However, bilateral kainic acid lesions of CA3 caused a learning deficit during acquisition of the same task although performance did improve with training with lesioned rats being only slower than normal on the first 12 of 36 trials. Moreover, measurements of the heading angle on the paths to the platform indicate that these lesioned rats were eventually heading for the platform as efficiently as controls. The lesions did not impair swim speed indicating normal motor control of swimming.

These results agree with conventional lesion studies showing that when the fimbria or complete hippocampus was aspirated the rats were not able to relearn the 8-arm radial maze tasks (Jarrard, 1978). These deficits with aspiration lesions indicate that the subiculum or other structures disrupted by the lesion, rather than the hippocampus itself, make a crucial contribution to spatial behaviour. However without using a naive control group it is impossible to know whether reduction in extra-

hippocampal damage or preoperative experience determined the varying results.

Similarly, Jarrard et al. (1984) found this temporary impairment on the radial maze with colchicine lesions of the dentate gyrus, but again, this is disputed by Sutherland et al. (1983) who found no improvement across trials with bilateral colchicine dentate gyrus lesions in the hidden platform task. This difference could be explained by the fact that Jarrard et al's. (1984) experiment included preoperative experience and/or different tasks were used in the two experiments.

The results using neurotoxic lesions support neither the cognitive mapping nor the working memory theory. They suggest that transmission through the intra-hippocampal circuit (dentate gyrus → CA3 cell field → CA1 cell field → subiculum) may not be necessary for spatial learning. The slight temporary impairment on the place task seen in some of the studies of neurotoxic lesions of the subfields is similar to that seen after ibotenate lesions of the hippocampus.

### 3.6 Lesions of the subiculum

The results from experiments showing that rats with ibotenate lesions of the hippocampus can achieve spatial learning on the radial arm maze could be explained if the rats solved the task using the subiculum. Electrophysiological data shows that the subiculum

contains directional cells, (i.e. cells that fire depending on the direction the rat is facing independent of the location of the rat - Taube, Muller and Ranck, 1987).

Both kainic acid or ibotenic acid lesions of the subiculum caused reference memory errors on the place task but did not affect performance on the cue task in Jarrard's complex place and cue procedure (Jarrard, 1986). The impairment was similar to that caused by aspiration lesions of the hippocampus which is as expected as the subiculum is the major output pathway from the hippocampus (Swanson, 1979) and the subiculum is the area where the majority of both cortical and sub-cortical hippocampal efferents originate (Rosene and Van Hoesen, 1977). These results implicate the subiculum as being important in spatial learning.

Five rats in Jarrard's (1986) experiment had hippocampal and subiculum damage. They were severely impaired on the place task (reference memory errors, working memory-correct errors, working memory-incorrect errors). Similar errors of reference and working memory was found with lesions to the extrinsic connections of the hippocampus. These results emphasise that combined hippocampal and subiculum damage is especially effective in disrupting spatial behaviour.

## 3.7 Lesions of other areas of the brain

Investigators have demonstrated that there is a

behavioural dissociation between the hippocampal formation and other brain areas. Electrolytic lesions of the frontal cortex, amygdala, caudate nucleus or mammillary bodies did not impair retention on the radial maze (Becker, Walker and Olton, 1980; Jarrard, 1983). Electrolytic lesions of dorsomedial thalamus, medial and lateral habenular nuclei or amygdala do not affect acquisition of the hidden platform task in the water-maze. Damage to the parietal cortex did not affect learning of the same task (Morris, et al. 1982) but these "control" lesions were incomplete. Lesions of the medial septum, slowed down learning of the hidden platform task (Hagan, Salamone, Simpson, Iversen and Morris, 1988). These results are evidence that damage to areas other than the hippocampal system do not affect spatial behaviour.

### 3.8 Other tasks

Tasks that differentially reinforce low rates of responding (DRL tasks) show a performance impairment after fimbria-fornix section (Johnson, Olton, Gage and Jenko, 1977), aspiration lesions of the hippocampus (Clark and Isaacson, 1965) and ibotenic acid lesions of the hippocampus (Sinden, Rawlins, Gray and Jarrard, 1986). This is an operant task where rats press a bar for food and then must wait a certain interval before pressing the bar again. The deficit involves an exaggerated rate of response and a reduction in the ratio of reinforced

responses to total responses (efficiency) although these characteristics are not entirely independent. Sinden et al. (1986) also show that efficiency of responding after ibotenate lesions of CA3 and also of the subiculum was unaffected by ibotenate lesioning, however the damage to the subiculum was limited to only half the cells. Some behavioural practises (DRL performance) are similar when lesions of the hippocampus are made conventionally or with ibotenic acid. However, ibotenate lesions of the hippocampus are less effective than conventional lesions. (18-second DRL requirement as compared to 12-second DRL requirement). This task has disadvantages in that it is not sufficiently sensitive to discriminate between different areas of damage in the hippocampal formation. This problem suggests that there may be several causes of DRL impairments following lesions. One suggestion has been that the hippocampus is involved in behavioural flexibility and because of this lesions of this area cause impairments on such tasks as DRL (Olton, 1978b).

This behavioural review shows that there is still controversy between the cognitive mapping and the working memory theories of memory. This is despite the use of a variety of tasks and methods on these tasks. This thesis attempts to look further at this controversy in an attempt to resolve it. The behavioural review also reveals differences in behaviour between conventional and neurotoxin lesions. This thesis looks at the effects of neurotoxin lesions in

order to provide more evidence of the effect of using this new method.

# CHAPTER 4

# RECOVERY OF FUNCTION

## 4.1 Introduction

Recovery of function following lesions in the hippocampal formation has been demonstrated in several studies. Partial lesions of the fimbria fornix cause a deficit in the radial arm maze which recovers with postoperative testing (Olton and Papas, 1979). Dorsal hippocampal lesions impair performance on a continuous alternation task but performance returns to normal after 60 days of testing (Dawson, Conrad and Lynch, 1973). Unilateral entorhinal cortex lesions disrupt alternation behaviour in a Y-maze, but this behaviour recovers within 10 days (Loesche and Steward, 1977).

However, there are other studies that show no evidence of recovery of function. Olton, Walker and Gage, (1978) have found that the performance of rats, with lesions of the extrinsic connections of the hippocampus, on the radial arm maze tested 50 days after surgery was virtually the same as the performance of rats tested five days after surgery. Rats with lesions of the fimbria fornix tested on a working memory experiment on a 4 arm task showed that rats' choice accuracy did not return to preoperative levels despite being given five times as many test sessions as in preoperative training (Olton and Feustle, 1981).

## 4.2 Variables that influence rate of recovery

Becker, Walker and Olton (1980) have studied some of the variables that influence rate of recovery. They have shown that recovery of function by rats with medial frontal cortex lesions, tested on the radial arm maze, was a function of task specific experience and not simply the passage of time following surgery.

Specifically, after preoperative training rats took about 15 sessions of postoperative testing to improve to the level of control rats, regardless of whether the rats had waited one or three weeks after surgery.

Handelmann and Olton's (1981) data for recovery of function after kainic acid lesions to the CA3 cell field in rats tested on a rewarded alternation task also showed that recovery of function can be facilitated by task specific experience. Furthermore, they showed that it can be facilitated by preoperative training. Rats who had received preoperative training performed poorly at the start of postoperative testing but gradually improved until after 30 tests they performed as well as preoperatively. Rats only trained postoperatively had an enduring impairment which showed no signs of recovery of function. Handelmann and Olton (1981) suggest that this result indicates there is some factor that limits the rate at which the processes underlying the behavioural recovery could be completed.

Other studies have suggested that recovery of function may be due to the elimination of diaschisis (see Finger and Stein, 1982, Ch 13) or by activation of specific neurochemical systems (Luria, Naydim, Tsvetkova and Vinarskaya, 1969; Braun, Meyer and Meyer, 1966) or the reorganisation of neuronal circuits (Finger, 1978). Also, general experience and interaction with the environment may alter the rate of recovery (Will and Rosenzweig, 1976). Furthermore, task specific experience may be important either to alter the damaged neurological system or allow the animal to develop a new strategy using other undamaged parts of the brain.

Handelmann and Olton (1981) also showed that this recovery of function in the maze was functionally specific and did not extend to later training in the open field. However, there was no preoperative training on the open field task. It is also possible

that open field activity may depend on some system in the hippocampus which did not undergo recovery.

## 4.3 Theories of recovery of function

LeVere (1975) considers that behavioural recovery following brain damage reflects the animals' reliance on the existing functions of the undamaged brain area. It is implicit in this theory that a brain structure can "stand in" for another. In addition, this idea of sparing allows for the possibility that learning may cause functional recovery.

Experimental evidence for sparing as the process of behavioural recovery comes from a brightness discrimination task (LeVere and Morlock, 1973). After preoperative training the rats were given lesions in the posterior neocortex and then retrained on either the original or a reversal of the brightness discrimination task. The results indicated that the reversal group were impaired relative to the group retrained on the original task showing that the preoperative neural mechanisms were spared and influenced the recovery of the visually guided behaviour. This proves that the underlying mechanisms were not lost in the first place. LeVere and LeVere (1982) consider that the behavioural deficit following lesioning is caused by the underutilisation of the spared capacity of the neural system. This spared neural system is prevented from functioning by the operation of the neural systems that remain intact. Experimental evidence for this is demonstrated by a visual decorticate rat who unlike controls ignored visual cues and only responded to nonvisual cues in order to avoid footshock before recovery of his visual behaviour. This evidence of underutilisation of spared neural mechanisms has led LeVere (1980) to propose that the behavioural deficit may reflect inappropriate compensatory behaviours

which cause severe interference. So LeVere suggests that the deficits occur because the crucial neural mechanisms are not utilised even though they are spared. However, compensation is not true restitution of function because the behaviour is not identical to that seen prior to the lesion. Instead animals use different stimulus cues, different muscles and different cognitive strategies although sometimes giving the superficial impression that full recovery has occurred.

A second theory for the recovery of function is known as "reactive synaptogenesis" which involves the growth of new processes towards a denervated cell following a lesion (ie. axon sprouting - (Steward 1982). Sprouting begins between 1-5 days post lesion after degeneration of cells and the migration of astrocytes, microglia and oligodendroglia to the lesioned area. Several days post lesion the phagocytes begin to atrophy and sprouting starts. This suggests that the clearing of degenerating synapses and sparing may regulate each other (Matthews, Cotman and Lynch, 1976). Raisman (1969) has shown that the septal nucleus is capable of axon sprouting after lesioning. Unilateral ablation of the entorhinal cortex causes sprouting originating in the contralateral enorhinal cortex (Steward, Cotman and Lynch, 1974), the septum (Lynch, Matthews, Mosko, Parks and Cotman, 1972) and in the CA4 area (commissural-associational system) (Zimmer, 1973). This synapse replacement only occurs over very short distances with hippocampal CA4 fibres sprouting 20-30 um in the dentate outer molecular layer (West, Deadwyler, Cotman and Lynch, 1975). The process takes about 60 days to complete. It is selective in that it only involves the fibres in the actual area or adjacent to it. Sprouting is not seen in all parts of the hippocampus. Studies looking at other areas of the hippocampus have found that after lesioning of the commissural and associational

systems, sprouting in CA1 was by interneurons, remaining Schaffer collaterals or associational fibres.

There is some suggestion that the sprouting of septal and commisural/associational inputs may increase the vulnerability of hippocampal cells to excitotoxic activity and so increase cell death. Experiments with older rats indicate a loss of plasticity which may reflect behavioural deficits. The results showed a similar response in the ipsilateral inner molecular layer, as seen in young rats but there was no response in the contralateral molecular layer. This was attributed to the increased stabilisation of the CNS. However, electron microscopic studies have not been able to rule out whether axon sprouting per se is occurring or merely the restructuring of dendrites or minor extensions of terminals.

Physiological studies have shown that sprouting following lesions to the hippocampus is functional. Steward et al. (1974) saw evoked potentials in a layer of the deafferented dentate, shown by autoradiography, to be the region where the new collaterals from the intact entorhinal cortex project. Behavioural evidence for functional axon sprouting comes from alternation behaviour in a T-maze (Loesche and Steward, 1979). After preoperative training rats were given unilateral entorhinal lesions. Rats showed a transient deficit for nine or ten days irrespective of whether testing started three days postoperatively or ten days postoperatively. Loesche and Steward (1979) hypothesised that this recovery of function was due to reinnervation of the dentate gyrus by the surviving axons from the contralateral entorhinal cortex. However, it seems more likely that the transient deficit was due to diaschisis. Certainly, it has not been possible to replicate these results (Ramirez and Stein, 1984).

Convincing evidence for rejection of this theory that

reinnervation underlies behavioural recovery comes from an interspecies comparison in an open field experiment by Lasher and Steward (1981). Their results show that a temporal correlation between the time course of reinnervation and the restitution of performance apparent in rats does not exist for cats. The performance in cats returns to preoperational levels at a similar rate to rats, depite the fact that the sprouting response takes longer in cats.

# CHAPTER 5

## LESION TECHNIQUES

The experiments to be reported in this thesis were conducted on animals which had been given lesions using the neurotoxin ibotenic acid. This section is intended as a brief summary of the reasons why this neurotoxin was used instead of

(a) conventional lesion techniques and
(b) other neurotoxins.

Only a lesion approach can determine which behaviours cannot be performed without a certain brain area (Olton et al. 1979). This interpretation of lesion studies has been criticised by Gregory (1961) and Stein (1979). Gregory (1961) suggested that the lesioned brain would process information differently from the normal brain. He gave the example of a radio humming when a transistor is removed and pointed out that the function of the transistor is not to inhibit humming.

Interpretation of lesion studies is further complicated by practical problems. Conventional lesions (electrolytic or aspiration) interrupt fibres of passage and invariably include damage to adjacent structures. In the case of the hippocampus, the damage generally interrupts the sub-cortical projections from the subiculum and often includes direct damage to the subiculum. Conventional lesions disrupt the afferent blood supply.

In addition, there are difficulties comparing the effects of lesions obtained in different studies and laboratories. The lesions may differ in their site and size and the behavioural paradigms may differ. However, this problem has not been solved by using neurotoxic lesions.

Neurotoxic Lesions

A new technique is the use of neurotoxins. This overcomes many of the practical problems associated with conventional lesions. The most important property of these neurotoxins is that they destroy cells in an area but leave intact fibres of passage and afferents that terminate in the area (Kohler and Schwarcz, 1983; Jarrard 1986). Therefore, for example the sub-cortical projections from the subiculum would remain in intact after a neurotoxin lesion of the hippocampus. Their mechanism of action is as a result of their depolarising action, which causes influx of sodium, calcium and water into the dendrites and their receptors (Olney, 1971). They are all synthetic analogues, structurally related to glutamate, and include kainic acid (Shinozaki and Konishi, 1970) ibotenic acid (Johnston, Curtis, De Groat and Duggan, 1968), quisqualic acid (Shinozaki and Shibuya, 1974) and N-methyl-D- aspartic acid. There are a variety of receptors that respond selectively to kainic acid, quisqualic acid and N-methyl-D-aspartic acid (McLennan, 1981; Watkins and Evans, 1981). These analogues do not

show similar neurotoxic effects (Zaczek, Nelson and Coyle, 1981; Zaczek, Collins and Coyle, 1981).

Kainic acid

Several experimenters have used kainic acid to lesion the hippocampus in behavioural studies (Handelmann et al. 1981; Munoz and Grossman, 1981; Sutherland et al. 1983; Whishaw and Sutherland, 1982; Jarrard, 1983). However, these studies have all had to take into account the problems associated with kainic acid. These include diffusion within the brain because kainic acid is metabolically inert (Zaczek, Simonton and Coyle, 1980). This problem is compounded by seizures making it difficult to separate the 'convulsant' effects of kainic acid from its direct action on vulnerable neurons (Schwarcz, Scholtz and Coyle, 1978; Ben-Ari, Tremblay and Ottersen, 1980). Prior treatment with anticonvulsants reduce both the convulsions and the neurotoxic action of kainic acid, but secondary damage is still obtained (Zaczek, Nelson and Coyle, 1978, Zaczek et al. 1980, Jarrard, 1983). An example of the extent of distant damage comes from Jarrard (1983) who found damage to the amygdala, midline thalamic nuclei, deep layers of the cortex, the claustrum and the olfactory region, after injection of kainic acid into the hippocampus. This illustrates that distant damage serves as a confounding factor in attempts to attribute change in behaviour to damage within the hippocampal formation.

Another drawback of kainic acid is that cells in the hippocampal formation vary in vulnerability, with more CA3 pyramidal cells being destroyed, followed by CA4 cells, then the subicular pyramids, then the CA1 cells, and then the cells in the dentate gyrus (Nadler, Perry and Cotman, 1978). This problem severely limits the use of this neurotoxin to lesion the hippocampal formation. This led to the use of ibotenic acid in experiments.

## Ibotenic Acid

Ibotenic acid is a naturally occurring heterocyclic amino acid extracted from the mushroom Amanita muscaria (Good, Muller and Eugster, 1965; Eugster and Takemoto, 1967). It is categorised as an agonist at 'N-methyl-D-aspartate' receptors (McLennan and Lodge, 1979; Watkins and Evans, 1981).

This neurotoxin was chosen in this study because of its selectivity. It produces only localised damage and produces perikaryal specific lesions and so does not destroy fibres of passage (Kohler et al, 1983; Jarrard, 1986; Schwarcz, Foster, French, Whetsell and Kohler, 1984). It doesn't cause haemorrhagic necrosis nor any distant damage partly because ibotenate is at least a thousand times weaker a convulsant than kainate (Aldinio, French and Schwarcz, 1981). The pronounced variation in neuronal vulnerability to kainate within and between brain regions is also greatly reduced for

ibotenate. Especially important for this study is that hippocampal granule cells which are the least kainate-susceptible neurons in the limbic system degenerate rapidly after treatment with ibotenate (Kohler, Schwarcz and Fuxe, 1979).

An unusual aspect of ibotenate is its narcosis-potentiating property with animals sleeping for long periods of time (up to eight hours) even when low doses are given.

# CHAPTER 6

# AIMS OF THESIS

## 6.1 Aims of thesis in relation to behavioural literature

This section examines some issues arising from the behavioural review in relation to this thesis. In order to gain more information about the implications of Jarrard's (1986) result, with respect to the cognitive mapping theory and working memory theory, this thesis looks at spatial and non-spatial learning by animals with lesions of the hippocampal system in a different spatial situation. The results discussed in the behavioural review indicate how a factor such as task difficulty maybe a crucial determinant for the adequacy of a paradigm as a sufficient measure of hippocampal damage. This thesis questions whether the radial maze is a sufficiently sensitive measure for testing spatial memory by replicating Jarrard's (1986) neurotoxin lesions using the water-maze task.

While it has been established that normal animals performing in the water-maze generally use spatial strategies (Morris, 1981), it has also been determined that if normal animals are precluded from receiving distal stimuli they can adopt a response strategy which consistently gets them to the platform (Sutherland and Dyck, 1984). It is therefore necessary to take several behavioural measurements in order to determine which type

of strategy is used. In this thesis, I have measured (at different stages of training and testing) (i) the escape latency (ii) the time spent swimming in the quadrant in which the rats had previously been trained to find the platform, (iii) the number of crossings of the former platform position and (iv) the heading error. If lesioned animals are impaired on all aspects of performance then there is no problem of interpretation. Using the water-maze, response patterns were minimised by varying randomly the starting position on each trial ensuring that the rat had to swim in a different direction and for a different distance to reach the platform. This technique also prevents rats from learning to find the platform by remembering a sequence of movements (as is possible on the radial arm maze). Finally, a non-spatial task has been included (i) to act as a control for the spatial tasks and (ii) as previous results have shown that non-spatial tasks are unaffected by hippocampal lesions on the water-maze. (Morris et al. 1986).

## 6.2 Aims of thesis in relation to recovery of function literature

Although the possible neurological mechanisms underlying the behavioural recovery will not be looked at in detail in this thesis, the time course of recovery was manipulated as this has already been proved to make a

useful model for the study of recovery of function (Handelmann and Olton, 1981). Jarrard (1986) has shown recovery of function after 20 postoperative trials on the radial maze by animals with neurotoxic lesions of the hippocampus. This thesis addresses two important questions. Firstly, did this recovery occur due to time only? Or, secondly, did task specific experience affect the rate of recovery? In this thesis there are two postoperative testing periods. The first begins 12 days after surgery to determine the acute effects of the lesion. Extensive postoperative testing will determine if recovery of function is due to the testing experience. The second begins 3½ months later to determine if there is any recovery of function as a result of general experience.

# CHAPTER 7

# GENERAL METHOD

## 7.1 Subjects

The subjects were experimentally naive adult male hooded Lister rats. They were maintained on rat chow and water ad libitum. They were housed in individual cages in a temperature controlled room (22 + /-1°C), with a normal light/dark cycle.

## 7.2. Apparatus

The experiments were conducted in a large circular swimming pool (described in Morris, 1984). This was built as a method for motivating animals to learn through their fear of water (see Glaser, 1910; Wever, 1932; Waller, Waller and Brewster, 1960). It was a circular fibre glass tank, painted white, 2.14 m in diameter x 0.40 m in height, with a water depth of 0.25 m. four points (North, N; South, S; East, E; and West, W) were designated at particular positions around the perimeter of the pool, equal distances apart, and these were used as starting points. The surface of the pool was divided into four quadrants (north-east, NE; north-west, NW; south-east, SE; and south-west, SW).

The pool was situated in a laboratory and was surrounded by salient features such as blacked-out windows, doors, filing cabinet, a panel of monkey caging, blackboard, black and white paintings, and black curtains to provide cues for the spatial learning tasks. These extramaze cues have been shown to be essential for spatial learning as pulling the curtains right round the pool, thus hiding the cues from view, reduces spatial choice performance to chance levels (Morris, 1984). The black curtains were only pulled around the pool during a visual discrimination task (Experiment 4). There

were no constant sound sources in the laboratory.

Each day the tank was filled with water which was maintained at a temperature of 26 + /-1°C throughout the day (a sufficiently low temperature to motivate the rats to escape (N.B. The temperature must remain steady or learning rate is affected - Woods, Davidson and Peters, 1964). Dried skimmed milk (500 g "Five-Pints" brand) was mixed in to make the surface opaque (in order to render a platform "hidden" below the water level to ensure there were no cues coming from the platform to aid escape).

Rats escaped onto solid, circular, perspex platforms (9 cms. in diameter). They were painted white, matt grey or with black and white vertical stripes and used in different experiments (see Procedure below - Fig. 10). The platforms varied in height so that, in a hidden platform task, the platform lay 1 1/2 cm below the surface of the water. In addition, there were two floating platforms (also 9 cms in diameter) which were used in the visual discrimination task. They were made from perspex tubing and expanded polystyrene and anchored to a solid movable base. When touched by a rat, they submerged and so offered no form of escape. They were painted either matt grey or with black and white stripes to look similar to the rigid platforms used in this experiment. Reward by escape from water in this manner removes any requirement for food or water deprivation or electric shock.

During the place task, reflected illumination from the ceiling was provided by four 150 watt spotlights located on the floor of the laboratory by the side walls, and four car headlights were located round the pool edge. These were sufficiently bright to make the room cues fully visible. The car headlights and an additional four overhead lights were used for the visual discrimination task.

The behaviour of the rats in the pool was monitored using a

FIG. 10

Cross section of the pool showing the platforms for A - the place task, B - the cue task and C - the visual discrimination task (from Morris, 1984).

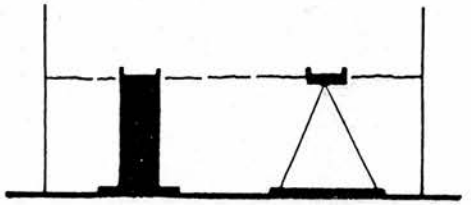
A

Hidden platform

B

Cueing procedure

C



Two platform task

closed-circuit video camera attached to the ceiling above the pool which relayed a picture to a television screen hidden behind the black curtain. A Sony C9 Betamax recorder was used to video-tape the trials. Latencies to escape were recorded with a stopwatch. Transfer test data was acquired using a tracking system (Morris, 1984). This consisted of an image-analyser (HVS Ltd, VP110) attached to a BBC Model B Microcomputer. The computer programme for collecting data was called "RATRUN" and for analysing it "RATN". During the transfer trial a T.V. screen showed the x and y coordinates of the rat's position and displayed the path taken. This was stored using floppy discs for later analysis. RATN was used to make calculations of path-length, number of times the exact previous location of platform was crossed (annulus entries) and latency in each sector of the pool. This system provided an objective technique for collecting data.

## 7.3 Procedure

### a. Surgery

The surgery was conducted by L.E. Jarrard (Washington and Lee University), F. Schenk (Universite de Lausanne) and R.G.M. Morris (University of St. Andrews). I anaesthetised, shaved and, after surgery, stitched up the rats.

The rats were food-deprived overnight before surgery. A solution of ibotenic acid (10ug/ul) (supplied by Sigma Ltd.) was made up in phosphate-buffered saline (pH 7.4). The rats were weighed and assigned to treatment groups of similar average weight (hippocampals 361 g (Range 296-470 g), subiculums 366 g (Range 295-432 g), controls 348 g (Range 266-392 g). The treatment groups

consisted of fourteen intended hippocampal rats, fourteen intended subiculum rats and seven neocortical rats, in addition to seven unoperated controls.

Rats were anaesthetised by a brief exposure to halothane in a desicator followed by an intraperitoneal injection of Avertin (10ml/kg). Further injections of Avertin were given during surgery as necessary to maintain anaesthesia. (The Avertin was made by adding 1.25 ml of a concentrate consisting of 62 ml tertiary amyl alcohol and 100 g of tri-bromo-ethanol crystals to a mixture of 62.5 ml 0.9% saline and 5.0 ml absolute alcohol.) The rats were placed in a Kopf stereotaxic instrument with the incisor bar set at the right level for the plane of the skull between bregma and lambda to be flat (at about 2 mm below the interaural line. The skin was cut in the midline from between the ears to the back of the head, folded back and held firmly in place with forceps. The surface of the skull was scraped clean. Holes were drilled using a dental drill and a wedge of skull over the required area was removed. The injections of ibotenic acid were made using 1 ul S.G.E. syringes. Volumes of ibotenic acid (typically 0.05 - 0.15 ul) were injected at the coordinates based upon those first used by Jarrard (1986) to give total lesions of all hippocampal cell fields, or the subiculum - see Tables 1 and 2. Injections were given over a one minute period at each site, and the syringe was left in place for another three minutes to prevent the ibotenic acid being drawn up the needle track. The neocortical lesion group had the dura removed and the neocortex damaged using the S.G.E. syringe at similar coordinates to the other lesion groups. After surgery, the animals were given an intramuscular injection of 1 ml of Ampicillin to guard against infections. They were left under lamps to keep them warm until they regained consciousness when they were returned to the housing room.

TABLE 1

COMPLETE HIPPOCAMPAL LESION

Using bregma as the point of reference the coordinates were as follows -

| AP | ML | DV | VOL(μl) |
|---|---|---|---|
| -2.4 | +1.0 | -3.4 | 0.05 |
| -2.4 | -1.0 | -3.4 | 0.05 |
| -3.0 | +1.4 | -3.0 | 0.10 |
| -3.0 | -1.4 | -3.0 | 0.10 |
| -3.0 | +3.0 | -3.0 | 0.10 |
| -3.0 | -3.0 | -3.0 | 0.10 |
| -4.0 | +2.6 | -3.3 | 0.05 |
| -4.0 | +2.6 | -2.3 | 0.05 |
| -4.0 | -2.6 | -3.3 | 0.05 |
| -2.6 | -2.6 | -2.3 | 0.05 |
| -4.0 | +3.7 | -3.0 | 0.10 |
| -4.0 | -3.7 | -3.0 | 0.10 |
| -5.0 | +4.4 | -7.0 | 0.10 |
| -5.0 | -4.4 | -7.0 | 0.10 |
| -5.0 | +3.8 | -3.3 | 0.05 |
| -5.0 | -3.8 | -3.3 | 0.05 |
| -5.8 | +4.1 | -3.8 | 0.10 |
| -5.8 | -4.1 | -3.8 | 0.10 |
| -5.8 | +5.1 | +5.8 | 0.0833 |
| -5.8 | +5.1 | -4.9 | 0.0833 |
| -5.8 | +5.1 | -4.0 | 0.0833 |
| -5.8 | -5.1 | -5.8 | 0.0833 |
| -5.8 | -5.1 | -4.9 | 0.0833 |
| -5.8 | -5.1 | -4.0 | 0.0833 |

TABLE 2

SUBICULUM LESION

Using bregma as the point of reference the coordinates were as follows -

| AP | ML | DV | VOL(μl) |
|---|---|---|---|
| -5.4 | +1.0 | -3.0 | 0.05 |
| -5.4 | -1.0 | -3.0 | 0.05 |
| -5.8 | +2.2 | -2.5 | 0.10 |
| -5.8 | -2.2 | -2.5 | 0.10 |
| -6.2 | +3.0 | -3.0 | 0.05 |
| -6.2 | +3.0 | -2.5 | 0.05 |
| -6.2 | -3.0 | -3.0 | 0.05 |
| -6.2 | -3.0 | -2.5 | 0.05 |
| -6.2 | +4.9 | -7.0 | 0.10 |
| -6.2 | -4.9 | -7.0 | 0.10 |
| -6.7 | +4.1 | -2.7 | 0.10 |
| -6.7 | -4.1 | -2.7 | 0.10 |
| -7.3 | +4.6 | -2.8 | 0.10 |
| -7.3 | -4.6 | -2.8 | 0.10 |
| -8.0 | +5.2 | -5.3 | 0.10 |
| -8.0 | +5.2 | -4.2 | 0.10 |
| -8.0 | +5.2 | -3.0 | 0.10 |
| -8.0 | -5.2 | -5.3 | 0.10 |
| -8.0 | -5.2 | -4.2 | 0.10 |
| -8.0 | -5.2 | -3.0 | 0.10 |

Seven rats of similar average weight as the lesion groups were chosen to act as unoperated controls. The ibotenate lesioned rats had poor appetites for several days after surgery (also noted by Jarrard, 1986) and so were fed Farex baby food mixed with water. They were weighed for a fortnight after the operations.

b. Behavioural procedure

Each group of rats was subdivided such that half of the animals began post operative training 12 days after surgery, the remainder 100 days after surgery. The subdivision was made such as to match the groups with respect to average weight. The 12 day groups consisted of 7 hippocampals, 7 subiculums, 4 neocortical controls and 3 unoperated controls. The 100 day groups consisted of 7 hippocampals, 7 subiculums, 3 neocortical controls and 4 unoperated controls. Both groups were run in the following four experiments in a similar fashion. Data sheets were prepared for each animal. The animals were tested in a fixed running order with one from each group following on from each other.

Habituation: Each animal was placed in the pool for sixty seconds without an escape platform. This served to familiarise them with the apparatus and assess their adequacy for the task. For although rats are natural swimmers, their ability to swim affects learning rate (Woods and Holland, 1961).

Training: A trial started when the rat was lowered into the pool facing and close to the side wall. This starting position varied quasi-randomly between the four points round the pool (N,S,E,W), so that no particular place, or turn strategy, could lead to successful performance. The rat was left on the platform for 30 seconds before being returned to its cage. If a rat failed to escape in 120 seconds, it was guided in the appropriate direction.

c. Data Analysis

Data were analysed by analysis of variance using ALICE statistical package on the University of St. Andrews mainframe VAX computer.

# CHAPTER 8

# HISTOLOGY

## 8.1 Method

### a. Perfusion Procedure

The rats were given a lethal dose of Euthatal (sodium pentobarbital) and cardiac perfusions of 0.9% saline followed by phosphate buffered formalin (pH7.4). The brains were carefully removed f om the skull and stored in formalin.

### b. Egg yolk embedding procedure

The egg yolk embedding procedure was chosen to keep the brain intact in order to cut horizontal sections on the cryostat. The brains were washed in three changes of distilled water and left soaking in distilled water overnight to remove all formalin (the formalir would make the egg yolk set). Then the brain surface was dried by dabbing with a tissue before being painted with fresh egg yolk (no albumen must remain). The egg yolk was pushed into the holes using a soft art brush. The brains were placed in "Peel-A-Way" moulds (the right hand side of the brain oriented with a notch in the right hand corner of the mould) and sufficient egg yolk was poured in to just cover them. The moulds were kept in the refrigerator until the brain had sunk to the bottom of the holder. To harden the egg yolk, the holder was put in a staining dish with a layer of 25% formalin covering the bottom and the lid sealed with "parafilm". This was left at room temperature. When the surface of the egg yolk was firm to touch, the sides of the mould were cut, to enable the formalin to permeate into the rest of the yolk. When sufficiently hard, the egg yolk could be cleanly lifted out of the mould (after about three days exposure to the 25% formalin), blocked

and notched in the right hand corner (corresponding to holder). The brain was then placed in 10% formalin overnight to ensure the complete denaturing of the egg yolk, followed by a week in 30% sucrose to reduce damage during cutting by ice crystals.

c. Sectioning

The brain was cut using an open top cryostat at -18° C. First the fast freeze was reduced to -30° C. The brain was placed on the chuck with "tissue-tek" and frozen quickly using dichloro-difluoro-methane and then in fast freeze in the cryostat for fifteen minutes (the period required for the cryostat temperature to fall to -18° C. Sections, 30 u thick, were cut right through the brain 2/10 were retained (one for cresyl and one for Fink Heimer) and transferred to distilled water ready for mounting.

d. Mounting

Slides were prepared by cleaning with industrial alcohol and then coating with chrome alum solution made from gelatine (1g of gelatine heated with 100 mls of water) and chromic potassium sulphate (0.1g). The solution was painted on with a brush and dried quickly on a hot plate. The sections were straightened out in distilled water using a large, soft brush and then mounted on to the slides (normally, three sections to each slide). Daubing with a tissue flattened wrinkles and the sections were left in the hot plate (37° C) until dry. Code numbers were scratched on the slides using a diamond, and the slides put in a slide tray ready for staining.

e. Staining

Both a cresyl-violet and a reduced silver stain were used.

Cresyl Fast Violet: Cresyl fast violet is a nissl stain useful for contrasting glia with live cells in order to determine an area of damage. The slides were washed for one minute, dehydrated through the alcohols to remove lipids and then rehydrated (70% industrial

alcohol, followed by 95% industrial alcohol, then 100% industrial alcohol or 74 O.P., absolute alcohol, 74 O.P. 95% industrial alcohol, 70% industrial alcohol) through 1% acetic acid in 74 O.P. solution and then left in water until it ran clear. The brain was stained for six minutes in 1% cresyl fast violet solution, given a quick wash and differentiated in 1% acetic acid in 74 O.P. solution. Slides were examined under the microscope while the sections were still wet. The sections were dehydrated quickly by going back up the alcohols (70%, 95%, 74 O.P., absolute alcohol) and removed from the absolute alcohol when the background looked white. Xylene was used to clear and to raise the refractive index (R.I.) of the specimen to that of the mounting solution DPX and the cover slip (1.524).

Reduced silver method: (Fink and Heimer, 1967). This is a metallic impregnation technique using silver. Despite optimal time for demonstrating fine terminal degeneration being only 4-5 days survival time, Jarrard (1986) has found degeneration after 12-14 weeks survival time. This method is advantageous in producing greater detail of the nature and extent of the damage. Four sections were chosen (at bregma levels -9.1mm, -6.6mm, -4.6mm) to reveal possible degeneration of fibres in the postcommissural fornix indicating the extent of damage to the subiculum.

The subiculum projects through the postcommissural fornix to the mammillary complex, hypothalamus and nucleus accumbens (Swanson, 1979; Kelley et al., 1982). Also, degeneration in the amygdala indicates subiculum damage (Swanson, 1979). Damage to CA3, CA4 cell fields would cause degeneration in the fimbria leading to the septum and nucleus of the diagonal band (Swanson and Cowan, 1977). In addition, this method was used to assess whether the perforant path remained intact.

The procedure was as follows:-

The sections stayed in 10% buffered formalin for fourteen days minimum. They were rinsed in distilled water (3 changes - 5 minutes each), left in 0.05% potassium permanganate solution for eleven minutes (the time necessary to "suppress" normal nondegenerated fibres) and then rinsed in distilled water just long enough to remove most of the obvious potassium permanganate. The sections were bleached for one minute to completely decolourise them in a solution of 1% oxalic acid and 1% hydroquinine volume/volume, followed by rinsing in distilled water (3 changes, 5 minutes each). Immersion in the following two solutions caused greater selectivity to reveal the degenerating boutons. The first consisted of 0.4 gms uranyl nitrate (N.B. radioactive and very toxic) and 2.0 gms silver nitrate in 400 ml distilled water, (immersion for sixty minutes); followed by a solution of 0.7 gms uranyl nitrate and 6.5 gms silver nitrate in 400 cc distilled water, (immersion for thirty minutes). Finally, the sections were again rinsed in distilled water (three changes, 5 minutes each).

Silver was laid down onto the degenerated fibres, for identification purposes, using ammoniacol silver nitrate solution for one and half minutes (2.5 gms silver nitrate in 100 ml distilled water to make the silver nitrate solution). Then 100 ml silver nitrate solution was added to 10 ml base mixture (nine parts 2.5% sodium hydroxide (45.5 ml) and 7 parts concentrated (S.P.G. = 0.88) ammonia water (27ml)). The tissue was put into reducing solution (distilled water (400 ml), 95% alcohol (33.2 ml), 10% formalin (4.0 ml) and 1% citric acid (18 ml)) to turn the silver nitrate to silver oxide. The sections were turned quickly for 1 minute until the tissue turned golden brown and transferred to a second container of reducing solution for a further 1 minute and then rinsed in several changes of distilled water. They were put into fresh 0.5% sodium

thiosulphate for 1 minute and then rinsed in distilled water (3 changes, 5 minutes each) and mounted from the solution composed of volume/volume 0.5% gelatin and 80% alcohol. The slides were then taken up through the alcohols (conc. of 90%, 95% and two changes of absolute alcohol), into xylene, coverslipped and labelled.

## 8.2 Results

### a. Preliminary description

Evaluations of the histological material of the first group of rats was carried out by L. Jarrard and subsequently discussed by R. Morris and myself. The second group was assessed by R. Morris and myself independently and then discussed. The lesions were evaluated without knowledge of the rats' performance.

The loss of cells that resulted from ibotenic acid injections was more selective than is possible with conventional lesion techniques. Jarrard (1986) points out that it is extremely difficult to selectively lesion the hippocampus with neurotoxins. Histological analysis indicated that many of the lesions were not successful. The damage in some of the animals was too limited, and in others chemical spread damaged adjoining structures. Of the 14 animals in the original hippocampal group (HIP), only 5 were considered as having sufficiently selective lesions which included most of the cells in the hippocampus (pyramidal cells and granule cells). In 7 rats, cells were destroyed in the dorsal hippocampus and also bilaterally in the subiculum. They formed a new group (HIP + SUB) and their data was considered together as a separate group. Of the 14 animals in the subiculum group (SUB), only 6 lesions were satisfactory (most of subiculum, parasubiculum and presubiculum damaged). In 4 rats there was damage to the subiculum and to the

ventral hippocampus. They formed a new SUB+HIP group. Photographs of selected sections showing lesions in a representative HIP, SUB, HIP+SUB, SUB+HIP, cortical control and control are presented in Fig. 11*.

In the HIP group there was usually shrinkage of the hippocampus (see Fig. 11) and this was more prominent in the HIP+SUB and SUB+HIP groups. In some animals there was mechanical damage at the site of the injection. In all groups there was considerable damage to the neocortex possibly due to leakage from the pipette. The cresyl violet stain revealed astrocytes (the final stage of healing) in the ibotenate affected areas. The Fink-Heimer stain showed degenerating fibres and fine terminal degeneration as reported with shorter survival times (Jarrard, 1986). However, the possibility that degeneration may occur in other areas at different survival times must be taken into account.

b. Detailed description

Fig. 11 (rat 5713) shows a typical hippocampal lesion. The cresyl violet stain shows bilateral pyramidal and granule cell loss medially (-5.1 mm to -4.6 mm). There was some sparing of dentate cells dorsally (-3.6 mm to 3.1 mm) and the cells in the ventral hippocampus survived the ibotenate injection. The subiculum was partially damaged ventrally. The Fink Heimer stain confirmed the loss of cells apparent in the cell stained sections. Fine terminal

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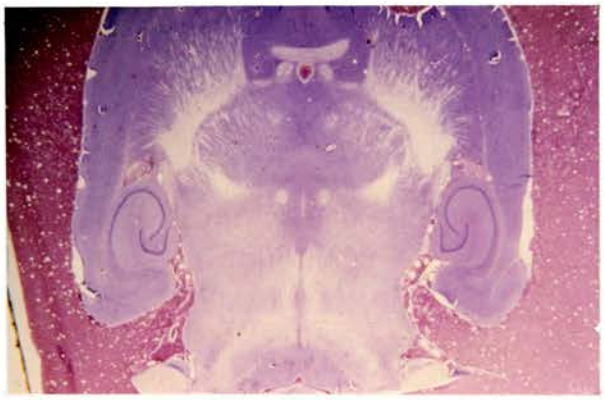
* Selection for the illustrations involved ranking the sections according to lesion size in the relevant area. A typical example would be the median along a continuum from maximum to minimum lesion size.

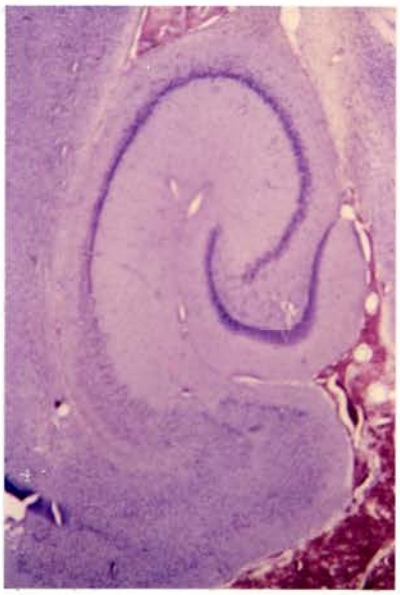
FIG. 11

Examples of the histology

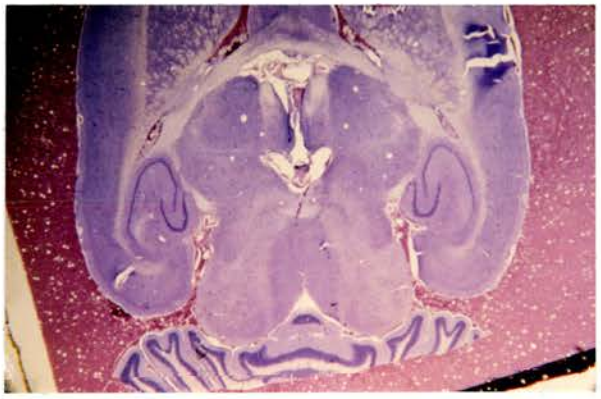
CONTROL RAT NO. 5746 CRESYL VIOLET STAIN

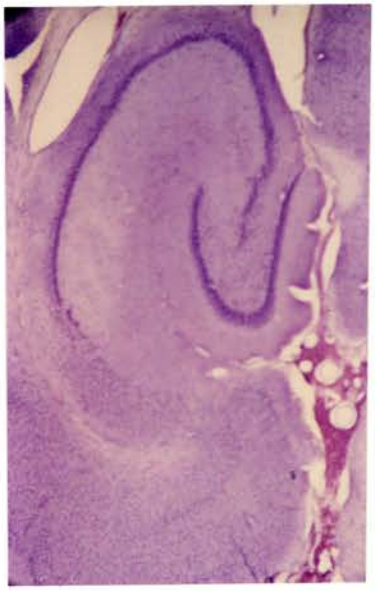
ventral



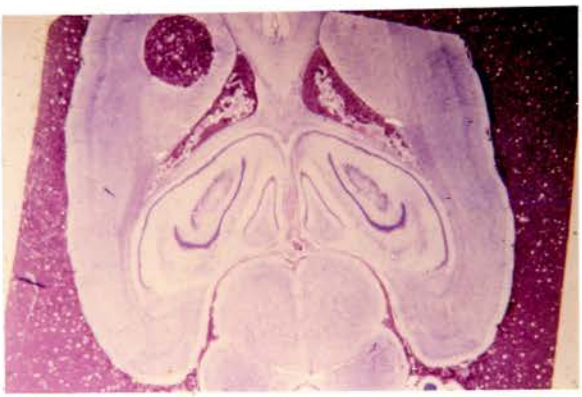


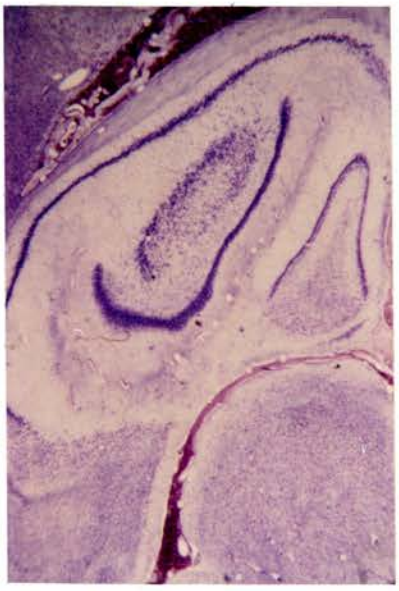
medial





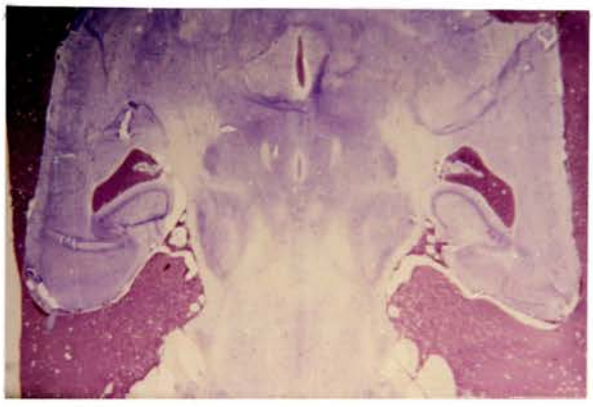
dorsal

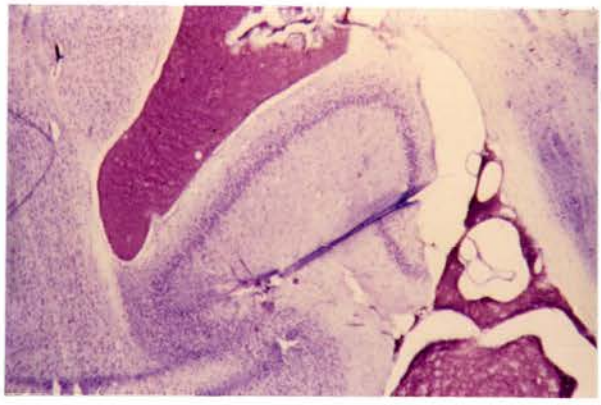




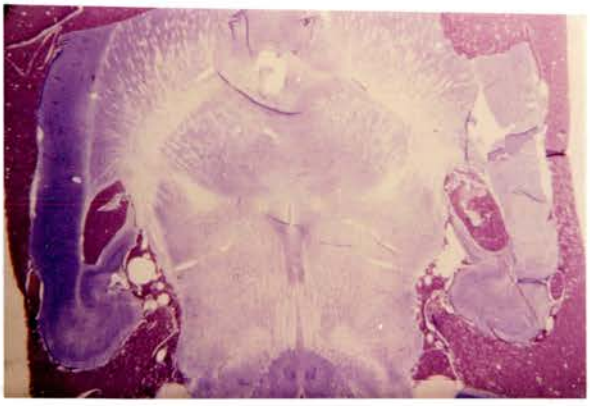
HIP RAT NO. 5713

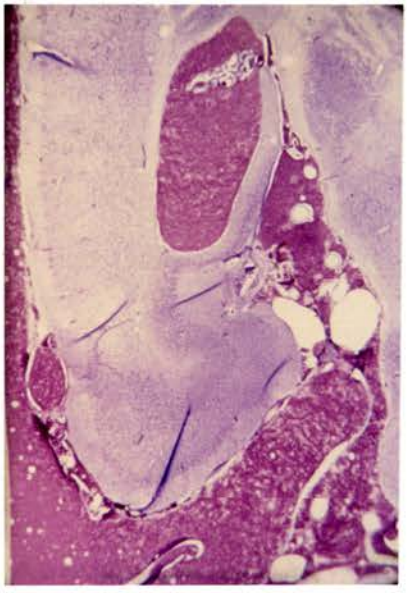
ventral



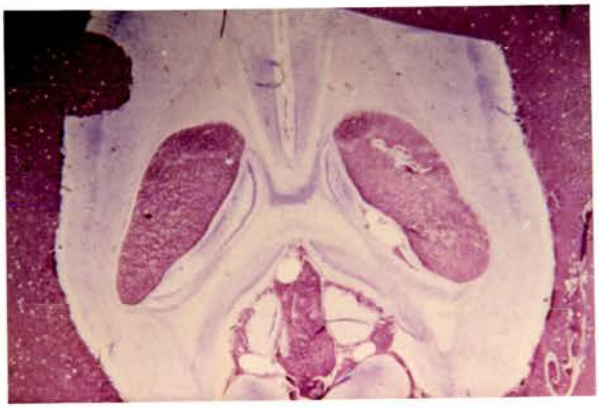


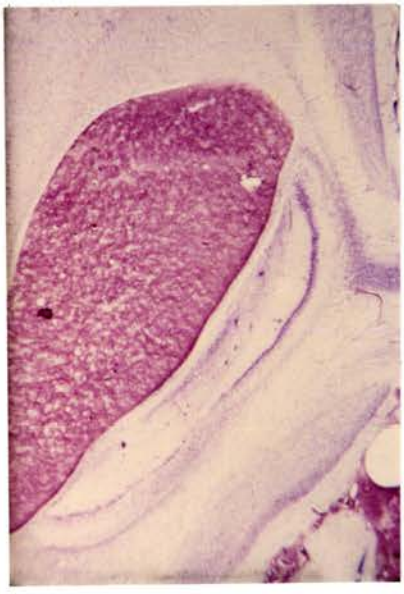
medial





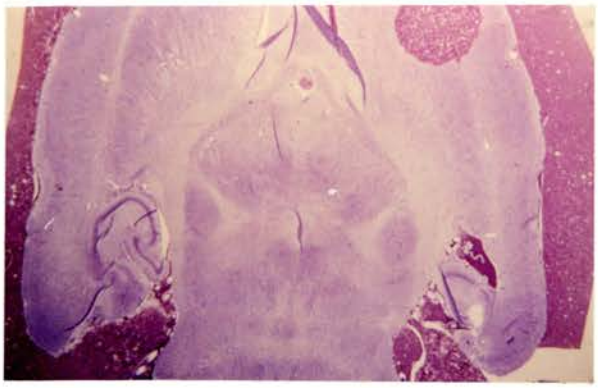
dorsal

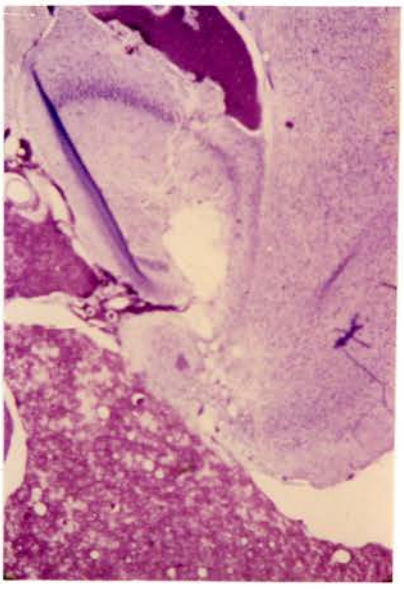




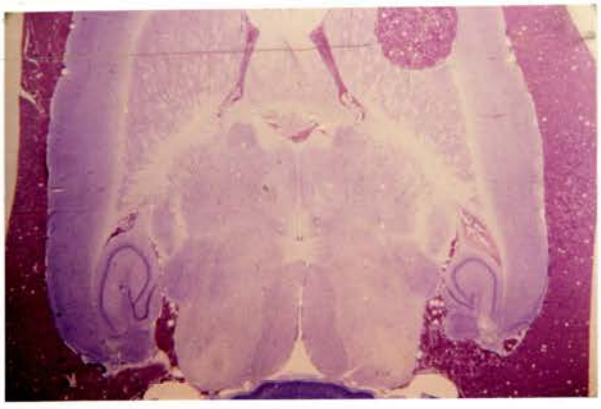
**SUB RAT NO. 5718**

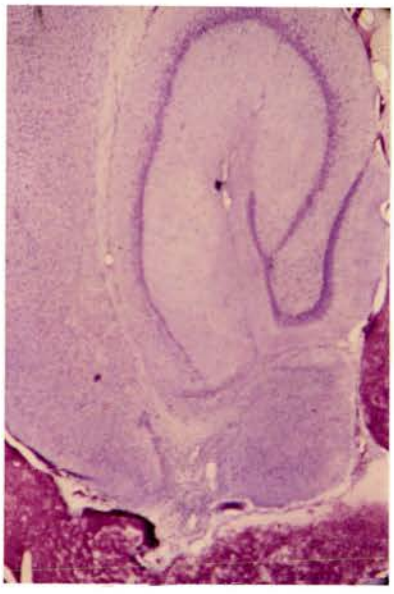
**ventral**



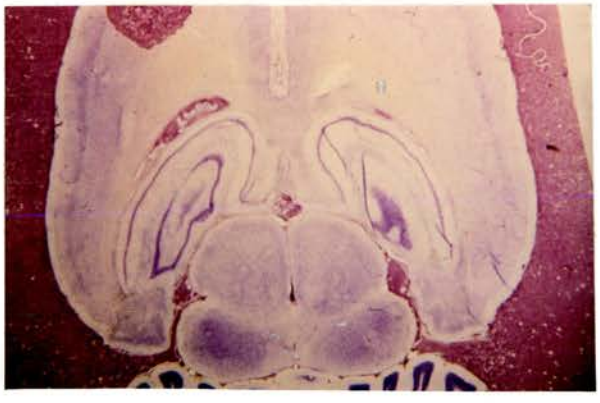


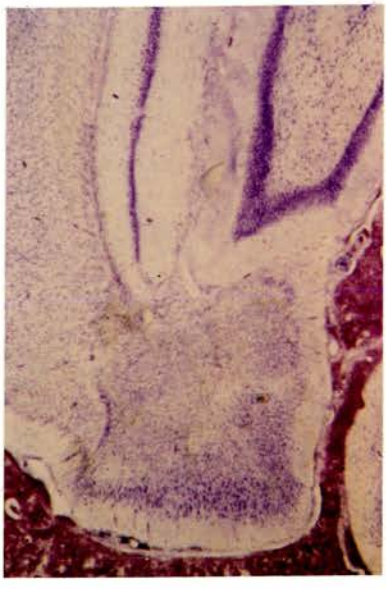
**medial**





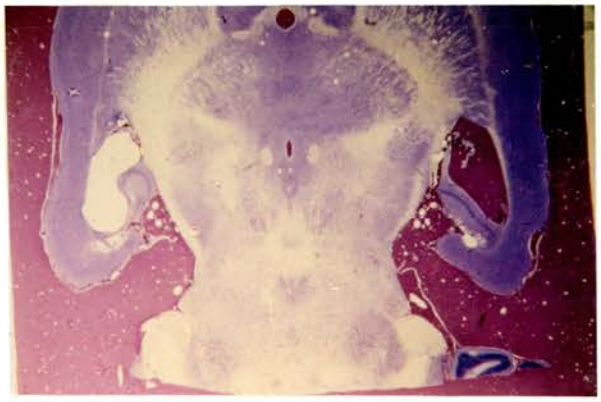
**dorsal**

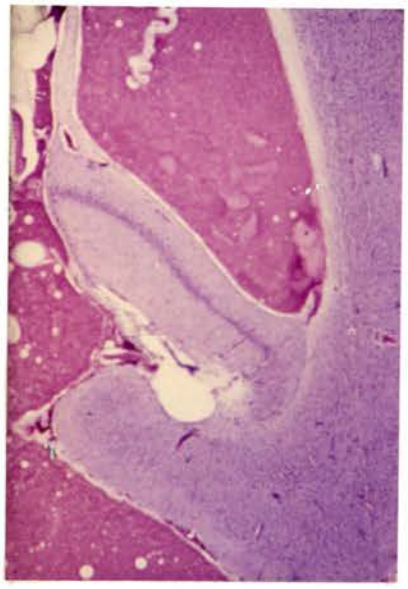




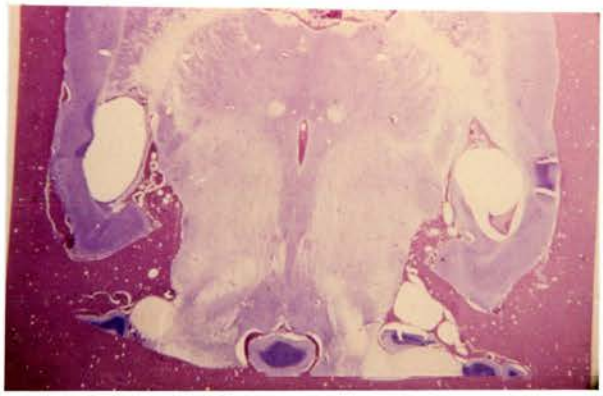
**SUBHIP RAT NO. 5739**

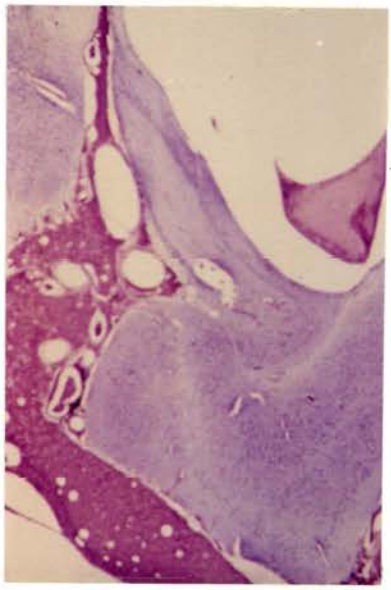
**ventral**



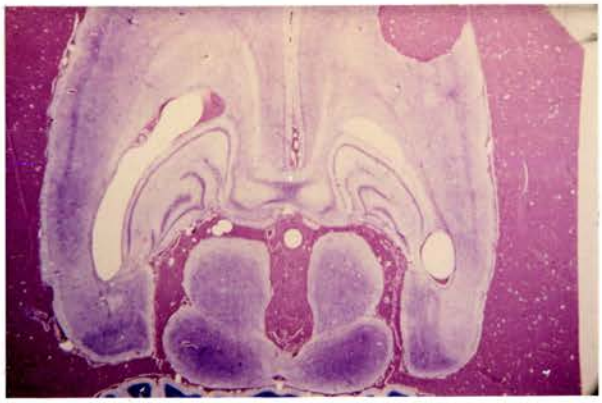


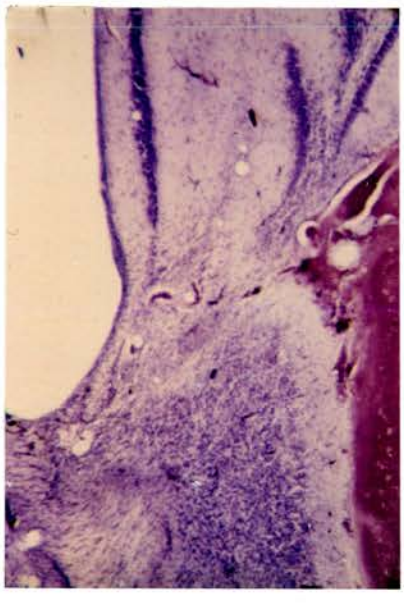
**medial**





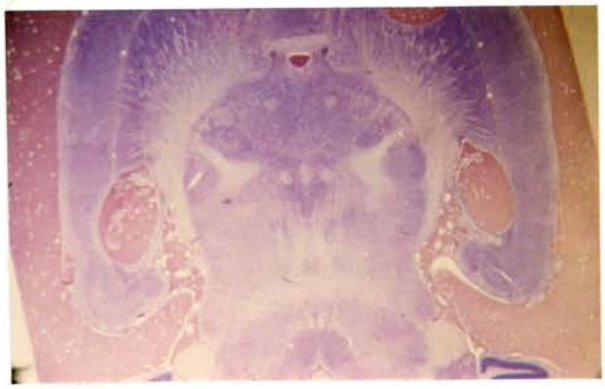
**dorsal**

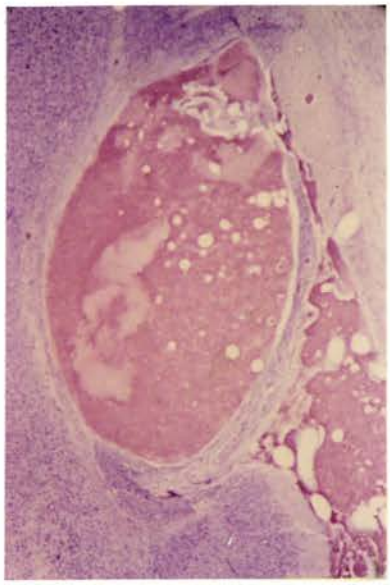




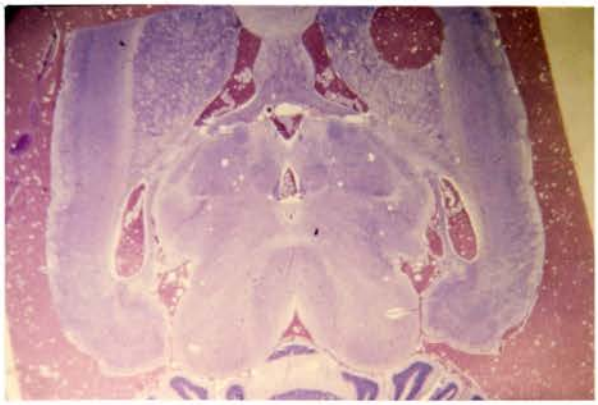
HIPSUB RAT NO. 5714

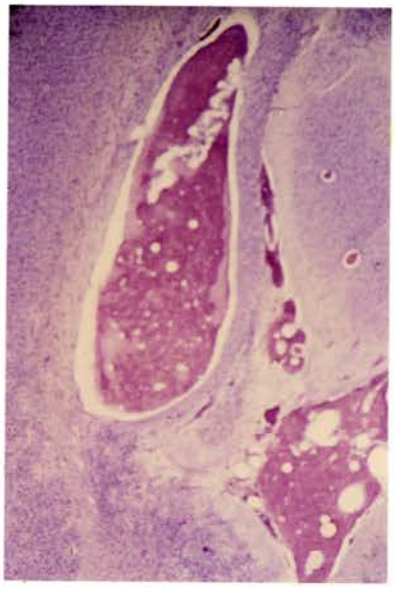
ventral



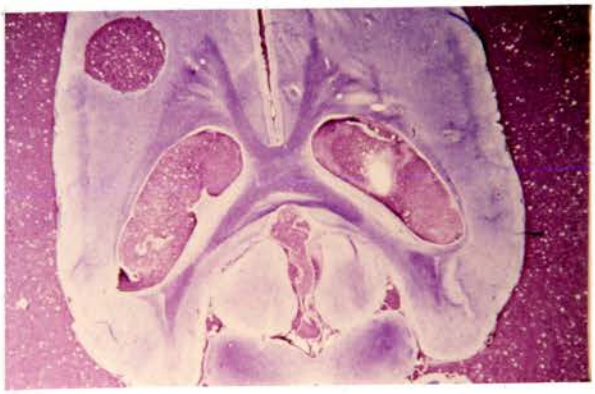


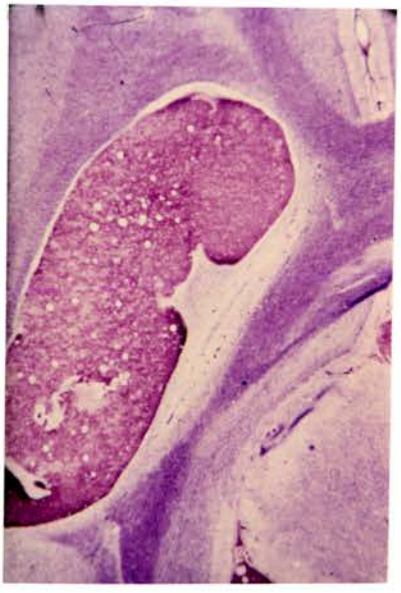
medial





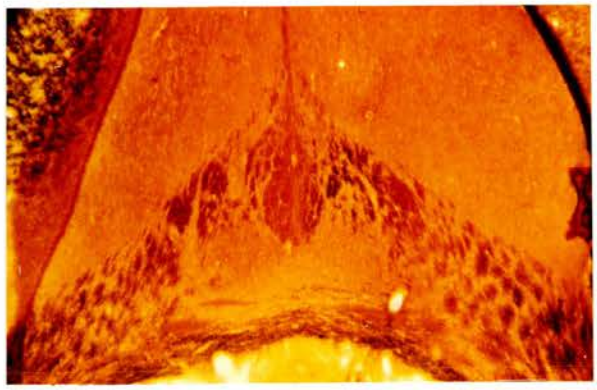
dorsal





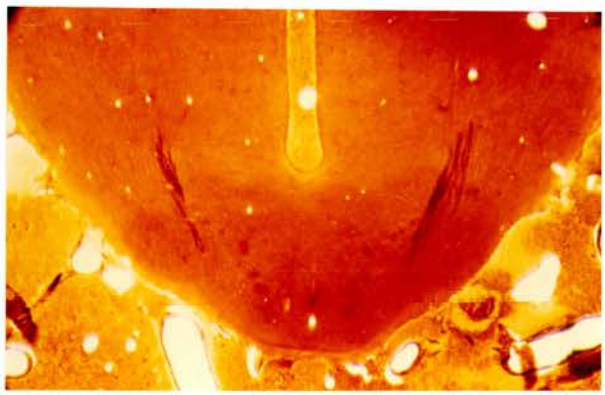
HIP RAT NO. 5713 FINK HEIMER STAIN

dorsal



SUB RAT NO. 5732

ventral



degeneration was found unilaterally in the lateral septum (at dorsal level -4.6 mm) confirming hippocampal damage. In addition, there was bilateral degeneration to septofimbrial nucleus, the hippocampal commissures and the fimbria at this level. Very slight bilateral degeneration of the fornix and the mammillary bodies suggest partial subiculum damage although it is possible that the damage in mammillary bodies may be secondary to damage in the septum, as the septum projects to the mammillary bodies (Meibach and Siegel, 1975; Nadler et al., 1978; Swanson, 1978). Fine terminal degeneration was present in the hippocampus presumably originating from other damaged pyramidal cells and unilaterally in the dentate gyrus which contains terminals of CA3 commissural fibres or could indicate damage to CA4 cells (Nadler, Perry Gentry and Cotman, 1980; Gottlieb et al., 1973; Hjorth-Simonsen, 1971; Swanson et al., 1977). Degeneration was present in the Schaffer collaterals and there was fine terminal degeneration in the subiculum (probably from deafferented hippocampal fibres).

The interruption of cortical connections caused degeneration in striatal fibres and in the pontine nuclei and also in the cerebral peduncle more dorsally. This damage was also present in the operated controls although no damage to hippocampal or subicular areas was evident.

Fig. 11 shows that the hippocampus was successfully damaged. The most important loss of cells was in the dorsal hippocampus, particularly of CA1 cells. This would destroy the fibres between CA1 and subiculum and therefore those to the septum and also the fibres projecting from the hippocampus to the entorhinal cortex (Swanson et al., 1977). Fibres of passage such as the projection from the subiculum to subcortical structures would also be destroyed.

A typical subiculum lesion is also shown in Fig. 11 (rat 5718).

The cresyl violet staining showed a bilateral subiculum and presubiculum lesion dorsally, with unilateral damage to the subiculum, presubiculum and parasubiculum but only partial subiculum and presubiculum lesion on the other side ventrally and medially. A fair amount of cell damage to the medial entorhinal cortex was present through all sections with slight damage to the hippocampus consisting of unilateral dentate damage ventrally and some CA1 cells medially. However, it was difficult determining the extent of the lesions in the subicular area with a cell stain. The silver stain proved to be more useful. This showed massive degeneration in the descending columns of the fornix, ventrally, heading into the mammillary bodies, indicating that the subiculum had been successfully lesioned (Fig. 11 rat 5732). Degenerated fibres stained in the fimbria dorsally. Fine degenerating terminals were present in the hippocampus and the subiculum unilaterally and in the entorhinal cortex bilaterally at a dorsal level. There was also striatal fibre damage in the external capsule medially. There was no evidence of damage to the terminal fields of the perforant path which courses through the subiculum to the dentate gyrus (Steward, 1976).

A typical example of the SUB+HIP group is rat 5739 in Fig. 11. This was intended as a subiculum lesion. However, inspection of the cell stained section shows that there was similar cell loss in the subiculum as in the SUB group but the damage also included ventral hippocampus (bilateral dentate gyrus, CA4, partial CA3, partial CA1) but with parts of dorsal hippocampus spared. Silver stain sections show both degeneration in the fornix and in the lateral septum indicating that both the subiculum and the hippocampus have been

damaged*. Other areas of degeneration include the hippocampal commissures, fimbria, hippocampus and subiculum, striatal fibres and a small part of the entorhinal cortex at the dorsal level.

Finally, Fig. 11 also shows sections from an animals in the HIP+SUB group (rat 5714). This was intended to be a HIP lesion but as the example shows the cell loss has occurred beyond the hippocampus and into the subiculum. The silver stain confirms this.

---

* Subicular damage alone could account for both the lateral septal degeneration and fornical degeneration.

# CHAPTER 9

# EXPERIMENT 1

## 9.1 Method

### a. Place Training - Reference Memory Task

Place training with the hidden platform consisted of 28 trials spread over the following five days (4 training trials on the first, second and fifth day, eight trials on the third and fourth days). For half the animals in each group, the hidden platform was placed at a precise point in the middle of the NE quadrant, and the other half in the middle of the SW quadrant (placed using a circle drawn on the video-monitor. Two locations were used in order to offset spatial bias known to occur in this pool). The top of the platform lay 1 1/2 cm below the water level. The time taken to find the platform was recorded.

### b. Transfer Test

A transfer test was conducted after the last training session to assess the animals' "spatial bias". The platform was removed, the tracking programme "RATRUN" initiated by pressing a switch at the starting position, and the rat was placed in the pool for sixty seconds. The "RATRUN" programme produced the x,y coordinates which were stored on floppy disc, from which RATN computed the time spent in each of the four quadrants of the pool and the frequency with which the rat crossed the former location of the platform (an annulus). These measure the accuracy of the rat's memory for the training quadrant.

## 9.2 Results

### a. Body Weights

An unequal analysis of variance of the animals' body weights, with the lesion condition (Gps = 5) as the between subjects factor and days ( N = 12, 1 pre- and 11 post-operative days) as the within subjects factor, revealed no significant difference between groups (F < 1). There were a significant changes in weight over the 12 days (F = 3.2, d.f. = 11/341, p < 0.0005), with the pre-operative mean weight of 367.9 g dropping to 363.3 g on the following day and then steadily climbing to 370.8 g by the twelfth day.

b. Escape Latencies on Place Training

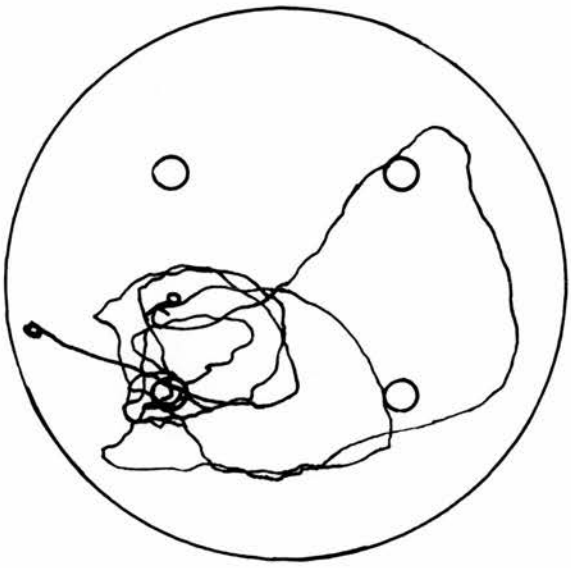
Qualitative Assessment of Experiment 1: Most of the controls initially swam all over the pool although some scraped at the edge. As they began to learn the task they increasingly spent more time in the training quadrant and passed quickly through the other quadrants depending on entry point and platform location. Performance of certain typical animals during transfer test 1 is illustrated in Fig. 12.

During training, the controls and operated controls learned the task rapidly (over the first 2 sessions), only reaching the 120 second time limit on a few trials. In contrast, the rats with hippocampal lesions (HIPs) had to be shown the platform more often and for more sessions. Their performance did improve over trials, however, and by the final session (trials 24-28) the rats had learnt to escape unaided. The rats with subiculum lesions (SUBs) and the rats with subiculum and hippocampal damage (SUB + HIPs) found the task more difficult with more 120 second trials and a less pronounced improvement in performance. The rats with hippocampal and subiculum damage (HIP + SUBs) performed most poorly, having to be guided to the platform on more trials and showing no consistent level of performance. Initially the HIP + SUB and SUB + HIP were thigmotaxic,

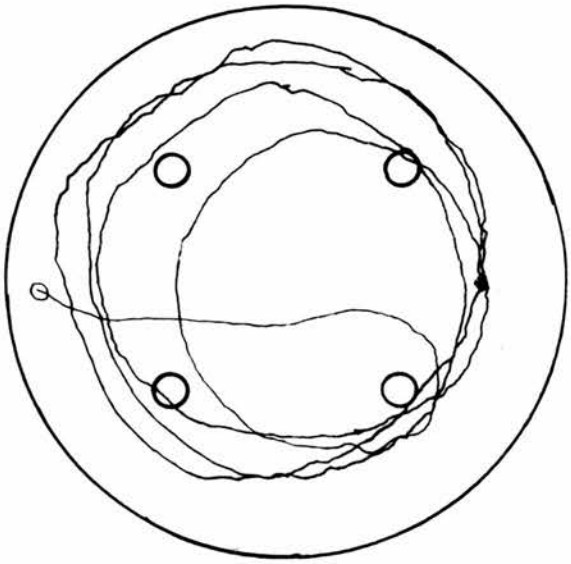
FIG. 12

The pathway taken by a typical rat from each group in transfer test 1. These rats were trained to the S.W.

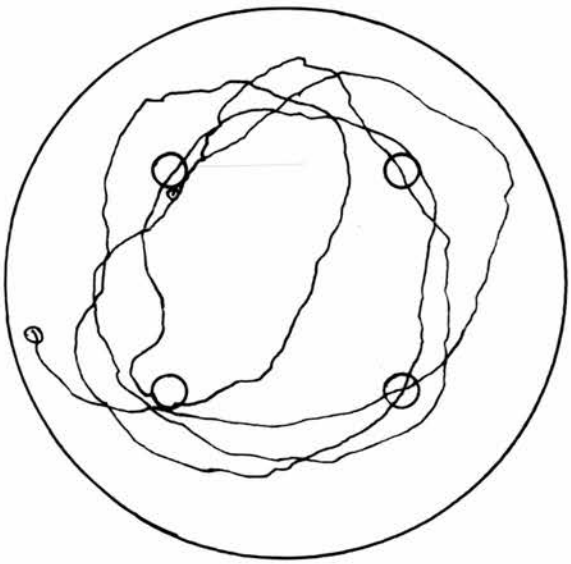
CONTROL



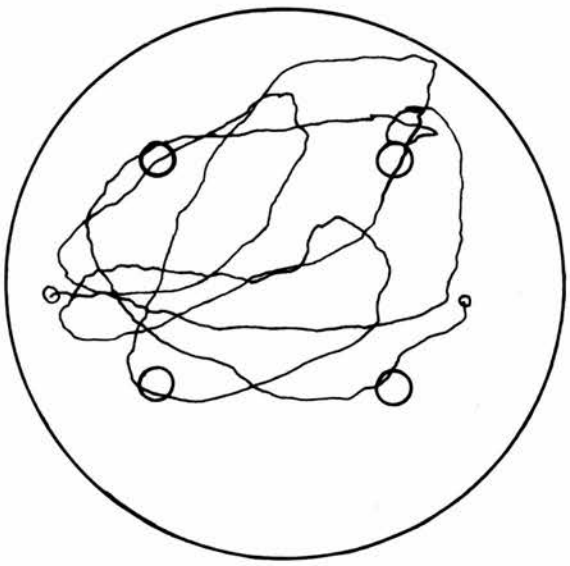
HIP



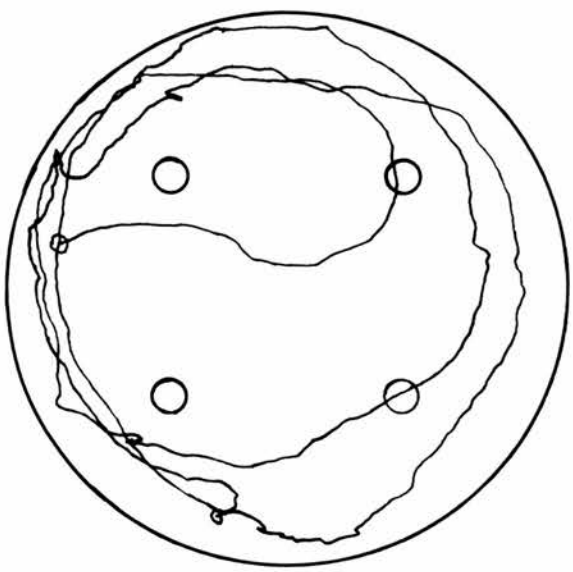
HIP SUB



SUB



SUB HIP



only swimming round the side walls. HIPs, HIP + SUBs, and SUB + HIPs developed a strategy of swimming in circles round the pool passing through all 4 quadrants and often crossing the annuli. The SUB group swam all over the pool in no particular pattern but spending more time in the adjacent left quadrant. A few of the ibotenate lesioned animals were noticably more irritable and difficult to handle with a tendency to leap off the platform. All the rats used the normal adult swimming posture (Schapiro, Salas and Vikovich, 1970), with none of the lesioned rats showing any sign of gross motor discoordination. Casual observation suggested no difference between groups in incidence of rearing and grooming.

Quantitative Analysis of Experiment 1: No difference in behaviour of cortical operated and unoperated control animals was discerned and they were therefore combined into a single control group (N =14). An unequal N analysis of variance was performed on escape latencies for the 5 groups with the data collapsed into blocks of 4 trials per block. Lesion condition was the between subjects factor, and trials and blocks were the within subjects factors. The results showed a highly significant groups effect ($F = 10.4$, d.f. = 4/31, $p < 0.0001$), together with an improvement over blocks ($F = 42.1$, d.f. = 6/186, $p < 0.0001$). In order to establish the differences between groups, post hoc analyses using the Scheffe Test (Myers, 1966) with the error level experiment-wise set at 10%, provided a reasonably conservative approach. This procedure is used routinely throughout the results section. Using the Scheffe Test, the mean latency (trial 1-28) of the HIP rats (43.8 secs) was significantly higher than the mean for controls (23.6 secs, $F = 10.55$, $p < 0.1$). The HIP group was performing significantly better than the HIP + SUB group (67.6 secs, $F = 11.6$, $p < 0.1$). The HIPs

were not significantly different from SUBs and SUB + HIPs ($F < 1$). Data (plotted in Fig. 13) indicated a decrease in latency for each group as training progressed, implicating an improved search strategy. Different rates of spatial learning in the groups is revealed in a significant groups x blocks x trials interaction ($F = 1.72$, d.f. = 72/558, $p < 0.0005$).

The escape latencies of rats trained 12 days after surgery were compared with the escape latencies of rats trained 100 days after surgery. With such small numbers of rats in each of the 4 experimental groups given lesions, the analysis was conducted by collapsing across groups for each of the 2 post-surgery recovery periods. The mean escape latencies of the 9 rats trained 12 days after surgery was 54.4 seconds, while that for the 12 rats trained after 100 days was 52.8 seconds; an analysis of variance confirmed that these did not differ ($F < 1$), that is, that there was no strictly time-dependent recovery of function.

Data (plotted in Fig. 14) was treated to an unequal N analysis of variance on time spent in each of the 4 quadrants (standardised to 60 seconds) with time spent in each quadrant as a within-subject factor and lesion condition as a between-subject factor. There was a highly significant interaction between groups and quadrants ($F = 5.36$, d.f. = 12/93, $p < 0.0001$), ascribed to the bias toward time spent in the training quadrant by the controls in comparison with time spent in all quadrants by the experimental groups. The average of the adjacent left quadrant mean, adjacent right quadrant mean and the opposite quadrant mean was subtracted from the training quadrant mean in order to determine spatial bias. Scheffe Tests (Myers, 1966) showed that the hippocampals were significantly poorer than controls ($F = 24.8$, $p < 0.1$). From Fig. 14, this result indicates that all other groups were also significantly different from the controls. An

FIG. 13

Mean latency of escape for the 28 trials of experiment 1. Error bars represent standard errors.

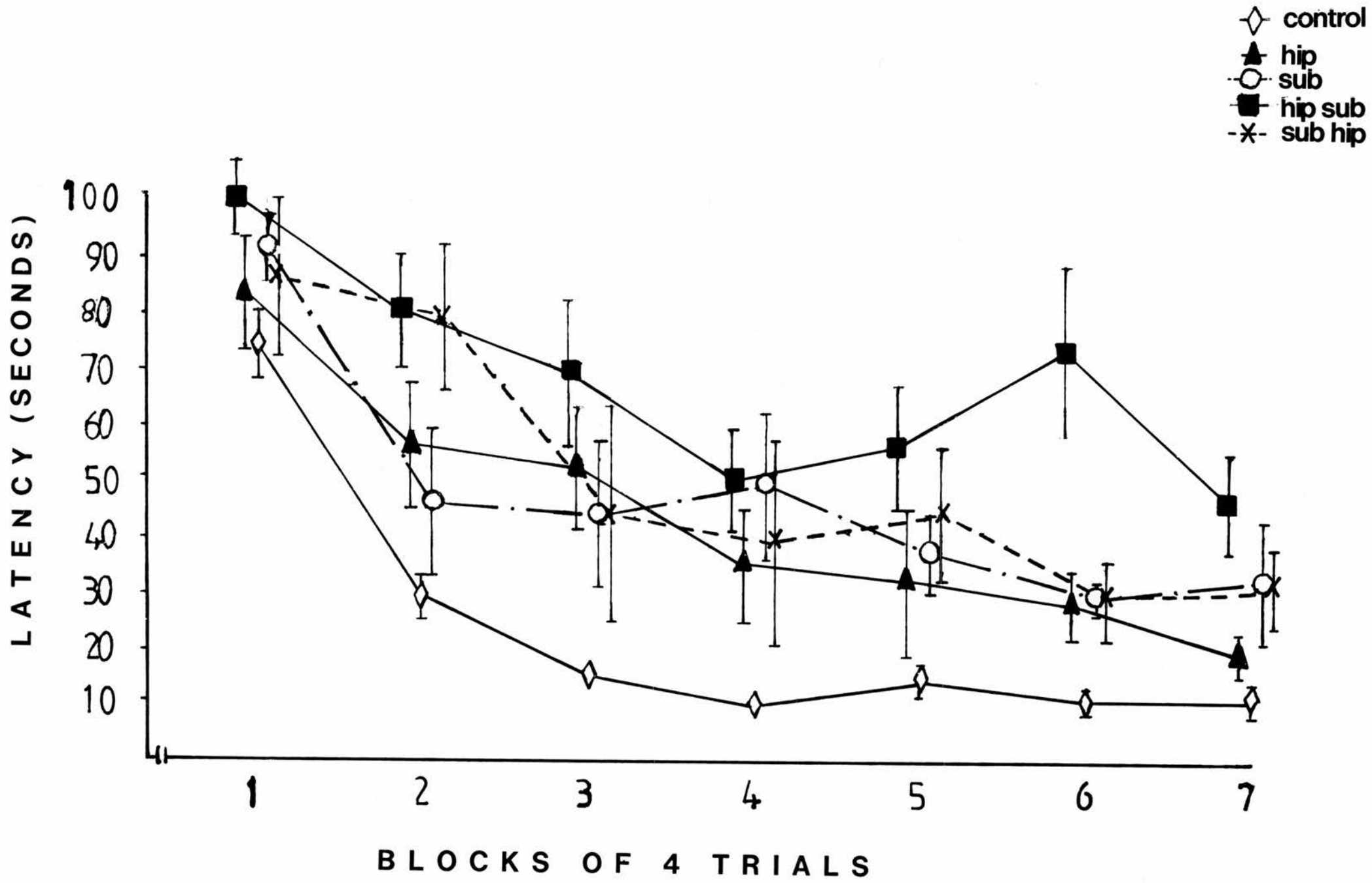
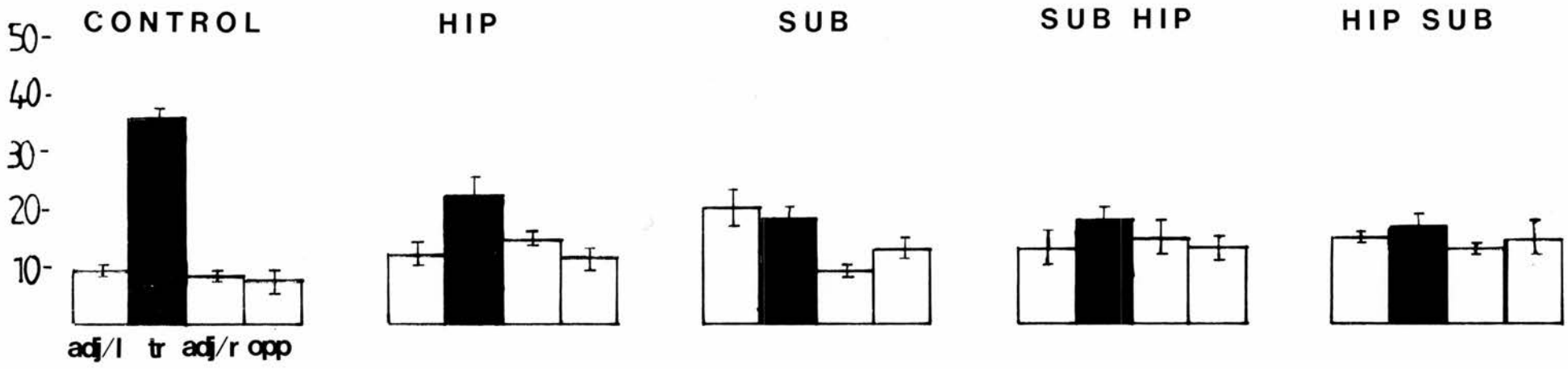

control
hip
sub
hip sub
sub hip
LATENCY (SECONDS)
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
BLOCKS OF 4 TRIALS

FIG. 14

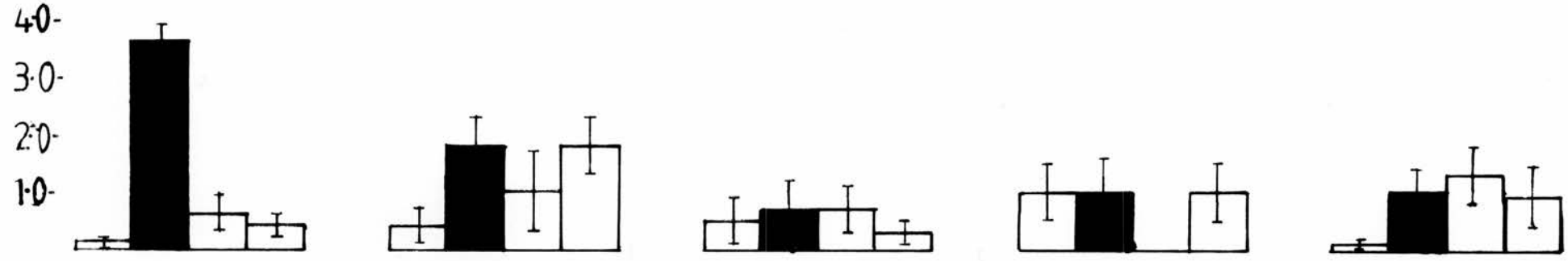
Transfer test data for experiment 1.
Error bars represent standard errors.

tr = training quadrant
adj/l = adjacent quadrant to the left of training quadrant as viewed from above.
adj/r = adjacent quadrant to the right of training quadrant as viewed from above.
opp = opposite quadrant to the training quadrant as viewed from above.

TIME SPENT IN EACH QUADRANT(SECONDS)




NUMBER OF CROSSINGS OF EACH ANNULUS



unequal N analysis of variance between time intervals indicated no difference between groups ($F = 1.97$, d.f. = 3/60, $p > 0.10$) on these transfer test scores also.

An analysis of variance was performed on the frequency each annulus was crossed as a second index of spatial bias. The between subjects factor was lesion condition, and the within subject factor the number of entrances of each annulus. There was a highly significant spatial bias ($F = 9.87$, d.f. = 3/93, $p < 0.0001$) which was distributed differently across groups (groups x annulus interaction; $F = 4.61$, d.f. = 12/93, $p = 0.0001$). Post hoc analyses using Scheffe Tests (after taking the average of the means of the other quadrants away from the mean of the training quadrant) showed that the hippocampal group was significantly different from controls ($F = 22.56$, $p < 0.1$). This indicates that all other groups were significantly different from controls. These results reveal that none of the ibotenate-lesioned groups showed any sign of spatial bias towards the training quadrant. An unequal analysis of variance between the 2 time periods revealed no recovery of function ($F < 1$).

# CHAPTER TEN

# EXPERIMENT 2

## 10.1 Method

Experiment 2 followed on from Experiment 1 and used the same rats. Its purpose was to explore the effects of extended training on the reference memory procedure.

The experiment involved running 48 trials over 6 days. This consisted of 8 trials per day, 4 of these being place learning and 4 being cue learning. Cue learning was included as a control measure to ensure that the place task was testing spatial learning per se rather than sensorimotor ability, motivation to escape or reinforcement. The order of cue vs place training varied over days. The animals were given a transfer test on the seventh day.

### a. Place Learning

This was a continuation of the training used in Experiment 1.

### b. Cue Learning

A taller platform was used for cue learning, protruding 1 1/2 cm out of the water, and painted with black and white stripes to be easily visible. It was placed on the exact former location of the hidden platform and the procedure followed that of place training.

## 10.2 Results

### a. Escape Latencies on Place Learning

Qualitative Assessment of Experiment 2: Several ibotenate lesioned animals (1 SUB, 4 HIP + SUB, 2 SUB + HIP) still had to be directed to the hidden platform on a few trials throughout this experiment. These animals also showed a remarkably inconsistent performance over trials between animals (as an illustration, scores

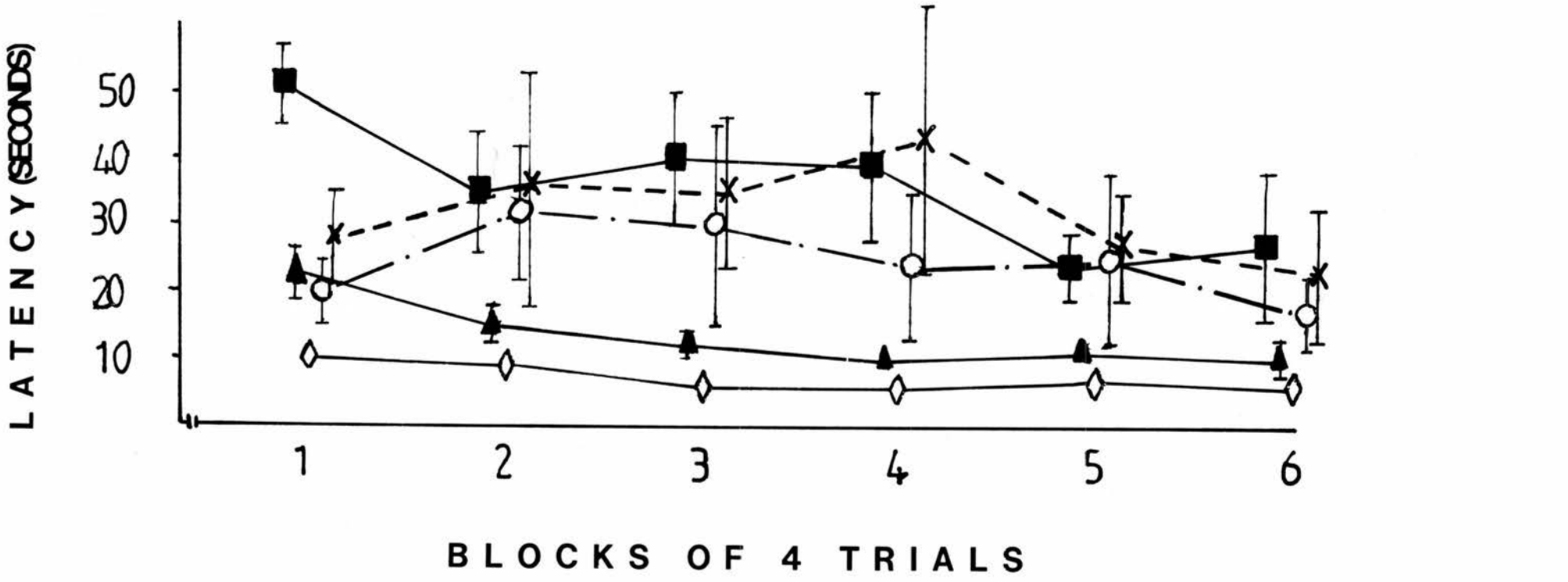
on trials 47-50 for rat 5724 in HIP + SUB group were 7.9, 51.9, 120.0, 13.8 seconds). The latencies for the control and hippocampal animals decreased over time but their ability to perform reached "floor" levels very quickly. While HIP + SUB improved slightly over blocks of trials, their performance and those of the SUB and SUB + HIP groups remained worse than that of the HIP and control groups. The controls and HIP groups took direct paths and so rarely crossed other annuli. This shows that the hippocampal rats have learnt more than swimming away from the side walls and how to escape. Therefore, the deficit is transitory. The SUB and SUB + HIP groups circled and looped across the pool passing through the training annuli more frequently than the other annuli showing some spatial bias. The HIP + SUB group showed no improvement from transfer test 1.

Quantitative Analysis: An unequal N analysis of variance was conducted on escape latencies during place training with groups as the between subjects factor and blocks of 4 trials as the within subjects variables (Fig. 15). Results reveal a significant groups effect for the place task ($F = 7.64$, d.f. = 4/31, $p < 0.0005$). Post hoc analysis using the Scheffe Test confirmed that the hippocampal group did not differ significantly from controls ($F = 1.15$, $p < 0.1$) but did perform significantly better than the mean of the HIP + SUB and SUB + HIP groups ($F = 12.33$, $p < 0.1$). The SUBs differed significantly from controls ($F = 12.12$, $p < 0.1$) but not from the hippocampals ($F = 2.92$, $p < 0.1$) nor from the mean of SUB + HIPs or HIP + SUBs ($F = 2.89$, $p < 0.1$). A significant result was found for the groups x blocks x trials interaction ($F = 1.93$, d.f. = 60/465, $p < 0.0005$) indicating subtle differences in rate of spatial learning between groups over sessions. These were not analysed further.

These results show that the hippocampal rats can learn a place learning task but require a large number of trials (76 in total) to

FIG. 15

Mean latency of escape for the 24 trials of the place task in experiment 2.
Error bars represent standard errors.


control
hip
sub
hip sub
sub hip
LATENCY (SECONDS)
50
40
30
20
10
1
2
3
4
5
6
BLOCKS OF 4 TRIALS

achieve comparable performance with controls. There are 2 possible reasons for slower learning: Firstly, it is possible that the deficit in initial place learning by the hippocampals is only transitory; or secondly, that the hippocampal rats have learned no more than to swim away from the side walls and also how to climb on to the platform to escape. The latency data on their own do not distinguish these alternatives.

An analysis of variance between the means of the 2 recovery periods showed that the 100 day old rats to be escaping faster than the 12 day rats ($F = 6.03$, d.f. = 1/20, $p < 0.05$). In view of this significant result, a further analysis of the 2 subgroups of hippocampal animals only was conducted. No significant effect of recovery interval was obtained ($F = 1.96$, d.f. = 1/3, $p > 0.25$). The mean escape latencies on place trials for the 2 hippocampal subgroups were 11.8 and 15.6 seconds (12 day, 3 and a half month respectively), and 9.4 and 12.1 seconds over the last 2 blocks of training, indicating that the improved performance was not due to time-dependent recovery of function. Thus insofar as there were any trends within the hippocampal group they favoured the rats trained for 12 days*.

* See Footnote next page

* This leaves open the question of groups involved. Further analysis of the groups also revealed no significant effect of recovery of the HIP + SUB group ($F = 3.83$, d.f. = 1/5, $p > 0.10$). However, analysis of the SUB group showed a significant effect of recovery interval ($F = 123.32$, d.f. = 1/4, $p < 0.0005$) with the mean for the first time-interval (63.5 sec) being greater than the mean for the second time-interval (16.8 sec). But this result must be treated with caution, as there was only 1 rat in the first time-interval and 5 rats in the second time-interval. Analysis of the SUB + HIP group also revealed a significant effect of recovery interval ($F = 73.52$, d.f. = 1/2, $p < 0.05$) with the mean for the 12 day time-interval (44.7 sec) being greater than the mean for the 100 day time-interval (19.4 sec). However, there were only two rats in each time-interval.

b. Analysis of Transfer Test 2

Quadrant Scores: The control group's performance was similar to the transfer test 1 (see Fig. 16). The HIP, SUB and SUB + HIP groups all showed a consistent improvement with the HIP + SUB group remaining the same. The groups x quadrant interaction was highly significant ($F = 4.26$, d.f. = 12/93, $p < 0.0001$). Post hoc analysis with the Scheffe Test confirmed that the hippocampals showed a spatial bias which did not differ significantly from controls ($F < 1$). However, the HIP + SUB group did spend significantly less time in the training quadrant than the HIP group ($F = 24.79$, $p < 0.1$). Fig. 16 shows that the SUB and SUB + HIP groups spent less time than controls but more time than HIP + SUB in the training quadrant. These results show that the spatial localisation deficit remained in the case of the SUB + HIP and HIP + SUB groups even after trials with the visible platform, in which these lesioned rats had escaped relatively rapidly. Therefore, the initial place learning deficit of the HIP group (in Expt 1) was a consequence of a transient or gradually diminishing general learning impairment.

An unequal N analysis of variance between time intervals gave no significant results ($F < 1$).

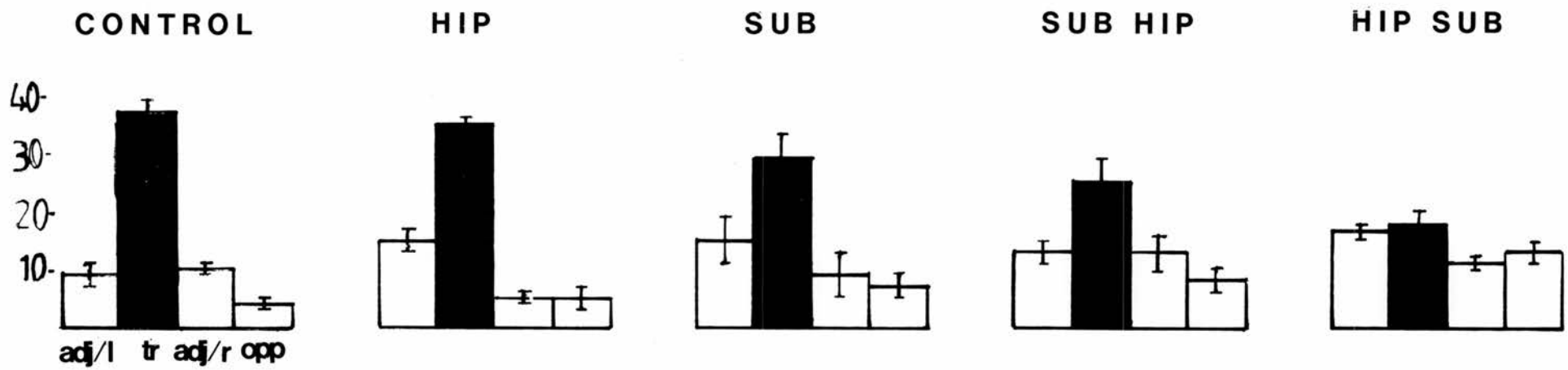
Annulus Scores: An analysis of variance for the groups of experiment 2 established a significant annulus effect ($F = 16.65$, d.f. = 3.93, $p < 0.0001$) reflecting a disproportionate crossing of each annulus, and a significant groups x annulus interaction ($F = 2.20$, d.f. = 12/93, $p < 0.05$). A post hoc analysis revealed that the spatial bias of the HIP group was not significantly different from the controls ($F = 5.26$, $p < 0.1$), nor different from the SUB group ($F = 5.03$, $p < 0.1$). However, the control and HIP + SUB groups did

FIG. 16

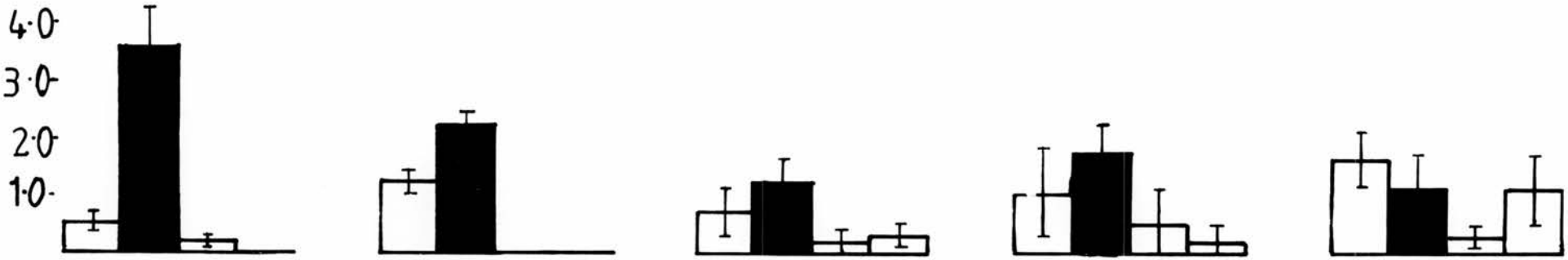
Transfer test data for experiment 2.
Error bars represent standard errors.

tr = training quadrant
adj/l = adjacent quadrant to the left of training quadrant as viewed from above.
adj/r = adjacent quadrant to the right of training quadrant as viewed from above.
opp = opposite quadrant to the training quadrant as viewed from above.

TIME SPENT IN EACH QUADRANT(SECONDS)




NUMBER OF CROSSINGS OF EACH ANNULUS



still differ ($F = 27.34$, $p < 0.1$).

An unequal analysis of variance revealed no significant difference between time periods ($F = 1.18$, d.f. = 1/20, $p > 0.10$).

Heading Angle: Heading angles were analysed at 50 cm and 100 cm distance along path over the last session (4 trials) before the transfer test. These data are tabulated in Table 3. At 50 cm into the path, control rats were deviating from the platform by, on average, no more than 11.9°. The HIP group were also accurate in their approach to the platform (14.6°) while the HIP+SUB group were deviating from the "correct" path by a mean of 42.7°.

An unequal analysis of variance showed a statistically significant difference between the groups both at 50 cm ($F = 7.4$, d.f. = 4/31, $p < 0.0005$) and 100 cm ($F = 5.6$, d.f. = 4/31, $p < 0.005$). Further analysis using the Scheffe Test (Myers, 1966) revealed that the hippocampal and control groups were not significantly different at 50 cm ($F = 1.8$, $p < 0.1$) or at 100 cm ($F = 1.3$, $p < 0.1$). These results show that the hippocampals were as accurate as the controls in heading for the platform at the end of Experiment 2.

The means for the other groups indicate that they were heading at an acute angle away from the platform. The SUBs were more accurate than the SUB + HIP or the HIP + SUB but all these groups had high standard errors for each trial indicating a more random approach. This implies that although the SUB, SUB + HIP and HIP + SUB groups could learn that escape was possible they were unable to swim accurately to the platform from a distance, showing that they had difficulty learning where the platform was located. These points are illustrated in Fig. 17 which plots the last 4 trials for typical rats from each group. Note that the Control and HIP rat headed

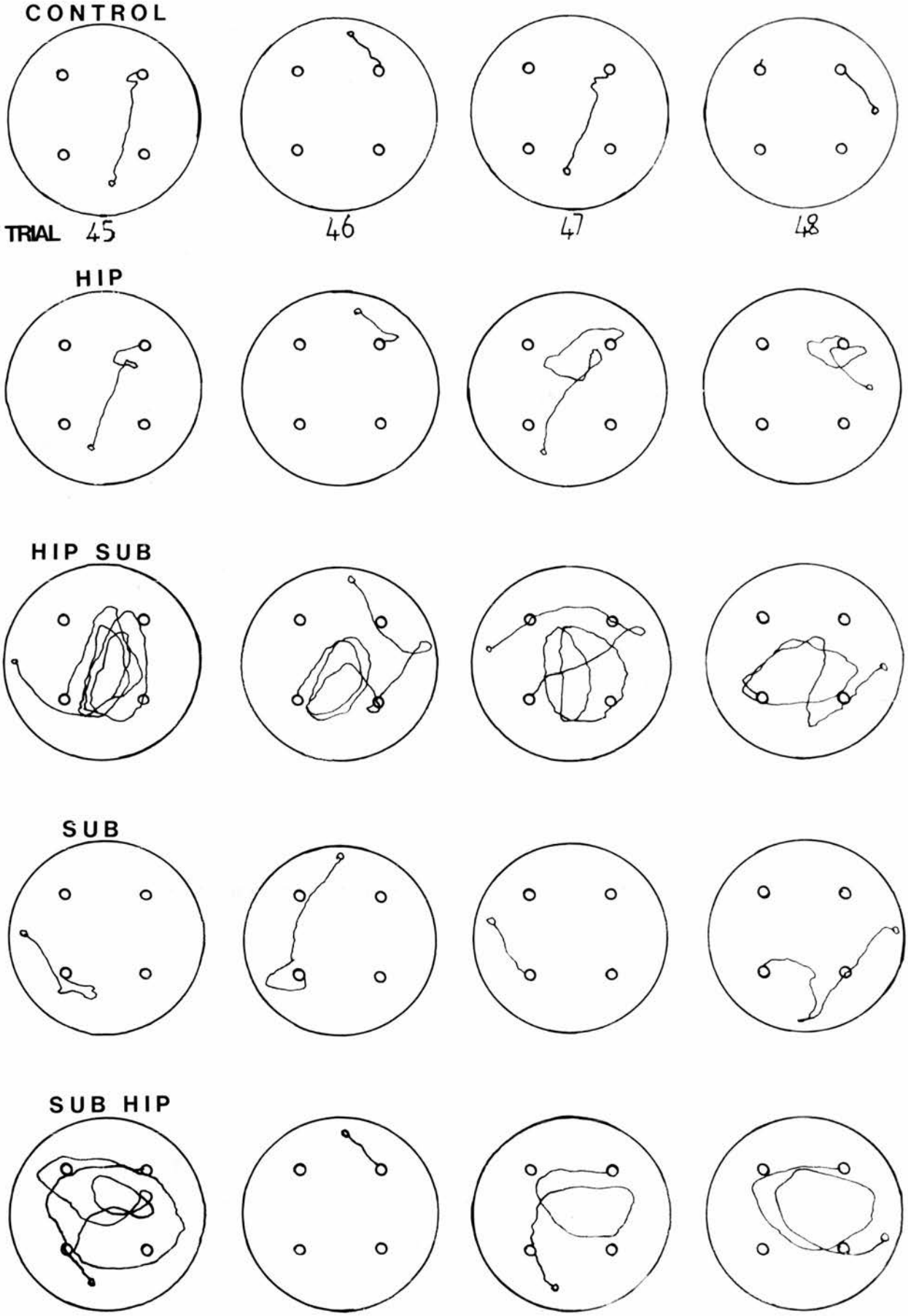
TABLE 3

Mean heading angle in experiment 2.

| | GROUP | | | | |
|---|---|---|---|---|---|
| | CONTROL | HIP | SUB | SUB+HIP | HIP+SUB |
| at 50cm | 11.900 | 14.610 | 24.533 | 40.138 | 42.707 |
| at 100cm | 10.657 | 19.690 | 34.433 | 40.275 | 45.321 |

FIG. 17

Pathways taken by each group over the last 4 place trials of experiment 2.


CONTROL
TRIAL 45
46
47
48
HIP
HIP SUB
SUB
SUB HIP

accurately towards the platform from each of 4 starting positions. The SUB rat also does well, but is inaccurate on the last trial. Other SUB rats also showed variability of performance. The HIP + SUb and SUB + HIP rats show much more varied paths.

Swimming Speed: The mean swimming speed was 25 cm/sec and the range of mean swimming speeds across groups was from 22.2 to 29.8 cm/sec. An unequal analysis of variance on speed of swimming gave a significant groups effect ($F = 4.3$, d.f. = 4/31, $p < 0.01$), the HIP + SUB group being the fastest swimmers. This is probably not due to a direct effect of the lesion. The HIP and controls did not differ (means of 25.9 and 24.7 cm/sec). The means indicate that only the SUB and SUB + HIP groups were slower than controls. However, post hoc analysis using the Scheffe Test (Myers, 1966) showed that they were not significantly slower ($F = 2.0$, $p < 0.1$).

There was a 20% difference in swimming speed between controls and HIP + SUB group but in the opposite direction from the difference between these groups with respect to latency. Therefore swimming speed cannot be a factor in explaining the poorer place-learning performance of the HIP + SUB group.

b. Escape Latencies on Cue Training

Qualitative Assessment: All groups showed a faster and more consistent performance with the visible platform than in place training. Ibotenate lesioned animals were slower than controls over all blocks, but the impairment was very small.

Quantitative Assessment: An unequal analysis of variance was conducted on escape latencies in the cue task. A significant group

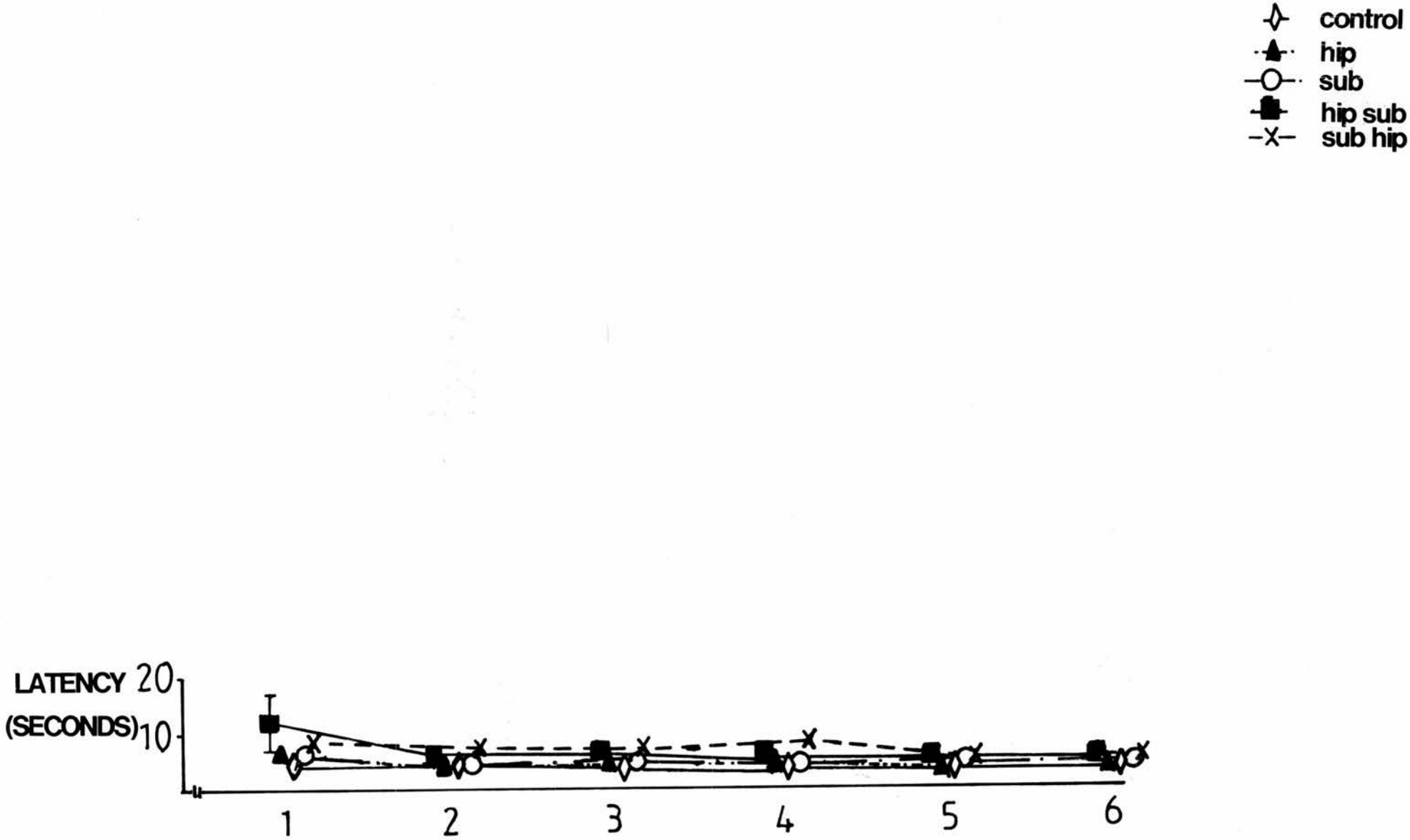
effect was discovered ($F = 5.37$, d.f. = 4.31, $p < 0.005$), illustrated in Fig. 18, indicating that some lesioned animals showed a deficit in the cue task.

Post hoc analysis using the Scheffe Test revealed that HIPs were not significantly different from controls ($F < 1$), nor from SUBs ($F < 1$), but were escaping significantly faster than HIP + SUB ($F = 9.92$, $p < 0.1$). SUBs did not differ from controls ($F = 2.11$, $p < 0.1$), nor from SUB + HIPs ($F = 3.90$, $p < 0.1$). However, SUB + HIPs were slower than controls ($F = 12.40$, $p < 0.1$). This result shows that the HIP and SUB groups did not suffer from sensorimotor deficits. Bearing in mind that the visible platform remains in the same position in each trial, the HIP + SUB and SUB + HIP rats have probably been disadvantaged in this task by their inability to orientate in the pool because of their large spatial learning deficit. Although the effect of blocks was significant ($F = 7.23$, d.f. = 5/155, $p < 0.0001$), there was no groups x blocks nor groups x trials interaction indicating that performance did not improve.

Unequal analysis of variance between the 2 time intervals showed no significant difference ($F = 1.15$, d.f. = 1/20, $p > 0.25$) indicating no recovery of function as a function of time interval.

FIG. 18

Mean escape latency over the 24 trials of the cue task in experiment 2.
Error bars represent standard errors.


control
hip
sub
hip sub
sub hip
LATENCY (SECONDS)
20
10
1
2
3
4
5
6
BLOCKS OF 4 TRIALS

# CHAPTER 11

# EXPERIMENT 3

## 11.1 Method

Experiment 3 followed on from Experiment 2 and used the same rats.

Spatial 'Matching to Sample' Procedure - Working Memory Task

This task involved finding the hidden platform, with 4 trials a day, for 16 days. The platform position was changed daily with the locations varying quasi-randomly between N,S,E,W (at a short distance from the pool edge), NW, NE, SW, SE (in middle of quadrant). The important factor in this task is the latency to escape on trial 2 relative to trial 1. Morris (1983) showed that rats remember the position of the platform on trial 1 and so are quicker on trial 2 because they use a win-stay strategy.

For the first eight days the four trials of each day were scheduled at a 30 second intertrial interval. During the second eight days, the intertrial interval between trials 1 and 2 was the 30 seconds on the platform, plus 0 seconds, 3 minutes, 24 minutes or 3 hours. Each rat had two sessions at each interval. During the shorter intervals the rat was left in a black bucket beside the pool, while for the longer intervals they were kept in their cages, but briefly placed in the black bucket before resuming the session. The purpose of extending the intertrial interval between trials 1 and 2 was to see if this differentially affected the working-memory of any of the groups. Trials 3 and 4 were given to control for any improvement in latency, between trials 1 and 2, being due to motivational or non-specific differences.

## 11.2 Results

a. Qualitative Observations

All rats performed better as the trials progressed from daily trials 1-4 (see Fig. 19). This reflected improved searching strategy as well as working memory for the preceding trial. However, even by the fourth trial the lesioned rats had not learnt the task (the SUBs did best at a poor 30 seconds). The HIP + SUB group showed little or no improvement across trials.

On the initial sessions, some HIP rats were biased towards the quadrant in which the platform had been located during Experiments 1 and 2. However, as the sessions progressed, the lesioned groups tended to circle the pool, with some rats becoming thigmotaxic. Some lesioned rats brushed past the platform but did not climb on and some became irritable (ie. difficult to handle).

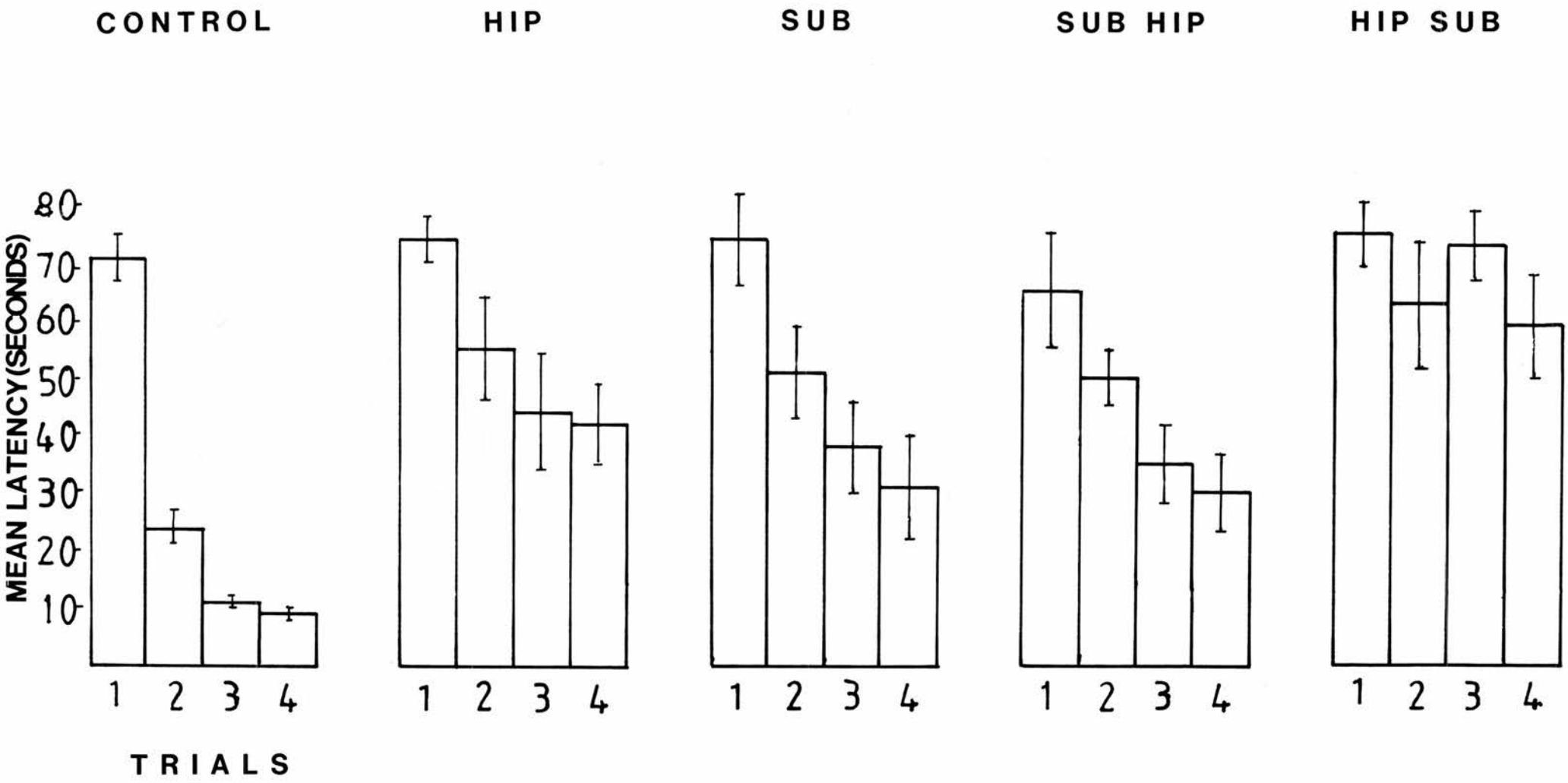
On subsequent sessions the controls would often search in the vicinity of the previous day's platform position on the first trial. This was not seen in the lesioned groups.

b. Quantitative Analysis

An unequal N analysis of variance was conducted on the data with groups as the between subjects factor and blocks of trials as the within subjects factor. The results revealed a highly significant groups effect ($F = 7.93$, d.f. = 4/31, $p < 0.0001$), and a groups x trial interaction ($F = 4.22$, d.f. = 12/93, $p < 0.0001$) showing that the groups differed significantly over trials. An analysis of variance of trial 1 showed no significant difference between groups ($F < 1$). For trials 2-4 there was a significant groups effect ($F = 11.97$, d.f. = 4/31, $p < 0.0001$). The Scheffe Test showed that hippocampals were significantly worse than controls ($F = 22.92$, $p < 0.1$) but not significantly different from the HIP + SUB group ($F = 6.08$, $p < 0.1$) nor from the SUB and SUB + HIPs ($F = 1.11$,

FIG. 19

Mean latency to escape on the working memory task.
Error bars represent standard errors.


CONTROL
HIP
SUB
SUB HIP
HIP SUB
MEAN LATENCY(SECONDS)
80
70
60
50
40
30
20
10
1 2 3 4
TRIALS

$p < 0.1$).

An analysis of variance between time intervals (12 vs 100 day rats) indicated no significant difference ($F < 1$).

## 11.3 Results - Part 2

### a. Qualitative Analysis

The controls, although performing well on trial 2 at each intertrial interval, did not show forgetting over time-intervals. Poor performance predominated in the lesioned groups with no apparent pattern as intertrial interval increased. Fig. 20 indicates that even with the 3 hour delay, the latency of the second trial was shorter than the latency of the first trial for the control, HIP, and SUB groups only.

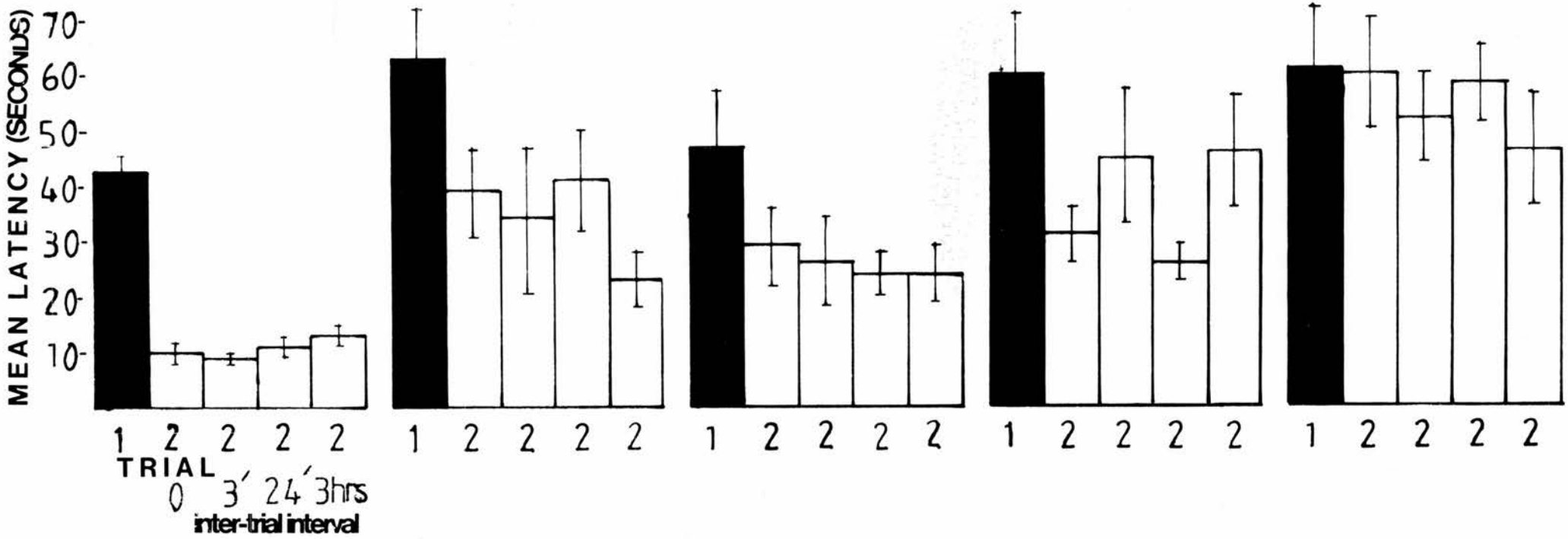
### b. Quantitative Analysis

An unequal N analysis of variance with groups as within subjects factor and trials and conditions (intertrial interval) as between subjects factor showed a significant groups effect ($F = 6.86$, d.f. = 4/31, $p < 0.0005$) and a significant groups x trials interaction ($F = 2.99$, d.f. = 4/31, $p < 0.05$) showing that the groups differed in their pattern of improvement across trials as in part 1 of this experiment. There was no significant intertrial interval effect ($F < 1$) suggesting that the control group was as good, and the lesioned groups as bad, at remembering the position of the platform 3 hours after trial 1 as 30 seconds after trial 1.

Analysis of variance between the 2 recovery time intervals (12 vs 100 days) gave no significant result ($F = 2.20$, d.f. = 1/20, $p > 0.1$).

FIG. 20

Mean latency to escape over 3 different intervals between trials 1 and 2.
Error bars represent standard errors.


CONTROL
HIP
SUB
SUB HIP
HIP SUB
MEAN LATENCY (SECONDS)
70
60
50
40
30
20
10
1 2 2 2 2
TRIAL
0 3′ 24′ 3hrs
inter-trial interval

# CHAPTER 12

# EXPERIMENT 4

## 12.1 Method

Experiment 4 followed on from Experiment 3 and used the same rats.

### Visual Discrimination Task

This was a non-spatial task conducted in the same apparatus. Including this task allows a comparison between spatial and non-spatial learning ability. It is also a useful test to establish whether sensorimotor or motivational factors are affecting the performance of the lesion groups.

However, there were some procedural differences. The rats had to discriminate between a rigid platform and a floating platform on the basis of pattern and brightness. For half of each group of rats, the rigid platform was painted with black and white stripes and the floating platform with matt grey (and vice versa for the other half). The platforms were randomly assigned to one of the eight compass points (ie. N, NE, E, SE, S, SW, W, NW) on each trial, either near the centre or at the pool edge. The rigid platform protruded 3 1/2 cms out of the water while the floating platform protruded 2 cms out of the water. The black curtains, which were hanging at one side of the pool during the place task, were now pulled right round the pool excluding all extramaze cues.

The rats were given 10 trials per day. The choice (rigid platform correct) and first-choice latency were recorded. A choice was defined as touching a platform with either the forepaws or snout while incidents of brushing against a platform in passing were excluded. The rats were considered as completing the experiment when they reached a criterion performance of 9/10 correct within a

day.

12.2 Results

a. Qualitative Observations

This task proved more difficult to learn than the place discrimination with all rats becoming difficult to handle. The controls took 16 days to reach the criterion of 9/10 correct choices, ie. 160 trials, compared to the 16 trials (or thereabouts) to learn the place task (cf Figs. 21 and 13). However, three of the 5 HIP rats performed no better than chance with the other 2 reaching criterion. One of the HIP + SUB group also only achieved chance levels. All the SUBs took 17 days and the SUB + HIPs 19 days to reach criterion. The number of animals that had reached criterion over days is shown in Table 4. Fig. 21 indicates that the HIP rats were poorer at learning this visual discrimination task.

Many of the rats made their initial choice by swimming to the middle of the pool and then heading off towards either the float or platform. Hippocampal rats sometimes tried the float several times while the controls usually abandoned it after one attempt.

b. Quantitative Analysis

An unequal N analysis of variance was performed on correct choice percentages with groups as the between subjects factor and days as the within subjects factor. The data were collapsed over trials. This revealed a significant groups effect ($F = 4.03$, d.f. = 4/31, $p < 0.01$) and a highly significant days effect ($F = 25.67$, d.f. = 20/620, $p < 0.0001$). Post hoc analysis of the groups effect using the Scheffe Test (Myers, 1966) revealed that the hippocampals were significantly worse than controls ($F = 106.71$, $p < 0.1$) and SUBs ($F = 81.22$, $p < 0.1$). However, HIPs were not significantly different from HIP + SUB ($F = 6.26$, $p < 0.1$). The SUB + HIP did not differ

FIG. 21

Graph of percentage correct on the visual discrimination task.

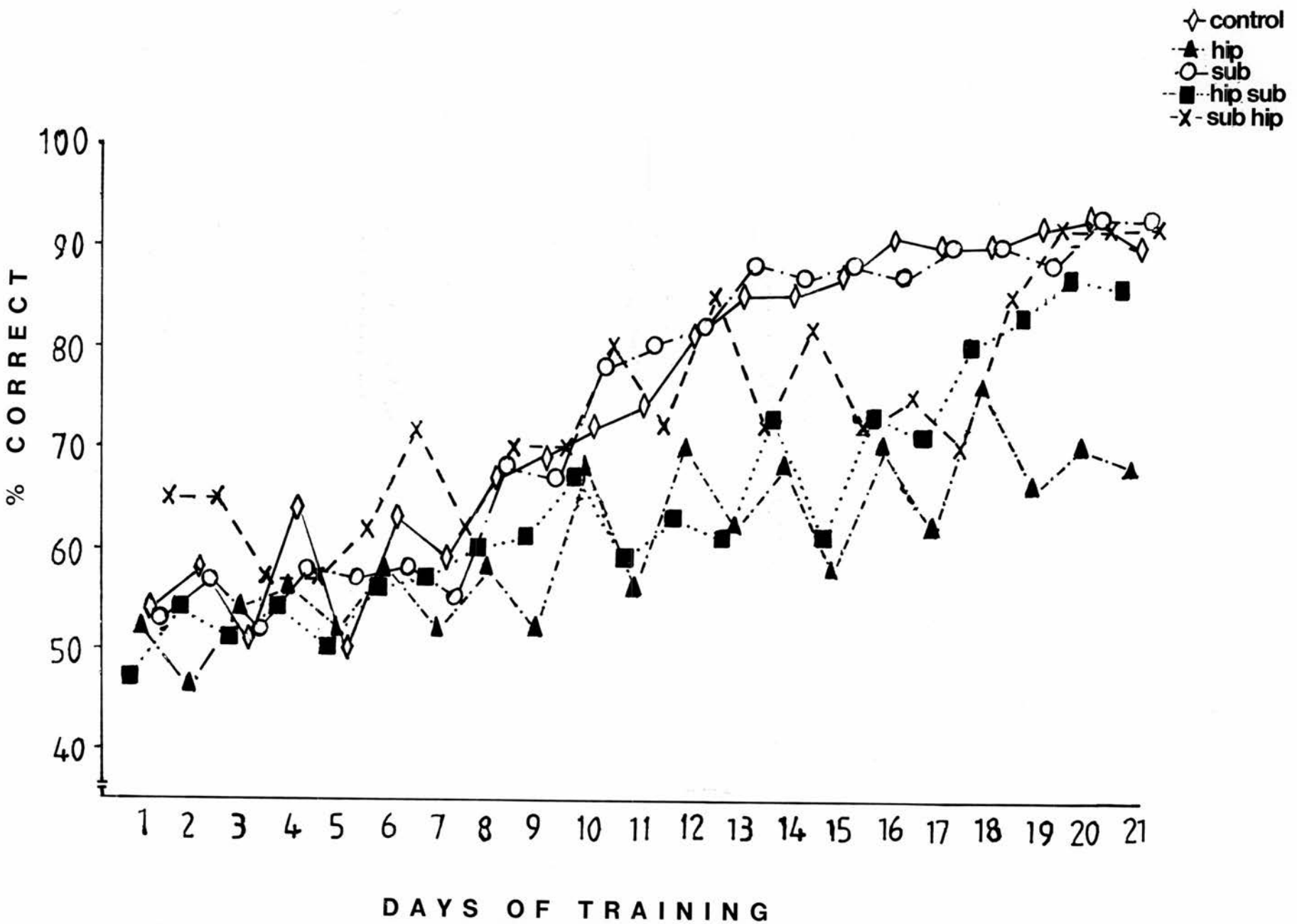

control
hip
sub
hip sub
sub hip
100
90
80
70
60
50
40
% CORRECT
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
DAYS OF TRAINING

TABLE 4

Number of rats in each group to reach criterion throughout the 21 days of training.

| GROUP | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | DAY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HIP | | | | | | | | | | | | 1 | | | | | 1 | | | | | |
| SUB | | | | | | | | | 1 | 2 | | 1 | 1 | | | | | | | 1 | | |
| HIP + SUB | | | | | | | | | | 1 | | | | 1 | | | 1 | 1 | | 1 | 1 | |
| SUB + HIP | | | | | | | | | 1 | | | | | | | | | 1 | 1 | | | |
| CONTROL | | | | 1 | | | | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 2 | | | | | | | |

significantly from controls ($F < 1$) but did differ significantly from SUBs and HIP + SUBs ($F = 8.79$, $p < 0.1$). These results are summarised in Table 5.

An analysis of variance between recovery time periods gave no significant result ($F<1$).

TABLE 5

SUMMARY OF RESULTS FOR EXPERIMENT 4

| GROUP \ compared to group | HIP | SUB | HIP + SUB | SUB + HIP |
|---|---|---|---|---|
| HIP | —— | —— | —— | —— |
| SUB | +(F=81.22, p<0.1) | —— | —— | —— |
| HIP + SUB | NS(F=6.26, p<0.1) | -(F=52.25, p<0.1) | —— | —— |
| SUB + HIP | +(F=59.51, p<0.1) | NS(F<1) | +(F=35.28, p<0.1) | —— |
| CONTROL | +(F=106.71, p<0.1) | NS(F<1) | +(F=72.72, p<0.1) | NS(F<1) |

\+ Performed significantly better than

\- Performed significantly worse than

NS Performed the same as

# CHAPTER 13

## GENERAL DISCUSSION

The main findings of the series of experiments reported in this thesis are as follows:

1 Rats with lesions of the hippocampus, subiculum and of both the hippocampus and subiculum were found to be impaired in the postoperative acquisition of a reference-memory place learning task.

2 With extended training, rats with hippocampal lesions were found to improve their performance on this same task to control levels.

3 Rats with combined hippocampal and subiculum lesions failed to show recovery to control levels during extended training.

4 All rats with lesions, including animals which had shown recovery on the reference-memory task, showed a persistent deficit in a spatial working-memory task relative to controls.

5 Rats with lesions restricted to the hippocampus were impaired in the learning of a visual discrimination task relative to controls.

6 Little or no evidence was obtained for recovery of performance in any of these tasks as a function of the time between surgery and training.

Each of these conclusions should be treated with some caution, principally because of the small group size. Nevertheless, the first of these findings is inconsistent with one aspect of Olton et al's. (1979) theory because the impairment was shown on a reference-memory task. The second of these findings poses problems for O'Keefe and Nadel's (1978) cognitive mapping theory. It supports

Jarrard's (1986) finding that neurotoxin lesions restricted to the hippocampal formation do not cause a sustained impairment of a spatial task and, in particular, shows that the improvement in performance is not task-specific. The recovery is unlikely to be due to incomplete lesions because, as summarised in the fourth main finding, these same animals subsequently showed a sustained deficit on the working-memory task. The fifth finding is in contrast to many published studies which indicate that visual discrimination performance is generally unaffected by hippocampal lesions. However, it may be that proactive interference from the earlier training tasks has contributed to this deficit.

These findings will now be discussed in detail, focussing upon their implications for the cognitive-mapping and working-memory theories, but also in relation to the processes of interference and behavioural-inhibition (Gray 1982).

## 13.1 Implications for "cognitive-mapping" theory

A prediction from cognitive-mapping theory is that rats with hippocampal, subiculum or combined hippocampal plus subiculum lesions should show severe impairments in the post-operative acquisition of a spatial task. The results of Expt 1 of this series bear out this prediction; indeed, over the 28 trials of training, the performance of the rats with combined hippocampus plus subiculum lesions

was broadly comparable to that of the rats with aspiration lesions of the hippocampus reported by Morris et al (1982). This finding implies that the hippocampus plays some important function in spatial learning.

However, the further training of Expt 2 suggests that the ibotenic acid lesions used in this study caused less severe behavioural consequences than conventional aspiration lesions; further experimentation with conventional lesions would be necessary to confirm this. After 76 training trials, the 5 rats with lesions restricted to the hippocampus were showing (a) rapid escape latencies, (b) relatively direct paths to the escape platform from any starting point around the pool, and (c) a distinct spatial bias to the training quadrant during the transfer test. This pattern of performance indicates that the hippocampus is not essential for spatial processing.

An interpretation of these results is that information essential for the effective performance of this task can be acquired in the absence of the hippocampus but that, normally, the hippocampus participates in some aspect of the processing involved in spatial learning. Rats with hippocampal lesions were, on average ten times slower to learn the task than controls. It is possible that a guidance strategy using extra-maze cues learned during the visual cue task could account for the improvement in performance. This possibility would

not require that the task be solved using a spatial map but instead could be solved using vectors (c.f. Collett, Cartwright and Smith, 1986).

A second prediction of the cognitive mapping theory is that rats with hippocampal lesions are able to solve non-spatial tasks. The results do not support this aspect of the theory. However, the rats given subiculum lesions or subiculum plus hippocampal lesions performed as well as controls suggesting that other factors such as behavioural inhibition, task complexity and possibly differences in relative importance of proximal versus distal cues etc., contributed to the deficits shown by rats given hippocampal or combined hippocampal plus subiculum lesions.

A third prediction of the cognitive mapping theory is that the map should be updated on each trial. This prediction is supported by the control animals who were seen returning to the previous day's platform location on trial 1 of a day's session in the working memory experiment.

## 13.2 Implications for "working-memory" theory

A prediction of the working-memory theory is that lesions of the hippocampus, and of the subiculum, should each cause a deficit in a short-term memory task in which information must be retained for only a single testing session. The result of Expt 3 bears out this prediction.

Whereas control rats escaped much faster on trial 2 of each day, all rats with lesions showed a relative impairment. This finding contrasts with Jarrard's (1986) results using the radial arm maze in which rats with either hippocampal or subiculum lesions showed no sustained impairment relative to controls. At first sight, this comparison suggests that an impairment in working-memory after a neurotoxin lesion may be task-specific.

However, a comparison of neurotoxin and aspiration lesions on radial-arm maze and swimming-pool working-memory tasks indicates that Jarrard's (1986) finding with ibotenic acid is unique. Fimbria-fornix lesions cause an impairment of working-memory in both the radial arm maze (Olton et al, 1978) and the swimming-pool (Morris, 1983); complete-hippocampal removal causes an impairment in both tasks also (Jarrard, 1986; Morris et al, 1986); ibotenic acid lesions, however, appear to cause an impairment in the swimming-pool but not in the radial-arm maze. What might be the explanation of this discrepancy?

The possibility that the lesions were different in the present study from that reported by Jarrard (1986) can be dismissed because (a) the same coordinates were used and (b) Jarrard performed some of the operations for this study (indeed he did the operations for 2 of the 5 rats in the hippocampal group). A further experiment to test the present rats on a radial maze would have been useful, but

time precluded this possibility. It is hard to see how certain differences in the procedure of the two tasks (e.g. swimming vs running) could have caused the different results in the two experiments. However, other differences (e.g. the 30 second inter-trial interval in the swimming-pool task, the necessity for inter-trial handling) may be contributory factors.

One feature of the present procedure is that there were 4 trials per day to a single platform position. The procedure is therefore ambiguous because it could, in principle, be solved by using reference memory within a single day. In the second part of Expt 3, control rats showed no forgetting of the first-trial position over an interval of 3 hours and, on occasions, these animals returned to this position at the start of the first trial of the following day. These findings imply that control rats may have been using reference-memory to solve the task. If this interpretation is correct, then the apparent "working-memory" impairment of the rats given lesions is nothing more than the same impairment as that shown in Expt 1, ie. an impairment in the speed with which a reference memory task can be learned. Consistent with this interpretation is the fact that the rats with hippocampal or subiculum lesions did show a steady decline in escape latency across the 4 trials of each day and that the hippocampus plus subiculum group showed little improvement within each day. Moreover, this

interpretation solves the apparent discrepancy between the effects of ibotenic acid lesions in the radial arm maze task and the swimming pool task. These results indicate that reference memory is involved in short term memory processing which questions the distinction between reference and working memory.

## 13.3 Implications for "behavioural inhibition" theory.

The predictions of the "behavioural inhibition" hypothesis (Gray, 1982) with respect to Expts 1-3 are unclear. This hypothesis was derived from experiments in which rats with septo-hippocampal damage showed impairments in extinction but not acquisition, ie. they failed to inhibit previously acquired behaviour. Here, no preoperative training was given to the animals so the impairments in Expts 1-3 are all impairments of acquisition. Arguably, these impairments fall outside the scope of the theory. On the other hand, rats do show certain "strategies" in the swimming-pool at the start of training, eg. swimming around the side-walls and it could be that rats with hippocampal lesions are impaired in the task because of a failure to inhibit these and other inappropriate behavioural strategies.

However, one aspect of the results does seem relevant to the behavioural inhibition hypothesis, namely, the

deficit shown by the hippocampectomised rats on the visual discrimination task. Specifically, there was some evidence of difficulty in controlling the choice response by some animals; that is, within a single trial, such rats would keep returning to the floating platform after their initial incorrect choice. This tendency might reflect a failure of "behavioural inhibition" and thus explain the lack of improvement by individual animals in the hippocampal group beyond chance levels. However, this "perseverative" tendency was much more pronounced in the rats with combined hippocampal and subiculum lesions, but this group performed better than the hippocampal group in their initial choice response. This suggests that the impairment in visual discrimination learning by the hippocampal group was not due to "behavioural inhibition".

However, an alternative view is that perseverative responding was secondary to inability to solve the problem and that frustration induced fixation of behaviour may contribute to the deficit. Certainly the animals were more difficult to handle at this stage. This irritability seen in the hippocampal rats dispels Gray's (1982) theory claiming that the hippocampus is the "centre" for controlling anxiety. Other errors may have been caused by a "proximity effect" (Morris et al, 1986) with the rat swimming to the nearest platform in an attempt to minimise the time spent in the water. The proximity effect only emerges as a strategy if the animals fail to solve the

problem, but once established, it may prove difficult for rats to abandon the habit.

## 13.4 Implications with respect to recovery of function after brain damage

Compensation by other areas of the brain (possibly the subiculum) with training, rather than recovery of function of the hippocampus per se, alleviated the impairment seen in the rats given hippocampal lesions in Expt 1. However, lesions of the hippocampus still caused a slight although non significant residual deficit indicated by a longer latency to escape and poorer accuracy in locating the exact position of the platform in Expt 2. This suggests that other areas of the brain cannot compensate for all aspects of performance. The actual time interval between surgery and training was not significant indicating that the temporary impairment seen in Jarrard's (1986) animals was not due to time alone.

## 13.5 Prospects for future experiments

This thesis provides further evidence that neurotoxin lesions may produce less behavioural impairment than conventional lesions. This fact is important for the future of research in this area and suggests that many of the classic experiments using conventional lesions will have to be redone using neurotoxin lesions.

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