The Neurobiology of Olfactory Learning in the Rat

by

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In accordance with the requirements of the University of Edinburgh, regulation 3.4.7, this thesis has been composed by myself and the work presented herein is my own.

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Abstract

It has been proposed that the spectacular olfactory learning capabilities of the rat may prove useful in the development of rodent models of human amnesia. In particular, it has been suggested that rats show a "primate-like" learning capacity when tested with olfactory (rather than visual or auditory) cues; and that this learning is sensitive to damage to brain structures considered critical in the human amnesic syndrome.

This aim of this thesis is to evaluate and exploit these claims in the investigation of the neurobiology of rodent olfactory learning.

In a series of experiments, an automated "olfactory maze" is developed for the demonstration and measurement of rodent olfactory learning capacity, and parallels between rodent and primate learning capabilities are investigated. It is concluded that the suggestion that rats form "primate-like" learning sets (and therefore learn complex abstract rules) when trained on a series of novel olfactory problems is unlikely to be correct.

Investigation of the effects of hippocampal and dorsomedial thalamic nucleus (DMN) lesions on olfactory learning do not support the hypothesis that olfactory learning is sensitive to damage to the structures considered critical in human amnesia: hippocampal lesions are without effect, and DMN lesions appear to cause a perceptual, rather than cognitive, abnormality. Infusion of the N-methyl D-aspartate receptor antagonist AP5, widely used as a tool to investigate the role of synaptic plasticity in learning, is also without effect. Hippocampally lesioned animals are, however, demonstrated to be impaired in a spatial reference memory task.

On this basis, it is concluded that rodent olfactory learning does not constitute a useful model for the investigation of the biology of human amnesia.

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"It is a common experience that some old views and statements show a remarkable tendency to outlast the tenability of the original observations on which they were based. The conceptions survive almost like proverbs, and become proclaimed as long-established truths and accepted as such by those who practise in the particular field of knowledge concerned."

Alf Brodal, *The Hippocampus and the Sense of Smell*, 1947.

CHAPTER 1 - INTRODUCTION

Human Amnesia

Efforts to develop a general, biologically based theory of memory have exercised many neuroscientists in the last few decades. One important element in this effort has been the attempt to develop animal models of human amnesia. The aim of this thesis is to evaluate, both theoretically and experimentally, recent attempts to create a rodent model of human amnesia utilising rats' alleged spectacular olfactory learning capabilities. Chapters 1 and 2, whilst brief, seek to indicate the critical features of viable animal models of anterograde amnesia, taking account of both psychological and neurological considerations and with special reference to the role of the hippocampus.

Neuropathological and neuropsychological studies of the human amnesic syndrome have provided the basis for many investigations of the neurobiology of memory in non-human species. The syndrome is briefly outlined here. It is characterised by an anterograde memory impairment of varying severity (an inability to learn and remember new material), usually accompanied by a variable degree of retrograde amnesia (failure to recall events occurring prior to the onset of amnesia), in the setting of a clear sensorium with preserved intellectual and language function. In contrast to the anterograde amnesia, certain forms of learning remain conspicuously intact, and this apparent dissociation between spared and impaired learning capacities has proved of particular interest in the development of current concepts of the neurobiology of memory. In implying the existence of at least 2 (and perhaps multiple) memory systems, this dissociation has formed a central issue in the development of animal models of human amnesia.

The Neuropsychology of Human Amnesia

The Nature of Human Amnesia

Clinically significant memory impairment (amnesia) sometimes occurs in the absence of other intellectual deficits and in the face of intact short term memory, and as such may provide useful information about organisation of memory in normal subjects and its associated neural basis. This 'amnesic syndrome' is a severe and pervasive disorder of learning and memory that affects both verbal and non-verbal material, apparently irrespective of the modality of stimulus presentation. The syndrome can arise as a consequence of a variety of brain insults: temporal lobe surgery, encephalitis, ischaemic episodes, traumatic head injury, electroconvulsive therapy, chronic alcohol abuse, tumours and certain toxaemias. The most striking and disabling feature of the disorder is the afore-mentioned inability to learn new material. The well known, and intensively studied amnesic patient 'H.M.', rendered amnesic iatrogenically following bilateral temporal lobectomy in an effort to treat severe intractable temporal lobe epilepsy (Scoville and Milner, 1957) provides a useful example: "(His) memory impairment can extend to words, digits, paragraphs, faces, names, maze routes, spatial layouts, geometric shapes, nonsense patterns, nonsense syllables, clicks, tunes, tones, public and personal events, and more. H.M. does not know his age, the date, the place where he lives, or the recent history of his mother and father" (Cohen, 1984).

In the face of this devastating disability, it has also been shown that H.M. and other amnesic subjects can, in certain special circumstances with special classes of material, demonstrate an impressive learning capacity and indeed maintain the acquired performance over long intervals.

Assessment of Amnesia

The routine clinical assessment of memory disorders (of which the 'amnesic syndrome' is only a subset) is reviewed by Lishman (1987). This most basic assessment is designed for rapid and convenient use at the bedside and acts as a screening device with the aim of detecting patients with organic brain damage. The routine assessment consists of tests of immediate memory (e.g. digit span) which is characteristically unimpaired in amnesia; and delayed free recall (recall of a passage of prose, or a name and address) and more remote memory (recollection of famous people and public events) which are impaired in the amnesic syndrome. Tests of concentration and general intellectual function are also included. Such simple testing can thus detect in a general way many of the clinical features of the amnesic syndrome. More formal testing, using such neuropsychological tests as the revised Weschsler Memory Scale (WMS-R), the Weschler Adult Intelligence Scale (WAIS) and the National Adult Reading Test (NART) may be used clinically to confirm and further refine findings quantitatively. For research purposes, more detailed neuropsychological testing and experiment has been used to determine more precisely the nature of the deficits encountered in amnesia.

Anterograde Amnesia

With regard to anterograde amnesia, many of the theories proposed to account for the psychological features of the disorder have been be broadly classified according to the stage or aspect of hypothesised memory processes considered impaired: it has been variously suggested that failure of acquisition processes, such as 'encoding' and 'consolidation'; and retention processes such as 'forgetting' and 'retrieval', may be responsible for the variety of deficits encountered (reviewed by Butters and Cermak, 1980)

Encoding theories propose that registration of information is in some way impaired in amnesia. It has been suggested, for example, that while the direct 'sensory' properties of stimuli can be encoded normally, 'meaningful' or semantic aspects of the 'to be remembered material' are incorrectly processed, a view supported by the poor performance of some amnesics on tasks normally facilitated by direction to the semantic rather than phonological aspects of verbal learning tasks (Cermak and Reale, 1978). Consolidation deficits are hypothesised to interrupt processing between 'immediate' memory and longer term memory, given that immediate memory is characteristically intact in amnesia. Accelerated forgetting has been demonstrated, by comparison of retention scores over a variety of intervals, to contribute to some amnesic states such as those following electroconvulsive stimulation, but not others, such as Korsakoff's syndrome (Squire, 1981). Hypotheses centering on the possibility that amnesia may arise from deficits in proposed retrieval processes draw support from the observation that amnesics sometimes respond erroneously to memory tests with 'correct' material from earlier tests, even though such responses had not been given correctly at the time of the original testing, (Warrington and Weiskrantz, 1973) and the fact that retrieval cues can improve performance. Such findings have been taken to imply that information

has been registered, but can only be re-accessed by certain favourable testing procedures.

Retrograde Amnesia

Retrograde amnesia, the inability to remember events experienced prior to the onset of amnesia, is frequently encountered in the amnesic syndrome. The phenomenon is central to the notion that an impaired retrieval process may account for the amnesic syndrome as a whole. However, the characteristic temporal gradient of retrograde amnesia, with relative sparing of more remote memories, is difficult but not impossible to explain in these terms. Weiskrantz (1985) has, for example, argued that the memory traces of premorbid events will have been subjected to an increase in strength as a consequence of "recoding processes", from which new events cannot benefit. On this view, the recall of premorbid events need not be as severely affected by a global retrieval deficit as the recall of new events. Alternatively, it has been suggested that the anterograde and retrograde components of the amnesic syndrome are truly dissociable, in that not only does the severity of one fail to correlate with the other, but their improvement or deterioration may occur independently (e.g. Goldberg, Hughes, Mattis and Antin, 1982). A consolidation hypothesis has been proposed to account for some aspects of retrograde amnesia (Squire, Cohen and Nadel, 1984), but this does not easily explain the wide variety in the length of the retrograde period.

Part of the problem surely lies in the fact that from both clinical and experimental perspectives, retrograde amnesia is difficult to assess and measure accurately given that much of the apparently forgotten (and the *allegedly* remembered) material must necessarily be personal to individual subjects. Despite this, tests of memory for famous faces and public events have been used in an effort to overcome such difficulties. The obvious drawback of tests of this kind is that by virtue of their fame, well known people and events may be 'encountered' outside their original temporal context. This problem has been addressed in some measure by the use of questionnaires relating to television series shown only once, but to a wide audience in the United States (so-called 'one season soaps') as a test for American subjects (Squire and Cohen, 1979). Using this technique, Squire and Cohen (1979) were able to suggest that the susceptibility of memory to disruption by electroconvulsive stimulation decreased as time passed after initial learning, in keeping with a 'consolidation' process.

In contrast to efforts to explain the nature of amnesia in terms of the interruption of particular stages of a *unitary* information processing system, much recent work has instead taken as its point of departure the concept of 'multiple memory systems', differentially affected in amnesia. Dissociations between impaired and spared learning capacities in amnesia have led some investigators to the viewpoint that memory is not a 'monolithic entity' (e.g. Squire and Zola-morgan, 1983), and that attempts to understand anterograde and retrograde amnesia in terms of global encoding, consolidation and retrieval hypotheses are perhaps insufficient in themselves to provide a coherent explanatory framework. It may be possible, for example, to re-frame the surprising finding that amnesics sometimes respond erroneously to memory tests with 'correct' material from earlier tests, even though such responses had not been given correctly at the time of the original testing, by postulating differentially impaired and spared domains of learning and memory in amnesia, rather than in terms of a global retrieval deficit. In other words, some aspect of learning and memory, *unaffected* in amnesia, accounts for the evidence that memory impaired subjects do appear to be influenced by prior exposure to material that is not freely recalled in conventional tests. This is not to suggest that a 'multiple memory system' view necessarily competes with an 'interruption of serial processing' view. It may be the case that each of any number of proposed memory systems may individually analysed in terms encoding, consolidation and retrieval functions. It is possible, rather, that a 'multiple system' view may adequately explain the neuropsychological findings in amnesia without the need to the postulate (for example) a global retrieval deficit.

Preserved Learning in Amnesics.

It has long been suggested that not all forms of learning and memory are impaired in human amnesia. Claperede's (1911) report constitutes an early anecdotal account which may represent preserved memory function in amnesics, in which he describes a Korsakoff patient who avoided shaking hands with him despite her inability to explicitly recall a previous episode in which he had hidden a pin in his hand and pricked her with it. More contemporary experimental reports are exemplified by studies of dissociations between different kinds of memory performance in amnesic subjects, in which performance is unimpaired on tasks that do not require conscious recollection of the original learning episode in the face of poor performance on tests of explicit recognition and recall of recently studied material. The spared capacities in human amnesia fall into 2 main classes of phenomena: 1. intact ability to acquire and retain a variety of motor, perceptual and cognitive skills, despite poor memory for the learning episodes and despite impaired memory-test performance for the facts that are normally accumulated in using the skills; 2. normal facilitation or other alterations in the ability to perform certain processing tests based upon prior exposure to or priming of the to-be-tested stimulus materials, despite impaired recall or recognition memory for these materials.

Amnesics have been shown to learn a variety of new perceptuo-motor skills. These include mirror tracing (Milner, 1962), a skill which the amnesic patient H.M. steadily learned across a period of 3 days improving both in terms of accuracy and time required to complete the task; and the rotary pursuit task (Corkin, 1968), in which H.M. gradually increased his 'time on target' over a seven day period and retained the skill for at least a further 7 days. Although H.M.'s performance did not reach the levels attained by control subjects, Korsakoff and post-encephalitic

amnesics have been shown perform as well as controls in the learning and retention of rotary pursuit and jigsaw puzzle completion tasks (Brooks and Baddeley, 1976). Similar findings have been reported in subjects rendered amnesic by electroconvulsive stimulation (Cohen, 1984).

Amnesics have also been compared with controls on more purely perceptual tasks, such as the reading of mirror reversed word triads (e.g. Cohen and Squire, 1980). In this task, the speed with which mirror reversed words are read gradually improves and and the time required to read repeated items can be compared with the speed to read novel mirror reversed words over a variety of intervals. Amnesics improve in overall performance at a rate comparable to control subjects and retain the skill over a period of at least three months. Performance is facilitated in both amnesics and controls by repeated exposure, but amnesic patients fail to recall individual learning episodes. The findings have been interpreted to indicate that amnesic patients can learn and retain aspects of such tasks which do not require explicit recall of learning episodes.

Of particular interest is the claim that the discrepancy between normal acquisitition of skills and impaired memory test performance for 'specific item' information can also be demonstrated in the cognitive domain. Much of the evidence for this proposal lies in Cohen's unpublished doctoral dissertation (data and figures reproduced in Cohen, 1984). In this study, the Tower of Hanoi puzzle was taught to 12 amnesic patients, and their performance compared with control subjects. The puzzle consists of five wooden blocks and three pegs (see fig 1.1). At the outset, the five blocks are arranged on the leftmost peg in order of size, the largest block at the bottom. Subjects are asked to move the blocks form the leftmost peg to the rightmost peg, moving only one block at a time and without placing a larger block on top of a

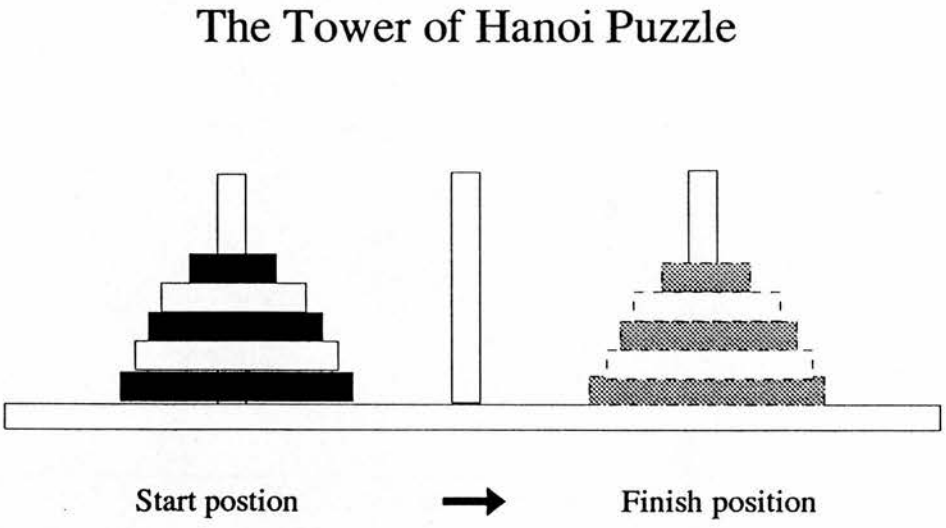


Fig. 1.1

Schematic diagram of the "Tower of Hanoi puzzle, showing the inital state (start) configuration of the task, and the "goal" or final position. See text for details.

smaller one. In order to complete the task, blocks must be shuffled from peg to peg, using all three pegs. The optimal solution to the problem, (i.e. the fewest number of moves required) is 31 steps, and is a unique sequence. In Cohen's experiment, subjects performed the task four times a day on four consecutive days, with the aim of learning the optimal solution. Amnesic subjects showed normal acquisition of the task over the four testing days, despite little or no recollection of having performed the task. Although unable to distinguish task configurations lying on the optimal 'path' to solution from non-optimal (and often never previously encountered) configurations, the amnesic subjects were able to complete the task from a variety of stages as well as controls. In an especially interesting manipulation, subjects were asked to perform the task with the middle peg (rather than the rightmost peg) as the goal. The amnesics had no difficulty in completing this 'transfer' task when tested after learning the regular version. In the case of H.M., performance was found to be normal even after one year. Cohen interprets these findings as a demonstration of normal acquisition and retention of a knowledge of the 'deep structure' of the problem in the absence of explicit recall of the learning events - the preserved learning and memory of a cognitive rule or procedure. This experiment is highlighted here, as the findings play an important role in the interpretation of later animal studies by Eichenbaum Fagan and Cohen (1986), to be discussed in chapter 3.

Other observations of the preserved ability in amnesics to learn and remember cognitive rules or strategies have been made. Wood, Ebert and Kinsbourne, (1982) demonstrated the ability of Korsakoff amnesics to learn a rule which permitted them to predict successive numbers in a Fibonacci sequence, which the patients retained for 17 weeks. Again, the patients were unable call to conscious memory ever having performed the task.

A second class of putative preserved learning is the 'repetition-priming' effect. This is a facilitation of the ability of amnesic and normal subjects to perform certain processing tasks by prior exposure to test materials, even when the materials cannot be recalled or recognised by the amnesic patients. Typical examples are the facilitated ability of amnesic patients to identify fragmented drawings (Warrington and Weiskrantz, 1968), or words (Warrington and Weiskrantz, 1970), having been presented previously with the complete picture or word cue. The question initially arose as to whether this type of cued recall simply had the effect of allowing weaker memory traces in amnesic subjects to be more efficiently retrieved, as opposed to reflecting the operation of an intact memory system dissociable from those impaired in amnesia, given the fact that amnesic subjects, though facilitated by the procedure, sometimes did not perform equally well as controls. Graf, Mandler and Squire (1984), however, further refined the observations (and have gone some way in resolving this issue) by comparing amnesic and normal subjects in their ability to complete three letter word stems following presentation of the complete words, in a variety of test conditions. Specifically, though both groups were biased to complete words on the basis of those presented previously (rather than generating new words when completing the stems), performance of the control subjects varied depending on the instructions given. If the groups were instructed to complete the word stems with words previously seen (cued recall), then controls performed better than amnesic subjects. However, if the groups were instructed to complete the stems with "the first word that comes to mind," then the biasing effect of prior exposure to completed words persisted, but the control subjects produced rather less of the previously seen words than in the cued recall condition. The amnesics, on the other hand, performed at a comparable level to that seen before. As a consequence of this, both groups now performed equally well. Furthermore, performance in the task

declined equally in the 2 groups over increasing test delay intervals up to 2 hours. In contrast, in a test of free recall of the complete items originally presented control subjects significantly out-performed amnesic subjects. These findings constitute a further example of preserved learning in the face of profound amnesia, with the additional demonstration that direction of control subjects away from explicit conscious recollection of material tends to match their performance with that of amnesics. This lends weight to the notion that a specific form of learning, dissociable from explicit recall (and that can be demonstrated in normal subjects), is intact in amnesics.

Classical conditioning phenomena have also been demonstrated to remain intact in amnesic subjects. In the first experimental demonstration of this, Weiskrantz and Warrington (1979) showed the successful conditioning of the eyeblink response to a tone in 2 amnesic subjects. The association was retained for 24 hours by the subjects, despite the fact that they failed to recognise the testing apparatus used. The observation has subsequently been replicated, with the additional finding that amnesic patients may acquire conditioned responses as rapidly as normal subjects (Daum, Channon, and Canavan, 1989).

The characterisation of multiple memory systems

While conceptualizations of the organisation of multiple systems in human memory are not, of course, guided exclusively by the study of amnesic humans, such investigations have proved to be of considerable value in this regard. As Tulving points out in a recent review of the issue (Tulving, 1987) "Theoretical ideas concerning classification of learning and memory are related to but not identical with theoretical ideas concerning the nature of amnesia. Amnesic patients may provide evidence regarding dissociations, and hypothetical classificatory schemes may be useful in making sense of observed disassociations, but the link is flexible." As discussed below, relationships between empirical findings in amnesic humans and associated theoretical conceptualizations of multiple memory systems have provided an important stimulus to research, both in studies of human amnesia and animal analogues of human amnesia.

A number of conceptual frameworks have been proposed. Tulving (1987) has tabulated several contemporary classificatory schemes for purposes of comparison, reproduced in table 1.1 (see Tulving 1987 for references):

Table 1.1

Level	Tulving	Weiskrantz	Cohen and Squire	Kinsbourne
III	Episodic memory	Event memory	Declarative memory	Episodic memory
II	Semantic memory	Knowledge systems		Semantic memory
I	Procedural memory	Associative memory priming	Procedural memory	

The ranking of the various subsystems is intended to reflect both their 'power' (in Tulving's terms, capacity for representation and flexibility), and their presumed sequence of evolution and development. More powerful and sophisticated systems, and those appearing later in evolution and development are at the top, less powerful and phylogenetically and ontogenetically earlier ones at the bottom.

With respect to human amnesia, spared capacities have been subsumed by many investigators under the broad theoretical term 'procedural memory' (level I in the table above), while there is general agreement that the impaired capacity is (at least) best characterised as explicit, episodic or event memory (level III). There is considerable disagreement, however, over the status of constructs assigned to level II, 'semantic memory' or 'knowledge systems' in human amnesia. Cohen and Squire, as the above table indicates, propose a dichotomous approach to the classification of memory systems, with concepts such as 'semantic' and 'episodic' memory considered together as 'declarative' memory, distinct from 'procedural' memory. This is reflected in Squire's assertion (Squire, 1986) that both episodic and semantic memory are impaired in amnesia. As the table indicates, this view is not universally accepted, and considerable debate has arisen regarding the evidence supporting the various viewpoints. Important sources of disagreement include variations across studies in the patient populations examined (both in terms of the aetiology and the severity of the amnesia - see Weiskrantz, 1985); and the nature of the tasks used.

However, there is clearly a degree of consensus regarding these various concepts of memory organisation. It can be seen from the Table 1.1 that spared capacities are generally considered to lie in the domain of skills and procedures, while the impaired capacities are considered to represent memory for the events and experiences usually available for conscious recollection. Taken in the context of the neuropathology of

human amnesia, the general features of such distinctions between impaired and spared capacities have proved particularly attractive, in that efforts to map capacity to brain structure have enjoyed modest success. It appears that damage centered on limbic structures, either the medial temporal lobe, or the diencephalon, may often result in amnesic states. In consequence, impaired capacities (variously characterised as episodic memory, declarative memory, event memory and so on) have been described together as a 'limbic' memory process, while spared capacities (procedural memory, memory for skills) have received the designation 'non-limbic.'

The Neuropathology of Human Amnesia

Diencephalic Amnesia

The commonest form of human amnesia is seen in the Wernicke-Korsakoff syndrome, most frequently a sequel to chronic alcoholism, though the syndrome may result from any situation in which thiamine deficiency occurs, such as chronic malnutrition or malabsorption. Neuropathological surveys of such subjects consistently reveal damage to diencephalic structures, particularly the dorso-medial nucleus of the thalamus and the mamillary bodies. The earliest studies (reviewed by Corsellis and Janota, 1985) established that the periventricular grey matter was selectively damaged in patients dying from alcoholic poisoning (Wernicke 1881, in Corsellis and Janota, 1985), prior to Korsakoff's (1890, in Corsellis and Janota, 1985) description of the illness, in which "together with the confusion, a profound memory disturbance is nearly always observed, although at times the disorder of memory occurs in pure forms." A few studies published in the following decade reported pathological changes in the region of the mamillary bodies in patients described as suffering from "alcoholic neuritis with mental disturbance", and 'Korsakoff's psychosis', but the work was initially ignored, most neuropathologists of the day being convinced that the responsible defect must be located in the cerebral cortex. Gamper (1928, in Corsellis and Janota, 1985) challenged this view, again demonstrating lesions in peri-aqueductal grey matter and around the third and fourth ventricles, and especially involving the mamillary bodies. Though this was not immediately accepted, repeated replication of the finding, associated with the realisation that Wernicke's description of a confusional state and Korsakoff's description of a memory disorder had a common aetiology (thiamine deficiency) eventually resulted in contemporary understanding of the disorder now known as an

acute illness (Wernicke's encephalopathy) upon which a chronic state (Korsakoff's psychosis) may supervene, perhaps best described as the Wernicke-Korsakoff syndrome. Malamud and Skillicorn (1956), studying the relationship between the Wernicke and Korsakoff elements of the syndrome further implicate the periventricular grey matter and emphasise the marked vulnerability of the mammillary bodies. Victor, Adams and Collins (1971), while accepting these findings generally, suggest that the most consistent factor is disorganisation of the dorso-medial nucleus of the thalamus.

Studies of patients who have sustained brain damage to diencephalic structures as a consequence of surgical or mechanical injury (reviewed by Parkin, 1984) extend, but do not conclusively resolve the debate as to the site of the critical lesion. Kahn and Crosby (1972, in Parkin, 1984) describe two patients rendered amnesic following tumour resection largely restricted to the region of the mammillary bodies, while Squire and Moore (1979) detail the amnesic effects of a stab wound destroying, (as far as can be determined on the basis of radiological evidence) the left dorso-medial nucleus of the thalamus in the patient 'N.A.' The precise neuropathology, and in particular, the minimal lesion required to produce the amnesic state of the chronic Korsakoff condition is still the subject of debate today but it is generally agreed the damage required is most often bilateral and lies somewhere in the diencephalic sector of the limbic pathways.

Medial Temporal Amnesia

A second, less common cause of the amnesic syndrome is Herpes Simplex encephalitis. This rare but severe form of acute necrotising encephalitis shows a predilection for medial temporal lobe structures. Post-mortem and radiological studies reveal extensive lesions in hippocampus, amygdala and uncus, whilst leaving diencephalic structures intact (Parkin, 1984). Such patients therefore share similar pathology to those unfortunate enough to have undergone bilateral temporal lobe surgery in an effort to treat otherwise intractable epilepsy associated with a temporal lobe focus. As in those amnesic subjects with diencephalic damage, the minimal lesion essential to the amnesic syndrome following temporal lobe damage has proved difficult to determine. Scoville and Milner's (1957) post temporal lobectomy series suggested, following analysis of operative procedures, that all amnesic subjects had both hippocampus and amygdala removed. There is evidence that amygdalectomy alone does not cause amnesia (Parkin, 1984) while the amnesic case 'R.B', described by Zola-Morgan, Squire and Amaral (1986) was shown to have damage essentially restricted to a bilateral lesion of the CA1 cell field of the hippocampus (demonstrated by an extensive *post mortem* neuropathological survey). The lesion in this case was caused by an ischaemic episode.

Comparison of Diencephalic and Medial Temporal Amnesias

Although superficially similar, the neuropsychological consequences of medial temporal, and diencephalic damage have been suggested to differ in detail (Parkin, 1984). This is likely to be due, in part, to the variable sequelae of additional damage incurred dependent upon precise aetiology. In Herpes Simplex encephalitis, for example, damage can be so extensive that the Kluver-Bucy syndrome occurs, which

incorporates a variety of disturbances such as visual agnosia, hyper-orality and altered sexual behaviour in addition to amnesia, deficits never seen after diencephalic damage. Similarly, patients suffering from the Wernicke-Korsakoff syndrome following prolonged alcohol abuse are frequently found to have widespread cortical atrophy (presumably as a consequence of the toxic effects of prolonged alcohol consumption, and not specific to the Wernicke-Korsakoff syndrome) and occasionally evidence of repeated head injury, these factors conspiring to extend neuropsychological deficits beyond the 'core' amnesic syndrome and exaggerating perceived neuropsychological differences between diencephalic and medial temporal syndromes. In particular, deficits on frontal tasks are frequently reported in Korsakoff patients. Despite these potential confounds, there is some evidence that differences exist. Parkin (1984) reviews a series a of studies bearing on this issue and draws particular attention to the fact that patients with diencephalic amnesia often have a less well circumscribed retrograde amnesia, and that temporal lobe amnesics may forget new information more rapidly than diencephalic amnesics. Squire (1986), on the other hand, reviews studies showing cognitive deficits in Korsakoff patients rarely found in bitemporal amnesics (such as impaired 'meta-memory skills', failure to release from pro-active interference, disproportionately large impairments of judgement of temporal order, and 'source' amnesia) which do not correlate with the degree of anterograde amnesia, and are therefore perhaps unrelated to the 'core' syndrome. Squire questions whether remote memory impairment (perhaps a dissociable sub-component of the more extensive retrograde amnesia seen in Korsakoff patients) should be considered in the same light.

It is important to appreciate that patients suffering from apparently circumscribed disorders of memory form a heterogonous group, differing both in detailed aspects of neuropsychological function, and in the aetiology and neuropathology of their condition. The study of brain damage leading to human amnesia in an effort to elucidate the biology of memory will always be hampered by the vagaries of 'uncontrolled' illness, varieties of clinical presentation and deficits additional to postulated 'core' or 'critical' damage. Animal models of the amnesic syndrome may permit some of these difficulties to be overcome.

CHAPTER 2

Animal Models of Amnesia

The Nature of Animal Models.

Animal models permit experimental intervention impossible or unethical in the human domain. In using animal models to explore the human amnesic syndrome, investigators are afforded the opportunity of making precise lesions of supposed critical brain structures in a way that accidents of nature rarely provide, and controlling the exposure to material that is to-be-learned in a precise and easily repeatable way.

Overmier and Patterson (1988) have considered in general terms the nature and value of animal models. They propose that models "assert a structural congruency between sets of causally related variables in two different domains." Central to their analysis is the concept of "analogy", which is taken to represent relationships between two domains of interest. They illustrate their concept by drawing on examples of animal models of human psychopathology, showing how analogies can be drawn between clinical psychopathological constructs in human psychiatric disorder (e.g. psychomotor retardation in depression), and animal behaviour (e.g. locomotor passivity). They point out that, in general, models may be exploited by demonstrating that relationships between *additional* variables in one domain are paralleled by relationships between corresponding variables in the second domain. For example, in the case of animal models of human depression, they note that human depression may be treated with mono-amine oxidase inhibitors, which are believed to have an effect on human mono-amine systems. An analogy is drawn

between these relationships and the fact that mono-amine depletion in rats induces locomotor passivity which is reversed by mono-amine oxidase inhibitors. This "structural congruency" between aspects of human and animal behaviour and their relationships to mono-amine function (the "model") can then be used in a variety of ways to directly investigate or predict further relationships in the 2 domains.

Overmier and Patterson make an important distinction between 2 different types of analogy, which they designate "formal" and "material" respectively. "Formal" analogies are held to represent mappings between the *relationships* amongst elements in the 2 domains of interest, while material analogies constitute similarities between individual elements *themselves* across domains. In the example given above, the parallel relationships between an aspect of human behaviour and mono-amine system modulation, and animal behaviour and mono-amine system modulation may be considered to constitute a formal analogy; while similarities between psychomotor retardation in humans, and locomotor passivity in animals (e.g. paucity of movement) may be considered to constitute a material analogy. It does not follow, of course, that simply because a formal analogy exists, that any material analogy may confidently be made - this will require additional supporting evidence. In considering the validity of animal models, it follows that 1. the adequacy of the analogy made is clearly of considerable importance, and 2. formal and material analogies be evaluated independently.

In providing a means of specifying the nature of analogies being made, Overmier and Patterson's framework constitutes a useful conceptual tool with which to evaluate the adequacy of animal models of human amnesia. For example, as outlined earlier (p.15), there are reasons to believe that human amnesia might be characterised by the observation that limbic damage impairs of some forms of

learning, but not others. In parallel, it may be shown that limbic damage in rats also impairs some kinds of learning and not others. These parallel dissociations would represent a "formal analogy", in that it is the *relationships* between forms of learning (impaired or spared) that constitute the analogy. Whether the *kinds* of learning so dissociated are *in themselves* similar in humans and animals (i.e. whether a "material analogy" can also be drawn) is a separate issue requiring independent evaluation and which has important consequences for the validity of the model as a whole. It is clearly of value, therefore, to specify in some detail the formal and material analogies (and the assumptions that underlie them) in considering the validity of an animal model.

On this view, and following on from the characterisation of amnesia outlined in chapter 1 (p.13-16), a convincing animal model of amnesia must demonstrate evidence of both impaired and spared learning and memory capacities in the species of interest (a formal analogy), following lesions to structures analogous to those considered critical in the human syndrome. To be of explanatory value, it should be possible to analyse the types of learning processes so dissociated, and to draw some realistic (material) analogy between such processes and those considered to be of interest in the human syndrome.

As Morris (1984a) points out, it may be important to establish *in advance* (say, of lesion studies) the nature of the learning processes of interest in order to avoid circular argument. Morris (1985, p.455) gives an example of this kind of reasoning: "(1) Humans use explicit memory in recognition tasks. (2) When asked to explicitly remember, amnesic patients do worse than normals. (3) Hippocampus- and amygdala-lesioned monkeys do badly on recognition tasks. (4) Therefore, monkeys explicitly remember. The claims may be correct, but the argument is circular." This

viewpoint underlines the value of specifying the nature of the analogies being made in order to evaluate an animal model.

Primate models of Human Amnesia

Efforts to produce a non-human analogue of amnesia have been dominated by two important considerations: 1. the kinds of test required to demonstrate impaired learning and memory in animals, and 2. the nature of brain damage required to produce such deficits; recapitulating in many ways the issues discussed in relation to human amnesia in the preceding chapter. While early attempts to localise memory function in animals were unsuccessful (Lashley, 1929), later studies consistently demonstrated, initially in primates and later in other mammals, that specific cortical damage could cause deficiencies in the acquisition and performance of discrimination tasks. Such tasks involved the discrimination of simultaneously presented cues, one of which was consistently associated with reward. Lesions were initially made in the inferotemporal cortex (non-primary, visual association areas), and the deficit produced was specific to visual discrimination learning (Blum Chow and Pribram, 1950). Subsequent studies demonstrated analogous isolated deficits in tactile (Wilson, 1957) and auditory (Neff, 1961) modalities, placing lesions in the relevant cortical association areas. Control tasks used in these studies demonstrated that the deficits were associative in nature, and not due to impaired sensory or motor function. While the studies generally supported the principle that specific brain areas might subserve aspects of learning and memory, the findings did not mirror the pattern of global, multi-sensory deficit seen in human amnesia. Efforts to produce a global amnesia in primates and lower mammals by destroying the limbic areas to which the cortical association areas project (and which are damaged in some amnesic humans) were initially disappointing, as the kinds of discriminative tasks

used were largely, although not entirely, unaffected (Mishkin, 1954; Orbach, Milner and Rasmussen, 1960; Correll and Scoville, 1965).

Significant progress was made, however, following the development of new types of memory task. These new tasks, initially developed by Gaffan (1974), and modified by Mishkin and Delacour (1975), differed form the earlier tasks in employing trial unique visual stimuli necessitating single trial acquisition of information. A version of this new class of task, 'Delayed Non-Match to Sample' (DNMS) was found to be sensitive to limbic lesions (Mishkin, 1978).

This task, which is carried out in the Winsconsin General Testing Apparatus, consists of 2 phases. In the first ('sample') phase, the monkey is presented with a distinctive object, under which it finds a reward. The object is then removed and after a variable interval, the second phase ('choice') begins. The animal is now confronted with two objects, one of them the object seen earlier, the other an unfamiliar object. The food is now concealed under the new object and the monkey must choose to displace it rather than the familiar object to obtain reward. Each trial makes use of a new pair of objects, such that the information needed to perform successfully changes from trial to trial, with none of the cues repeatedly associated with reward. Normal monkeys performed the task with greater than 90% accuracy over an interval of 1-2 minutes between the sample and non-match phases of the trial, while animals with combined amygdalo-hippocampal lesions performed almost at chance. Importantly, however, the impairment does not occur in lesioned animals when the delay between sample and non-match phases is short (less than 20 seconds), indicating not only that sensory and motor systems are intact, but also that the 'rule' of choosing the unfamiliar object is successfully learned and remembered. The effects of limbic damage on this task (Mishkin, 1978) are not restricted to the

visual modality. Similar impairments have been made observed in tactile versions of the task (Murray and Mishkin, 1983), suggesting that the learning deficit is global.

In sharp contrast, and in confirmation of the earlier work discussed above, repeated-trial visual discrimination learning (where cues are repeatedly presented and consistently associated with reward) is largely unimpaired in lesioned monkeys, even at long delays between individual trials. Mishkin and his colleagues have shown that lesioned monkeys can learn a concurrent visual object discrimination task as well as controls, even if the individual presentations of each pair of objects are separated by 24 hour intervals (Malamut, Saunders and Mishkin, 1980). In this task, 3 sets of 20 pairs of easily discriminable objects were used, one of each pair being consistently associated with reward. Each of the 20 pairs in the first set was presented daily until the animals reached a criterion of 90 correct responses in five 20 trial sessions. The second and subsequently the third set were then presented. Monkeys with combined amygdalo-hippocampal lesions required the same number of sessions to reach criterion on each of the sets of concurrent discriminations as the control monkeys. The same monkeys were profoundly impaired on a single trial association task (Malamut and Mishkin, 1981). In the 'acquisition phase' of this task, a rewarded and an unrewarded object were presented successively with a 10 second interval between them. After a further 10 second interval, both objects were presented again, this time simultaneously (the 'choice phase') and the monkey received a reward for choosing the previously rewarded object. Twenty of these trials were presented daily, with a new pair of objects appearing on each trial. The order of presentation in the acquisition phase and the position of the objects in the choice phase was determined pseudo-randomly. The fact that lesioned animals failed completely in the task is of particular interest, given that the task is formally very similar to the concurrent discrimination procedure they had earlier successfully

performed. Both tasks use 20 pairs of objects per day, and both tasks require the subject to choose a previously rewarded object from 2 simultaneously presented objects. Mishkin argues that the important difference between the 2 tasks lies in the fact that in the former, the choice must be made on the basis of a single acquisition trial, while in the latter several repeated acquisition trials have been presented (Mishkin, Malamut and Bachevalier, 1984).

In interpreting these findings, Mishkin and his colleagues (Mishkin et al, 1984) have proposed the operation of 2 learning systems, only one of which is impaired by limbic lesions. The impaired system is considered to subserve both recognition memory (as measured by the DNMS task) and associative recall (e.g. 1-trial object-reward association). The spared system is viewed as "involving the gradual development of a connection between an unconditioned stimulus object and an approach response, as an automatic consequence of reinforcement by food" (Mishkin et al, 1984). Mishkin designates this particular capacity as "habit formation," which he describes as a "non-cognitive" form of learning operating independently of limbic structures and therefore unaffected by limbic lesions. An important implication of this viewpoint is that *both* systems are likely to operate in the intact animal, and their contributions to any learning task may not be easily distinguished. For example, it is conceivable that the "non-cognitive" system *could* subserve 1-trial object-reward association if a stimulus-response connection were formed sufficiently rapidly so as to be complete in one trial. Mishkin concludes that it is not the differential speed with which tasks are learned (e.g. "slow" versus "rapid" learning) that characterises their sensitivity to limbic lesions, but rather the crucial difference lies in whether or not cues are repeatedly presented (Mishkin et al, 1984 pp 71-73).

However, not all tasks utilising repeated cue presentation are unaffected by limbic lesions in monkeys. Mahut, Moss and Zola-Morgan (1981) have reported marked effects of hippocampal lesions on a task requiring delayed retention of object discrimination, in which cues were presented repeatedly. In this task, monkeys were taught a simultaneous object discrimination to a criterion of 9 correct responses in 10 trials. After an interval, the monkeys were trained again to the same criterion using the same pair of objects. Monkeys with hippocampal or amygdalo-hippocampal lesions learned the discrimination without difficulty, but made almost 4 times as many errors as control subjects when re-tested at retention intervals of 1 hour or 24 hours. Furthermore, in contrast to the lack of impairment described on the 24 hour concurrent learning task outlined above (Malamut et al, 1980), Moss, Mahut and Zola-Morgan (1981) have reported significant impairment following hippocampal lesions on a concurrent discrimination learning task employing *shorter* inter-trial intervals. In this version of the concurrent learning task monkeys learned simultaneously 8 different discriminations, the delay between trials with the same pair of objects being 3 minutes, rather than 24 hours. Monkeys with hippocampal lesions made more than twice as many errors as normal monkeys in reaching criterion. Similar findings on concurrent learning task performance following lesions to the medial temporal lobe were also reported by Correll and Scoville (1965) in which 6 different discriminations were used with an interval of approximately 5 minutes between trials with the same pair.

Although these tasks involve repeated cue presentation, they may still be accommodated generally within a framework seeking to distinguish between qualitatively different types learning and memory system if it is assumed that concurrent discrimination tasks conducted at short intervals do, in fact, engage rather different processes than formally similar tasks at long intervals. In a review of the

effects of limbic lesions on learning in monkeys, Squire and Zola-morgan (1983) argue that concurrent learning tasks generally are likely to increase the demand for "data-based" learning (a learning system analogous to Mishkin's "cognitive" system), given that "the monkeys must remember day to day a substantial amount of information concerning which objects are rewarded", and concurrent tasks are therefore more likely to be sensitive to limbic lesions than simple discrimination tasks. The apparently paradoxical finding that concurrent discrimination with 24 hour inter-problem intervals is unaffected by limbic lesions (Mahut et al, 1980) may be resolved by proposing that *normal* monkeys cannot use the limbic lesion sensitive learning system to solve this particular version of the task. Though they do not consider Malamut et al's (1980) report itself, Squire and Zola-Morgan (1983) have argued that earlier discrepancies in the literature may be similarly resolved, suggesting, for example, that even simple discrimination tasks may differentially engage different learning systems, based on the discriminability of the cues used and reflected in (though not necessarily a consequence of) the speed with which the tasks are learned. They draw attention to the fact that simple pattern discrimination tasks are more slowly learned by monkeys than object discrimination tasks, and propose that "the role of data-based, explicitly presented information would play a proportionally larger role" in object discrimination learning. They cite the study of Mahut et al (1981; detailed above) as evidence that *object* discrimination tasks may indeed, under certain circumstances, be sensitive to limbic damage, in contrast to their demonstration that *pattern* discrimination learning is unaffected (Squire and Zola-Morgan, 1983), though they note that the evidence is not conclusive and that other studies have reported contradictory findings (e.g. Orbach et al, 1960: see Squire and Zola-Morgan, 1983, fig 6.8 for additional references). Squire and his colleagues (Cohen and Squire, 1980) have used the theoretical distinction between declarative memory (memory based on facts or data) and procedural memory

(memory based on skills) discussed in relation to characterisations of human amnesia in the previous chapter, to explain findings in monkeys with limbic damage. They suggest that simple pattern discrimination learning in monkeys exemplifies a type of learning more akin to "skill learning" than "data-based learning", and in this sense their viewpoint is broadly in agreement with the formulation proposed by Mishkin et al (1984) in which "habits" and "memories" are distinguished. In extending the evidence supporting the proposal that skill learning is spared in amnesic states, Zola-Morgan and Squire have demonstrated normal learning in monkeys with limbic damage on a series of motor-skill tasks. In one such task, monkeys are required to retrieve a "Life-Saver" (a sort of American "Polo-Mint" counterfeit) which has been threaded onto a thin metal tube with a right angle bend. The time taken to obtain the "Life Saver" is recorded across repeated sessions, and normal monkeys typically become increasingly proficient at the task, taking approximately 20 seconds during the first session, and 5 seconds by the eighth. Monkeys with limbic lesions not only learn at the same rate, but retain the skill as well as normal monkeys over a period of at least 1 month (Zola-Morgan and Squire, 1984).

Overall then, it would seem that following limbic damage monkeys are impaired on recognition memory tasks, although the evidence is insufficient to show that it is recognition memory that is *exclusively* impaired in such circumstances. Rather it is some process, some form of memory which in intact subjects permits high level performance on both one trial object recognition and one trial 'unique object'/reward association learning that is impaired. However, the latter task may, in some circumstances, also be efficiently supported by (unusually rapid) simple conditioning processes apparently unaffected by limbic damage, and is therefore ambiguous in this regard. In other words, monkeys with limbic damage will usually

be impaired on recognition memory tasks, may be impaired on tasks requiring single trial acquisition of information, and are less likely to be impaired on tasks which involve the repeated presentation of cues, given that the last two tasks can be supported to a greater or lesser extent by a non-limbic neural process. On this view, it follows that (a) the precise nature of the memory process impaired in amnesic monkeys is as yet inadequately characterised though most sharply delineated by one trial object recognition tasks; (b) its *unambiguous* demonstration cannot be achieved using standard object-reward association tasks, especially if the object-reward association is repeatedly presented to the subject; and (c) "every piece of learning will have to be analysed and re-analysed carefully for contributions to it by not just one, but two qualitatively different types of processes" (Mishkin, Malamut and Bachevalier, 1984). It may be that rapid discrimination learning (such as that seen in object discrimination, as distinct from pattern discrimination learning) represents just such a case of a combination of recognition and habit memory, perhaps accounting for the variable results of limbic lesions found in primate studies (Squire and Zola-Morgan, 1983). There is, however, no *a priori* reason, nor consistent experimental evidence, for attributing special status to 'rapid' discrimination learning generally. The learning of motor skills and procedures appears to be preserved in amnesic monkeys, and the normal performance of monkeys with limbic lesions on the DMNS task at sufficiently short intervals may represent the normal acquisition of a cognitive procedure, the "non-match" rule.

While there is general agreement that one-trial object recognition tasks are sensitive to limbic damage in monkeys, the nature of the critical limbic lesion is disputed. In Mishkin's (1978) original report, combined bilateral damage to both the amygdala and hippocampus was required to produce a severe delay dependent deficit in the DNMS task, the degree of impairment being significantly greater than that produced

by damage to either structure alone. This result was taken to indicate that circuits through both the hippocampus and amygdala contribute to those aspects of recognition memory which are assessed by the DNMS task. However, in the creation of the combined hippocampus and amygdala lesion, peri-allocortex ventrally adjacent to both structures was removed. Interpretation of the experiment was therefore confounded by damage to this additional tissue.

In an effort to determine the relative contributions of these various structures to the memory impairment, Murray and Mishkin (1986) compared the effects of damage to the cortical tissue subjacent to both hippocampus and amygdala combined with either a) bilateral hippocampal lesions, or b) bilateral amygdala lesions. They found impairment after both lesion combinations, with greater impairment seen in the condition involving the amygdala. The finding was taken to support the notion that damage to both amygdala and hippocampus was necessary, given that removal of the cortical tissue in condition 'b' would have effectively de-afferented the hippocampus.

In further consideration of this issue, Zola-Morgan, Squire and their colleagues have recently conducted a series of studies examining the performance of monkeys with a variety of selective lesions on the DNMS task (reviewed by Zola-Morgan, 1990). They have developed a useful notation to indicate the nature of the various lesions: 'H' refers the hippocampus, 'A' to the amygdala, and the optional suffix '+' to adjacent cortical damage, such that Mishkin's original combined lesion would be designated 'H+A+'. The lesion 'H+' includes, for example, the hippocampal formation and much of the parahippocampal gyrus but excludes the most anterior portions of the entorhinal cortex. This lesion caused a significant delay dependent impairment on the DMNS task, but less severe than that seen with the 'H+A+' lesion, consistent with Murray and Mishkin's (1986) result. The 'A' lesion constitutes a

lesion of the amydaloid complex, sparing the surrounding cortex (peri-amygdaloid, entorhinal and peri-rhinal cortices), while the 'A+' lesion includes all of these structures. Monkeys with the selective 'A' lesion performed normally on the DNMS task, while monkeys with the 'H+A' lesion were significantly impaired, but no more so than monkeys with the 'H+' lesion alone (Zola-Morgan, Squire and Amaral, 1989). Further studies examining the effects of lesions restricted to peri-rhinal ('PR') cortex and peri-hippocampal ('PH') gyrus alone (referred to as the PRPH lesion; Zola-Morgan, Squire, Amaral and Suzuki, 1989) resulted in performance deficits apparently as severe as those seen in the 'H+A+' lesion, but could not be directly compared as the monkeys required a modification of the DMNS procedure in which the sample stimulus was presented twice in succession prior to the choice phase of the trial. The same subjects performed normally in pattern discrimination. Taking these findings together, Zola-Morgan and his colleagues suggest that the deficit seen following the 'H+A+' lesion results from damage to the hippocampal formation and related cortex, rather than to the hippocampus and amygdala as proposed by Mishkin's group. Furthermore, because the PRPH lesion may cause a greater deficit than the H+ lesion, Zola-Morgan has concluded that the impairment cannot simply represent a hippocampal disconnection phenomenon, and suggests that these cortical areas are implicated in aspects of normal memory function in their own right (Zola-Morgan, 1990).

In response, Murray and Mishkin (1990) have maintained their view that the amygdala plays an important role in recognition memory despite these findings, citing earlier work (Bachevalier, Parkinson and Mishkin, 1985) showing that combined lesions to the fornix and amygdalo-fugal pathways which largely spare the afore-mentioned cortical pathways impair function on the DNMS task in a delay dependent manner. Furthermore, other studies suggest that hippocampus and

amygdala may, in fact, make independent contributions to different aspects of memory. For example, amygdalar, but not hippocampal lesions have been shown to impair a cross-modal DNMS task (Murray and Mishkin, 1985). In the sample phase of this task, cues are presented in the tactile modality (by presenting the cues in the dark), but in the choice phase the same cues are presented in the visual modality. The monkey must therefore use information gained via touch in the recognition of a visually presented object in order to perform successfully. Conversely, the hippocampus plays an important role in tasks requiring the use of spatial information, but the amygdala does not. Monkeys trained pre-operatively to associate objects with locations performed at near chance levels following hippocampectomy, while their amygdalectomised counterparts performed as well as they had on the task prior to surgery (Parkinson, Murray and Mishkin, 1988).

The effects of lesions to diencephalic structures have also been studied in monkeys performing the DNMS task. Aggleton and Mishkin (1983) found that monkeys with surgical lesions which removed the medial and thalamic nuclei were markedly impaired on the DNMS task at delays of greater than 10 seconds. The animals were unimpaired at short ITIs on the DNMS task, on pattern discrimination learning and a spatial delayed response task. These findings were proposed as a model of human diencephalic amnesia. Drawing together their various findings, Mishkin and his colleagues (1984) have proposed a neural circuit for memory linking diencephalic structures with parallel circuits in the temporal lobe, involving the hippocampus and amygdala respectively.

Overall, the above findings can be broadly mapped with some success onto contemporary conceptualizations of human amnesia. Both impaired and spared learning and memory capacities can be demonstrated, and the nature of the impaired capacity - the ability to recognise as familiar a recently presented cue - accords with

some features of so-called declarative or episodic memory considered impaired in human amnesics, while the unimpaired ability to acquire a strategy or procedure - the 'non-match rule' required in the DMNS task (evidenced by the good performance of lesioned monkeys at short inter-trial intervals) parallels the preserved cognitive skill learning in human amnesics proposed by Cohen and Squire (1980). Mishkin's concept of intact 'habit formation' provides a further example of the class of spared capacities in the model, sharing features with instrumental conditioning, while normal motor skill learning has been observed in both human amnesics and monkeys with limbic damage. The anatomical location of the brain damage (medial temporal and diencephalic) involved appears similar across the species, though specification of the critical or minimal lesion required to produce the amnesic state is not yet fully resolved.

The validity of the model is further supported by the poor performance of human amnesics (principally, but not exclusively Korsakoff subjects) on similar tasks to those used with monkeys, such as delayed matching (Aggleton, Nicol, Huston and Fairbairn, 1988) and non-matching tasks (Squire, Zola-Morgan and Chen, 1988).

Rodent Models of Human Amnesia

Given the ethical objections that some hold to the use of higher mammals in research, and the fact that that monkeys are expensive, require specialised facilities and cannot therefore be easily used in large numbers, there are a number of good reasons for developing non-primate models of human amnesia. In recent years many studies of learning and memory in rats have been concerned with hippocampal function, and much effort has been directed to the development of tasks sensitive to

hippocampal disruption and their analysis. A number of competing theories have been proposed in the course of this work, including O'Keefe and Nadel's (1978) 'spatial mapping' theory which proposes that the hippocampus is involved in the construction of an allocentric map of space used by animals in navigation, exploration and place learning tasks; and Olton's 'working memory' hypothesis (Olton, Becker and Handelmann, 1979), which asserts that the septo-hippocampal system together with the entorhinal cortex constitutes a flexible memory system holding information (not exclusively spatial) for only one trial. Although the theories are apparently mutually incompatible, both have been offered as models of amnesia. Neither is entirely satisfactory, given that human amnesia can occur in the absence of hippocampal damage. However, as Morris (1983) points out, the study of memory in animals need not be exclusively concerned with the modelling of human amnesia, and "animal research offers the opportunity of searching for patterns of functional breakdown only indirectly related to amnesia" in an effort to elucidate the neural mechanisms which underlie memory function generally. Considerable advances have been made, for example, in the study of hippocampal function and its relationship to spatial learning and memory in rats. In particular, recent work relating to the role of the N-methyl D-aspartate receptor in hippocampal long-term potentiation and its involvement in spatial learning (Morris 1986, 1989) has proved of considerable interest. This kind of work clearly has enormous potential for the investigation of basic neural mechanisms underlying learning and memory, but as yet has few direct points of contact with the human amnesic syndrome.

Part of the difficulty lies in the nature of the tasks used to test learning and memory in rats, as they are generally very different from those used in studies with humans and monkeys. Spatial learning tasks are not routinely used in the investigation of either human or monkey learning, thus making it difficult to compare findings across

species, while studies of learning in monkeys are now beginning to influence the neuropsychological assessment of human amnesia. For example, the recently developed "Cambridge Automated Neuropsychological Testing Battery (Cantab)" (Morris, Evenden, Sahakian and Robbins, 1987) employs a computerised version of the kinds of matching tasks used extensively in monkey studies for use with human amnesics. Efforts have therefore been made to use similar tasks with rats. Aggleton (1985) has used a 'Y' maze with multiple, visually distinctive goal boxes to successfully train rats on a non-match to sample task, while Rothblat and Hayes (1987) have studied similar learning using 'junk' visual objects in apparatus based on the WGTA, adapted for rats. However, these tasks naturally rely on the rats' visual capabilities. It has been suggested that the use of the rats' dominant sensory modality, olfaction, might be a more useful candidate stimulus mode for studies of rodent learning and amnesia (Eichenbaum, Fagan and Cohen, 1986). Several independent research groups studying rodent olfactory learning have claimed that rats demonstrate a "primate-like" learning capacity when tested with olfactory cues, and in the course of studying the effects of lesions to structures considered critical in human amnesia, have attempted to create rodent models of human amnesia. These studies are reviewed in detail in the next chapter.

By way of a summary and conclusion to this section, the characteristics of potentially valid animal models of amnesia are proposed. The development of primate models of amnesia provides a useful guiding framework, and has been dominated by 2 important considerations: first, the nature of the tasks used in determining and interpreting the behavioural competences of the species of interest; and second, the neurological considerations relating to brain structures hypothesised to be relevant in learning and memory function.

Psychological considerations:

The concept of multiple dissociable memory systems has proved heuristically useful (and enjoys a measure of empirical support) in the both analysis of human memory disorders and the interpretation of deficits following experimental lesions in primates as reviewed above. Precise specifications of the types of memory spared and impaired remain elusive, being variously characterised as dispositional, procedural, habit or skill memory; and episodic, event, knowledge, propositional or declarative memory respectively. An important aim in developing non-primate animal models of Human amnesia has been the attempt to design and formally analyse useful tasks which lie within the behavioural competences of the species of interest in order to predict, study and interpret the effects of brain damage. Contemporary formulations centre on the possibility that information may be differentially represented in the brain under particular circumstances, despite the fact that tasks may be formally similar (Squire, Psych. Rev., **99**, 1992 p.204). From this perspective, it is possible to develop hypotheses to account for the variable effects of limbic lesions on similar tasks across species as reviewed above. A simple object discrimination problem

could, for example, be solved either dispositionally by habit formation (a memory system insensitive to limbic lesions), or propositionally by conscious memory of which object was rewarded and which was not (a memory system sensitive to limbic lesions).

An important factor which underlies comparative studies of memory is the expectation that human and non-human investigations will reciprocally inform one another, such that the psychological constructs currently used to characterise aspects of cognitive function in each species will co-evolve. In this spirit, the construct 'declarative memory' may be usefully considered to extend beyond the more restricted notion that it refers to the ability to "declare knowledge verbally", to some of the cognitive operations of non-verbal animals. Indeed, "declarative memory includes memory for faces, spatial layouts, and other material that is declared by bringing a remembered image to mind rather than by verbalising" (Squire, 1992). On this view, the task at hand is to forge points of contact between characteristics of human memory, and ideas about memory systems derived from work with experimental animals. In this way, it is hoped that general, cross species principles of memory organisation and their relationship with brain function can be investigated and determined.

In the recent experimental and theoretical literature a number of the characteristics of non-human memory (generally in rodents) have been considered to share important features with those characteristics of human memory which are impaired in amnesia: examples include the speed with which certain tasks are accomplished (Squire and Zola-Morgan, 1988), the flexibility of the learning process (Squire, 1992), and the relational (Eichenbaum et al, 1988) and configural aspects of such learning

(Sutherland and Rudy, 1989).

The work reported here is concerned with the evaluation of a specific example of this general approach. As reviewed in detail in the following chapter, certain features of "higher order learning" have been hypothesised to lend themselves to the study of both episodic and procedural learning in non-primate species (Slotnick and Kaneko, 1981; Eichenbaum et al., 1986, Staubli et al., 1987a). In particular, the formation of "learning sets" has proved of special interest. An important and influential interpretation of the phenomenon of learning set formation has been that in the course of solving a series of novel 2-item discrimination problems of the same general class, animals develop an abstract understanding (Restle, 1958)) or rule of the form "win-stay, lose-shift" (Levine, 1959) which can be applied generally across problems. This accounts for the progressive ease with which problems are solved, resulting in both very *rapid* solution of novel problems (in one trial), and the *flexible* "transfer" of the rule to related procedures (Schusterman, 1962). The analogy with episodic and procedural memory has been formulated as follows: in using the *procedure* (the "win-stay, lose-shift strategy) to solve discrimination problems, individual *episodes* (the *events* and outcome of individual trials) determine behaviour. Staubli et al (1987a) make this point explicitly: "Learning sets seem to require both procedural and knowledge memory. In these problems, the animal must learn how to solve the problem, something which takes several days, and then on each day to acquire information about the valence of specific cues used on that particular day alone." Furthermore, discrimination learning performed after learning set acquisition differs from 'simple' discrimination learning (which occurs, for example, at the outset of learning set training but prior to learning set acquisition), despite the fact that for any given problem the cues to be discriminated, procedure

and so on are identical: discrimination learning following learning set acquisition deteriorates with increasing intertrial interval, while simple discrimination learning, if anything, is enhanced by increasing the intertrial interval (Bessemer and Stollnitz, 1971 - in: *Behaviour of Non-human Primates*, **4**, 1-58). The question therefore arises as to whether these different kinds of discrimination learning are differentially susceptible to limbic damage, given that performance following learning set acquisition may share features with human episodic learning, such as speed of learning, flexibility and a special relationship with individual events or episodes.

Neurological Considerations

Many of the features of non-human memory which have been considered analogous to human "episodic" memory described in this chapter have been derived from the analysis of deficits in task performance following damage to limbic structures in animals. Psychological considerations therefore cannot easily be divorced from neurological factors when considering the issue as a whole. Problems arise, however, (as noted earlier) when one factor is used to validate the other and *vice versa*, resulting in circular argument. In this study, psychological investigation precedes the examination of the effects of lesions.

As reviewed above, there is considerable evidence implicating the hippocampus and related cortical and sub-cortical structures in the neuropathology of human amnesia, though the relative importance of individual components and the precise roles that they play in learning and memory remain the subject of continuing study. With regard to comparative issues, the hippocampus (which has a stereotyped internal structure) is comparable and probably homologous across mammalian species

(Shepherd, 1988) and has therefore attracted considerable attention. However, relationships between cortical sensory projections and entorhinal cortex, a major source of primary afferents for the hippocampus, vary in different species. An important exception, particularly with regard to this thesis, is the olfactory modality (Staubli et al, 1984; Lynch, 1985). The relationships between olfactory projections, the entorhinal cortex and the hippocampus are largely preserved across mammalian species, including primates.

In developing valid models of anterograde amnesia, therefore, critical factors include, first, a plausible *a priori* mapping between the features of human memory impaired in amnesia and the nature of the psychological process engaged in the tasks performed by experimental animals as described above. Specification of these features must take the form of testable hypotheses, given that *precise* specification is the very information that animal models, in concert with human studies, might reasonably serve to generate. Second, such learning should be sensitive to the effects of limbic damage, in the face of a spared learning capacity. Investigation of the phenomenon of learning set has the particular advantage that both episodic and procedural learning are hypothesised to occur, and that these elements may be dissociated by manipulation of intertrial interval. Assuming that learning set formation can be demonstrated in the species of interest, then a prediction can be made about the effects of limbic damage on each of these components as a test of the hypothesis: first, that the learning of the 'win-stay, lose shift' procedure be unaffected, while second, the learning of individual events, trial to trial, be impaired.

CHAPTER 3

The Olfactory Model

The general features of the rodent olfactory model of human amnesia proposed in recent years is summarised briefly and then examined in some detail.

Summary of the Olfactory Model:

The model has three main elements:

First, rodent olfactory discrimination learning appears to share some features of primate visual object discrimination learning. A number of investigators have noted that olfactory learning in rats is very rapid and have argued that rats can form so called 'learning sets' when tested with serial novel 2-odour discrimination problems. This has been taken to imply that not only can rats acquire new information rapidly after minimal exposure (a feature of 'declarative' or 'explicit' memory) but also that rats can acquire the 'complex abstract rules' thought to underlie learning set formation in primates, an example of a form of cognitive procedure. (e.g. Otto and Eichenbaum, 1991). The analogy with certain characterisations (discussed above in relation to primate models) of the spared (cognitive procedures - procedural memory) and impaired capacities (rapid acquisition of new information) in human amnesia is explicitly made by Staubli, Fraser, Faraday and Lynch (1987a).

Second, from a neuroanatomical and neurophysiological point of view, the rodent olfactory system is considered by some to be 'phylogenetically fully evolved' in comparison with the olfactory systems of higher mammals (Otto and Eichenbaum, 1991) and 'conserved across mammalian species' (Staubli et al 1987a), and has the advantage that olfactory cortex projects directly to brain structures considered critical in human learning and memory such as the hippocampus and dorso-medial nucleus of the thalamus (Lynch, 1986). Combined with its relatively simple structure, and therefore the relative ease with which it can be investigated, these features have led to the apparently persuasive argument that rodent olfactory learning constitutes "an ideal model system for the investigation of the biology of memory" (Otto and Eichenbaum, 1991).

Third, there is considerable evidence that lesions to structures considered critical in human amnesia affect olfactory discrimination learning in rats in a variety of ways.

Each of these factors will be reviewed in turn.

1. *The Psychology of Rodent Olfactory Learning - Analogies with Primate Learning.*

The ability of primates to acquire abstract rules in the course of solving serial novel visual discrimination problems has long been known (see Mackintosh, 1974). Following initial observations of progressive improvement across visual discriminations culminating in 'one-trial' learning in monkeys (Harlow, 1949) ('learning set formation'), a number of theories were proposed to account for the phenomenon, focussing on the possibility that primates were capable of developing 'hypotheses' or 'strategies' facilitating problem solution (Restle, 1958). Theoretical and experimental analyses suggested that a learning set, once acquired, resulted in

the ability of monkeys to "use an abstract understanding of an LS [learning set] experiment, transcending the 'stimulus-response' rubric familiar in most theories of learning" (Restle, 1958). The acquisition of a particular strategy ('win-stay, lose-shift') (Levine, 1959) was principally proposed to underlie learning set formation. The adoption of this strategy implies that in the course of learning a series of discrimination problems, monkeys remember the outcome of the preceding trial as being either rewarded ('win') or unrewarded ('lose'), and learn to choose on the next trial the same cue if previously rewarded ('win-stay') or to select the alternative cue if unrewarded ('lose-shift'). The proficiency with which this strategy is acquired is demonstrated by the high level of performance achieved on the second trial of any new problem. In further support of this view, Schusterman (1962) reported that such a strategy, however acquired (e.g during serial reversal learning), was sufficient to support the one-trial learning characteristic of learning set formation; and that training procedures designed to discourage the development of such a strategy (e.g. object alternation) *retarded* the development of learning set formation in monkeys.

Having found that the rate of learning and asymptotic performance in non-primate species tested for learning set acquisition were significantly inferior to those achieved by primates (Warren, 1965), attempts were made to rank species in terms of 'intelligence' determined by the degree to which learning sets could be formed. The conventional measure used was the probability of a correct response on trial 2 of a novel problem, and in initial visual discrimination studies Rhesus monkeys achieved almost 90% correct responding on trial 2 after exposure to some 400 discrimination problems. Rats, on the other hand, barely achieved scores greater than chance (50% correct responding on trial 2 of novel problems) after 1200 problems, while cats achieved almost 70% correct responding after 1000 problems (Warren, 1965).

It is difficult, however, to compare different species on comparable tasks given the fact that differences in performance may be determined by differences in sensory capacity, motor capacity or other "contextual variables", rather than cognitive capacity (Macphail, 1982). Dolphins, for example, performed extremely well on auditory learning set tasks, but rather more poorly on visually based tasks (Herman, Beach, Pepper and Stalling, 1969).

In view of the fact that the rats dominant sensory modality is olfaction, studies of olfactory rather than visual discrimination learning were performed. The first of these studies showed that rats progressively improve in performance across a series of olfactory problems (Jennings and Keefer, 1969), in a manner rarely seen in rodent visual discrimination learning (Nigrosh, Slotnick and Nevin, 1975). This observation has been repeatedly confirmed (Nigrosh, et al, 1975; Slotnick and Katz, 1974; Eichenbaum, Fagan and Cohen, 1986; Otto, Schottler, Staubli and Lynch, 1987), resulting in the currently widely repeated claims that rats do indeed form learning sets, are therefore capable of learning "complex abstract rules" (Otto and Eichenbaum, 1991) such as the 'win-stay, lose-shift' strategy (Slotnick and Katz, 1974) and thus demonstrate a "human-like" learning capacity (Otto and Eichenbaum, 1991) when tested with olfactory problems. Strikingly, it has been suggested that a learning set can be "completely" (Eichenbaum et al , 1986) established after training on only 3 problems, in contrast to the 400 or so problems required by Rhesus monkeys.

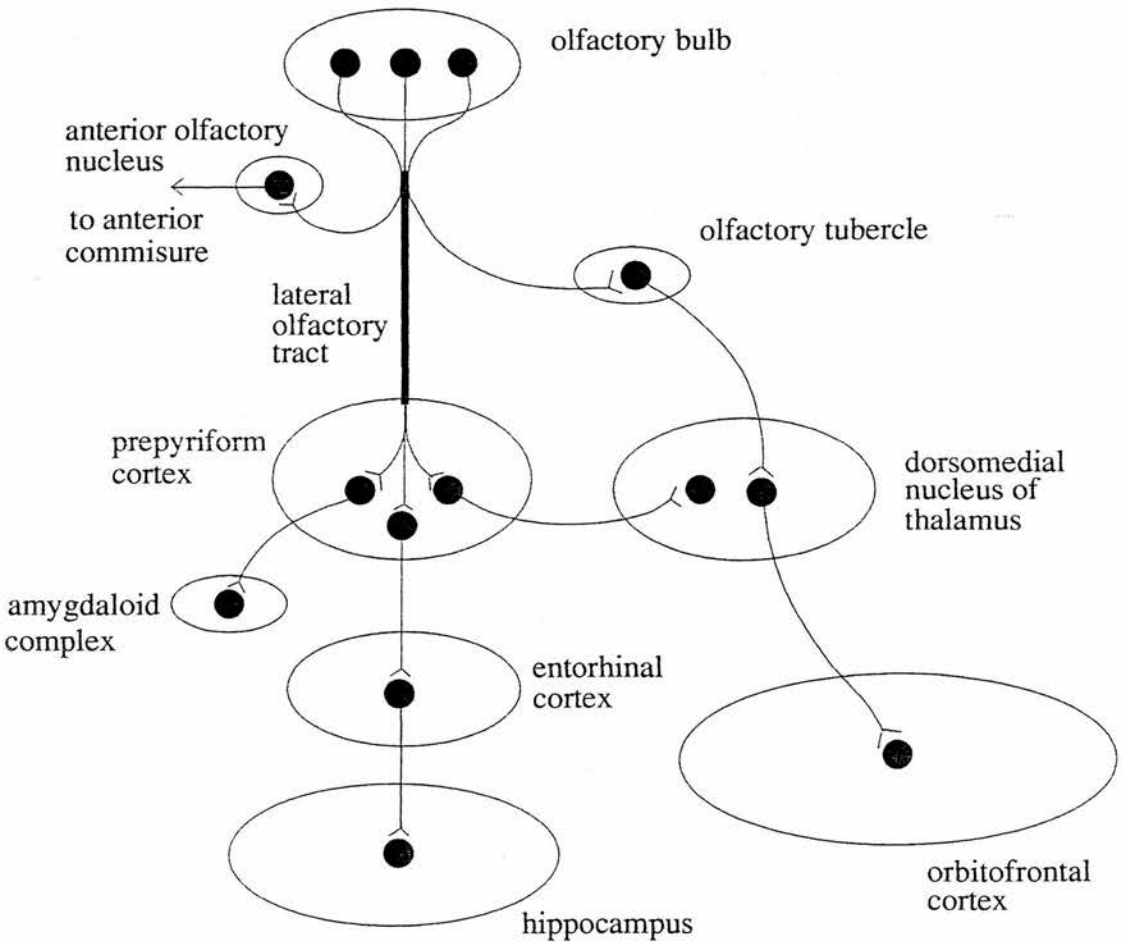
These observations on rodent olfactory learning are intriguing, but they must be interpreted with caution for 2 reasons.

First, although it has been shown that olfactory discrimination learning in rats is indeed rapid, and that progressive improvement occurs when rats perform a series of novel problems, the studies have not taken account of the fact that the observation of progressive improvement alone is *insufficient* to demonstrate the acquisition of an abstract rule. There are other potential sources of transfer in the solution of a series of novel discrimination problems (see Mackintosh, 1974, pp 612-614), aside from the development of the abstract "win-stay, lose-shift" strategy. General factors, such as familiarity with the testing apparatus and a consequent reduction in anxiety, or suppression of error generating behaviour such as position habits (where position is an irrelevant factor), which may contribute to or even account entirely for the early improvement in trials to criterion on novel problems. It was generally appreciated in the development of learning set theories that not all sources of progressive improvement were necessarily of special interest, as they could be explained without appeal to higher-order or abstract processes. As Restle (1958) pointed out: "If we had available *only* the observation that monkeys improve in successive discrimination problems, it would be tempting to try an explanation in terms of the simple processes characteristic of naive rats" [italics added].

Second, none of the studies of rodent olfactory discrimination learning described above have used the conventional measure of learning set acquisition - the percentage of correct responses made over the *earliest* trials of any new problem (e.g. trial 2 or trials 2-5). This is surprising, given that it was the finding that monkeys were eventually able to solve visual discrimination problems in only one trial (Harlow, 1949; Restle, 1958; Levine, 1959) which originally generated interest in learning set formation. Slotnick and Katz (1974), for example, only report the percentage of correct responses made by rats performing serial odour discrimination problems over *20* trials.

Other investigations of learning set formation in non-primate species have taken account of these issues. In a study of learning set formation in Blue Jays for example, Kamil, Jones, Pietrewicz and Maudlin (1977) demonstrated not only that performance on the second trial of any new problem was considerably above chance, but also that transfer to learning set occurred from other training procedures designed to encourage the formation of an abstract 'win-stay, lose-shift' strategy (in a manner analogous to Schusterman's (1962) study). In addition, they controlled for the operation of non-specific factors in the study by comparing the performance on novel problems of experienced birds trained previously on a series of different problems, with birds who had only been trained continuously on a single problem prior to transfer to the novel problem. Their findings were modest - Blue Jays *could* develop an abstract strategy to some degree, but only after some 160 problems and, at an asymptotic level, performance was considerably lower than that seen in equivalent primate studies.

There are, therefore, some grounds for caution in accepting that the progressive improvement seen in rats performing a *few* serial 2-odour discrimination problems truly represents the development of an abstract 'win-stay, lose-shift' strategy as seen in primate studies. The fact remains that this hypothesis has never been appropriately tested, and an attempt to settle the issue constitutes a main feature of this thesis.



Schematic diagram of rodent olfactory pathway (after Shepherd, 1988)

2. *Anatomical Considerations*:

The rodent olfactory system is intimately connected with those limbic structures considered of interest in human amnesia. Briefly, the olfactory bulbs project monosynaptically to layer 1A of piriform cortex and the lateral subdivision of adjacent entorhinal cortex via the lateral olfactory tract. These areas, designated primary olfactory cortex, then project monosynaptically to several of the amygdaloid nuclei, the hippocampus and the dorsomedial nucleus of the thalamus. Further projections from amygdala, dorsomedial nucleus of the thalamus, and intra-cortical afferents from the olfactory bulb itself, reach the prefrontal cortex (see Shepherd, 1988 pp. 244-246 for a review).

Otto and Eichenbaum (1991) describe these rodent olfactory-limbic connections as fully evolved with respect to primate sensory-limbic connections (see Lynch, 1986 for a similar view). It is worth considering briefly the implications of this assertion. It is certainly true, in very general terms and from a purely anatomical point of view, that primary olfactory cortex in rats projects to limbic targets in analogous fashion to primate sensory-limbic systems. There are, however, fundamental differences between the olfactory system and other sensory systems in *both* rodent *and* primate brain. First, in either olfactory system, there is no thalamic relay between the sense organ and primary cortical targets. Second, the primary cortical targets are not true neocortex, but 3-layer paleocortex. Third, from this point, limbic projections are relatively direct, and therefore sensory information is less highly processed with respect to other sensory systems.

It has been argued that these factors may be usefully exploited in the study of the neural basis of learning and memory, given the relative ease with which such a "simplified" system may be studied (Lynch, 1986). Given the conservatism of olfactory anatomy across mammals (Lynch, 1986), it would seem to be implied that not only is the olfactory system ideal for study in rodents, but by the same argument its relative simplicity across species should also make it a useful target of investigation in primate learning and memory. It must be appreciated, however, that this argument is strictly anatomical rather than functional, and there is no *a priori* reason to assume that *despite* the differences between olfactory and non olfactory systems mentioned above, that their limbic projections are *functionally* equivalent with respect to learning and memory - and indeed, there is evidence to the contrary.

This evidence lies in the study of olfaction in human amnesics. If it is indeed true that the structure and function of the olfactory system is conserved across mammalian species and that it constitutes a useful system for the study of learning and memory by virtue of its privileged limbic connections, then it must be predicted that human amnesics, who by definition have sustained damage to the structures of interest in learning and memory, will show deficits in olfactory learning in the absence of intellectual and sensory dysfunction, just as they do with material presented to other sensory systems. This consideration is analogous to efforts to validate primate models of human amnesia by studying the performance of human amnesics on tasks used in the primate studies (Aggleton et al, 1988; Squire et al, 1988).

Eichenbaum, Morton, Potter and Corkin (1983) conducted a detailed study of the olfactory capacity of the noted bi-temporally amnesic patient 'H.M.'. Although H.M. demonstrated normal performance on a battery of tests of odour detection, intensity

discrimination and adaptation, he was unable to discriminate or identify odours in same-different combinations and in immediate, *not* delayed matching to sample tasks. Despite the fact that he could name common objects using visual or tactile cues, he could not identify them by smell. Eichenbaum et al (1983) concluded that the perceptual phenomena of odour detection and discrimination were dissociable by cerebral damage, and that structures in the medial temporal lobe play an important role in odour discrimination. Eichenbaum et al (1983) make the point that H.M.'s odour discrimination impairment "is not attributable to his well documented memory deficit" and point out that none of the tests used in the study (with the exception of odour and object naming tasks) required the use of information outside the patient's intact, immediate memory. The deficit was clearly modality specific, and H.M. performed normally on visual analogues of the olfactory tasks. Neither his anterograde nor retrograde amnesia could account for the poor performance on odour naming (identification) tasks - all the odours used were "items of common experience to people in childhood" and he could easily name by sight or touch the objects he could not identify by smell. Thus, H.M.'s deficit is perceptual not cognitive.

Qualitatively similar, though less severe deficits have been observed in patients with unilateral temporal lobectomy (whether right or left lobe was excised) (Rausch and Serafetinedes, 1975; Eskenazi, Friend, Cain, Lipsitt, Rabin and Novelly, 1981), but the patients described in these studies are not amnesic. Although a later study by Rausch, Serafetinedes and Crandall (1977) describes deficits on olfactory delayed matching to sample tasks, the finding is unlikely to imply a cognitive deficit, given that impaired discrimination performance at short intervals (a perceptual deficit) would clearly preclude good performance in delayed matching tasks, and in any case, the subjects tested were not amnesic.

Patients with diencephalic lesions, mainly subjects with Korsakoff syndrome, have also been studied. Again, marked deficits in odour discrimination have been reported, though frequently in association with impairments in odour detection and intensity discrimination (Jones, Butters, Moskowitch and Montgomery, 1978; Mair, Capra, McEntee and Engen, 1980). It is also clear from these studies that olfactory perception is specifically affected, and is not a consequence of the patients' memory dysfunction. Interestingly, in the latter study recognition memory for very easily discriminated odours (which the Korsakoff patients were able to discriminate to some extent) was compared with recognition memory for faces and consonant trigrams. Over a thirty second interval, olfactory recognition memory remained unaffected, while memory for the other classes of material became impaired in a time dependent manner. This was not simply a 'floor' effect with respect to the olfactory findings, as the subjects scored well above chance in the olfactory test, and were much more markedly impaired on simple matching tests (even at very short intervals) when tested with odours which control subjects found less easy to discriminate. One can conclude from this finding that, if anything, *memory* for odour cues is relatively preserved with respect to other classes of material, despite the fact that an olfactory perceptual deficit is evident. Similar olfactory perceptual deficits have also been found in subjects with frontal lesions (Potter and Butters, 1980), Parkinson's disease, Alzheimer's disease and other dementias, in addition to schizophrenia and depressive illness, although such patients do not suffer from amnesic states (reviewed by Harrison and Pearson, 1989).

In summary, a number of studies demonstrate that medial temporal, diencephalic and frontal damage all cause deficits in olfactory *perceptual* function to varying degrees in human subjects. Although detection of odour presence *may* be spared, and

intensity discrimination intact, odour quality discrimination is often markedly disturbed, such that patients cannot tell one odour from another. This is not a learning deficit, and severity is uncorrelated with the severity of the amnesia. The deficits occur in the setting of normal perception in other modalities.

To conclude, then, it appears that while limbic damage in humans may result in learning deficits for non olfactory material, it also reliably causes perceptual deficits in the olfactory modality. This finding has, in fact, recently been exploited in the development of tests of olfactory perception for use diagnostically in brain damaged subjects (Harrison and Pearson, 1989).

It follows from the above that if similar perceptual deficits are encountered in rodents with limbic damage, then some weight is lent to the notion that olfactory function is conserved across species, but the concept of a rodent model of human amnesia utilising olfactory stimuli as cues may prove difficult to sustain. Conversely, if perceptual deficits are not found, then the original arguments relating to conservation of function across mammalian species become difficult to accept.

3. *Lesion studies*:

i) *Lesions of DMN, and LOT/Piriform Cortex.*

Early olfactory studies were concerned with the differentiation of brain structures involved in olfactory perception rather than the learning and memory of olfactory cues. Initial experiments produced largely negative results. Swann (1934) found that rats with large temporal lobe lesions had no deficits when trained to respond in a T-maze to follow odour cues, though Allen (1941) has pointed out that the olfactory cues used may have stimulated both olfactory *and* trigeminal pathways. Allen (1941) examined the effects of temporal lobe lesions in dogs on a series of tasks mediated by olfactory cues. He found that extensive bilateral extirpation of the pyriform-amygdaloid areas and adjacent neocortical neocortical tissue, with or without additional complete damage to the hippocampus, had little or no effect on the animals' ability to detect and react to odours as assessed by a conditioned foreleg response. The animals were also unimpaired in selecting a package containing meat from other empty packages of like size and texture while blindfolded. Allen did observe, however, effects on an olfactory discrimination task in which three dogs with the above lesion responded positively to one odour but were unable to withhold responding in the presence of another - the dogs tending to respond positively to all odours in a perseverative fashion. Slotnick (1985) has commented that, with hindsight, it is unclear whether the failure of discrimination was due to olfactory deficits, or to the well established deficit of animals with amygdala lesions to inhibit punished responses, given that no non-olfactory control task was used. In fact, Allen himself felt that olfactory discrimination was probably intact in his subjects, observing that on making an error of commission the dogs would "brace themselves or cry as if in expectancy of punishment." He suggested, as later authors would claim

without acknowledgement, that the lesions may interrupt "a very high order of olfactory synthesis" drawing attention to similar discrimination deficits observed in dogs following removal of the prefrontal areas (Allen, 1940). It appears, however, that what Allen meant by a 'higher order of olfactory synthesis' was the ability to discriminate one odour from another, as opposed to the ability to detect odours - both perceptual rather than cognitive deficits - and in this sense his statement about a "higher order of olfactory synthesis" is somewhat vague and contradictory. In Swann's (1934) and Allen's (1941) studies the hippocampus was either bilaterally destroyed or deafferented, and a review of the anatomical, physiological and behavioural data of the period led Brodal (1947) to conclude that there was "no support for the conception that the hippocampus has important relations to the sense of smell in mammals." The weight of the early evidence indicated that frontal areas were of primary importance in olfactory function, while the temporal projections of primary olfactory cortex were not concerned with olfactory function and in particular did not appear to be essential to support simple olfactory learning.

More recent studies have tended to support this finding. Eichenbaum et al (1980), noting that the dorso-medial nucleus of the thalamus (DMN) receives direct input from olfactory cortex and projects to the frontal cortex of the rhinal sulcus (RS) and medial wall of the frontal neocortex (MW) in rats, studied the effects of lesions to these brain areas on a variety of odour detection and discrimination tasks in thirsty rats motivated by a water reward. Olfactory threshold and detection ability was not affected by any of the lesions (as assessed by the learning of a go/no-go task requiring discrimination between odourised and non- odourised air flow at a variety of odour intensities) was not affected by any of the lesions. Discrimination between different odours (go/no-go 2-odour discrimination learning) was, however, disrupted by DMN and RS lesions, but not by MW lesions. In addition, although deficits were

seen in the re-acquisition of problems learned pre-operatively, more severe deficits were seen on the learning of novel discriminations post-operatively, and the most severe deficits were seen on those odour pairs considered more difficult to discriminate as judged by human observers. All odour problems were, however, eventually learned to criterion by lesioned animals.

These results largely accord with findings in humans with Korsakoff syndrome (see p48-49), save that in some studies Korsakoff patients also sometimes have detection threshold deficits (although they are by no means anosmic). Eichenbaum et al (1980) also make this point in their report, but then go on to state that "thus, the cognitive defects associated with this (Korsakoff's) disease might be interpreted as similar to those observed in rodents with MD (DMN) lesions". The point has already been made that the olfactory deficits seen in Korsakoff's syndrome need not be considered 'cognitive', and cannot, in particular, be attributed to a memory deficit. Indeed, the patients deficits are clearly perceptual, and their poorer performance in discriminating odour pairs judged as relatively more similar in quality by controls follows from this (Mair et al, 1980). It is striking that Eichenbaum et al (1980) made precisely the same observation in rats. They preferred, however, to interpret the finding as an "associative deficit" rather than a perceptual deficit, suggesting that the more similar odour pairs are 'associatively, not psychophysically similar'. The meaning of this claim is unclear, given that the psychophysics of odour *quality* poorly understood. Eichenbaum et al's (1980) evidence for the distinction is the fact that their human subjects tended to place the more similar odours used in the experiment in pairs in terms of subjectively similar categories (e.g. floral, tar-like) - but the relevance of these human *associations* (as opposed to perceptions) to rodents is debateable. A parsimonious view would seem to be that DMN lesioned rats have a perceptual deficit which is most clearly observed when they are required to

discriminate odours which are perceptually more similar - i.e harder to discriminate - just as is found with humans who suffer form Korsakoff's syndrome. In Eichenbaum et al's (1980) study, all three groups of rats were unimpaired in a discrimination between odour and 'no odour' (perhaps equivalent to a 'very easy' discrimination, given that it is unlikely that a true 'no odour' condition can be achieved in olfactory testing apparatus short of a vacuum) and thus the lesioned rats clearly had no deficit in associating an odour cue with reward.

Slotnick and Kaneko (1981) found rather different results in a study of the effects of DMN lesions and lesions of the lateral olfactory tract (LOT) at the level of the anterior amygdala. They examined olfactory discrimination reversal learning, using a similar water rewarded go/no-go schedule as that used by Eichenbaum et al (1980). On the first, post-surgical, 2-odour olfactory discrimination problem, no impairment was found in either the DMN or LOT lesioned groups in contrast to Eichenbaum et al's (1980) findings. However, clear differences began to emerge on the first and subsequent 5 reversal problems. While sham and LOT lesioned animals progressively improved across the reversals in terms of errors made in reaching a learning criterion of 90% correct responding in blocks of 20 trials, the DMN lesioned animals made almost 4 times as many errors on the first reversal, gradually improving over subsequent problems to a level equivalent to their initial discrimination score, still making considerably more errors than the other groups. Slotnick and Kaneko interpreted these findings as showing that DMN lesions affect "complex olfactory learning", while lesions to olfactory limbic projections have no effect on either complex or simple olfactory discrimination learning. It is notable, however, that the rats with DMN lesions did improve across reversals, at a *rate* comparable to control subjects, but made many more errors on each individual problem.

Although Slotnick and Kaneko described the normal performance of their LOT lesioned animals as "surprising" (given the massive olfactory projection to limbic areas), perhaps their most striking finding lies in the performance of the control animals on reversal learning. Not only did these subjects improve across reversals but "most normal animals show(ed) positive transfer on the first reversal". This particular observation is unusual, for although improvement across a series of reversal problems has been previously described in rodents with visual cues (e.g. Mackintosh, McGonigle, Holgate and Vanderver, 1968), positive transfer by rats on the first reversal has not, as far as I am aware, ever been observed before (or since) outside of Slotnick and his group's olfactory discrimination studies (e.g. Nigrosh, Slotnick and Nevin, 1975, p.292).

Unfortunately, it is this positive transfer on the first reversal to which Slotnick and Kaneko refer when they discuss "complex olfactory learning" - the only behaviour affected by lesions in their study. Were the reversal finding to be shown to be unreliable, it would be difficult to accept Slotnick and Kaneko's interpretation of their findings.

In fact, this level of performance was *not* observed by Slotnick in a later paper (Slotnick and Risser, 1990) in which lesions to the DMN and posterior LOT were again studied in rats performing novel olfactory discriminations and reversal discriminations. Rats were trained pre-operatively on an odour detection task using a go/no-go schedule, and then on each of 4 novel 2-odour discrimination problems. Following this, each of the 8 odours were presented in a quasi-random order in a go/no-go discrimination task. This task was run in 3 sessions of 200 trials each, and in the last 2 sessions reinforcement probability was reduced to 0.3 for responses on S^+ trials. The rats were then re-tested 10-12 days later on the same task, except that

responses to 3 of the 4 previously rewarded odours were not reinforced (the fourth being reinforced as before to maintain responding) in an effort to obtain a "pure" measure of odour memory. Following this the rats were operated, receiving DMN, LOT, combined DMN and LOT, and sham lesions. After recovery, the last task (8 odour memory task) was performed again. The rats were then trained on 3 novel 2-odour discriminations, followed by a reversal of the final problem. In the pre-operative memory test, it was established that rats could retain responding to rewarded odours over a 10-12 day period, the animals attaining "perfect or near perfect scores". This performance was compared with that measured following surgery in the same task. Only the group with combined LOT and DMN lesions showed any deficit, the remaining groups performing as well as before. Interestingly, the 3 groups performed relatively well on the discrimination of the 3 novel 2-odour problems, with marginal but significant impairments seen on only 1 problem for the DMN group and 1 (different) problem for the combined DMN/LOT group. However, substantial differences were noted on the final reversal problem, in which both the DMN and combined DMN/LOT groups made many more errors in reaching criterion. The sham operated, and LOT lesioned animals performed similarly, but even these rats made at least 6 times as many errors on the reversal than they had on the immediately preceding novel discrimination.

This last finding shows that positive transfer did *not* occur on the first reversal problem in control rats, as had been observed in the earlier study described above (Slotnick and Kaneko, 1981). Of course, the 2 experiments differ considerably, particularly with respect to the amount of prior training received by the animals in the latter study. However, comparing absolute levels of performance across studies, the reversal error scores of the animals with the more extensive training were, in fact, about 4 times higher than those made by the rats in the earlier study which had

received less training. Were it the case that rats had adopted a 'win-stay, lose-shift' strategy in the course of training, one would predict the opposite result - that extended training should minimise errors on reversal problems. It might be argued that the 8-odour concurrent problem memory test performed by the rats in the latter study (in which odours were randomly re-paired, and 3 out of 4 previously rewarded odours now not rewarded) would have discouraged a 'win-stay, lose-shift' strategy, thereby accounting for the discrepancy across studies. However, there are 2 reasons to doubt this interpretation. First, if this were true and, as Slotnick and Katz (1974) have claimed, the rapid learning seen in discrimination of novel problems is also mediated by a 'win-stay, lose-shift' strategy, then discouraging the development this strategy should have resulted in poorer performance on novel problems than the near errorless performance actually observed in the control group. Second, there are a number of other studies employing reversal learning in which the rats are not exposed either to extensive pre-operative training, or to tasks in which a 'win-stay, lose shift' strategy might be discouraged, in which reversal error scores are greatly in excess of those obtained on the preceding novel discrimination (e.g. Eichenbaum et al, 1986; Eichenbaum et al, 1988; Staubli et al, 1987b, further discussed below). In other words, it seems possible that the observation of positive transfer on the first reversal of an olfactory discrimination problem by rats is spurious, and perhaps cannot be replicated.

In any case, from the point of view of modelling human amnesia, the finding that DMN lesions impair 'rule learning' (the implication of Slotnick and Kaneko, 1981), but have no effect on the retrieval of previously acquired information in rats (Slotnick and Risser, 1990) is exactly at odds with the findings in both human diencephalic amnesia and primate models of this syndrome. Moreover, the impaired performance of the DMN lesioned rats on reversal problems is inconsistent with the

view that rapid forgetting of olfactory information occurs following DMN lesions in the rat as claimed by Staubli, Schottler and Nejat-Bina (1987) (discussed below).

Further to this, Slotnick and Risser (1990) have pointed out that the bilateral LOT transection used in their study deprives the hippocampus of its major olfactory input and concluded, on the basis of their findings of no impairment in any of the tasks examined, that the hippocampus itself was not essential for olfactory discrimination learning or memory as tested in their apparatus. These findings are consistent both with the early work of Swann (1934) and Allen (1941), and more recent studies showing that LOT transection at the level of the anterior amygdala does not cause anosmia as determined by performance on an intensity discrimination task (Slotnick and Berman, 1979) or 2-odour quality discrimination tasks (Slotnick, 1985). Slotnick and Risser (1990) also stated that the failure of rats with combined LOT/DMN lesions to perform well when re-tested on a task originally learned pre-operatively indicates that retention of olfactory information requires the integrity of both olfactory/limbic and thalamocortical projections. They argued that neither projection alone was essential for retention as individual bilateral lesions of either the LOT or DMN were without effect on the retention task. It is notable however, that the combined lesion group performed most poorly (i.e. made most errors) in learning the final reversal task, implying that they had no difficulty in recalling the previously positive odour learned *post*-operatively. This casts doubt on the notion that the animals with combined lesions have an enduring deficit in their ability to remember odour cues - and the post operative 'retention' finding is perhaps better characterised as a 'retrograde' deficit. Unfortunately, Slotnick and Risser (1990) appear to have confused "impaired long term memory" with "retrograde amnesia" throughout their report, and consequently failed to comment on this issue.

Staubli et al (1987b) have also studied the effects of DMN lesions on olfactory learning. They found that both pre-trained and experimentally naive rats with lesions of the DMN were profoundly impaired on water rewarded, simultaneously presented (rather than go/no-go) 3-odour olfactory discrimination problems when compared to sham lesioned animals. However, if lesioned animals were given extensive training, (a maximum of 100 trials on each problem, rather than the 25 trials per problem in the 'standard' schedule) the rats were eventually, after three 2-odour problems, able to perform almost as well as controls. Unfortunately, it is difficult to evaluate the findings presented in this report because the absolute performance scores given in the text bear little relation to the scores illustrated in the figures (e.g. fig 3 p.122, sham group score, problem 1 = approx 35 trials to criterion; text p. 122, para 2, same score =27). I suspect that some confusion has arisen between error score and trials to criterion scores, given that on p.121, fig 2b, control subjects are shown to be reaching criterion with less than 3 trials, while the text states that the learning criterion is 8 correct trials within a 10 trial block - i.e. *minimum* criterion score must be 8 trials to criterion on any problem. The general trend of the findings is, however, largely consistent with the other studies - DMN lesions tend to impair olfactory discrimination learning, though not consistently.

In this study (Staubli et al, 1987b) DMN animals were more impaired on "difficult" discriminations, consisting of odourant cues made by combining odourants (e.g. a+b v. a+b+c), consistent with the above interpretation that a sensory deficit may account for poorer performance, given that an odourant a+b is more likely to be perceptually similar to odourant a+b+c, than (say) odour a is to odour b, since in the former condition, more components are shared within the pair. Staubli and her colleagues, however, choose a cognitive interpretation of their data - describing the initial very poor performance of the intensively trained DMN group as a "transient procedural

deficit", and describing the later, better performance as representing a mild "anterograde learning deficit" to account for the fact that the lesioned animals never quite matched the performance of the control subjects. These interpretations necessarily rely on the assumptions that 1) rats form learning sets (Staubli et al, 1987b: p. 117), thereby acquiring a 'cognitive rule'; and 2) that there is no perceptual deficit. Neither of these assumptions are systematically evaluated in the study, although as noted above, the performance of lesioned rats on multi-component odour pairs does, in fact, suggest that they have difficulty in discriminating more alike members of individual pairs. The claim that the rats have an 'anterograde' impairment is supported by the statement that lesioned subjects show little evidence of savings upon re-presentation of a previously learned problem on consecutive days, but this data is not shown in the paper. Overall, the concluding remark that "it appears that the widely held notion that the DMN plays an important role in the establishment of memory in humans at the time of learning also applies for olfactory memory in rats" is misleading, given that a marked "procedural" impairment - the main implication of the study - is *not*, as noted above, characteristic of the *type* of learning deficit seen in human diencephalic amnesics.

In the same study, Staubli et al (1987b) examined the effects of partial piriform ablations on olfactory learning in rats. These lesions were placed more rostrally than those discussed in relation to LOT lesions in the studies reviewed above (e.g. Slotnick and Risser, 1990), at the level of the anterior olfactory nucleus extending caudally into piriform. They were therefore likely to de-afferent much of piriform cortex as well as destroy part of it. Similar behavioural results were obtained to those found following DMN damage, and a role in "procedural" learning was ascribed to piriform cortex. Unlike the DMN lesioned animals, however, extensive pre-operative odour discrimination training had a pronounced effect, the post-operative

performance of such animals being much improved though still poorer than sham operated animals. The animals were, however, more markedly impaired on 'compound odour' training, consistent with a persisting perceptual deficit. While it is difficult to account for the selective effects of pre-operative training, it seems likely that at least part of the deficit observed in these animals may be related to de-afferentation of the DMN and prefrontal cortex, the rostrally placed lesion probably destroying both intra-cortical afferents to prefrontal cortex as well as deafferenting those areas of piriform cortex subsequently projecting to DMN which in turn project to pre-frontal cortex.

Thus far, it can be seen that DMN damage tends to interfere with olfactory discrimination learning in the rat, but to a variable degree, ranging from inconsistent impairment (Slotnick and Risser, 1990; Eichenbaum et al, 1980), to considerable deficit (Staubli et al, 1987b). Important factors appear to include the discriminability of the odours chosen and prior training experience (Eichenbaum et al, 1980; Staubli et al, 1987b). Overall, the patterns of deficit described are rather similar to the perceptual deficits encountered in human Korsakoff subjects, and inconsistent with findings made in other modalities in either human diencephalic amnesia, or in primate models of human diencephalic amnesia. The studies cannot therefore be considered to represent rodent models of human diencephalic amnesia, despite the claims of Eichenbaum et al (1980) or Staubli et al (1987b), quoted above. In addition, with regard to modelling human bi-temporal amnesia, the studies reviewed so far have tended to minimise the role of the hippocampal formation and/or amygdala in either olfactory learning or memory, suggesting that deafferentation by posterior LOT transection or destruction of more caudal piriform cortex produces no effect. More rostral LOT lesions, likely to interrupt projections to prefrontal cortex, produce similar patterns of deficit to those seen following DMN or frontal lesions,

which are more akin to perceptual than cognitive deficits.

ii) *Lesions of fornix, and entorhinal cortex*

Eichenbaum and his colleagues (Fagan, Eichenbaum and Cohen, 1985; Eichenbaum et al, 1986) have made further attempts to develop a rodent model of human bitemporal amnesia, similar to those developed with non-human primates. Setting their work in the context of the claims that rats show a 'primate-like' learning capacity when tested with olfactory cues and rapidly develop 'learning sets', they examined the effects of fornix damage on 'learning set' formation and reversal learning. Groups of rats were trained on 3 novel (successive go/no-go) discrimination problems, followed by a reversal of the final problem. Prior to training, rats received either sham surgery, fornix lesions, amygdala lesions or combined fornix and amygdala lesions. The groups rapidly and equally improved across the 3 novel problems. Differences emerged on reversal training, in which animals with fornix lesions (both the fornix, and combined fornix and amygdala groups) solved the reversal problem more rapidly than sham treated animals or animals with amygdala lesions alone. Eichenbaum and his colleagues interpreted the results as showing: 1) preserved procedural learning (intact learning set formation), in the face of 2) impaired declarative memory (faster reversal learning, implying "forgetting" of previous stimulus associations - Fagan et al 1985, p.510), in the groups with fornix lesions. The case was made that these findings therefore represented the development of a useful rodent model of human amnesia, demonstrating analogues of the capacities believed to be impaired (declarative memory) and spared (procedural memory) in human amnesia. It is worth noting that this study was the principal inspiration for the experimental work outlined in this thesis.

In a later study, however, (Eichenbaum, Fagan, Mathews and Cohen, 1988) rather different results were obtained - despite the use of identical apparatus, training schedules, strain of rat, odour cues and lesion technique to those described above. The earlier experiments were repeated as part of a larger experiment in which odour task parameters were varied in further experiments. In addition to examining performance on a successive cue, go/no-go 2-odour discrimination schedule as above, 2 further tasks were used employing the same odour cues: a simultaneous-cue, go-left/go right task (essentially a conventional 2-odour discrimination task in which cues are presented simultaneously); and a successive-cue, go-left/go-right task (in which rats had to 'nose-poke' in either a left or right located 'nose port' conditional upon which of 1 of the 2 odours in the particular discrimination problem was presented to them from both nose-ports).

In the go/no-go task, identical to that reported in Eichenbaum et al (1986) (save for the fact that the final reversal problem was not used) fornix lesioned rats now significantly *out-performed* the sham operated animals on each of the 2-odour discriminations problems. This finding is used to support a new interpretation of the effects of fornix lesions on olfactory discrimination learning in rats, inconsistent with the 'amnesia' interpretation offered earlier (though the fact that the findings are different in the 2 experiments is not acknowledged). The further 2 experiments show that while performance on a successive-cue go/no-go schedule is facilitated by fornix lesions, performance is impaired on both *simultaneous*-cue, 2-odour discrimination problems and successive-cue, go-right/go-left discrimination problems. In particular, in the simultaneous condition, the fornix lesioned rats are not only impaired on each of the 3 problems presented, but do not show progressive improvement across problems. This is clearly at odds with the notion presented earlier that progressive

improvement represents the acquisition of a cognitive skill, *unimpaired* in fornix lesioned rats.

While this discrepancy is also not acknowledged in print, Eichenbaum and his colleagues present a new theory of hippocampal function to account for their findings. The theory proposes that the hippocampus is concerned with the 'relational' processing of cues, such that simultaneous-cue discrimination which is presumed to require "multiple comparison between cues" (and hence requires 'relational processing') is impaired by fornix lesions, while successive-cue discrimination does not require 'relational processing' and is therefore not impaired. It is argued that successive-cue discrimination is, in fact, facilitated in fornix lesioned animals because an intact relational processing strategy hampers the performance of *intact*, sham operated animals. In a further study (Eichenbaum, Mathews and Cohen, 1989) the hypothesis is elaborated by studying the effects of 'mis-pairing' cues from previously learned, simultaneously presented 2-odour discrimination problems, such that cues which had previously been scheduled as (say) A+ v B- and C+ v D-, were re-presented as A+ v D- and C+ v B-. While sham-operated rats rapidly learned the new pairing, presumably benefitting from their previous experience with the conserved individual cue-reward association, fornix lesioned rats performed less well, apparently treating the new pairing as a new problem. Eichenbaum and his colleagues suggest that the fornix-lesioned rats treat the simultaneously presented problems differently from intact animals, responding to a compound cue rather than individual cues, and basing their discriminative performance on whether the cues are presented [A+/left, B-/right] as a compound to which the correct response is a 'nose-poke' to the left, and [B-/left, A+/right] as a different compound, to which a right-directed nose-poke is required to obtain reward. Richard Morris (personal communication) has commented that, if anything, it is the lesioned animals which

are performing 'relational processing' (rather than being impaired in this respect), while by Eichenbaum et al's account, the control animals are processing cues individually. In my view, the problem lies in the fact that the construct 'relational processing' is insufficiently specified to be useful in interpreting the data presented.

In a later review of these studies (Otto and Eichenbaum, 1991), the authors draw attention to similarities between the findings detailed above and related studies by Staubli, Ivy and Lynch (1984), in which the effects of lateral entorhinal cortex lesions on simultaneous 2-odour discrimination in rats was examined. The point is made that both fornix and entorhinal lesions interfere with hippocampal function, and may produce similar effects on olfactory learning and memory, assuming that the hippocampus plays a prominent role in rodent olfactory learning analogous to that in human (bi-temporal) global amnesia. Comparison with the work of Staubli is permitted in the context of the simultaneous 2-odour discrimination study described above, as Staubli used simultaneous 2-odour discrimination problems in her experiment. In their study, Staubli et al (1984) showed that lesioned rats performed poorly on simultaneous olfactory discrimination problems when trials were separated by a delay of 3-10 minutes. Otto and Eichenbaum (1991) state that this is consistent with their findings, describing the result as an "exacerbation" of impairment by long inter-trial intervals. Their reading of the study is incorrect, however, given that at short delays (less than 3 minutes) the rats in Staubli's study were, in fact, unimpaired. As Eichenbaum and his colleagues used an inter-trial interval of 10 seconds in their own experiment (i.e. well within the interval in which rats were unimpaired in the Staubli study), the studies are clearly inconsistent with one another, in that Staubli et al's (1984) data conflicts with the 'relational processing' theory proposed by Eichenbaum et al (1988), while Eichenbaum et al's data cannot support the crucial time-dependency findings central to Staubli et al's (1984) model of amnesia.

This raises an important issue concerning the adequacy of these respective findings to be convincing models of human amnesia. While it must be acknowledged that the 2 research groups each use a different lesion site (and their results may differ on this account alone) it is clear that their findings cannot both serve the same theory. In fact, Eichenbaum and his colleagues appear to have abandoned attempts to model human amnesia to concentrate on a theory of hippocampal function at odds with their earlier data, but consistent with later findings (Eichenbaum et al, 1988 v. Eichenbaum et al, 1986). This shift in emphasis is not, however, made explicitly: from a reading of Otto and Eichenbaum's (1991) review it would appear that the 2 (mutually exclusive) interpretations are held simultaneously.

In addition to lesion studies, Staubli, Thibault, DiLorenzo and Lynch (1989) have examined the effects of intraventricular infusion of the N-Methyl D-Aspartate (NMDA) receptor antagonist, D-aminophosphono-valeric acid (AP5) on olfactory discrimination learning in rats. Blockade of the NMDA receptor by AP5 has been shown to suppress the induction of Long-Term Potentiation (LTP)(Collingridge, Kehl and McLennan, 1983), a form of long lasting synaptic facilitation believed to be involved certain forms of learning (Bliss and Lomo, 1973). Chronic administration of AP5 via intraventricular infusion has been observed to cause selective spatial learning deficits in rats (Morris et al, 1986), in a dose-dependent manner (Davis, 1990). Noting that olfactory pathways project to NMDA receptor rich telencephalic targets, such as piriform cortex, entorhinal cortex and hippocampus, coupled with the observation that entorhinal cortex lesions appear to affect olfactory discrimination tasks in rats (Staubli et al, 1984), Staubli et al (1989) hypothesised that chronic infusion of D-AP5 might affect olfactory learning. They used a water rewarded, simultaneous 2-odour discrimination task at short (2 minute)

and long (10 minute) inter-trial intervals. It was found that AP5 infused animals tended to make significantly more errors than control animals at long ITIs, but did not differ from controls at short ITIs. In contrast, when trained on a reversal of a previously learned problem 24 hours later, the same AP5 infused animals performed equally with respect to controls. Staubli et al (1989) concluded that administration of AP5 impaired the acquisition, but not retention of olfactory memory. Curiously, however, these results were only obtained at "weak" odour concentrations. Using "standard" concentrations, no effect of ITI was seen, while using an 8-fold dilution of the odourants produced the effect described above. It is not clear from the report what a "standard" concentration actually represents, as no details are provided in the text. Staubli et al (1989) acknowledge that AP5 may have an affect on odour perception itself, but draw attention to the fact that the AP5 infused animals are unimpaired at short inter-trial intervals even in the weak odour condition.

The performance of control subjects in this study is also of interest with respect to whether or not rats rapidly develop olfactory learning sets. Although by no means the aim of the experiment (and therefore no formal comparisons are made) it appears from inspection of the figures (Staubli et al, 1989, p.57) that the performance of the control subjects was equivalent at both short (2 minute) and long (10 minute) inter-trial intervals. This, in addition to the fact that in both conditions the rats' performance on trials 2-5 (averaged across 4 problems) appears to be less than 70% correct argues against the notion the the rats have acquired a "learning set" in the course of learning the series of discriminations, given that: 1. sensitivity to inter-trial interval is commonly observed in learning set studies (e.g. Mackintosh, 1974, p.614); and 2. performance on the second trial of a novel discrimination is usually about 90% correct (see this chapter, p.41).

A surprising general feature of the studies discussed above is the fact that despite clearly discrepant findings, each research group cites the others' work with approval, maintaining the impression that their studies form an logically consistent story. For example, Staubli et al (1984, p.5887) mis-cite the study of Eichenbaum et al (1980) as having shown the effect of DMN lesions on 'learning set formation' in rats - in fact no progressive improvement was seen across discriminations in the Eichenbaum et al (1980) study and therefore there was no evidence that learning set formation had occurred (see fig 4 p. 264, Eichenbaum et al, 1980) - and thereby incorrectly infer that the study is consistent with that of Slotnick and Kaneko (1981).

CHAPTER 4

General Methods

In order to evaluate the olfactory model of amnesia outlined in the previous chapter, an olfactory maze was designed and built with the particular aim of achieving very rapid olfactory learning by rats.

The Olfactory Maze:

A number of different types of apparatus have been used in the study of olfactory discrimination learning in animals. None have been quite as elegant as that proposed by Heath-Robinson (fig. 4.1), but it is worth considering here some of the methods used.

Early experimenters (e.g. Allen, 1941) used relatively crude methods. In the report cited, the ability of blindfolded dogs to "go to a certain pan by smell and select and open a paper package containing meat from three paper packets of like size and texture" was used as a test of the olfactory sense in the face of pyriform-amygdaloid and hippocampal lesions. More recent work, conducted largely with rats, has employed more conventional equipment. Jennings and Keefer (1969) used a modified Grice box, consisting of a start box separated from 3 'choice' alleys (used two at a time) by a perforated guillotine door. The alleys were odourised by air drawn through absorbent material patches connected via plexiglass tubing to the end walls of the alleys by a fan system pulling air out through the start box. In this way simultaneous 2-odour discriminations were presented to thirsty rats for water reward

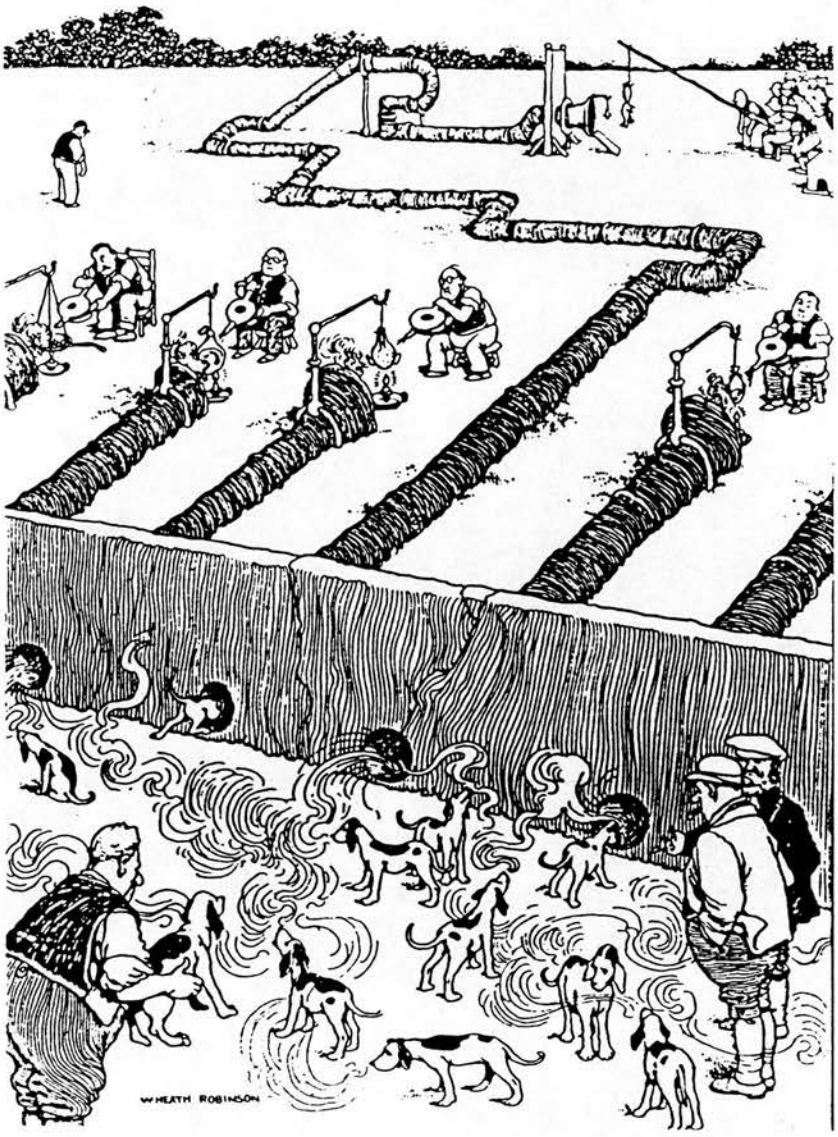


Fig 4.1

Early olfactory discrimination apparatus

by hand. Langworthy and Jennings (1972) developed a novel method of creating olfactory discriminanda in which ping-pong balls were saturated with food flavourings by keeping them sealed in jars of commercially available flavouring essences until required for use. They were then suspended in front of food wells such that they had to be displaced by hungry rats seeking food reward. Thompson (1980) used a system consisting of 4 compartments (a start box, choice area and 2 goal boxes). Rats were trained to displace plastic boxes containing cotton wool saturated with odourants to enter 1 of the 2 goal boxes in order to escape painful electric shock. Staubli, Ivy and Lynch (1984) used a modified 8 arm radial arm maze designed to present simultaneous 2-odour discriminations. Three of the arms were permanently blocked off, and 2 arms chosen at random from the 5 remaining arms were filled with odourised air streams for each discrimination trial. Rats were required to select and enter the odourised arm designated 'correct' in order to receive a water reward. Odours were delivered by directing pressurised air through odourised solutions and into the choice arms. Slotnick and Katz (1974) and Eichenbaum, Fagan and Cohen (1986) have used a different approach, employing sequential odour presentation in the form of a 'go, no-go' discrimination task. In this set-up, the water deprived rat had to respond to the 'positive' odour by making a sustained nose poke into an odourised nose port to obtain water reward. Responses to the 'negative' odour were unreinforced. Again, odour cues are created by bubbling pressurised air through or over odourous solutions, using olfactometer systems of varying sophistication. It can be seen then that methods used vary principally in terms of the response required by the rat, the manner of odour cue production, and the form of the discrimination task and its reinforcement.

The apparatus developed for the studies detailed here shares many features of the equipment described above. Water reward was chosen as reinforcement for 3 reasons. First, as most other studies use this method of reinforcement, the results obtained would be more widely comparable. Second, water was judged to be less odourous than a food reward, and therefore less likely to provide additional odour cues to reward. Third, it was possible to use the same kind of solenoid valve components to deliver the water reward as were used to deliver the odourised airstreams, thereby minimising both the costs and complexity of the system. Odour cues were produced by odourising airstreams controlled by solenoid valves as this was relatively easy to automate, and again, was a common strategy used by other investigators. A simultaneous 2-odour discrimination task requiring the rat to approach an odour source (as used by Staubli et al, 1984) was chosen for 3 reasons. First, this arrangement appeared to encourage the most rapid learning in rats (determined by comparing learning rates across the studies reviewed in chapter 3); second, simultaneous discrimination problems have generally been used in learning set studies; and third, simultaneous olfactory cue presentation (as opposed to successive cue presentation) appears to be most likely to be sensitive to hippocampal damage in rats (Staubli et al, 1984; Eichenbaum et al, 1986; Eichenbaum et al, 1988).

In outline, the basic task used here was a simultaneous 2-odour discrimination for water reward. The apparatus took the form of a three arm 'Y' maze, based on an earlier version of the equipment used by Staubli et al (1984), and conceived in prototype by Richard Morris. Odour cues were produced by controlled airflow through odourous solutions. The equipment was automated with both operation and data collection controlled by computer.

In more detail, the final version of the maze consisted of an enclosed, symmetrical three arm 'Y' maze constructed from acrylic plastic (see figs 4.2 and 4.3). Each arm (75 cm long), radiating from a central choice area, terminated in a goal box. Each goal box contained a water delivery spout and a photocell/lamp arrangement (RS components) so arranged that the arrival of a rat at the water spout located in centre of the end wall of the goal box could be detected automatically. The rat could then be confined in the chosen maze arm by means of sliding doors operated by electronic solenoid bolts (RS components). The doors were perforated to allow odours to pass through them. Air streams were odourised by directing compressed air through electrically controlled solenoid valves (RS components) to odour solutions contained in specially modified 500ml specimen jars. The odourised air was then fed into the goal boxes via 'Tygon' brand surgical grade plastic tubing. A centrally mounted fan (RS components) ensured a continuous passage of air from the maze arms to the central choice area, and from there the air was exhausted into a continuously ventilated room. Two odours could be used at any one time, and each odour could be directed independently to each of the arms. The operation of the maze was controlled automatically by a BBC 'B' microcomputer and 'Spider' interface system (Paul Fray Ltd.). Programs were written in BASIC.

This description of the apparatus refers to the maze in its final form. A number of important considerations encountered in the development of the maze are detailed in the following chapter.

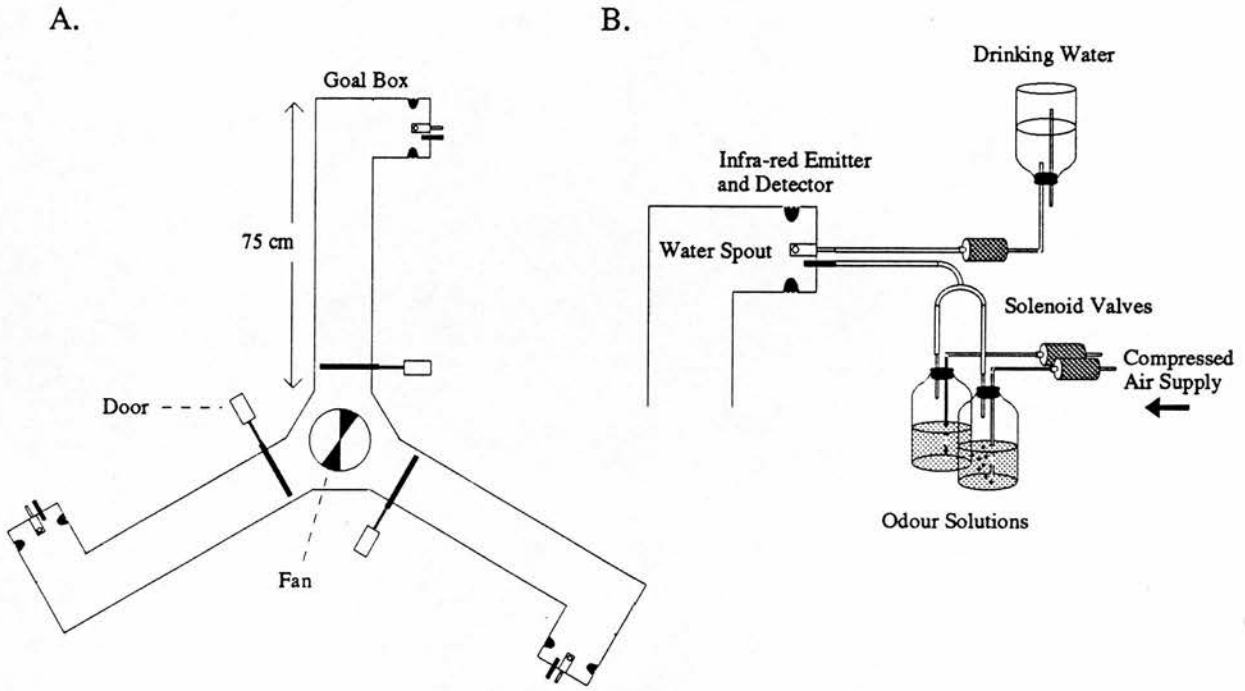
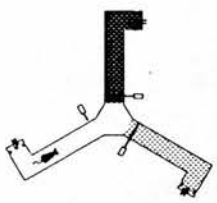
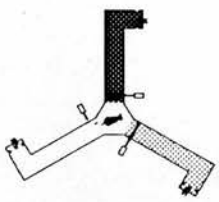


Fig. 4.2

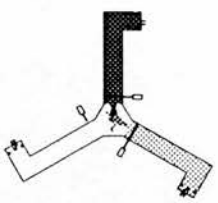
A) Schematic diagram of the olfactory maze, as seen from above. B) Details of odour delivery system, and goal box arrangement. This figure illustrates the final version of the maze - see text for details of development.



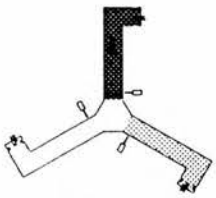
a. Trial commences.



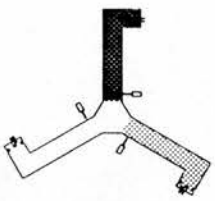
b. Rat enters central choice area.



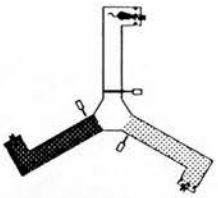
c. Rat samples odours flowing through perforated doors.



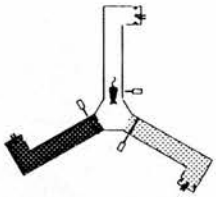
d. Doors open simultaneously, and rat enters chosen arm.



e. Rat enters goal box and is detected by photocell beam. Rat rewarded if 'correct'. Door to chosen arm closes.



f. Odour stream to choice arm is switched off, odour streams now directed to the other 2 arms.



g. After intertrial interval, sequence begins again with 2 new choice arms.

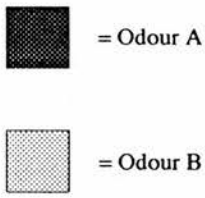


Fig 4.3

Illustration of events occuring in the maze in the course of 1 trial.

Maze Operation:

The maze was programmed to present simultaneous 2-odour discrimination problems in the following manner (see fig 4.3): on any trial the rat proceeded from the last chosen goal box to the central choice area where he could sample odourised air being drawn into the central area from each of the other two arms. To ensure sampling of the odour streams, the rat was delayed for 5 seconds in the central area before the doors allowed entry to one or other of the choice arms. On reaching the end of an arm, the rat's presence was detected by the photocell, the door behind him closed and, if a correct choice had been made, a water reward (0.15ml) was delivered immediately. Incorrect choices were unreinforced. No correction procedure was used. The 2 odour streams were switched off 5 seconds later, and then immediately redirected to the now unoccupied arms according to a pseudorandom schedule. These arms now became the choice arms for the next trial. To ensure extraction of preceding odours and optimal odourisation of the choice arms, a minimum intertrial interval of 60 seconds was scheduled.

The rats were generally run for a maximum of 31 trials each day or until a criterion score of 8 consecutively correct responses was achieved in which case the session was terminated and a new discrimination problem commenced the following day. If criterion was not achieved in a session, the subject continued on the same problem the following day. Errors made in reaching criterion were recorded for every problem. The computer recorded a rat's choices and response times and these data were saved to disc at the end of each session for analysis.

Between every rat's session the maze was thoroughly cleaned with alcohol.

Odour Materials:

Readily available household materials were used as odour stimuli. These included crushed herbs, flavouring essences and so on (see table 4.1 for details of all odour pairs used). The materials were dissolved or suspended in water. Odour solutions were allocated in pairs, and individual pair members were diluted until judged to be of equivalent intensity. Each pair constituted a 2 odour problem. Liquid odorants were added to cold tapwater to a volume of 50 ml, while solid odorants were crushed in a mortar and pestle prior to being dissolved or suspended in 50 ml of cold tap water. To minimise interproblem generalisation, the members of each pair of odours were selected to be of similar odour quality, as judged by human observers, such that 'fruity' odours were paired together (e.g. strawberry, lemon), 'herb-like' odours paired together (e.g. mint, cloves) and so on (see table 4.1). Odour materials were chosen on the basis of low cost and ready availability. No attempt was made to use pure odourants as this was deemed unnecessary given the type of experiments performed. Air streams were odourised by bubbling air through the solutions at a fixed rate controlled and monitored by needle valves and flow meters. The air streams were directed into the maze via surgical grade plastic tubing which was replaced for each novel odour.

Pretraining:

In each experiment, a pretraining procedure was used. Generally, water deprived rats were permitted to explore the maze in the absence of odours with water reward available at the end of each arm. Rats had to move from one arm to another in order to obtain further rewards - repeated consecutive visits to the same arm were unrewarded. Rats who failed to make more than five arm visits in any 30 minute

pretraining session are excluded from further training, but this was a very rare occurrence, and detailed in individual experiments where relevant.

A variety of different pretraining schedules were used initially, varying mainly in the amount of exposure to the apparatus received prior to the introduction of odour discrimination problems. The following schedule was finally adopted: rats were given 3 daily sessions of 30 minutes duration pretraining. On the first day, the rats were permitted to explore the apparatus freely, receiving a 0.15 ml water reward for each new arm choice and goal box entry. On the following 2 days, the doors became operational, directing the rats left and right on a pseudorandom schedule in an effort to extinguish position habits. A 30 second 'inter-trial' interval was used. On the third day this was increased to 60 seconds. Odour discrimination problems began the following day.

Water Deprivation:

Rats were permitted free access to water for 30 minutes each day. Deprivation was commenced 2 days before pretraining began, and was continued throughout the experiment, the animals receiving access to water on return to the home cage following the day's training session. Because of this, rats were housed individually. This schedule is similar to that used by both Eichenbaum et al (1986) and Slotnick and Katz (1974). Rats were weighed daily, and monitored for signs of distress or ill-health as a consequence of the deprivation schedule. Initially, water intake during the access period was measured, to ensure that rats were not becoming sated during the training periods. Maximum reward volume (which varies depending on the type of experiment being carried out) was 4.65 ml (31 = max correct trials x 0.15 ml = reward volume). In the free access period, rats drank between 12 and 18 ml, and

usually completed drinking within the first 10 minutes of the period. This indicated that variations in training performance (and therefore water received) were unlikely to result in significant variations in the state of deprivation, and that the maximum reward volume was considerably less than the total quantity drunk daily thereby maintaining motivation throughout any individual training session.

Animals:

Throughout the experimental programme, male Lister Hooded rats were used. They weighed between 200 - 250g at the start of each study. The animals were supplied by the local Home Office approved breeding unit in the Department of Pharmacology, University of Edinburgh. Animals were caged individually. They were housed in a temperature controlled room, on a normal, consistent day/night lighting schedule (14 hours on, 8am to 10 pm). Food was freely supplied, and during training, access to water was restricted as described above. Animals were inspected daily.

Table 4.1

Odour pairs used throughout the experimental work.

almond / chocolate

almond / vanilla

basil / oregano

butterscotch / raspberry

coffee / ginger

fennel / cumin

lemon / strawberry

mint / chives

nutmeg / coriander

onion / sage

orange / apple

ovaltine / coconut

parsley / dill

rum / rum

vanilla / vanilla

(Not all odour pairs were used in all experiments)

CHAPTER 5

Initial Studies

A series of discrimination experiments were performed in order to evaluate the apparatus, using both serial novel olfactory discrimination problem learning and serial reversal learning. The results of the early studies, though strictly pilot experiments, proved of considerable importance to the design of the experiments which make up the main body of this thesis. They are therefore described in detail.

Having established that the basic mechanics of the maze were operating satisfactorily and that the animals were able to perform reasonably well within it (that is to run down the arms, approach the reward area in the goal box, receive rewards, and apparently reach criterion levels in 2-odour discriminations), a series of 'transfer' experiments were conducted. The rationale for this series of experiments is described in full in chapter 6 (p.101-105), and is only briefly outlined here. As a first test of the validity of claims that rats could establish a 'win-stay, lose-shift' strategy in the course of solving a series of novel olfactory discriminations, three groups of rats were trained in a 'learning set' acquisition phase on three different types of olfactory problem series: one group, ('Novel') was trained on a series of novel olfactory discrimination problems; the second group was trained on serial reversals of a single discrimination problem ('Reversal'); and a third group was trained continuously on a single discrimination ('Single') for as many trials as animals in Group Novel took to complete their series of problems.

Each group of animals was then transferred to a further 4 novel problems (now the same for each group) with a reversal of the fourth of these problems. In this way, the effect of different training experiences on the learning of novel olfactory problems, and a reversal problem could be compared. The acquisition phase training experiences of Group Novel and Group Reversal were intended to encourage respectively 'learning set' and 'reversal set' formation and the development of a 'win-stay, lose-shift strategy'; while the the acquisition phase training of Group Single was intended to act as a control condition to reveal the non-specific effects of exposure to simple discrimination learning in the apparatus. It was predicted that animals acquiring a 'win-stay, lose-shift' strategy should learn both novel and reversal problems more rapidly than animals who had not had the opportunity to acquire this strategy.

Finally, to ensure that only the intended odour cues were guiding performance, all rats were tested on a 'control discrimination', in which 2 identical odours were delivered by the apparatus which was otherwise set up as if for a regular 2-odour discrimination, the expectation being that the rats' performance should fall to chance.

Pilot Experiment 1:

9 water deprived male Hooded Lister rats were used, 3 per group.

Procedure:

Pre-training as detailed in chapt. 4

	Acquisition Phase	Transfer Phase
group 1 (Novel)	8 novel problems	(all groups)
group 2 (Reversal)	novel problem + 8 serial reversals	4 novel problems, followed by a reversal of the fourth problem, and ending with the control task
group 3 (Single)	continuous single discrimination (matched for trials with animals in Group Novel)	

Results: (See fig 5.1)

Acquisition Phase

Problem 1, All groups: All rats successfully completed the three days of pre-training. On the first problem, the rats quickly learned to sample both odour streams in the choice area, and were observed to wait by the door of the chosen arm until it opened. They would then run rapidly down the arm and into the goal box. Choice latency was generally uniform. The rats averaged 11 seconds between being allowed to enter the choice area and reaching the chosen goal box, including the compulsory 5 second delay period during which the doors to the choice arms remained closed. Infrequently, individual rats would remain in a goal box for long periods even after the door to the choice area had opened. Rarely, a rat would reverse out of a chosen arm and return to the choice area before reaching the goal box. If a correct choice had been made, the rats drank the entire water reward at once. At this early stage, the rats were sometimes observed to 'flinch' or otherwise become distracted by the mechanical sounds produced by the apparatus (such as door closure, switching of odour streams and so on); and occasionally became agitated during the inter-trial interval when trapped in the chosen arm, especially if a reward had not been obtained. In the course of learning the problem, some rats (but by no means all) would follow simple position habits for a while, producing for example, a series of left turns or a series of left/right alternations. In general, these potentially disruptive behaviours reduced in frequency with continued training.

All rats reached criterion on their first problem (mean errors to criterion scores: Group Novel = 35; Group Reversal = 30; Group Single = 44).

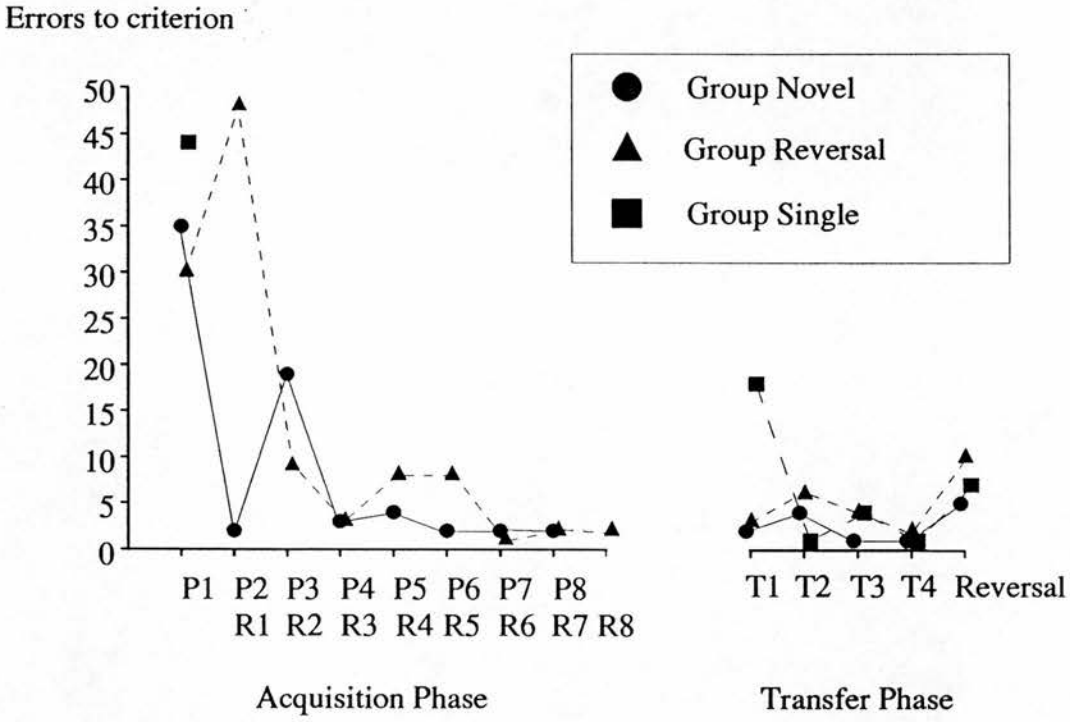


Fig 5.1

Graph showing mean errors to criterion across odour problems for the 3 groups (Novel, n=3; Reversal, n=3; Single, n=3) in experiment 5.1. P=problem number R=reversal problem number, T= transfer problem.

Group Single: Having reached criterion, the rats in this group were trained continuously on the same problem for as many trials as the animals received in Group Novel. The rats continued to choose correctly throughout this phase, performing with greater than 95% accuracy.

Group Novel: This group solved the second novel problem much more rapidly than the first, making a mean of only 2 errors in reaching criterion. The third problem was solved more slowly (mean errors to criterion = 19), but the remaining 5 problems were rapidly solved (mean error scores < 5)

Group Reversal: On the first reversal, animals in this group initially responded to the previously correct, but now incorrect, odour. They eventually reached criterion after making more errors than on the first problem (mean errors = 48). The remaining 7 serial reversals were solved more rapidly, the final reversal being solved with a mean of 2 errors to criterion.

The rats in groups Novel and Reversal therefore appeared to show evidence of progressive improvement across problems, making an average of only 2 errors in each of these groups in reaching criterion on the eighth novel discrimination and on the eighth reversal problem respectively, while having made mean errors scores of 35 (group Novel) and 30 (group Reversal) on the initial discrimination problem.

Transfer Phase:

Groups Novel and Reversal continued to perform at low error rates following transfer to a further series of novel problems (mean of 2 errors and 3 errors respectively on the first transfer problem), while Group Single performed less well initially, making a mean of 18 errors on the first novel transfer problem, but reaching comparable levels of performance to those rats in the other groups for the subsequent 3 problems. All three groups performed the final reversal with relatively few errors with respect to initial discrimination scores, reaching criterion with mean error scores of 5 (Novel), 10 (Reversal) and 7 (Single).

Control Task: The first rat to reach the control task phase of the experiment reached criterion almost immediately (making 1 error) rather than peforming at a chance level as had been expected. This finding, suggesting that factors other than those under experimental control were guiding the rats' performance, was to prove central to the subsequent development of the project.

Discussion:

The progressive improvement by Groups Novel and Reversal to levels of performance where little more than 2 or 3 errors occurred per problem in the acquisition phase was consistent with previous reports in the literature (Slotnick and Katz, 1974; Slotnick and Kaneko, 1981; Eichenbaum et al, 1986). Furthermore, the predicted effects of the different kinds of training given in the acquisition phase on transfer phase performance were largely borne out: Rats in groups Novel and Reversal performed better on the first transfer problem than rats in group Single.

However, the first control task finding strongly suggested that cues other than those intended were guiding the rats' high levels of performance.

The original aims of the experiment were consequently abandoned, and the remaining experienced rats were used in experiment 2 to try and determine the nature and location of the spurious cue(s), given that it was likely that the remaining rats had also detected it.

Pilot Experiment 2

The purpose of this experiment was to try to identify and eliminate any cues to reward additional to those intended. Possible sources considered included olfactory cues produced by contamination of apparatus by odour substances; and/or auditory cues produced by the movement of the mechanical components of the maze, or by the airflow through the valve system.

Procedure:

A series of manipulations of the apparatus (see fig 5.2) were performed with each of the remaining animals on their reaching the control task phase of training.

Tests Used:

1. The control task as outlined above with no modification
2. Disconnection of the air supply to the valves
3. Disconnection of the air supply to the odour bottles
4. Direct connection of clean air to the valves
5. Direct connection of clean air to the valves, with replacement of the tubing between the valves and the goal box
6. Replacement of the 2 odour flasks with one flask feeding both valves at each goal box
7. Reversal of valve/reward assignment
8. Replacement of odour solution with distilled water

The rationale behind these tests was to try to find a manipulation that would

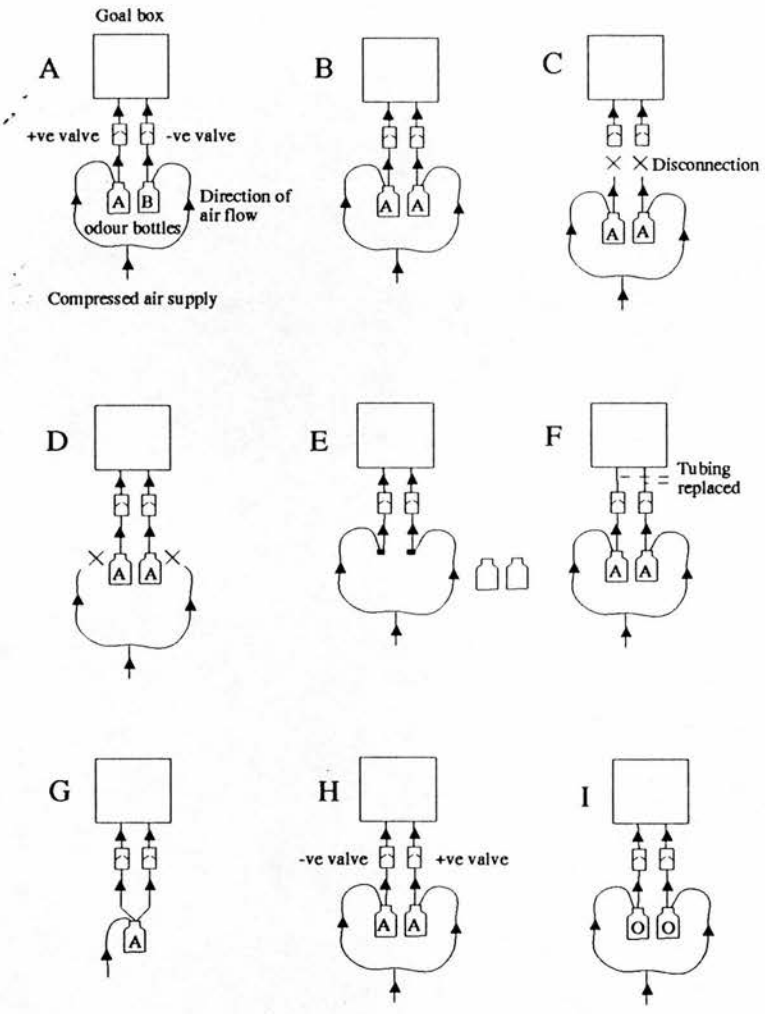


Fig 5.2

Schematic illustration of the tests used to determine the source and nature of unintended cues to reward in the odour delivery apparatus. Each element represents the arrangement at a single arm.

A: Original arrangement of apparatus (A= odour 1, B= odour2)
B: As A above, but with identical odours in each flask
C: Disconnection of air supply from valve system
D: Disconnection of air supply from odour bottles
E: Direct connection of clean air to valves, bypassing odour bottles
F: Replacement of tubing between valves and goal box
G: Replacement of both bottles with one bottle feeding both valves
H: Reversal of reward valency/valve assignment
I: Replacement of odourant with distilled water

eliminate the spurious cues (such that the animal performed at chance) in an effort to identify the source and nature of the cues. All rats were tested on the standard control task (condition **a.** above) and then given 10 to 20 trials on a variety of the other tests.

Results:

On the standard control task, 2 out of the 3 rats from each of the serial novel discrimination group (Group Novel) and serial reversal group (Group Reversal) reached criterion rapidly (mean % 'correct' responses = 82% over 20 trials; chance = 50%). None of the rats in group Single reached criterion (mean % 'correct' responses = 56% over 20 trials). Of all the further manipulations attempted (tests b. to h. above), only disconnection of the air supply from either the valves or the flasks resulted in disruption of the animals ability to reach criterion (mean % 'correct' responses =58% and 60% respectively). Valve reversal (test g.) depressed scores *below* chance (mean % 'correct' responses = 20%).

Discussion:

In view of the pattern of results, it was concluded that 1. the unintended cue was in some way related to the valves controlling airflow, and 2. the cues required active airflow to operate. It was hypothesised that either the cue was auditory in nature (due to airflow whistling differently through each of the valves) but outside the range of human hearing, or that the valves had become contaminated (acquiring characteristic odours), or both. Given that at this stage in the development of the apparatus that three of the valves consistently carried the positive odour and three others the negative odour, the 2 sets of valves themselves might have become identifiable.

Pilot Experiment 3

In an effort to eliminate consistent valve/reward associations, the programming of the apparatus was modified to randomise valve/reward assignment from session to session - i.e. for any 31 trial session either of the two valves at each arm could carry the 'positive' odourised air stream. This was varied on the basis of a modified Gellerman schedule from 31 trial session to 31 trial session, but not trial to trial. In addition, a white noise generator was used.

Water deprived male hooded lister rats were used, 3 per group as in pilot experiment 1. The procedure was identical to that employed in pilot experiment 1 with the exception of the modifications outlined above, and that only three transfer problems were used, with a final reversal of the third.

Procedure:

Expt. 3

Pre-training as detailed in chapt. 4.

	Acquisition Phase	Transfer Phase
group 1 (Novel)	8 novel problems	(all groups)
group 2 (Reversal)	novel problem + 8 serial reversals	3 novel problems, followed by a reversal of the fourth problem, and ending with the control task
group 3 (Single)	continuous single discrimination (matched for trials with animals in group Novel)	

Results:

(See fig 5.3)

Acquisition Phase: Rats in Group Novel made a mean of 39 errors to criterion on the first discrimination problem, and showed general inter-problem improvement, making a mean of only 3 errors in reaching criterion on the eighth problem. Group Single reached criterion with a mean of 42 errors, thereafter performing consistently with a post-criterion score of >95% correct. Group Reversal animals made a mean of 41 errors in reaching criterion on the first problem. One of the rats in the group failed to reach criterion on the first reversal problem, despite completing 450 trials before being discontinued on reversal problems. The two remaining rats made a mean of 118 errors and 101 errors on the two subsequent reversals respectively before being discontinued on reversal training because of their poor performance, and all three were transferred to only 1 of the novel problems in the transfer phase.

Transfer Phase: Group Novel performed the 3 novel problems with few errors (mean scores of 7, 9 and 5 errors), and took a mean of 58 errors to complete the final reversal problem. Group Single made mean scores of 5, 29 and 14 errors on the novel transfer problems, and 71 errors on the final reversal. On their single transfer problem, group Reversal made a mean of 10 errors. These rats received no further training.

Control Test: Only rats from Groups Novel and Single performed this test, given that rats in group Reversal were felt to be unlikely to have been guided by extraneous cues in view of their poor performance. None of the 9 rats tested reached criterion on the control task over 30 trials, and all were at chance (50%) levels of

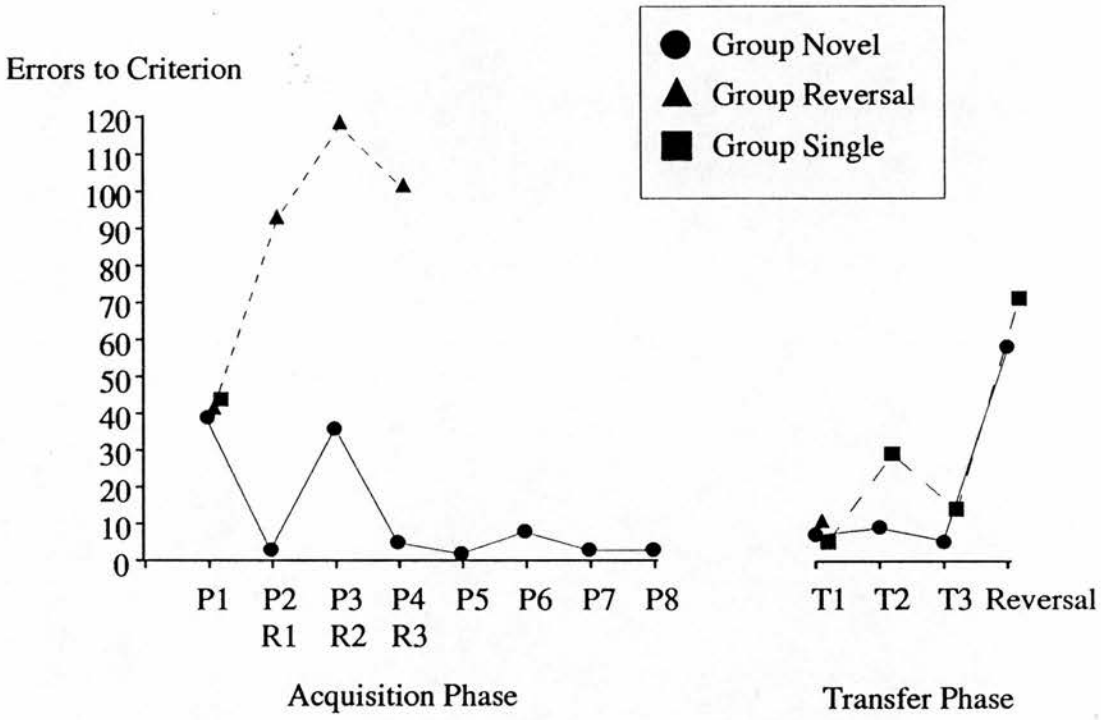


Fig 5.3

Experiment 3

Graph showing mean errors to criterion across odour problems for the 3 groups (Novel, n=3; Reversal, n=3; and Single, n=3) in experiment 5.3. P = problem number R = reversal problem number, T = transfer problem number.

performance.

Discussion:

Rats in group Novel showed evidence of progressive improvement across problems, despite the changes made to the apparatus. Rats in group Single, however, transferred almost equally well to novel problems, with scores in a comparable range to those recorded for group Novel, despite *not* having had the opportunity to develop a true 'learning set'. Interestingly, Group Reversal failed to show progressive improvement across reversals and performed much less well on individual problems than in the earlier pilot experiment, presumably as a consequence of the modifications made to the operation of the apparatus. Strikingly, however, they performed comparably with the other groups on their single transfer problem, despite having clearly failed to develop a 'reversal set'. The fact that animals in groups Novel and Single failed to perform above (or below) chance levels on the control task suggested that the high levels of performance seen were only mediated by the intended odour differences and not by extraneous (and uncontrolled) cues.

There are therefore 2 reasons for doubting that progressive improvement observed across a series of novel problems represents the development of a learning set, and that rapid olfactory learning is mediated by a 'win-stay, lose-shift' strategy. First, exposure to a series of novel problems did not appear to be necessary in the development of rapid learning, as shown by the transfer phase performance of Group Single. Second, animals who had shown no indication of progressive improvement, and were therefore unlikely to have acquired any strategy, appeared to learn novel problems as rapidly as those who *had* shown progressive improvement, as indicated by the performance of Group Reversal and Group Novel respectively.

In view of this unexpected finding, the experiment was repeated with more subjects, and with complete training to criterion for the animals in Group Reversal.

Pilot Experiment: 4

The purpose of this experiment was to try to replicate the findings of experiment 3 with more subjects. In addition a more stringent design was used, with counterbalancing of the order and reward valency of the individual odour problems in order to confound the possibility that inter-problem transfer might be mediated by odour similarities across odour pairs. The number of reversals in group Reversal was reduced by 1 (i.e 1 novel discrimination followed by 7 serial reversals of that discrimination). The original aim was to run 24 rats in three groups of 8 (Novel, Single, and Reversal) in 2 replicates each with 12 animals (4 per group).

Procedure:

Experiment 4

Pre-training as detailed in chapt. 4

Semi-counterbalanced odour series (see tables 5.1-2)

	Acquisition Phase	Transfer Phase
group 1 (Novel)	8 novel problems	(all groups)
group 2 (Reversal)	novel problem + 7 serial reversals	3 novel problems
group 3 (Single)	continuous single discrimination (matched for trials with animals in group Novel)	

Table 5.1

Odour pairs used in experiment 5.4

(Acquisition phase)

Odours	Code
butterscotch + raspberry -	= 1
nutmeg + coriander -	= 2
onion + sage -	= 3
almond + chocolate -	= 4
fennel + cumin -	= 5
basil + oregano -	= 6
orange + apple -	= 7
parsley + dill -	= 8

(Transfer phase)

mint + chives -	= 9a	chives + mint -	= 9b
lemon + strawberry -	= 10a	strawberry + lemon -	= 10b
coffee + ginger -	= 11a	ginger + coffee -	= 11b
ovaltine + coconut -	= 12a	coconut + ovaltine -	= 12b

(Control Pairs)

rum + rum -	= 13
vanilla + vanilla -	= 14

Table 5.2

Single discrimination group (acquistion phase)

Replicate 1

Rat	Odour pair	(continuous training beyond criterion)
A1	1	
B1	2	
C1	3	
D1	4	

Replicate 2

E1	5
F1	6
G1	7
H1	8

Novel discrimination Group (acquisition phase)

Replicate 1

	Problem						
Rat	P1	P2	P3	P4	P5	P6	P7
A2	1	2	3	4	5	6	7	8
B2	2	3	4	5	6	7	8	1
C2	3	4	5	6	7	8	1	2
D2	4	5	6	7	8	1	2	3

Replicate 2

E2	5	6	7	8	1	2	3	4
F2	6	7	8	1	2	3	4	5
G2	7	8	1	2	3	4	5	6
H2	8	1	2	3	4	5	6	7

Table 5.2 (continued)

Reversal discrimination group (acquisition phase)

Replicate 1

Rat	odour pair (with subsequent reversal of reward valency)
A3	1
B3	2
C3	3
D3	4

Replicate 2

E3	5
F3	6
G3	7
H3	8

Transfer Phase (all rats)

Replicate 1

	Problem				
Rat	T1	T2	T3	control	T4 (not used)
A1,2,3	9a	10a	11a	13	12a
B1,2,3	9b	10b	11b	13	12b
C1,2,3	10a	12a	9a	13	11a
D1,2,3	10b	12b	9b	13	11b

Replicate 2

E1,2,3	11a	9a	12a	14	10a
F1,2,3	11b	9b	12b	14	10b
G1,2,3	12a	11a	10a	14	9a
H1,2,3	12b	11b	10b	14	9b

Replicate 1.

Results: (See fig 5.4)

Acquisition Phase: Animals in Group Novel made a mean of 20 errors in reaching criterion in the first discrimination problem, and improved across the subsequent 7 problems, making a mean of 5.5 errors on the eighth problem. Group Single made a mean of 22 errors in reaching criterion, and thereafter performed with >95% accuracy during continuous training on this problem. Group Reversal committed a mean of 35 errors in reaching criterion on the initial discrimination, and failed to improve on this score across the series of 7 reversals, making a mean of 47 errors on the final reversal problem prior to transfer. Absolute performance scores on the first problem of the series were better in all groups than that observed on the first problem in pilot experiment 3.

Transfer Phase: Each group of animals transferred equally well to the first transfer problem (Group Novel: mean of 21 errors to criterion; Group Single: mean of 25 errors; Group Reversal: mean of 22 errors to criterion); though the absolute error scores were greater than those observed in experiment 5.3., and Groups Single and Novel made more errors on this problem than on the first acquisition phase problem. The remaining 2 transfer problems were solved more rapidly by each of the groups (mean scores - Novel: 12, 6.7 errors; Single: 6.7, 16 errors; Reversal 15, 6.5 errors).

Control Test: Unfortunately, and to my considerable disappointment, a proportion of the rats in each group reached criterion on the control test. (2 subjects in group Novel, 1 subject in group Reversal, and 2 subjects in group Single.

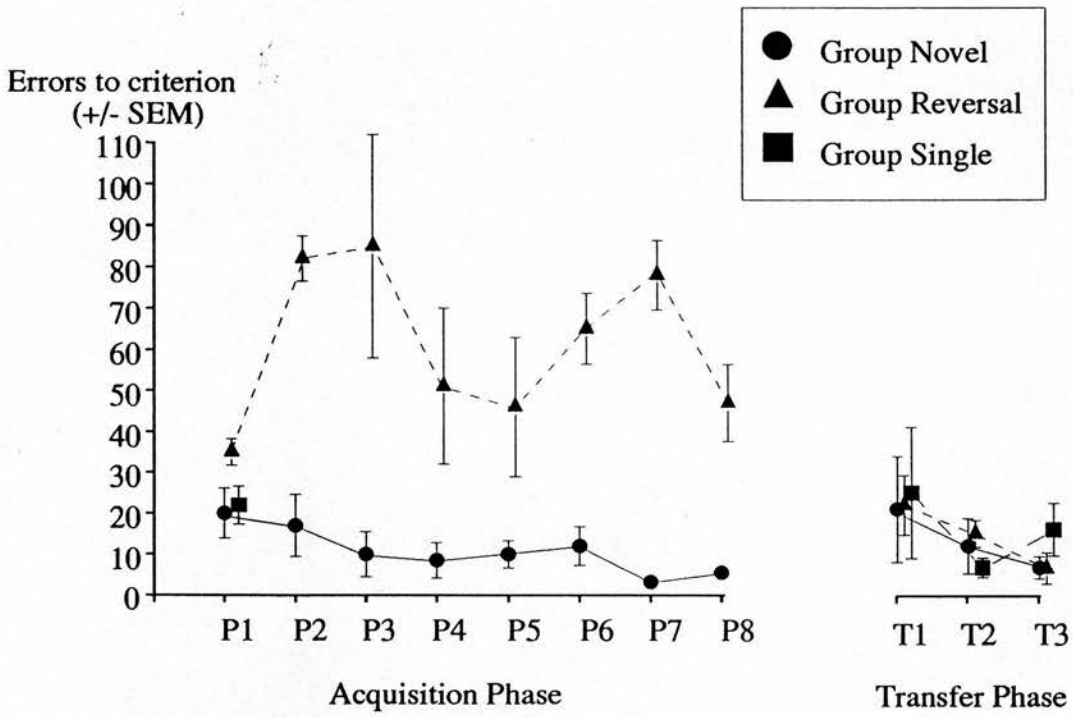


Fig 5.4

Graph showing mean errors to criterion across odour problems for the 3 groups (Novel, n=3; Reversal, n=3; and single, n=3) +/- 1 SEM in experiment 5.4.

Discussion:

The pattern of results was broadly in supportive of the conclusions drawn in pilot experiment 5.3, in that despite different training procedures in the acquisition phase, equivalent transfer performance was observed in each group. There were, however, some notable differences. First, performance on the first acquisition problem was better than previously observed; second, performance on the first transfer problem was poorer than previously observed; third (and most importantly) a significant proportion of animals were able to reach criterion on the control task.

It was clear that efforts to ensure that performance was guided solely by intended odour differences had failed, and as consequence, no firm conclusions could be drawn. In light of this, replicate 2 of the experiment was abandoned, and the equipment re-examined.

The odour delivery system was taken apart, and the solenoid valves taken apart and examined. On inspection, it was clear that the valves were heavily soiled and discoloured internally by odour material, one valve actually containing solid pieces of odourant. Efforts made to thoroughly clean the valves proved unreliable. In consequence, all the valves were discarded, and the odour delivery system completely redesigned and rebuilt such that odourised air-streams no longer passed through the valve system, which was entirely replaced with new equipment (see fig 5.5).

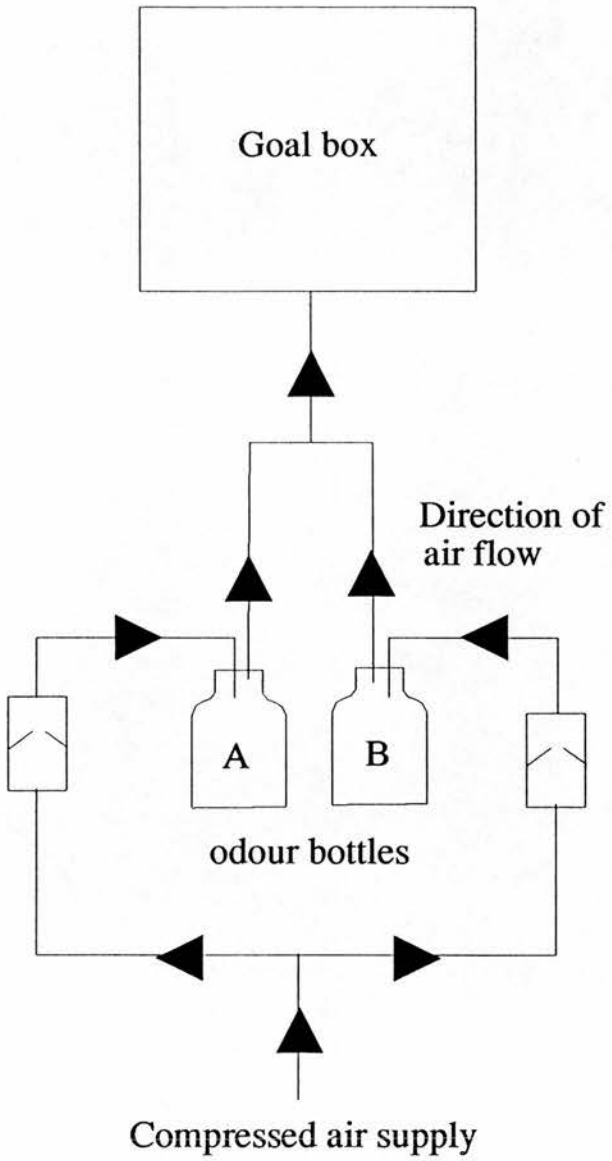


Fig 5.5

Schematic diagram illustrating the new odour delivery system arrangement. Either valve may now direct the 'positive' odourised airstream. Note that the odour no longer passes through the valve. All tubing is replaced for individual odours. Compare with fig 5.2A.

Pilot Experiment: 5.5

The aim of this experiment was to test the apparatus in its new form. Given the considerable time taken to run the full transfer experiment, combined with the uncertainty as to whether the modifications would work, it was decided that a shorter discrimination experiment should be carried out prior to running the main study.

Procedure:

Following pre-training, 4 rats were trained on 4 novel discriminations to criterion in the usual manner, and then tested on the 'identical odours' control task continuously for 50 trials. A semi-counterbalanced odour series was used.

Results: (See fig 5.6)

All subjects showed a progressive decline in the number of errors to criterion across problems as observed in earlier pilot experiments. No rat reached criterion on the control task. Mean responses to the rewarded and unrewarded 'identical' stimuli did not differ significantly from one another (paired t-test, $p > 0.3$, two tailed), nor from chance.

Discussion:

In view of the control task result, it was concluded that efforts to bring cues to reward under experimental control had been successful.

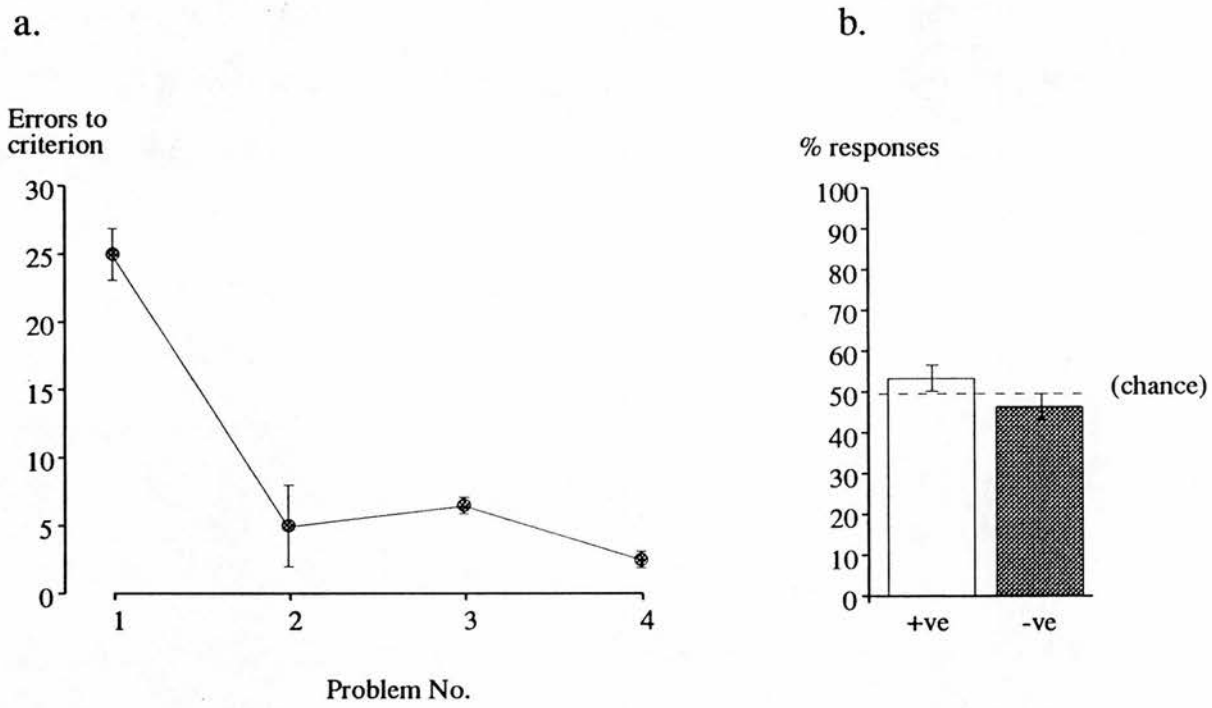


Fig 5.6

a) Graph showing mean errors to criterion (+/- 1 SEM) for 4 animals trained on 4 problems.

b) Mean % responses (+/- 1 SEM) to rewarded (+ve) and unrewarded (-ve, filled column) 'identical' stimuli after 50 trials.

General Discussion:

The main finding from this series of pilot experiments is the fact that it proved extremely difficult to ensure that performance in 2-odour discrimination problems was guided *exclusively* by *intended* odour differences. It was generally concluded that contamination of the valve systems responsible for automatically directing specific odours to specific arms of the maze was in some way able to provide consistent reward cues to the rats. Randomisation of odour assignment to individual valves was, in fact, partially successful in that in pilot experiment 5.3 no animal reached criterion on the control task, and in experiment 5.4 only 41% of the animals did so. Odour assignment could only be varied on a session to session rather than a trial to trial basis without including more valves in the system, and under such circumstances the rats apparently still had the opportunity to detect consistent uncontrolled extraneous cues to reward. The precise nature of this cue to reward remained undetermined, although the pattern of results obtained in pilot experiment 5.2 pointed clearly to the valve system. It seemed sensible to take the attitude that the apparatus should always be considered potentially flawed unless there was an empirical demonstration to the contrary, and it was decided that in every experiment subsequently performed, an 'identical odours' control task should be used. This was underlined by that fact when the results of the control task procedure in experiment 5.1 were available, a number of the lab staff were invited to try and reproduce the rats' performance using their own 'sensory apparatus' on an identical odours test, indicating their choices of computer designated 'correct' goal boxes by triggering the photocells manually. (It had already been established that human volunteers could perform 'true' 2-odour discriminations to criterion using the maze by sniffing the inside of the goal boxes during an earlier stage of the development of the apparatus.) None of the human volunteers scored above chance on the identical odour task,

demonstrating that the cue to reward detected by the rats was not immediately obvious to human subjects.

Nevertheless, the original efforts to remove the unintended cues by randomising valve assignment, while only partially successful, did radically alter the performance of rats in the various Reversal Groups. Progressive improvement in reversal learning was no longer seen over the number of reversal problems given. This finding (while at this stage admittedly unreliable) was at odds with the results of Slotnick and Kaneko (1981), who had previously found that even the first reversal of an olfactory discrimination can sometimes be attained more rapidly than the initial discrimination. This unusual finding (which has not been reported in other rat discrimination studies) might be explained by the presence of extraneous cues to reward. On this view, when apparently learning A+, B- rats are actually learning AC+, BD- such that a reversal becomes BC+, AD-. If C and D are at least as prominent as A and B, a "reversal" is actually a continuation of the original problem.

The use of control tasks in olfactory learning studies is rarely reported. Exceptions include the work of Slotnick and Katz (1974) who had earlier reported the use of a similar control task to the identical odours task used here (as a 'go, no-go' task rather than a simultaneous discrimination) in which the rats' performance was observed to fall to chance over many trials. However, this task was not reported in the study of olfactory reversal learning (Slotnick and Kaneko, 1981) and was used only on a small proportion of the subjects in the 1974 experiment. As I had already found, the control task had to be used in every experiment and on all animals, as uncontrolled cues to reward could develop insidiously over time (See pilot experiments 5.3 and 5.4). Eichenbaum, Shedlack and Eckmann (1980) also reported the use of a control task, but the task was run *prior* to training in the apparatus.

As Pilot experiment 5.5 and the following and subsequent chapters demonstrate, the rearrangement of the odour delivery system eliminated the unintended cues such that the performance of all well trained subjects fell to chance on the identical odours control task. Ensuring that odours did not pass through the solenoid valves was a crucial element in the design of apparatus of this kind. As far as can be determined from diagrams of apparatus used in the existing olfactory literature, no other odour discrimination equipment has employed this particular design feature.

A more general implication of the findings outlined here is that perhaps any study alleging "spectacular" (Eichenbaum et al, 1986) learning performance should include some logical control manipulation which ensures that subjects fall to chance.

CHAPTER 6

Serial Olfactory Discrimination Learning in Rats- Progressive Improvement or Learning Set Formation?

Introduction:

It has long been believed that rats have an acute, highly developed sense of smell. They seek food, find mates and determine territory on the basis of olfactory cues (Barnett, 1963). This has led some investigators (as outlined in chapter 3, p.40-44) to consider the possibility that rats may be able to perform qualitatively more complex tasks when using olfactory cues than when tested on tasks with visual, auditory or somatosensory cues. Specifically, it has been suggested that rats may be able to achieve levels of performance previously considered to be largely (though not exclusively) attainable by primates in the formation of 'learning sets.' Eichenbaum, Fagan and Cohen (1986, p.1876) have, for example, recently suggested that rats develop a "complete learning set" when presented with a short series of novel olfactory discrimination problems, while Slotnick and his colleagues have made similar claims for evidence of 'higher order' processes in the course of both serial novel olfactory discrimination learning (Slotnick and Katz, 1974) and serial reversal learning (Slotnick and Kaneko, 1981). The first report of apparent learning set formation by rats presented with serial olfactory discrimination tasks was made by Jennings and Keefer (1969), while Staubli, Fraser, Faraday and Lynch (1987a) have more recently made similar claims.

All have observed that, when presented with a series of novel discrimination problems, rats show progressive improvement from problem to problem. The finding has been interpreted to represent the acquisition of a "win-stay, lose-shift" strategy (Slotnick and Katz, 1974), a form of higher-order learning (Slotnick and Kaneko, 1981) which requires the use of a "complex abstract rule" (Otto and Eichenbaum, 1991).

These interpretations are based on Restle's (1958) formulation that *primates* can acquire an abstract or 'higher order' understanding of the general class of problems to which they have been exposed, which Levine (1959) has ascribed to the development a "win-stay, lose-shift" strategy. The adoption of this strategy is taken to imply that, in the course of learning a series of discrimination problems, subjects learn to remember the outcome of the preceding trial as being either rewarded ('win') or unrewarded ('lose'), and on the next trial choose the same cue if previously rewarded ('win-stay') or select the alternative cue if unrewarded ('lose-shift'). Having acquired this strategy, novel problems can be rapidly solved such that very high levels of performance are observed as early as the second trial.

It is important to make clear the difference between learning based on conventional principles of instrumental learning and that based on the adoption of a higher-order strategy such as 'win-stay, lose-shift.' According to the former, choice behaviour in a discrimination task is based on the relative associative strengths of the 2 available cues (S+ and S-). Associative strengths accumulate gradually over a series of trials as a result of pairings between the response to each stimulus and the outcome of a given trial. Thus, at any moment in time, choice performance is based on the cumulative consequences of numerous trials and does not depend on memory for the outcome of the immediately preceding trial, or, indeed, on explicit memory of the

particular sequence of preceding trials. Whereas, according to the latter, the animal gradually develops a strategy in which explicit memory of the preceding trial is the sole or major determinant of performance on a given trial. It must remember 2 items of information: what *stimulus* was presented (S1 or S2), and what outcome prevailed. These 2 items are conjoined with the higher-order rule 'win-stay, lose-shift' to determine whether, on the present trial, the animal should stay with S1 or shift to S2 (or vice versa). Notice that according to this strategy, S1 and S2 are not said to accumulate "associative strength" even though they are differentially reinforced.

Development of the abstract, 'win-stay, lose-shift' strategy will, of course, result in progressive improvement in the course of learning a series of novel discrimination problems. However, as noted in chapter 3 (p.43), there are a number of potential sources of progressive improvement in discrimination problem performance which need not be mediated by higher order or abstract processes. Other sources of progressive improvement include the gradual abandoning of disruptive response tendencies, (e.g. position habits), and reduction in anxiety consequent on increasing familiarity with the testing apparatus. The observation of progressive improvement is therefore, by itself, insufficient to indicate that an abstract strategy has been acquired.

There are therefore 2 possibilities. One is that the progressive improvement in rate of learning can be explained in terms of existing principles of instrumental learning. The second is that use of the olfactory modality in rats opens the way to very rapid assumption of a higher order solution based on memory of the preceding trial. An experimental design was therefore developed to distinguish these 2 hypotheses. The key feature of this design was to arrange conditions across a series of groups so as to favour or limit the likely development of a "win-stay, lose-shift" strategy. Following such training, the groups were then transferred to a novel problem to examine whether there was any difference in performance on the early trials and the ultimate rate at which it was learned.

The design permitted consideration of whether the following characteristics of learning set acquisition occurred:

1. Progressive improvement across problems.

2. Transfer to novel problem learning from serial reversal learning (after Schusterman, 1962; see this thesis, p. 41).

3. The gradual increase in the percentage of correct responses made on trials 2-5 of novel problems.

In addition, efforts were made to control for non-specific sources of progressive improvement by training a group of animals continuously beyond criterion on a single discrimination problem for as many trials as those required by the animals training on a series of novel discriminations. It was assumed that disruptive response tendencies would be extinguished equally in both conditions, and therefore the

contribution of this phenomenon to progressive improvement could be quantified by comparing the performance of the 2 groups on further novel discrimination problem learning (after Kamil et al, 1977; see this thesis, p.44).

In the light of the results obtained in pilot studies (see chapter 5), a control task was used to ensure that the cues guiding performance were under experimental control.

Methods:

Subjects:

Male hooded Lister rats were used (N=40), weighing between 200 and 250g at the start of each experiment. Animals were water deprived, receiving 30 minutes free access to water following training each day. Deprivation began 3 days prior to the start of the experiment.

Apparatus: (see fig 4.2, chapt. 4)

2 olfactory maze systems were used, each as described in chapter 4.

Errors made in reaching criterion were recorded for every problem, and the mean percentage correct responses made during the early trials (2-5) of novel problems were noted for each group. The computer recorded a rat's choices and response times and these data were saved to disc at the end of each session for analysis.

Odour Materials:

Odour materials were prepared as described as in chapter 4, p.73. Actual odour pairs used are detailed in table 6.1.

Table 6.1

Odour pairs used.

Group Novel (n=8): (acquisition phase)

2 series of 6 novel problems, running in reverse order with respect to one another. 4 rats were allocated to series 1 and 4 to series 2.

	series 1	series 2
P1	onion^{+}/sage^{-}	mint^{+}/cloves^{-}
P2	lemon^{+}/strawberry^{-}	fennel^{+}/cumin^{-}
P3	coffee^{+}/ginger^{-}	ovaltine^{+}/coconut^{-}
P4	ovaltine^{+}/coconut^{-}	coffee^{+}/ginger^{-}
P5	fennel^{+}/cumin^{-}	lemon^{+}/strawberry^{-}
P6	mint^{+}/cloves^{-}	onion^{+}/sage^{-}

Group Reversal (n=6): (acquisition phase)

A series of serial reversal discriminations, each rat in the group (n=6) using one each of the six odour pairs listed above.

Group Single (n=16): (acquisition phase)

The 16 rats were divided into 2 sub-groups of 8 such that 1 rat in each subgroup was matched to 1 of the 8 rats in group 'Novel', with the reward assignment in the transfer problem (see below) for 1 rat of each pair being the opposite of that for the other. The full set of P1 to P6 odour pairs was used, with P1 and P2 used twice.

Group Control:

This group (n=6) performed only the transfer odour pair (see below).

Transfer Problem:

This odour discrimination, used in each of the 2 possible reward orientations (almond^{+}/vanilla^{-} or vanilla^{+}/almond^{-}) was performed by all rats. Half of the rats in each group performed one orientation, the remaining animals the other orientation.

Control Test:

The odour 'pair' used here was rum^{+}/rum^{-}.

Novel problem and reversal:

The odour pair used was the same for all rats - basil^{+}/oregano^{-} as the novel discrimination, and oregano^{+}/basil^{-} as the reversal.

Procedure: (see table 2)

Pre-training (Days 1-3): On the first day the animals were permitted to explore the maze freely for 30 minutes, with water reward briefly available whenever the rat moved into a new goal box. On the second day, the doors were operational, trapping the rat in each chosen goalbox for 30 sec before a further 'choice' could be made. The sequence of door closures was arranged such that the rats were directed to all arms of the maze on a pseudo-random basis. On the third day, the rats were trapped for 60 seconds between water rewarded visits. Of the original 40 animals, 4 were excluded for failure to traverse the maze rapidly during pre-training.

Discrimination Training and the Transfer Problem: The remaining 36 subjects were randomly allocated to 4 groups. Group Novel (N=8) were trained on a series of novel odour discrimination problems, group Reversal (N=6) were trained on serial reversals of a single discrimination problem, and group Single (N=16) trained continuously on a single discrimination problem, matched for total number of trials with animals in group Novel. On completion of their respective series of problems, groups Novel, Reversal, and Single were then transferred to the same novel discrimination problem. Group Control (N=6) performed this same problem as their first discrimination problem in order to examine 'naive' performance on the transfer problem. This problem was presented to all groups in each of the 2 possible stimulus-reward assignments - i.e 'a+,b-' or 'b+,a-'; with half the subjects in each group receiving the former, the remainder the latter.

The 8 subjects in Group Novel were individually matched for total trials received prior to the transfer problem with half of the subjects in Group Single in order to equate simple discrimination experience in the apparatus, and these 8 'matched pairs'

GROUP	INITIAL TRAINING (PROBLEMS 1-6)	PROBLEM 7	PROBLEM 8	PROBLEM 9	PROBLEM 10
NOVEL	serial novel discriminations	transfer problem	control task	novel problem	reversal of problem 9
REVERSAL	serial reversal discriminations	transfer problem	control task	novel problem	reversal of problem 9
SINGLE	continuous single discriminaton	transfer problem	control task	novel problem	reversal of problem 9
CONTROL	none	transfer problem	control task		

Table 6.2

Experimental Design

performed the same transfer problem in the same reward orientation. The remaining 8 subjects in Group Single were also matched trial for trial with subjects in Group Novel, but they performed the transfer problem in the opposite reward orientation. In this way, the possibility the transfer might be enhanced or retarded by generalisation between training and transfer odours was controlled.

Identical odours control task: Following completion of the transfer problem, all subjects were then trained on a control task involving 'discrimination' between two identical odours. The purpose of this test was to ensure that only intended odour differences were guiding performance and not inadvertently introduced cues.

Final Problem and its Reversal: Subjects in groups Novel, Reversal and Single were then tested on a further novel odour discrimination problem and a reversal of this problem in order to determine the effects of the various types of prior training.

Results

1. Qualitative

During pre-training the rats learned to run in the apparatus for water reward. Upon initial exposure to the odours in problem 1, some rats showed position habits (e.g. consistent left turns, position alternation) over short series of trials, but this was by no means universal or consistent. During acquisition of each odour problem and during the criterion run of trials, most animals briefly sampled both odour streams during the 5 second period that the 'choice' doors remained closed. They then tended to wait by the correct odour door until it opened, moving swiftly down the chosen arm to obtain reward. During the subsequent 60 sec inter-trial interval, the rats were observed to move back and forth between the goal box and the now closed exit door. There was some indication that the rats were initially disturbed by the various mechanical sounds made by the apparatus; but choice latencies were remarkably short (8-11 seconds) and consistent across trials.

An important feature of the experimental design was the use of a control task, late in training, when the rats were confronted by a choice between two identical odour streams. All rats 'failed' this task, and performance averaged over the 31 trials given to each of the 36 rats was 50.13%. This test established that the intended olfactory cues were the sole determinants of above chance performance in the maze.

2. Quantitative

Initial training: The primary measure of performance chosen was errors to criterion on each problem. An analysis of variance of performance on problem 1 showed that groups Novel, Single, and Reversal did not differ ($F<1$), and that they made a mean of 47 errors before completing their criterion run of 8 correct choices within a single session (fig. 6.1A) Thereafter, performance by group Novel improved over the five subsequent problem to a mean of 5.5 errors to criterion (repeated measures ANOVA, $F\ (5, 35) = 4.5$, $p < 0.001$). Group Reversal, on the other hand, failed to show improved performance over successive reversals ($F<1$). Rats of group Single continued their training after problem 1 with the same odour pair for as many trials as their matched counterparts in group Novel. Average post criterion score for this group was 93.2% correct.

Transfer Problem: (Fig 6.1B) Performance on the transfer problem was analysed in two ways. The first analysis considered whether the relative performance of the groups on this problem (problem 7) differed from that shown on problem 1. Accordingly, a repeated measures ANOVA was carried out in which Groups was the between subjects factor (groups Novel, Reversal and Single) and Problems the within subjects factor (problems 1 and 7). This revealed a highly significant improvement across problems ($F(2,27) =25.64$, $p<0.001$), but no difference between groups ($F<1$), nor any significant Groups X Problems interaction ($F<1$).

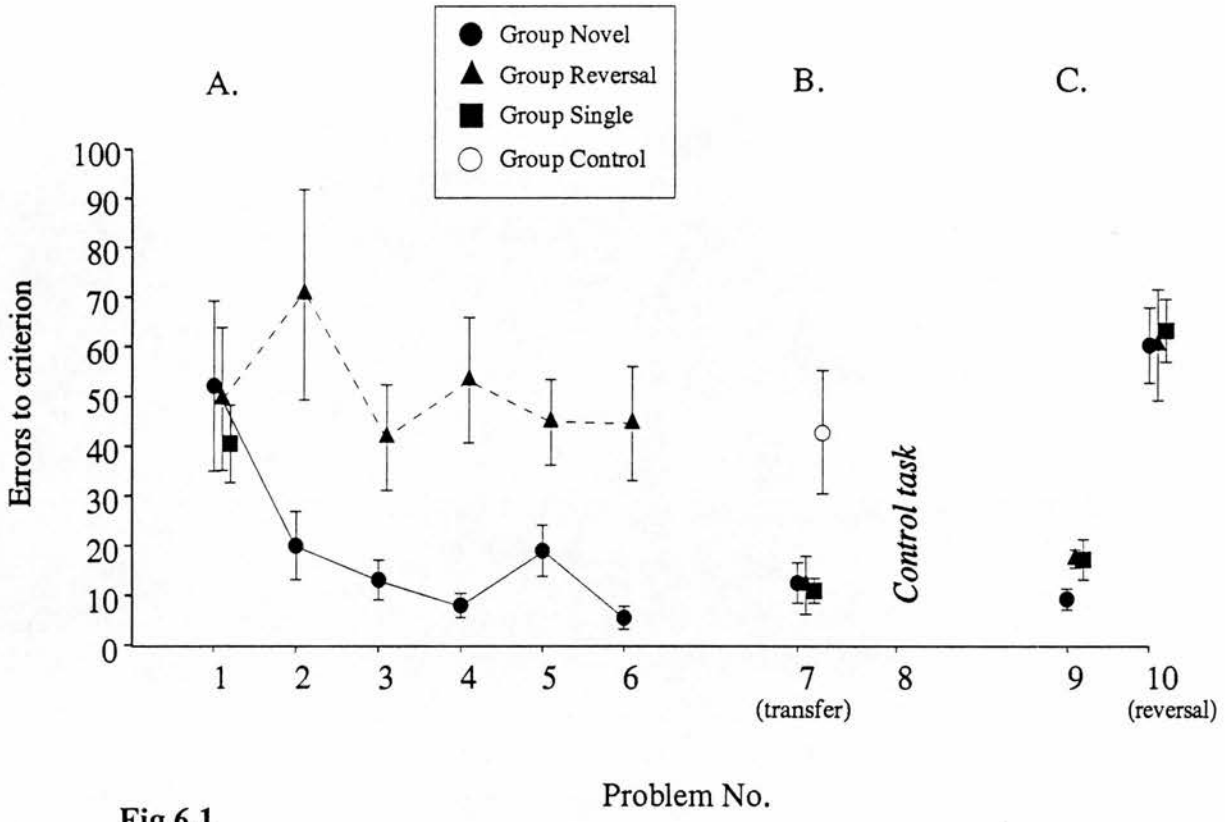


Fig 6.1

A; Mean errors to criterion (+/- 1 SEM) over problems in the acquisition phase for Groups Novel, Reversal and Single.

B: Mean errors to criterion (+/- 1 SEM) on the transfer problem for all groups.

C: Mean errors to criterion (+/- 1 SEM) for groups Novel, Reversal and Single on a further novel problem and its reversal.

The second set of analyses considered whether problem 7 was intrinsically easier than the other problems in the series. An repeated measures ANOVA of problem 7 alone, inclusive of group Control, showed a significant Groups effect ($F(3,32)=6.43$, $p<0.01$), which inspection of fig 6.1B clearly reveals can only be due to the relatively poorer performance of group Control. A further analysis established that when group Control's performance on problem 7 was compared with that of the other groups performance on problem 1, no significant difference was obtained ($F<1$).

Reversal of a novel problem: (fig 6.1C) Following completion of the identical odour control task (problem 8), animals in groups Novel, Reversal and Single were trained on a further novel problem, followed by reversal of that problem. A repeated measures ANOVA was conducted in the same fashion as that used to analyse transfer performance above, in which Groups was the between subjects factor, and Problems (in this case problems 9 and 10) the within subjects factor. A highly significant effect of reversal was found ($F(1,27)=117.54$, $p<0.00001$), but no differences between groups were revealed ($F<1$) and there was no significant Groups by Problems interaction ($F<1$).

Performance on early trials of novel problems: (fig. 6.2) The performance of group 'Novel' on early trials of novel problems (Trials 2-5) was neither significantly above chance nor did it improve over the course of training (Fig 6.2). Groups 'Novel', 'Reversal' and 'Single' averaged 54.9% correct on trials 2-5 of Problem 1, but performed no better on Problems 7 (50.5%) or 9 (58.7%); an analysis of variance of problems 1, 7 and 9 showed no difference between groups ($F(2/27)=1.54$, $p > 0.2$) nor any interaction with problems ($F < 1$); a separate analysis of trials 2-5 in Group 'Novel' over problems 1-6 also failed to show any trend towards improvement as

training progressed ($F < 1$).

Analysis of transfer problem reward orientation: Transfer problem scores of all subjects were compared with respect to the two transfer problem reward orientations (i.e. A+, B- or B+, A-). No significant difference was found ($F<1$), indicating that acquisition was equally easy on this problem when either odour served as the positive stimulus.

Control Task: (fig. 6.3) Responses to the arbitrarily designated positive and negative (but qualitatively identical) odours were analysed for all rats. No significant difference was found ($F<1$).

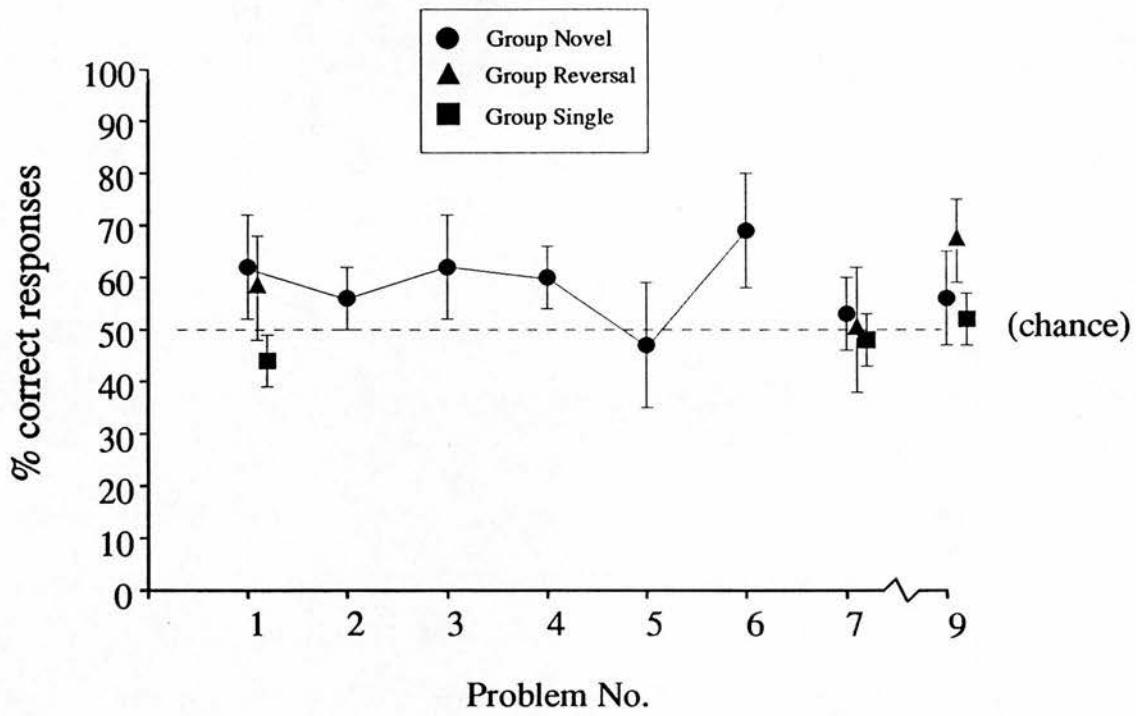


Fig 6.2

Mean percentage correct responses (+/- 1 SEM) on trials 2-5 of novel problems encountered by Groups Novel, Single and Reversal.

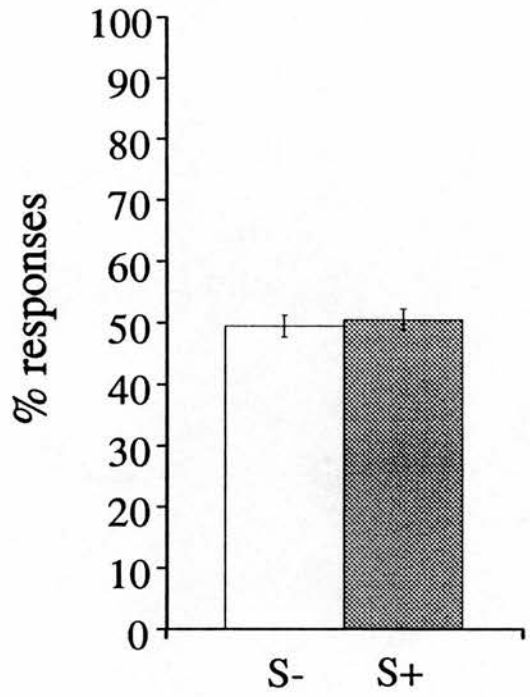


Fig 6.3

Mean percent responses (+/- 1 SEM) to the arbitrarily designated positive (S+) and negative (S-) stimuli for all subjects during the 'identical odours' control task.

Discussion:

The main finding of this experiment is that progressive improvement seen over a series of novel simultaneous odour discriminations is unlikely to be due to the acquisition of any 'higher-order' strategy because the same improvement is seen both in a group given extended training on only one discrimination task, and in a group which demonstrably failed to develop such a strategy despite prior exposure to serial reversal problems.

The first point to establish is that performance was guided strictly by olfactory cues. The high levels of accuracy shown by the single discrimination group day after day, session after session, demonstrated that all the odour stimuli used were reliably and replicably prepared and presented throughout the experiment. Furthermore, it was clear that the odours were consistently discriminable at every stage in the training process. The fact that all subjects in the experimental groups fell to chance levels of performance when presented with the control problem indicates clearly that performance was guided only by experimentally controlled differences in the odour stimuli, and not by inadvertent cues.

The second point is that progressive improvement across problems is shown by the novel discrimination group (Group Novel). This finding is consistent with previous reports in the literature. However, the fact that their performance on trials 2-5 of any new problem was little above chance, even on later problems in the series, casts doubt on the notion that they had acquired a "win-stay, lose-shift" strategy. Their failure to show faster reversal than the group trained continuously on a single discrimination problem (Single) also supports this view, given that one might expect reversal problems to be more effectively solved by such a strategy (e.g. Mackintosh,

1974 p. 610). This particular observation has also been previously reported by Eichenbaum (1986), where an increase in error score on reversal of a problem within a series was used as an index of how well the original problem was "remembered". Though not interpreted as such in the original article, Eichenbaum's finding might be construed as being inconsistent with a "win-stay, lose-shift" strategy.

In fact, subjects in the group Reversal failed to improve across serial reversal problems and in addition performed equally poorly on a further reversal of a novel problem. There was no evidence of the development of a "win-stay, lose-shift" strategy with respect to odour problems as a consequence of reversal training. The failure of Group 'Reversal' to show progressive improvement across serial reversal problems was puzzling for two reasons. First, Slotnick and Kaneko (1981) reported improvement in reversal learning across a series of 6 reversals, although such improvement is not universally obtained (Eichenbaum et al, 1986; Slotnick and Risser, 1990). While this discrepancy may reflect subtle differences in training procedure, the possibility that it reflects apparatus design should also be considered. Specifically, if there is any possibility, however remote, of uncontrolled cues guiding performance, a nominal reversal may involve unintended training with cues that have not, in practice, been reversed (see chapt 5, p.99). In this experiment, the failure of Group Reversal to show progressive improvement was coupled with chance performance on the identical odours control task. The second reason the reversal finding is puzzling is that rats have been reported to show progressive improvement across reversal problems in the visual modality (e.g. Mackintosh, McGonigle, Holgate and Vanderver, 1968). Whether much should be made of this difference between modalities is unclear; it may reflect no more than theoretically unimportant differences in experimental procedure or, more speculatively, a real

difficulty in changing the reward significance of an odour once acquired. Though present results do not discriminate between these possibilities, Slotnick and Brosvic (1987) have recently reported that rats fail to acquire a "reversal set" when presented with taste cues. It may be the case that learning via chemosensory systems in general is rapid (e.g. taste aversion learning), but relatively inflexible (with respect to changing the reward significance of cues once learned) in comparison with visual discrimination learning in rats.

All groups, however, transferred well to each of two novel discrimination problems, indicating that exposure to a series of novel problems was unnecessary for the development of high levels of transfer performance. Further to this, the excellent transfer of group Reversal underscored the suggestion that good transfer performance need not mediated by the development of a "win-stay, lose-shift" strategy, given that subjects in this group had demonstrably failed to acquire one.

It is important to note that the numerous observations of progressive improvement over a series of novel problems is replicated in our findings. However, the profile of the results raises an issue about the appropriate interpretation of such progressive improvement. A number of studies have assumed that progressive improvement is mediated by an acquired strategy; but it is clear that other interpretations may be equally valid. Possible sources of progressive improvement probed or controlled for in this study included, (in addition to the development of a "win-stay, lose-shift" strategy): 1. progressive increase in ease of odour problems across the series, including the first transfer problem; 2. similarities between earlier odour stimuli and transfer stimuli; and 3. non specific learning as a consequence of simple discrimination learning experience in the apparatus.

In view of the fact that animals in group Novel were divided into 2 subgroups, each performing the odour series in reverse order with respect to the other group, and that each subgroup showed progressive improvement, it would seem unlikely that later problems could be considered to be any easier than earlier problems. The transfer problem was acquired no more rapidly by naive subjects than the first problem in the series, and it was therefore not the case that the excellent transfer seen by all groups could be accounted for by the fact that this particular problem was in some way simpler than the rest. The possibility that the transfer odours could be considered to be in some way similar to odours encountered earlier in the experiment, thereby facilitating transfer, was discounted by the fact that it made no difference which of the cues in the transfer odour pair was rewarded - transfer was equally good to either of the reward assignments.

It would seem likely then, that an important determinant of transfer is experience in the apparatus. This may, of course, encompass a number of factors, including, for example, the development of selective attention to odour stimuli, reduced anxiety and consequent improvement in performance as a result of familiarity with the apparatus itself, and the abandoning disruptive response tendencies such as position habit. These factors were explicitly controlled for in this experiment (Group Single). There is no need to invoke "higher order" learning or "abstract processes" to account for the progressive improvement observed. The fact that such dramatic progressive improvement is rarely observed in other sensory modalities in rats may reflect no more than the relative ease with which rats learn olfactory discrimination problems. Specifically, in easy discrimination problems, disruptive response tendencies will contribute *proportionately* more of the total number of errors to criterion during the initial stages of learning than in more difficult learning procedures (such as, for rats, visual discriminations). Overcoming the same disruptive tendency in harder tasks

may, therefore, only marginally improve performance on subsequent problems. It follows that as such nonspecific factors are gradually overcome, rate of learning will appear to improve rapidly in the former case, but not in the latter, despite the fact that the underlying contribution to improved rate of learning is, in absolute terms, the same in the two cases.

This interpretation accounts equally well for previous observations of inter-problem transfer in olfactory studies. Indeed, some of the additional sources of transfer controlled for and discounted above have generally not been examined and may also have contributed to transfer in earlier studies. In fact, a number of studies have used a short, fixed odour series in learning set experiments (e.g. Eichenbaum et al, 1986). The possibility remains, therefore, that the problems were simply progressively easier to discriminate. More importantly, details of control tasks demonstrating control by odour cues rather than accidental but effective cues are rare. In the development of the apparatus used in this experiment (see chapter 5), elimination of additional cues guiding rat performance proved a most difficult task.

These findings therefore call into question the notion that the successive improvement represents the formation of a true 'learning set', involves the acquisition of rules or, indeed, requires principles beyond those underlying instrumental discrimination learning studied in other sensory modalities. Thus the recent claim that "within [this] appropriate stimulus modality, rats can learn complex abstract rules" (Otto and Eichenbaum, 1991) remains both unproven and unlikely. This is not to deny that rats, like primates (Levine, 1959) and certain avian species (Kamil et al, 1977), might be able to acquire a win-stay, lose-shift rule with more extensive training or other protocols.

In summary, the results of this study show that the progressive improvement seen in rodents performing simultaneous 2-odour discrimination problems need not be ascribed to the acquisition of a higher order strategy characteristic of "learning-set" formation in primates, given that: 1. subjects without this experience transferred to novel problems equally well; and 2. subjects who demonstrably failed to acquire such a strategy also transferred effectively; and 3. conventional measures of learning set acquisition (analysis of early trials on novel problems) revealed no evidence of learning set formation.

Appendix: Intraproblem Analyses

Data collected in the preceding experiment were re-analysed to examine changes occurring within individual problems during the reversal series (group Reversal), and during the first and transfer problems (all groups).

Reversal Learning

Figure 6A1 shows intra-problem learning curves for each reversal problem across days. The rate of reversal learning within problems did not change as a consequence of reversal problem experience (repeated measures ANOVA, main effect of problem $F<1$; main effect of blocks of trials, $F(5,25)=79.72$, $p<0.00001$; interaction $F<1$). When the rate of learning within the first reversal alone was compared with the last alone (fig. 6A2) in 10 trial blocks, again no significant difference was found (Main effect of reversal, $F<1$; main effect of blocks of trials, $F(14,70)=15.05$, $p<0.001$; interaction, $F<1$). Lack of change in performance across reversal problems (fig 6.1) is therefore also accompanied by a lack of change in learning profile within reversal problems. On both the first and last reversals, mean percent 'correct' score was below chance for the first 2 blocks of 10 trials (fig 6A2) indicating that stimulus perseveration accounted for most of the early errors made in each problem, and that this did not change as a consequence of reversal experience. Error type was classified for the first and last reversal. Errors were designated 'positional' if they occurred within a string of 5 consecutive left turns, right turns or alternations. The proportion of positional errors contributing to the first reversal error score (60.3%) did not differ significantly from the proportion

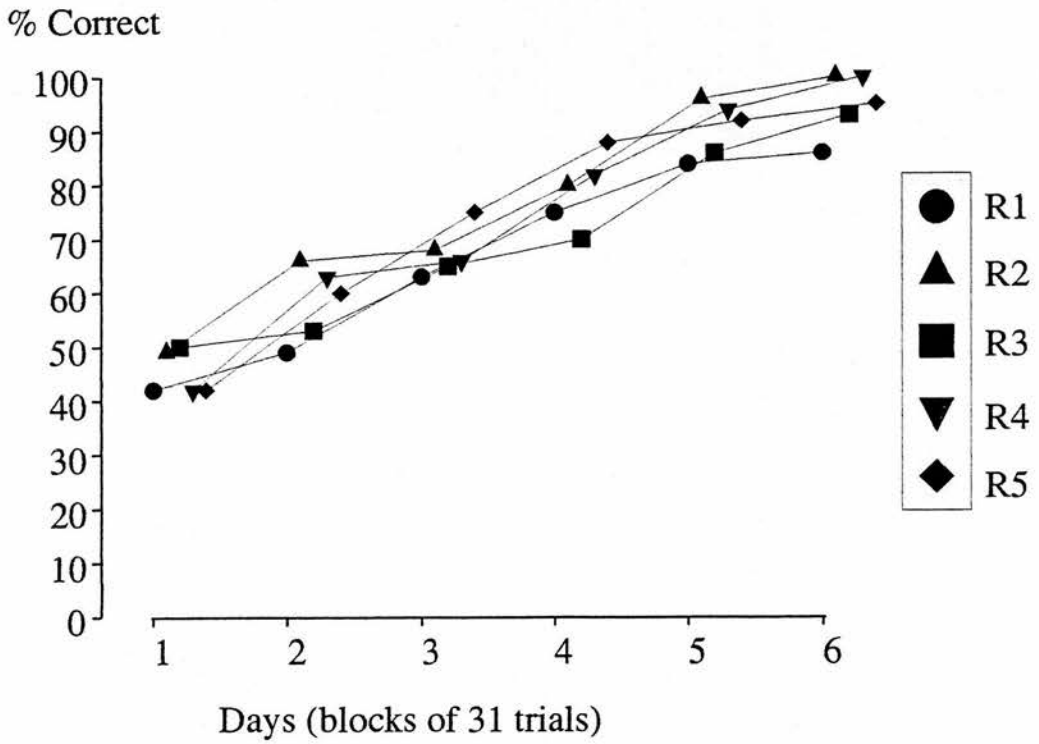


Figure 6A1

Intra-problem learning curves for each reversal problem across days

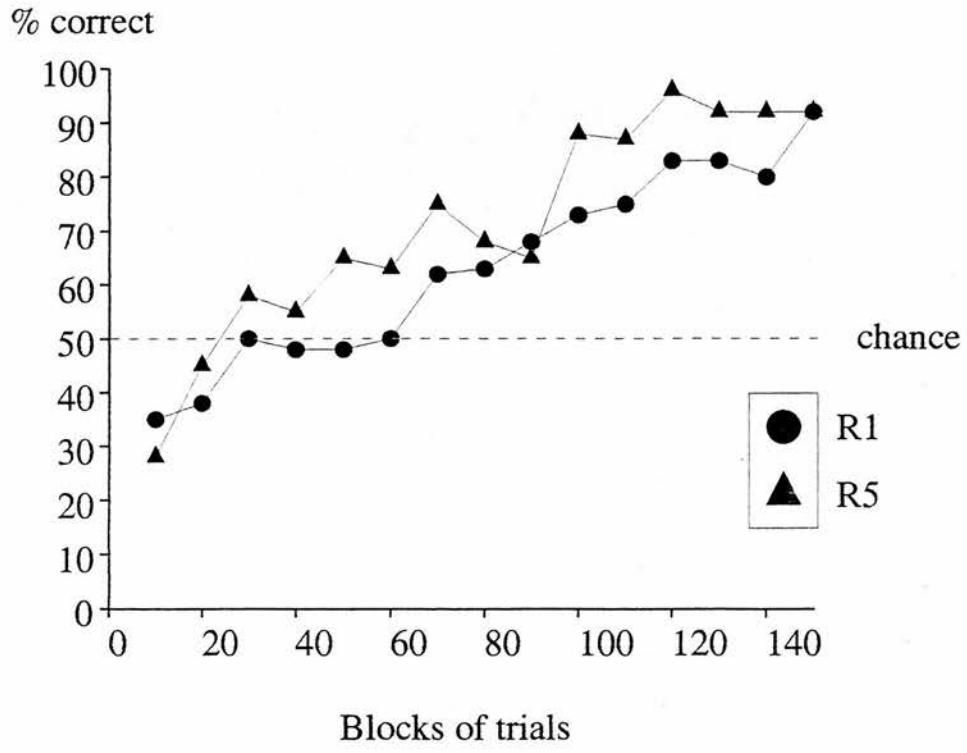


Figure 6A2

Percentage of correct responses per 10 trial block, within the first and last (fifth) reversal problems for subjects in group Reversal

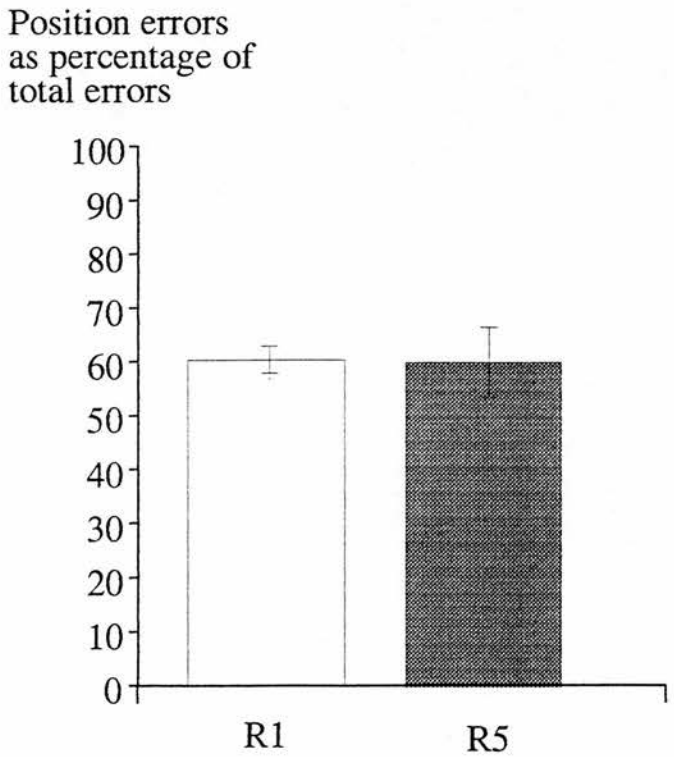


Figure 6A3

Proportions of positional errors committed during first and last reversal problems

(T=0.02, p>0.5, 2-tailed; see fig 6A3). It seems, then, that the rate of learning within problems, the type of error made and the distribution of errors made, do not change as a consequence of serial olfactory reversal learning experience in rodents. This is in marked contrast to brightness discrimination learning in rats (see chapter 6, Mackintosh et al, 1968), and supports the contention that odour-reward associations are extremely resistant to disruption in the rat. In particular, proactive interference does not appear to play an important role in rodent olfactory serial reversal learning, as indicated by the below chance performance on the first 20 to 30 trials of the final reversal problem.

The Transfer Problem

All experienced groups learned the transfer problem significantly more quickly than they had learned the first novel problem. Intra-problem learning curves for each group are shown, both for the first problem and the transfer problem (fig 6A4). Inspection of the figure shows that, although the *rate* of learning is similar throughout most of the learning curves for both the first and transfer problems, the earliest portion of the curve (trials 10 -30) is quite different (in all groups) when the first and transfer problems are compared. It is this initial difference which seems most likely to account for the difference in errors made in reaching criterion observed when the first and transfer problems are compared. Errors made within the first 30 trials were therefore analysed in detail. Figure 6A5 shows mean positional error score (classified as above) for each block of 10 trials for all subjects on problems 1 and transfer, along with the corresponding portion of the curves illustrated in the preceding figure. A repeated measures ANOVA confirmed

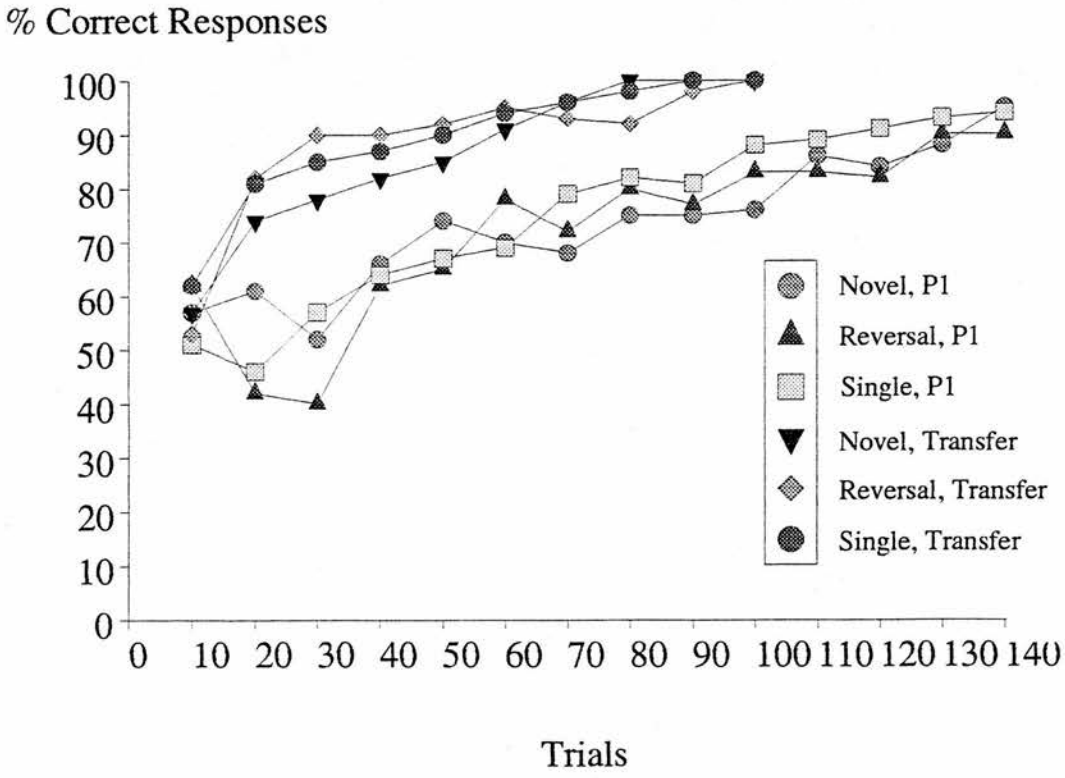


Figure 6A4

Intraproblem learning curves for each group, showing problem 1 and the transfer problem

that subjects made significantly less positional errors at the outset of training on the transfer problem than were made on problem 1 (main effect of problem, $F(1,58)=34.10$, $p<0.0001$; main effect of blocks of trials $F(2,116)=1.69$, $p>0.1$; interaction $F(2,116)=2.55$, $p=0.08$. Taken in the light of the analysis presented in chapter 6, this finding supports the view that reduction in disruptive response tendencies (for example, position habits), rather than the development of a 'higher order' abstract strategy, accounts for the transfer effects seen in this series of experiments.

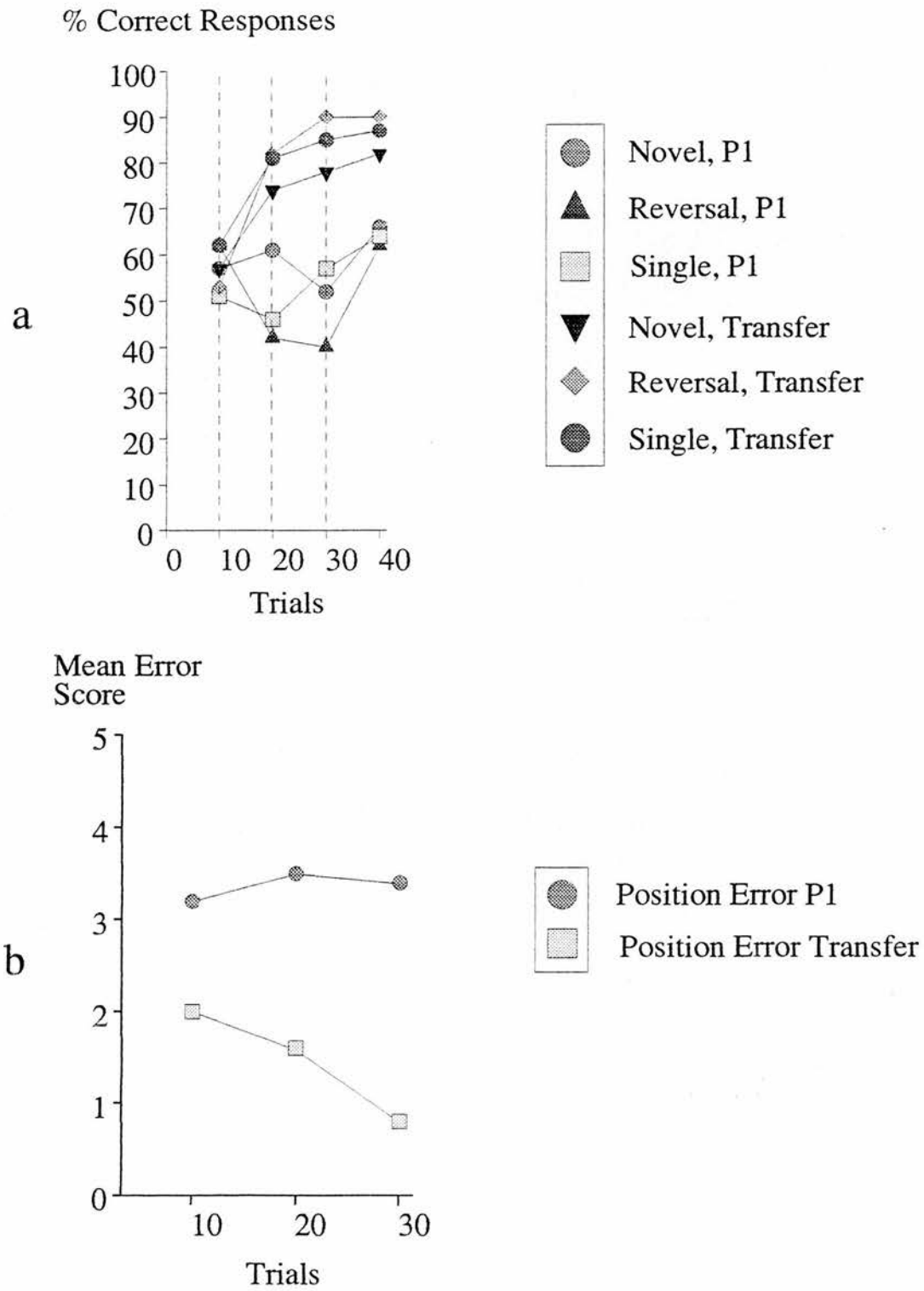


Figure 6A5

Early portion of intraproblem learning curves for problems 1 and transfer (a), and mean total positonal error score for each block of 10 trials (b)

CHAPTER 7:

The effects of Hippocampal and Dorsomedial Thalamic Nucleus Lesions, and Intraventricular Infusion of AP5, on Olfactory Learning.

Introduction:

Most recent studies of olfactory learning in the rat have been preoccupied with modelling aspects of human amnesia, and investigations have centred on the role of structures considered critical in human memory. The justification for such an enterprise has been drawn from a variety of fields, with investigators seeking parallels between psychological/behavioural and neuroanatomical variables in rats and primates, as discussed in the introductory chapters. As outlined in chapter 3, however, these studies have often proved inconsistent and may be subject to a variety of different interpretations.

In the preceding chapter, the proposed psychological parallels between rodent and primate learning were investigated in some detail, and it was concluded that the claim that rodents possess a 'primate-like' learning capacity when tested with olfactory cues may be misleading. In particular, it seemed unlikely that rats form olfactory 'learning sets' when faced with a series of novel odour discrimination problems, and that rodent olfactory learning, though rapid, should not be distinguished qualitatively from simple discrimination learning via other sensory modalities.

The question therefore arose as to whether such learning should be sensitive to hippocampal damage. Although direct, selective damage to the hippocampus itself has not been studied previously, lesions to important hippocampal afferents (lateral olfactory tract and lateral entorhinal cortex) and efferents (the fornix) have produced interesting but conflicting results, including impairment, facilitation, and 'no effect' on olfactory discrimination learning. Specifically, Staubli et al (1984) have suggested that lateral entorhinal cortex lesions do not impair the acquisition of simultaneous-cue discrimination problems, but cause rapid forgetting over a period of 1 hour; while Eichenbaum et al (1988) claim in contrast that successive-cue learning is facilitated by fornix lesions and simultaneous-cue learning is impaired over short intervals of less than 10 seconds. Slotnick and Kaneko (1981) and Slotnick and Risser (1990), studying the consequences of hippocampal denervation following lesions to the lateral olfactory tract, found no effect on successive-cue olfactory discrimination learning. Slotnick and his colleagues' findings are, in fact, consistent with an earlier study by Eichenbaum et al (1986), in which fornix lesions failed to affect successive cue discrimination learning. Although the report (Eichenbaum et al, 1986) conflicts with the later findings (Eichenbaum et al, 1988), this earlier study showed a further important finding - the facilitation of olfactory reversal learning in operated animals. It was suggested that the lesioned rats were 'amnesic' for the original configuration of the 'reversed' problem (see chapter 3 p.50-67 for a detailed review of these studies).

Drawing these varied findings together, the following outline experimental design was proposed for their further investigation: an examination of simultaneous cue learning in lesioned rats over a series of 5 novel olfactory problems, followed by a reversal of the final problem conducted approximately 24 hours after original learning of the problem to be reversed. This design had a number of advantages:

First, Staubli's model of amnesia could be tested in a similar fashion to the manner in which it was developed (excepting the fact that the retention interval is even longer than that used in her study); and second, Eichenbaum et al's (1988) representational theory (see chapt.3, p.62-64) could be tested by using the (simultaneous) cue configuration most consistently affected by damage to the hippocampal formation in their experiments. Third, Eichenbaum et al's (1986) earlier report of facilitated reversal learning could also be evaluated by using this strategy.

As Eichenbaum et al (1986), Staubli et al (1984), Slotnick (1985) and Slotnick and Risser (1990) had all used different lesions in their experiments, consideration was given to the nature of the lesion to be used in this study. Given that all three research groups related their findings to the presumed secondary effects of de-afferentation or de-efferentation of the hippocampus itself (rather than primary effects of extra-hippocampal damage) it seemed reasonable that selective hippocampal lesions should be the focus of the experiments described below. The use of this lesion might be expected to exaggerate the effects Staubli et al (1984) and Eichenbaum et al (1986, 1988) report, given their claim that the respective lesions produce a particularly selective impairment of hippocampal function.

The effect of intraventricular infusion of the NMDA receptor antagonist AP5 on this training procedure was also examined. Staubli et al (1989) have previously reported an effect of AP5 infusion on the acquisition of simultaneous 2-odour discrimination problems at long inter-trial intervals when "weak" odour cues are used. Retention of previously learned problems, as assessed by reversal performance 24 hours after original learning, was unaffected (see chapter 3, p65-67). Overall, the effects on acquisition reported were small but significant. These results (aside from the 24 hour retention finding) are qualitatively in accordance with Staubli et al's (1984)

observations of olfactory learning in rats with entorhinal cortex lesions, and it is possible that the effects of both entorhinal damage and AP5 infusion may be ascribed to interference with hippocampal function. Alternatively AP5 may act at the level of the olfactory cortex. In view of the contradictory effects of different aspects of hippocampal system damage on simultaneous olfactory discrimination learning (e.g. Staubli et al, 1987 in which entorhinal lesions were used; v. Eichenbaum et al, 1988 in which fornix lesions were used - see chapter 3 p 61-65), it was considered of interest to compare the performance of animals sustaining direct, selective hippocampal damage with those receiving chronic infusion of AP5 on the training schedule used here.

The role of the dorsomedial nucleus of the thalamus (DMN) in olfactory learning in the rat was also of interest. As reviewed in chapter 3 (p 50-61), variable and conflicting results have been reported following lesions to this structure. In summary, Eichenbaum et al (1980) observed less rapid olfactory discrimination learning in lesioned animals post-operatively when compared with control animals, while Slotnick and Kaneko (1981) observed no effect on the acquisition of a 2-odour discrimination problem, but a marked impairment in olfactory reversal learning. In a later study, Slotnick and Risser (1990) observed marginally but significantly impaired learning in DMN lesioned rats on only one of three post-operative discrimination problems. In contrast, Staubli et al (1987b) found that DMN lesioned rats were profoundly impaired on the post operative acquisition of a 2-odour discrimination problem, but after extended training on a further 2 problems the lesioned animals matched the performance of controls.

In order to further investigate the issue, DMN lesioned rats were trained in the same way as the hippocampally lesioned subjects (described above). In this way, the effect of DMN lesions on a series of 5 post-operative simultaneous 2-odour discrimination problems, followed by a reversal of the fifth problem, could be evaluated.

Finally, a number of the rats were trained on a spatial reference memory task, given that this task is reliably sensitive to the effects of hippocampal lesions.

In summary, three main interventions were used: ibotenate lesions of the hippocampus (group HPC), radiofrequency lesions of the dorsomedial nucleus of the thalamus (group DMN), and the intraventricular infusion of the NMDA receptor antagonist AP5 (group AP5). Rats receiving the lesions or infusion and sham (group SHAM) lesioned animals were then trained on a series of novel olfactory discriminations and a reversal discrimination; and a proportion of the lesion groups on a spatial task.

Methods:

General Procedure:

Groups of rats were run in a series of replicates in the following manner: Following pre-training (detailed in chapter 4, p.73), rats were run on a single olfactory discrimination problem to criterion and matched on the basis of performance prior to allocation to one of the 4 groups described above. The necessary surgical procedures were then performed and, following recovery, each animal was trained daily to criterion on a further 5 novel discrimination problems (problem order being counterbalanced across groups) followed by a reversal of the final problem. The control task of 2 identical odours (2 blocks of 31 trials, 1 block per day) described in chapter 5 (p.78) was then used. Animals receiving the AP5 infusion were sacrificed at this stage and brain samples taken for histological and pharmacological analyses. In later the replicates, rats in the remaining groups were then trained in a spatial reference memory watermaze task, following which they were sacrificed and brains removed for histological analysis. The protocol is summarised in table 7.1.

Subjects:

Male hooded Lister rats (n=54) were used and maintained as detailed in chapter 4, p.75.

Pretraining	Problem 1 (Novel problem)	Surgery	Problems 2-6 (Novel problems)	Problem 7 (Reversal of prob. 6)	Problem 8 (Control task)	Spatial Task (Watermaze task)
All animals	All animals	HPC DMN AP5 SHAM	HPC DMN AP5 SHAM	HPC DMN AP5 SHAM	HPC DMN AP5 SHAM	HPC DMN SHAM

Table 7.1

Experimental design. HPC = hippocampal lesion group; DMN = dorsomedial nucleus lesion group; AP5 = AP5 infusion group; SHAM = operated controls.

Drugs:

D-2-amino phosphonopentanoate (D-AP5)

A single concentration of D-AP5 (30mM) was used, having been previously shown to inhibit reliably the induction of LTP *in vivo* (Davis, 1990). A stock concentration of 100mM D-AP5 was made from the acid by dissolving the D-AP5 in 100mM NaOH and kept as frozen aliquots, and diluted when required to the appropriate concentration using artificial cerebrospinal fluid (aCSF). Each dose was "spiked" with NaOH (100mM) until it reached a pH of 7.4.

Artificial cerebrospinal fluid (a-CSF)

Modified aCSF was made up according to the methodology specified by the manufacturers of the osmotic mini-pumps (Alza) used to infuse the AP5. The final ion concentration in mM/l was: Na, 150.0; K, 3.0; Ca, 1.4; Mg, 0.8; P, 1.0; Cl, 155.0 (pH: 7.3 ± 0.1).

Tribromoethanol (Avertin)

Avertin was used as a recoverable anaesthetic during all surgical procedures. A stock concentration was kept at 4^{o} C in a dark container to avoid light degradation. A dilution of 1 in 55 was made up in absolute alcohol and saline (0.9%), 12 hours prior to surgery. The initially injected dose was 10ml/kg (0.29g/kg) body weight, supplemented by 0.5ml injections as required throughout surgery.

Osmotic mini pumps

Osmotic mini pumps, supplied by Alza (model 2002) were used for chronic delivery of the D-AP5 into the right lateral ventricle. The pump contained approximately 220 microlitres of drug which was pumped into the ventricle at a rate of 0.5 microlitres/hour over a 14 day period.

Surgical Procedures:

Implantation of mini pumps

Pumps were loaded with drug prior to implantation. An L-shaped cannula made from a 23 gauge syringe needle was placed in one of the stereotaxic manipulators. A length of silastic tubing (4.0cm) was placed on one end of the cannula which was then flushed through with 0.1 ml of drug solution. The pump was then attached to the other end of the silastic tubing via a flow modulator. Animals were anaesthetised with Avertin and placed in a Kopf stereotaxic device. A midline incision along the scalp was made to expose the scalp surface. This was scraped clear of connective tissue. The co-ordinates to place the cannula in the right lateral ventricle were measured relative to Bregma (Paxinos and Watson, 1982): AP: -0.9mm; ML: -1.3mm; DV (skull surface): -4.5mm. Holes were drilled in the skull for the cannula and for the placement of 3 stainless steel watchmaker screws which acted as anchors for the dental acrylic used to fix the cannula in place. The cannula was lowered into the ventricle and covered with acrylic. A subcutaneous pocket was created using a bone curette at the caudal end of the scalp incision extending posteriorly between the scapulae into which the body of the mini pump was inserted. The incision was then closed with a discontinuous suture, and the animal placed in a post-operative

recovery box for monitoring prior to return to home cage.

Hippocampal lesions

Ibotenic acid (Sigma chemicals) was used to make complete lesions to the hippocampus by multiple micro-injection, following a protocol adapted from Jarrard (1989). The acid was prepared to a concentration of 10mg/ml and pH 7.4 in phosphate buffered saline. Animals were placed in a Kopf stereotaxic frame and anaesthetised as described above with Avertin. An incision was made along the midline of the scalp and the skull exposed. Sections of skull overlying the hippocampal area were removed, and 12 microinjections of ibotenic acid made on each side of the brain using a 1.0 microlitre Hamilton syringe guided by a vertical manipulator on the stereotaxic frame. Injection volumes of between 0.05 and 0.1 microlitre were made (See table 7.2 for details of volumes and co-ordinates). The syringe needle was left in place for 1 - 2 minutes after each injection to prevent spread of ibotenic acid along the tract. Scalp wounds were then sutured and the animals allowed to recover prior to return to the home cage. A period of 14 days was allowed for recovery following surgery prior to behavioural training.

Table 7.2.

Coordinates for hippocampal lesion (mm from Bregma)

A-P	M-L	D-V
-2.4	1.0	-3.4
-3.0	1.0	-2.6*, -3.4*
-3.0	3.0	-3.0
-4.0	2.6	-2.3*, -3.3*
-4.0	3.7	-3.0
-4.9	3.9	-3.5*, -7.0*
-5.7	5.1	-4.0, -4.9, -5.8

0.10 microlitres ibotenic acid were injected at all sites except those marked with an asterisk, where 0.05 microlitres were injected.

Modified from Jarrard, 1989.

Lesions to the dorso-medial nucleus of the thalamus

Bilateral lesions to the dorso-medial nucleus were made using a Radionics radiofrequency generator and probe. In the development of this technique, 2 sets of co-ordinates were eventually used for stereotaxic probe placement in an effort to maximise the reliability of accurate lesion production.

Animals were placed in a Kopf stereotaxic frame and anaesthetised as described above with Avertin. An incision was made along the midline of the scalp and the skull exposed. RF probe placement was determined with respect to bregma, and bilateral access holes drilled in the skull. In early lesions, the probe was inserted at an angle of 18° from vertical on each side in order to avoid damage to the central venous sinus (AP: -2.8mm; ML: ±2.2mm; DV (18°): -6.2mm, with respect to skull surface). Probe temperature was adjusted to 90°C for 60 seconds. Subsequent histological analysis (see below) revealed that though the DMN was on occasion successfully targeted, the lesion would frequently be placed either too deeply or too superficially. It was felt that difficulty in maintaining accurate angling of the manipulator from vertical was often responsible for the error, and the lesion revised in the light of advice from Dr. John Aggleton of the Department of Psychology, University of Durham. In the revised version, a vertical probe penetration was used on either side of the central venous sinus, the vessel having been exposed by careful removal of a fragment of skull overlying the area calculated with respect to bregma (AP: -3.5mm; ML ±0.7mm; DV: -5mm). Post-operative care was carried out as described above.

Sham surgical lesions

All control subjects received sham surgical procedures. Animals were anaesthetised with Avertin as described above, and placed in the stereotaxic apparatus. Skull surface was exposed and dura penetrated only.

Behavioural Training:

Olfactory training

Rats were trained for water reward in the olfactory maze on a series of novel 2-odour discrimination problems as previously described (See chapter 4, p.73-75). In summary, the rats were run for a maximum of 31 trials each day or until a criterion score of 8 consecutively correct responses was achieved in which case the session was terminated and a new discrimination problem commenced the following day. If criterion was not achieved in a session, the subject continued on the same problem the following day. Errors made in reaching criterion were recorded for every problem. Odour pairs used are detailed in table 7.3.

Table 7.3

Odour Pairs

Odour pairs were used in 2 series, each in reverse sequence with respect to the other, and counterbalanced across groups.

	series 1	series 2	
P1	strawberry^{+}/lemon^{-}	vanilla^{+}/almond^{-}	
P2	coffee^{+}/ginger^{-}	mint^{+}/cloves^{-}	
P3	ovaltine^{+}/coconut^{-}	fennel^{+}/cumin^{-}	
P4	fennel^{+}/cumin^{-}	ovaltine^{+}/coconut^{-}	
P5	mint^{+}/cloves^{-}	coffee^{+}/ginger^{-}	
P6	vanilla^{+}/almond^{-}	strawberry^{+}/lemon^{-}	
P7	almond^{+}/vanilla^{-}	lemon^{+}/strawberry^{-}	(REVERSAL)
P8	rum^{+}/rum^{-}	rum^{+}/rum^{-}	(CONTROL TASK)

Spatial reference memory task

Rats from Sham, DMN and HPC groups were trained to locate an escape platform in an open field water maze over 5 days. The maze consisted of a large circular tank of water made opaque by the addition of powdered milk. Animals were placed in the water and allowed to swim in order to locate a hidden, fixed position escape platform using extramaze cues (Morris, 1981, 1984b).

The pool was 2m in diameter and 0.6m in height. The structure was made from glass fibre and placed on a wooden platform 0.6m from the floor in the centre of the testing room. The apparatus was plumbed into the laboratory water supply so that filling and draining could be accomplished automatically. The escape platform was constructed from a length of plexiglass tubing 10cm in diameter, weighted with stones to prevent it from floating. The platform and the tank sides were painted white. The room in which the maze was housed held a number of extra-maze cues, consisting of fixed location free-standing three dimensional objects (such as equipment racks) and distinctive posters on the walls.

The animals' behaviour in the pool was monitored by a ceiling mounted video camera. The pool area was evenly illuminated by 4 halogen flood lights so that animal movement could be tracked automatically by an image analyser (HVS, model 112) connected to the camera, detecting the contrast between the black head of the rat and the white pool surface. Information from the analyser was sampled using an Archimedes (Acorn) computer system and swim path, path-length, latency to find platform and time spent in each of the arbitrarily designated pool quadrants computed. The apparatus was designed and built by Dr. R.G.M. Morris, University of Edinburgh and programmes were written by Dr. Morris and Mr. Roger Spooner.

A video recorder enabled trials to be recorded, and analysed "off-line" if required. The Watermaze apparatus is illustrated in fig 7.1.

The spatial learning task consisted of a learning or acquisition phase, followed by a probe trial to test retention of the platform location. During the acquisition phase animals were trained to learn the location of a fixed (consistent) platform location by using the extra-maze cues in the room. Rats were trained over 5 days, 6 trials per day for the first 4 days, (acquisition phase) followed by a 'transfer test' (probe trial) on the 5^{th}. There was a maximum swim-time of 120 seconds per trial, with a 30 second ITI on the platform. Any animal failing to find the platform within 120 seconds was placed on the platform for 30 seconds at the end of that time. In the probe trial, the platform was removed from the pool and the animals forced to swim for 60 seconds. Typically, a normal animal would spend most of the time period searching for the platform in the quadrant of the pool it had previously occupied.

In all, 6 start positions were used, corresponding approximately to geographical north, south, east, west, northwest and southeast. 1 of 2 platform positions was used for the training of each rat: northeast or southwest. All start positions were used with each animal in a pseudorandom sequence. Platform location was counterbalanced across subjects in each group.

All trials were recorded on video and computer for subsequent analysis.

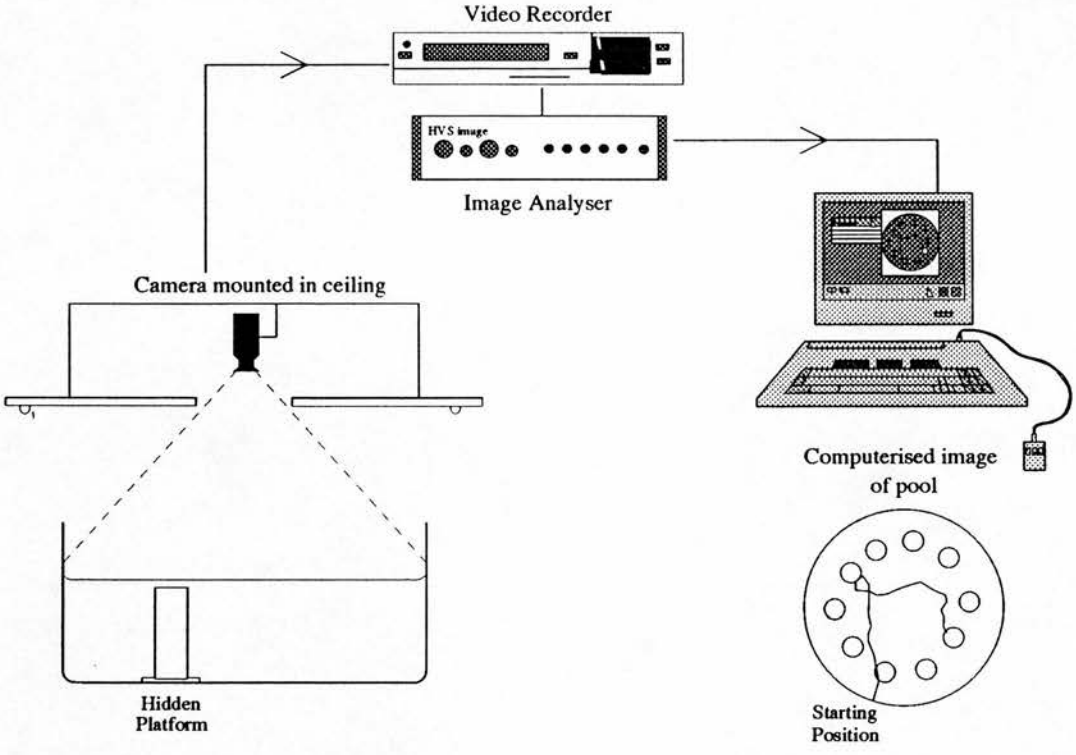


Fig 7.1

Schematic diagram illustrating the 'Water Maze' apparatus used in the spatial reference memory task. The tank is approximately 2m in diameter.

Histological Procedure:

At the end of training, animals were perfused transcardially with physiological saline and 10% formalin and their brains removed. 30 micron brain sections were stained with fast cresyl violet to assess lesion site and extent, or accuracy of cannula implantation.

Pharmacological analysis:

Tissue from the brains of rats undergoing intraventricular AP5 infusion was analysed to determine AP5 content using high performance liquid chromatography (HPLC).

Samples were taken from right and left hippocampus, and right and left piriform cortex. Tissue was homogenised in 1.0ml 0.6M perchloric acid to precipitate tissue protein. The homogenate was kept at -4^0C overnight, and then centrifuged at 10000g for 2 minutes. The supernatant was neutralised in potassium bicarbonate (2.0M) and centrifuged again at 10000g for 2 minutes. This preparation was diluted 1:10 with deionised water and injected onto the HPLC column for separation of amino acids and detection by fluorimetry. AP5 levels, expressed as nanomoles per mg wet weight of tissue were calculated with reference to a stock solution containing a known concentration of AP5. Each tissue sample was analysed twice, and the final value recorded as the mean of the 2 replicates. The HPLC analysis was carried out by Dr. S. Butcher and Mr. D. Bannerman of the Department of Pharmacology, University o Edinburgh. The theoretical background, equipment and procedure used is detailed in Davis, (1990).

Results:

Of the 54 animals entered in the study, 3 failed to complete pre-training, 5 died during surgery, and 8 animals were withdrawn from the analysis following histological assessment. 1 animal from the AP5 group was withdrawn following tissue analysis. This left 37 animals in total: SHAM = 12; HPC = 10; AP5 = 8; and DMN = 7.

Olfactory Learning: (fig 7.2)

Problem 1

Animals from the 4 groups were successfully matched for initial discrimination score (ANOVA $F<1$), making a overall mean of 22. 8 errors ($\pm$ 2.2 SEM) to criterion on the first problem.

Problems 2-6

Following surgery and recovery, all groups improved across the 5 subsequent novel problems, making a mean of 8.7 errors ($\pm$ 1.2 SEM) to criterion on problem 5.

An unequal n, repeated measures analysis of variance across the 5 novel problems for all groups indicated a significant effect of Groups ($F(3,33) = 5.65$, $p<0.005$), a highly significant effect of Problems ($F(4,132) = 10.51$, $p<0.0001$), but no significant interaction ($F<1$). Subsequent orthogonal comparisons showed that group AP5 did not differ from group HPC ($F= 3.61$, $p>0.05$) and that these 2 groups did not differ from sham animals ($F<1$). However, Group DMN differed highly

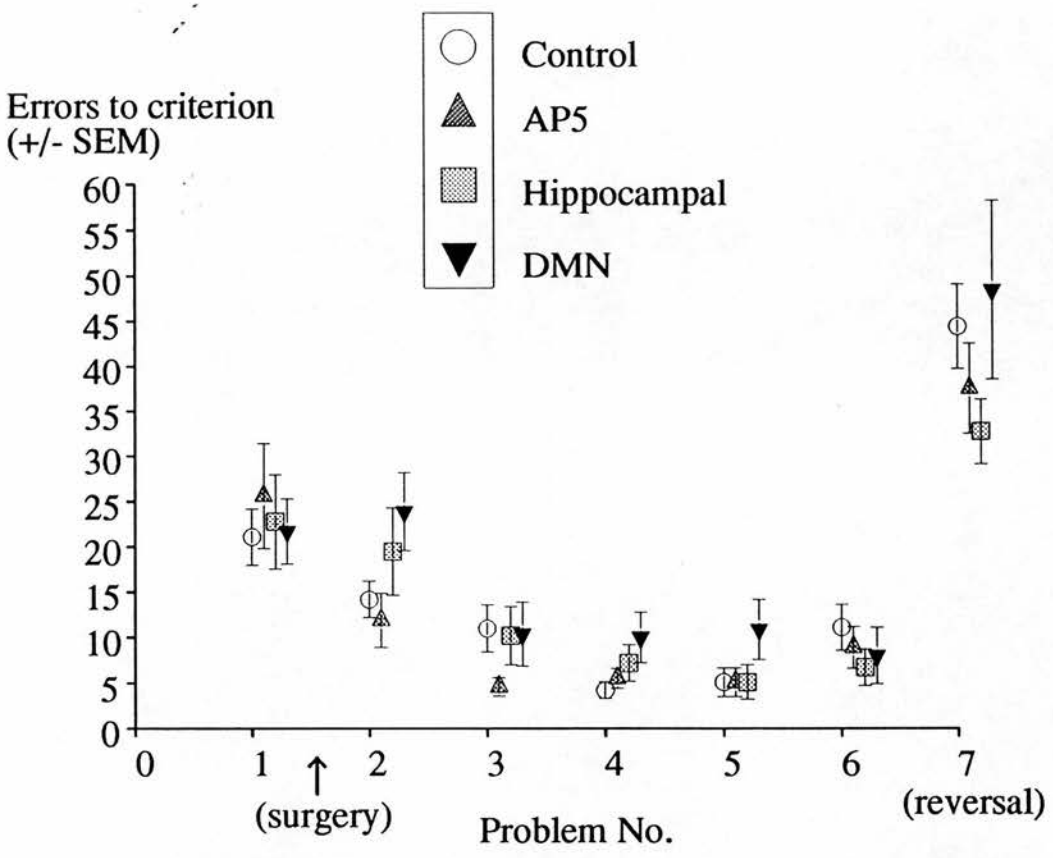


Fig 7.2

Graph showing mean errors made (+/- 1 SEM) by each group on each olfactory problem. Points have not been linked for clarity.

significantly from the other 3 groups ($F(1,33) = 11.07$, $p<0.005$), indicating that group DMN was responsible for the small (in absolute terms) but significant Groups difference obtained. Animals in Group DMN made more errors on average in solving each novel discrimination, but improved across problems at a similar rate to other groups (hence the lack of a significant interaction term). Although no learning impairment was detected in group AP5, these rats were noted to display some evidence of mild sensori-motor impairment as a consequence of the AP5 infusion, including an impaired 'righting reflex' and occasional slight ataxia. The animals appeared, however, to be perfectly capable of completing the olfactory tasks. In order to objectively measure any motor impairment, choice latencies were recorded for rats in both group AP5 and group SHAM on the first 30 trials of the first postoperative problem (problem 2). Mean choice latency for group AP5 was 31.65 seconds (+/- 14.3 SEM) per trial; and for group SHAM was 13.9 seconds (+/- 1.4 SEM) per trial. The distribution of latency scores in the AP5 group was, however, highly positively skewed, with only 2 of the subjects scoring outside the range of latencies recorded for group SHAM. Groups were therefore compared using the Mann-Whitney test and were found not to differ significantly ($U=35$; $N_A=12$, $N_B=8$; $p>0.1$).

It was noted that the rats in this experiment performed the first problem more rapidly than in earlier experiments (see chapter 6, p.111), possibly as a result of the different odour pair combinations used. Group SHAM was used to further examine the issue of learning set formation and performance on trials 2-5 of each novel problem was analysed. Performance on trials 2-5 for problem 1 was 57% correct ($\pm$9.1% SEM); and for problem 6, 54% correct ($\pm$ 6.7% SEM). A repeated measures analysis of variance indicated that the animals showed no evidence of significant improvement in performance on early trials across the 6 novel problems ($F<1$); see fig. 7.3.

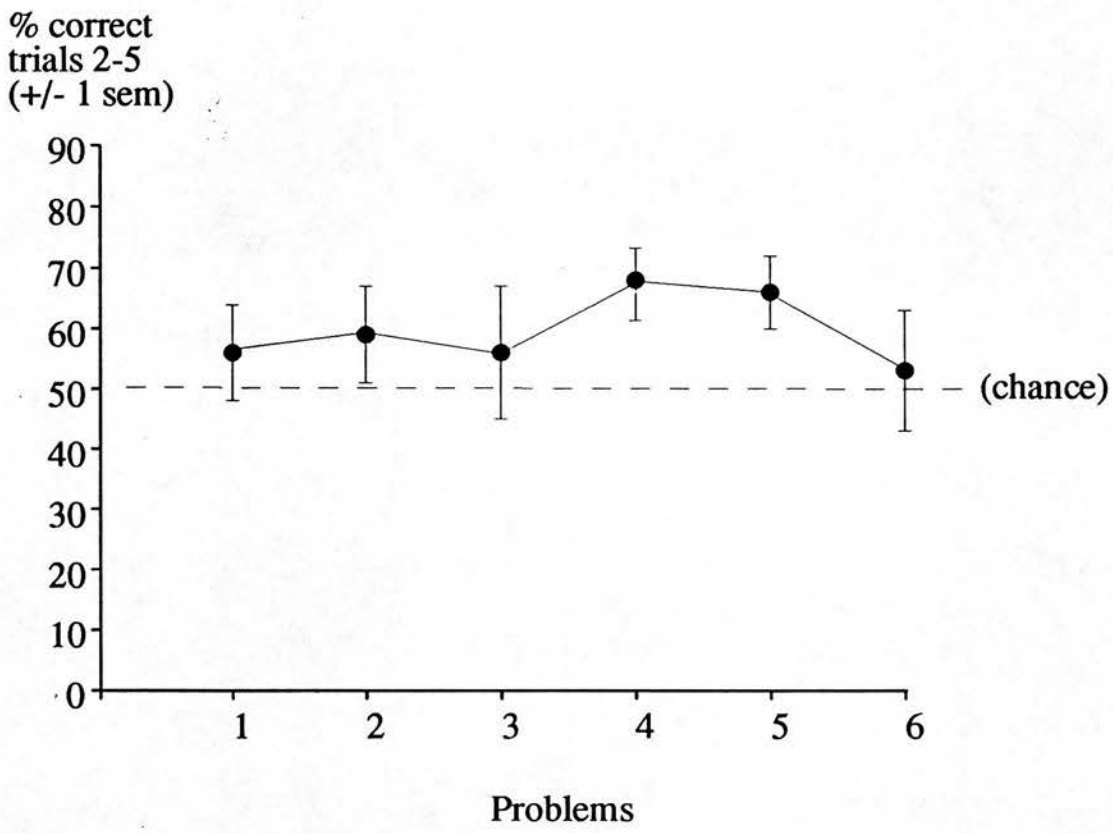


Fig 7.3

Graph showing the percentage of correct responses made on early trials of novel problems by group SHAM. Compare with figure 6.2.

Reversal Problem (problem 7, fig 7.2)

The reversal problem was learned equally and significantly more slowly by all groups with an overall mean of 40.8 errors made (repeated measures ANOVA, problem 6 - problem 7, Groups factor $F(3,33) = 1.4$, $p>0.1$; repeated measures (problems) factor $F(1,33) = 147.4$, $p<0.0001$; interaction $F(3,33) = 1.3$, $p>0.1$).

Although there was no statistically significant difference between groups in number of errors made in completing the reversal problem, there was a trend towards faster reversal by Group HPC. This trend may have arisen due to faster forgetting after hippocampal disruption (Staubli et al, 1984). However, an analysis of the percentage of correct responses on early trials (trials 1-10 and 11-20, see fig 7.4) on this problem failed to reveal group differences, though all groups made significantly less errors in the second block of 10 trials (ANOVA, Groups factor $F<1$; repeated measures factor (first 10 to next 10 trials) $F(1,32) = 23.7$ $p<0.0001$; Interaction $F(3,32) = 1.4$, $p>0.1$). (Group HPC reduced by 1 subject for this analysis - early trials data lost on account of computer failure during training).

Control Task

2 rats from the AP5 group were not tested on the identical odour control task as they barely completed reversal training within the 14 day period of osmotic mini-pump activity. They were therefore sacrificed prior to the control task stage of the experiment in order that pharmacological analysis of tissue AP5 levels might be conducted. 1 sham animal, and 1 animal from the AP5 group reached criterion on the control task. Given that large numbers of trials run on the control task, it is to be

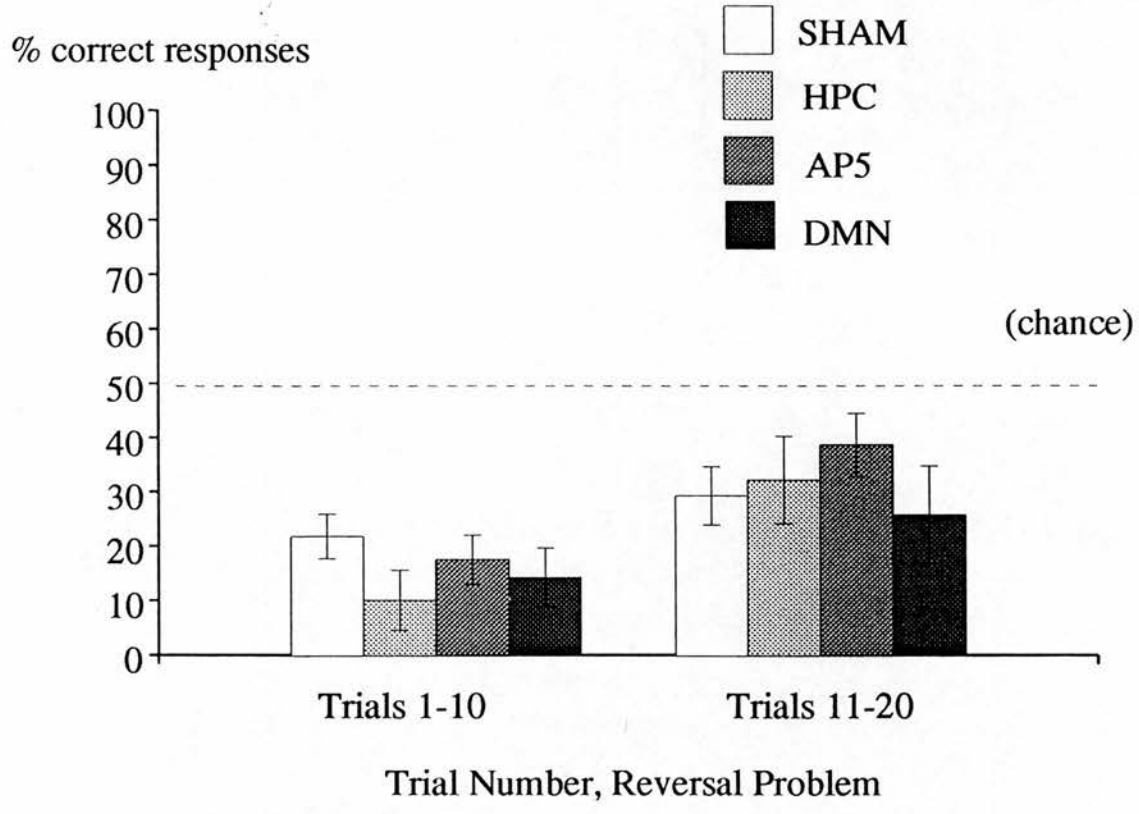


Fig 7.4

Graph showing the percentage of correct responses made on trials 1-10 and trials 11-20 of the reversal problem, for all groups.

expected that sequences of 8 consecutively 'correct' responses will occur by chance. To check whether criterion level performance was a chance finding, the 2 animals were trained again on the control task. They did not reach criterion over a further 32 trials and performed at chance levels. Mean total responses to '+ve' and '-ve' stimuli by the remaining subjects in the control task were 30.4 (± 0.8 SEM) and 31.6 (± 0.8 SEM) respectively over the 62 trials given to each subject, i.e. overall performance was at chance (49% correct).

Spatial Learning:

Spatial Reference Memory Task:

This component of the experimental procedure was used on later replicates of the study and the number of rats used in each group is consequently less than in the above section (HPC n=6; DMN n=7; sham n=8). AP5 infused rats could not be used in this experiment as mini-pump activity lasted only 14 days.

With respect to the probe trial, (see fig 7.5), animals in Group HPC appeared to perform randomly, distributing their time equally among the four quadrants. Rats from Groups DMN and SHAM tended to search for a greater proportion of the time in the correct platform location. An overall ANOVA showed a significant Groups by Quadrants interaction ($F(5,54)=3.53$, $p<0.01$, numerator degrees of freedom reduced by 1), indicating significant differences in the distribution of search times across quadrants amongst the groups.

Individual analysis of quadrant search times for each group indicated that Groups SHAM and DMN spent a significantly greater proportion of time in the 'correct'

quadrant ($F(2,21)=17.34$, $p<0.0001$; and $F(2,18)=8.45$, $p<0.005$ respectively), while Group HPC showed no such bias ($F<1$). The finding implies that while Sham and DMN rats were able to learn the spatial reference memory task, HPC rats were not. A further analysis of time spent in the 'correct' quadrant only (for all 3 groups) was carried out, showing a significant Groups difference ($F(1,18)=6.04$, $p<0.025$, and subsequent orthogonal comparison of F ratios indicated that group HPC mean time differed significantly from the mean of group SHAM ($F(1,18)=6.04$, $p<0.02$) and groups SHAM and DMN ($F(1,18)=11.7$, $p<0.005$).

AP5 Levels:

Mean tissue levels of AP5 are expressed as mean nmols/mg wet weight of tissue (+/- 1 SEM) for each of the 4 brain areas examined:

Right hippocampus = 0.92 (+/- 0.21); Left hippocampus = 0.62 (+/- 0.31); Right piriform cortex = 0.64 (+/-0.08); Left piriform cortex = 0.44 (+/- 0.08). The range of concentrations obtained overall was 0.21 - 1.70 nmols AP5/mg wet weight of tissue.

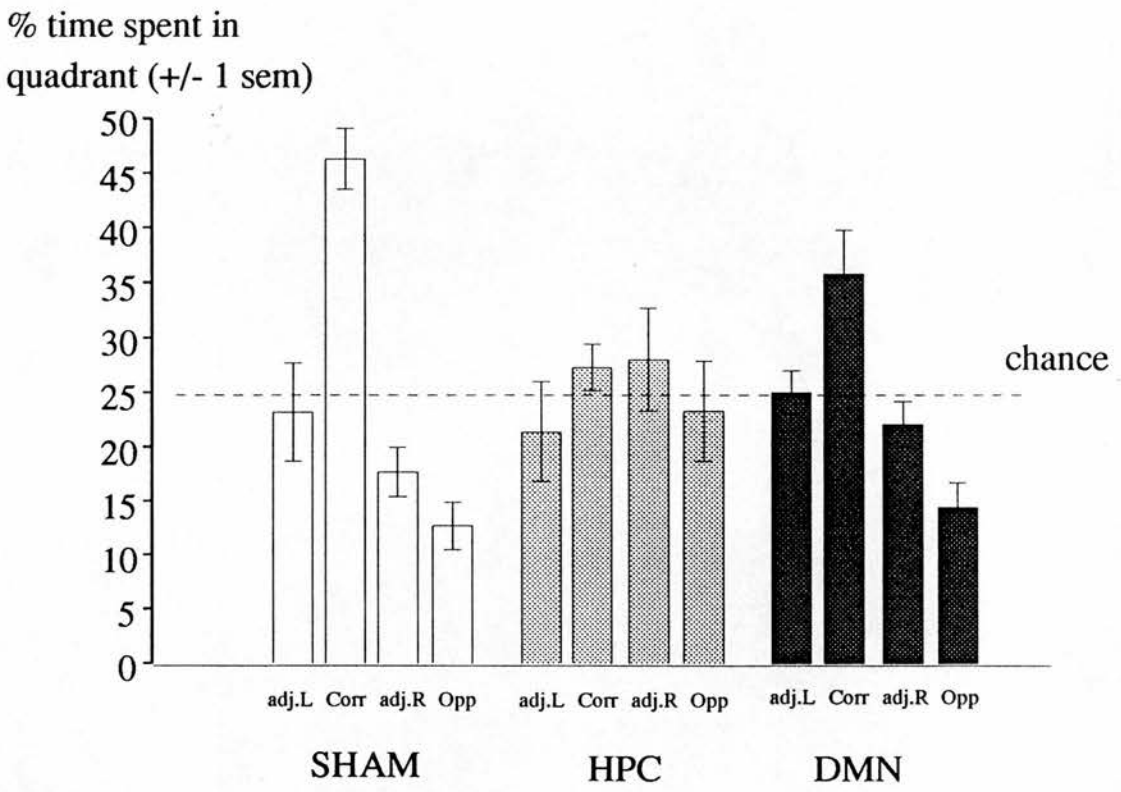


Fig 7.5

Bar graph showing the percentage time spent in each quadrant of the pool for each group during the spatial reference memory probe trial.
(adj. L. = quadrant adjacent to, and to the left of the quadrant to which the animals were trained; Corr = the 'correct' or training quadrant; adj. R. = the quadrant to the right of the 'correct' quadrant; Opp. = the quadrant opposite the correct quadrant.

Histology:

Hippocampal lesions

The extent of damage was at least 85-95% damage to the cell fields of the entire hippocampus (see fig. 7.6). In the majority of subjects, the entorhinal cortex and subiculum remained intact. Occasionally, as illustrated in fig 7.6, a small proportion of dentate gyrus was left undamaged, usually unilaterally. The extent of damage obtained was comparable to that reported in other studies (e.g. Davidson and Jarrard, 1989), and accords with the behavioural findings reported above with respect to performance on the spatial reference memory task.

DMN lesions

These lesions were in general large, destroying all but the most rostral and caudal components of the nucleus bilaterally. In most cases, the lesion fused across the centre (see fig 7.7) extending to and damaging other midline nuclei, including habenular and paraventricular nuclei. The extent of damage produced here was similar to that produced by lesions designated "large MD" by Slotnick and Kaneko (1981); and comparable to that reported by Slotnick and Risser (1990), Stokes and Best (1988), and Eichenbaum et al, 1980.

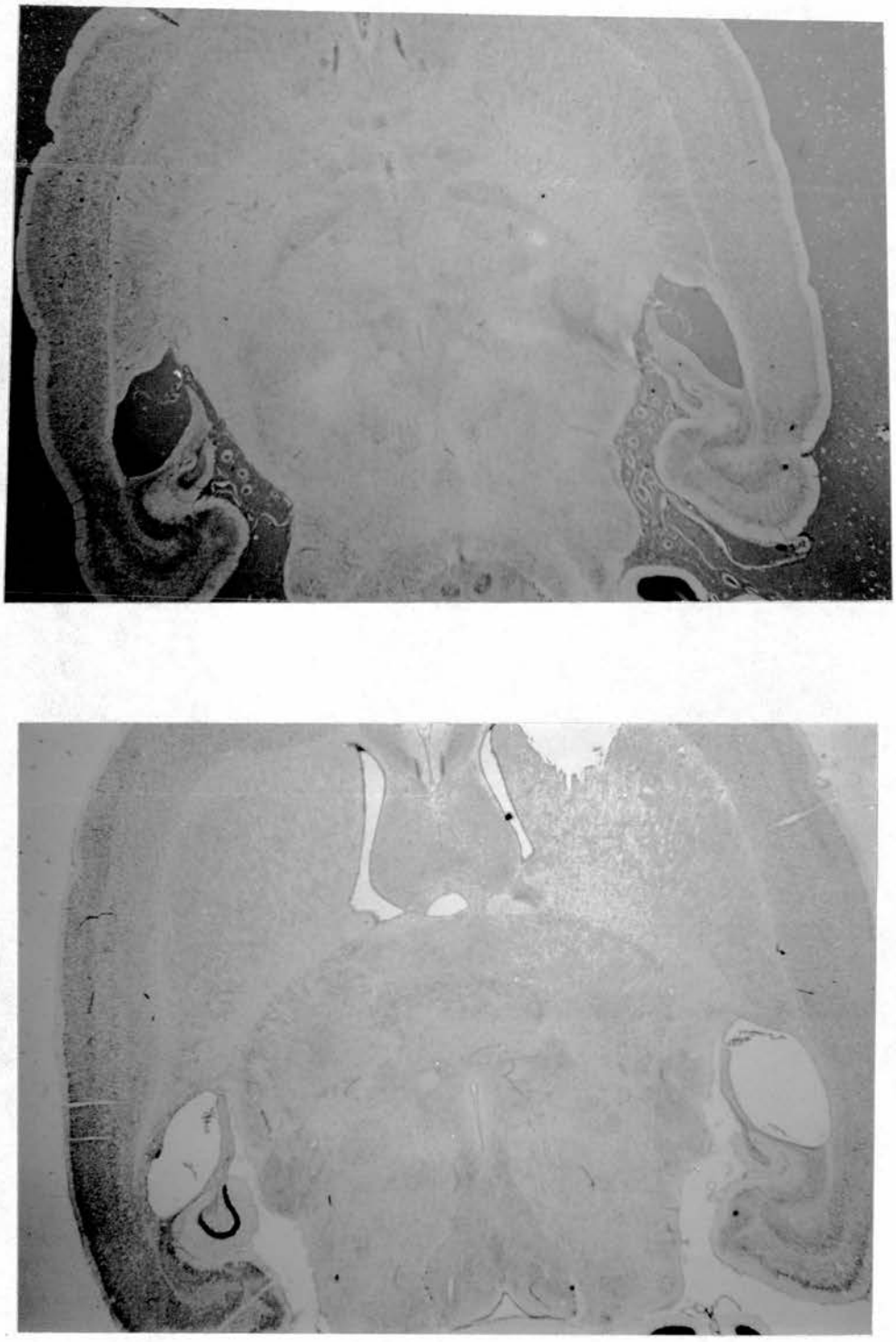


Fig 7.6

Photomicrographs showing representative hippocampal lesions

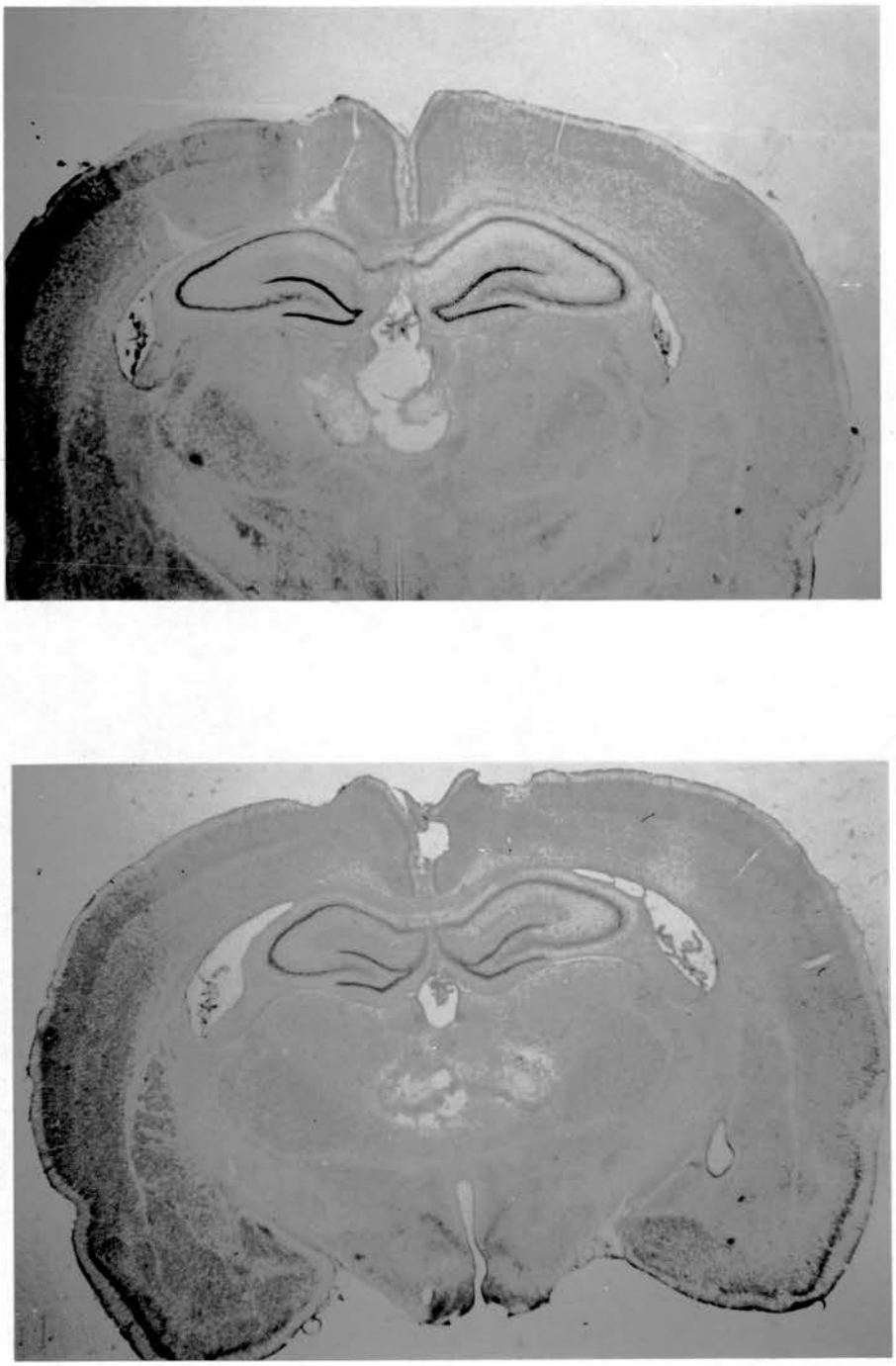
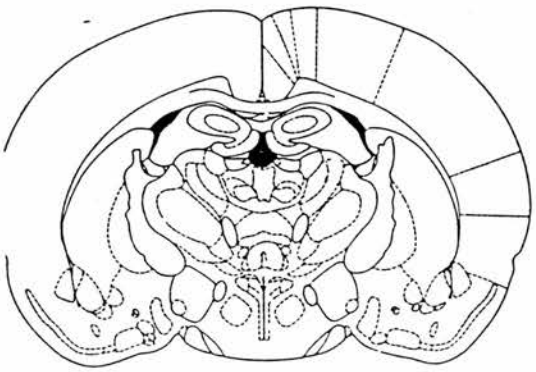


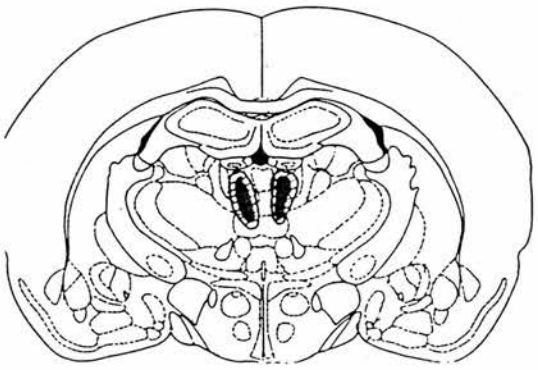
Fig 7.7

Photomicrographs showing representative DMN lesions

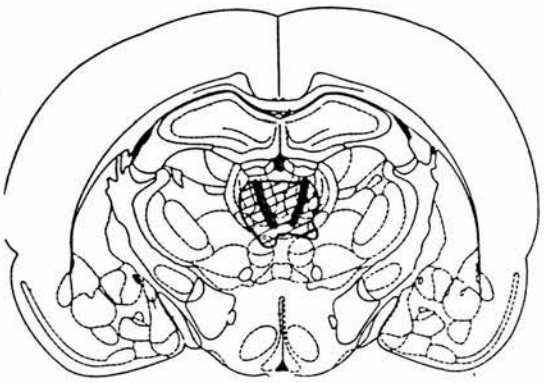
The following figures show diagrammatically the maximum (shaded area) and minimum extent (filled area) of the hippocampal and DMN lesions.



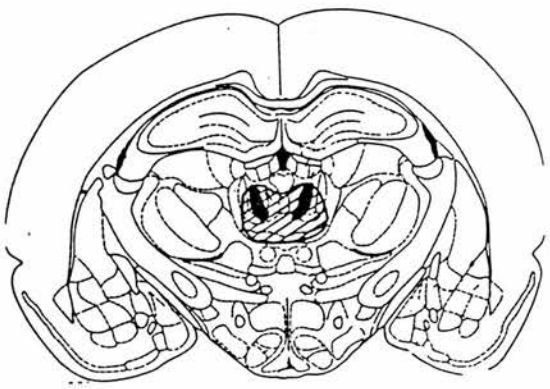
Bregma
-1.80mm



Bregma
-2.12mm

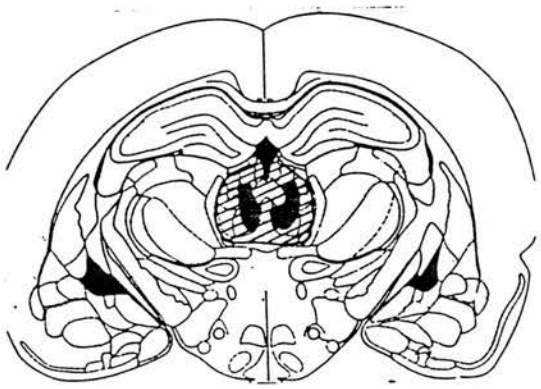


Bregma
-2.56mm

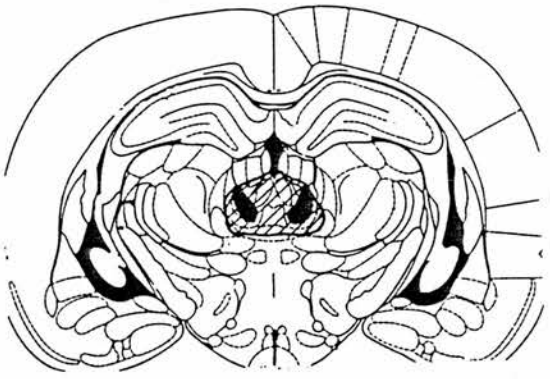


Bregma
-3.14mm

DMN Lesion



Bregma
-3.60mm



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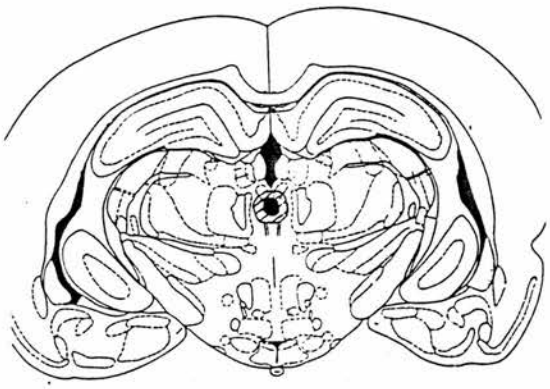
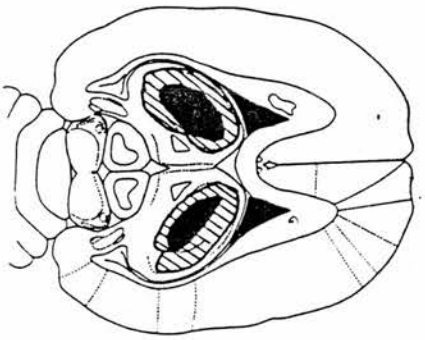
-3.80mm

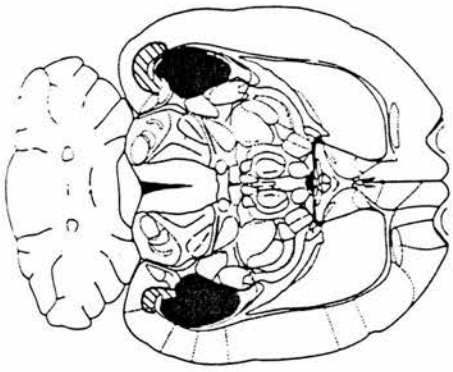


Bregma
-4.16mm

DMN Lesion



Bregma
-3.10mm



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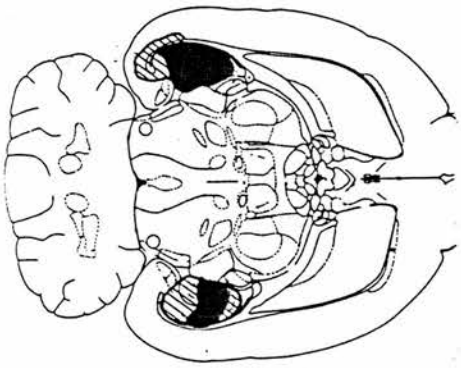

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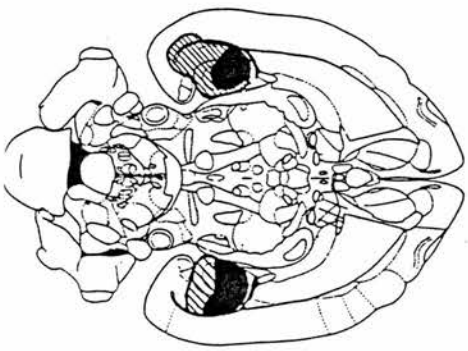

Bregma
-7.10mm

Hippocampal Lesion

Discussion:

Hippocampal lesions

While previous studies have examined the effects of indirect interference with the hippocampal system on olfactory discrimination learning - such as lesions to the fornix (Eichenbaum et al, 1986, 1988) entorhinal cortex (Staubli et al, 1984) or lateral olfactory tract (Slotnick and Risser, 1990) - this experiment focussed on the effects of selective lesions to the hippocampal formation itself. No evidence of either impairment or facilitation of the learning of novel discrimination problems was found. Furthermore, there was no evidence that hippocampal lesions caused forgetting of olfactory information over a 24 hour period. Although there was a non-significant trend towards faster reversal in the group with hippocampal lesions, analysis of early trials on the reversal problem indicates that the rats in this group initially responded to the previously correct cue (an index of 'remembering') as frequently as control animals, with initial scores below chance. Neither of these findings lend any weight to the notion that the learning of olfactory discriminations or the later remembering of such information in rats is dependent on intact hippocampal function. This stands in contrast to the finding of Eichenbaum et al (1988) that rats with fornix damage are impaired in learning 2 odour discrimination problems with simultaneously presented cues, but is in accord with the finding of Staubli et al (1984) of no impairment at short inter-trial intervals. Although the study does not address the effects of hippocampal system damage in discrimination problems in which cues are presented successively (go, no-go discrimination), it is worth noting Eichenbaum and his colleagues have reported two different findings despite identical experimental circumstances - that of facilitation of discrimination learning (Eichenbaum et al, 1988) and no effect (Eichenbaum et al, 1986). The claim

of Staubli et al (1984) that entorhinal lesions cause rapid forgetting of olfactory information by depriving the hippocampus of olfactory information cannot be sustained by the reversal findings outlined here with respect to damage to the hippocampal formation itself. Given that it is unlikely that rats form learning sets when presented with olfactory cues, as indicated in chapter 6, the claim that preserved learning of 'complex abstract rule' occurs in the face of fornix damage (Otto and Eichenbaum, 1991) is unlikely to be correct. The interpretation offered here is that olfactory discrimination learning, in common with discrimination learning via other sensory modalities in the rat, is largely unaffected by damage to the hippocampal system.

In contrast, the same hippocampally lesioned animals were markedly impaired with respect to sham lesioned and DMN lesioned subjects in a spatial reference memory task, performing essentially randomly on the probe trial. This confirmed that the lesions used were sufficient to cause deficits in tasks known to be reliably affected by hippocampal lesions. The learning deficit in the HPC group was therefore selective to spatial learning, and did not extend to olfactory discrimination learning.

AP5 Infusion

AP5 tissue levels were distributed in concentrations roughly in accordance with the distance of each brain area examined from the site of cannula implantation. Thus, the brain area closest to the site of implantation (the right hippocampus) had the highest mean concentration (0.92 nmols/mg) while the brain area furthest from the implantation site had the lowest mean concentration (0.44 nmols/mg). Even at the lowest tissue level (the lowest concentration obtained from a single brain area in an individual animal was 0.21 nmol/mg) the AP5 concentration should have been

sufficient to completely block hippocampal LTP (Morris, Davis and Butcher, 1990 p.98), although this was not be determined directly in the animals used here by electrophysiological techniques. The tissue level of AP5 required to block LTP in piriform cortex has not, to my knowledge, been determined.

Despite the evidence for mild sensori-motor disturbance, rats in group AP5 solved the 5 postoperative olfactory discriminations as quickly as animals in group SHAM and HPC. There was therefore no evidence of retarded acquisition of simultaneous 2-odour discrimination problems by rats in this group at the 60 second inter-trial interval used in this study. This is consistent with the findings of Staubli et al (1989), assuming that the odour intensity used in this experiment was comparable to, or greater than that used by Staubli and her colleagues. In addition, the learning of a reversed problem 24 hours after the original cue/reward configuration had been learned (problem 7) was unaffected, with AP5 infused rats initially responding to the previously correct (but now incorrect) cue and scoring below chance on trials 1-20. There was therefore no evidence for abnormally rapid forgetting in these animals over the 24 hour period, again consistent with Staubli et al's (1989) findings.

No attempt was made here to examine the effects of increasing the inter-trial interval, or diluting the odour concentration, on problem acquisition (as in Staubli et al, 1990). However, it should be noted that neither of these interventions were required to produce severe deficits in the acquisition of simultaneous 2-odour discriminations by rats with fornix lesions in Eichenbaum et al's (1988) report. In their study, the effects on odour problem acquisition were directly attributed to hippocampal system dysfunction. In the concentration used here, AP5 has been shown to have both significant effects on hippocampal physiology, completely blocking the artificial induction of LTP; and significant effects on behaviour,

impairing learning on a spatial task sensitive to hippocampal damage (Morris 1989). These effects of AP5 on both LTP induction and spatial learning have been shown to be closely correlated in a dose dependent manner (Davis, 1990). In this study, however, neither AP5 infusion, nor hippocampal lesions had any affect on odour discrimination learning in rats.

DMN lesions

Animals in Group DMN were mildly but significantly impaired across several of the 5 post-operative novel discrimination problems. They performed the reversal problem at a similar rate to the subjects in other groups. While both Staubli et al (1987b) and Slotnick and Kaneko (1981) have claimed that impairment on olfactory tasks seen in animals with DMN lesions represents some form of 'cognitive' disability, the data presented here is more consistent with a sensory deficit, akin to that described by Eichenbaum, Shedlack and Eckmann (1980) and indeed seen in human Korsakoff subjects with presumed DMN damage (Mair et al, 1980). Although the ANOVA detected both a significant Groups and Problems effect, no significant interaction was found. This implies that the animals improved across problems at a comparable rate, but tended to make more errors in the course of solving each problem with respect to the other groups. This might be expected if the rats suffered a (less than absolute) difficulty in discriminating one odour from the other. The fact that the rats performed the reversal problem at a similar rate to the other groups is also consistent with this view, given that all groups make more errors on this problem, thereby tending to obscure small differences, and that the DMN animals have by this stage experienced extended exposure to the cues giving them greater opportunity to successfully discriminate them.

Animals in group DMN were able to learn the spatial reference memory task, spending a significantly greater proportion of time in the "correct" quadrant on the "probe" trial. In comparison with group SHAM, however, they spent less time overall in the correct quadrant. A "double dissociation" (in which group HPC is impaired on spatial learning but not on olfactory learning; while group DMN is impaired on olfactory learning but not on spatial learning) cannot therefore be convincingly demonstrated in view of the superior performance of sham lesioned animals. Previous studies have produced conflicting results regarding the role of the DMN in spatial learning tasks. Kolb, Pittman, Sutherland and Whishaw (1982) found no effect of DMN lesions in rats on either radial maze or water maze spatial task performance. In contrast, Stokes and Best (1988), using a modified radial maze task such that visual cues were minimised by placing the maze in a large illuminated chamber lined with black cloth, found severe deficits in rats with DMN lesions. They argued that subtle deficits produced by DMN lesions could be more easily demonstrated in a "less enriched" spatial environment. Unfortunately, both control and DMN animals appeared to solve this version of the radial maze task using "response patterning" (e.g. by turning in a consistent direction and choosing every third arm, rather than attending to 'extramaze cues') to a considerable extent (Stokes and Best, 1988 p. 296-297). Control and DMN animals were observed to differ with respect to response patterning, and the impairment seen in the lesioned animals may therefore not have reflected an impairment of spatial learning per se.

Control Animals

Group SHAM was used to further examine the issue of learning set formation. As detailed in chapter 6, it was found that although rats performing a series of novel olfactory discriminations progressively improved across problems, their performance

on trials 2-5 of each problem was little above chance, even on the last problem of the series (see chapter 6, p.112-113). Group SHAM in the current experiment also performed a series of novel olfactory problems; and it was therefore of interest to see if the same result was obtained. The rats in all groups in this experiment showed significant progressive improvement across problems in terms of errors made in reaching criterion, but group SHAM scored little above chance on the early trials of novel problems, and did not improve across problems in this respect. This finding is consistent with the earlier observation.

Control Task

1 sham animal, and 1 animal from the AP5 group reached criterion on the control task. To check whether this criterion level performance was a chance finding, the 2 animals were trained again on the control task. They did not reach criterion over a further 32 trials and performed at chance levels. In contrast, animals trained beyond criterion on regular 2-odour discriminations were observed to perform with greater than 90% accuracy subsequent to criterion performance (see chapter 6, p.111). Taken together, the latter finding suggests that criterion performance on "regular" problems reflects problem "solution", while that shown by 2 animals on the control task, a chance finding.

A computer program was therefore developed to determine the likelihood of animals reaching criterion by chance across 31 trial blocks, using a random number generator to simulate random performance on a 2 choice task with a 50% chance of a correct choice. The computer recorded consecutively correct (chance) responses, and terminated sessions if the criterion of 8 consecutively correct responses was achieved and began a new session, or began a new session after 31 trials if not. In this way,

the experimental schedule was accurately modelled. The model continuously calculated the ratio of criterion scores achieved to sessions completed. The simulation was run for 36,275,620 trials. In the course of this, 1,180,419 sessions were completed (a 'completed' session being either 31 simulated trials, or a series of trials totalling less than 31 in which 8 consecutively correct responses were recorded). Criterion was achieved (by chance) 27,608 times. The ratio of criterion scores achieved to sessions completed was 0.023. Criterion was therefore achieved by chance every 43.4 sessions on average. Given that in this experiment a total of 70 (35 x 2) control task sessions were run, the computer simulation would predict that criterion should be achieved by chance approximately 1.6 times (0.023 x 70). Criterion performance on the control task was actually achieved twice, and therefore occurred no more frequently (and no less frequently) than would be expected by chance.

Summary:

In this study neither hippocampal lesions nor AP5 infusion had any effect the acquisition or retention of olfactory information in rats. The DMN lesion significantly retarded acquisition on some problems, but the deficit was mild. Only hippocampally lesioned animals were unable to learn the spatial reference memory task.

CHAPTER 8

Conclusion

Initial Studies:

The crucial experiments conducted in this project were those concerned with ensuring that the automated apparatus actually tested olfactory discrimination learning in rats. Given that rapid learning and progressive improvement across novel olfactory discrimination problems were considered central findings in olfactory 'learning set' discrimination studies, it seemed essential that some logical manipulation should demonstrate that rats performing in such a way were indeed attending to the odour differences under experimental control and not to other cues. The control task, in which identical odours were presented for discrimination, proved essential to the interpretation of all subsequent findings. In the study of 'learning set formation', the detection and elimination of unintended cues to reward in earlier versions of the apparatus radically changed the nature of the results obtained. In particular, the reported ability of rats to rapidly form olfactory 'reversal sets' (Slotnick and Kaneko, 1981) was apparently confirmed in early versions of the apparatus, but challenged following modifications to remove the uncontrolled cues to reward. With hindsight, it is difficult to evaluate findings produced using similar apparatus in studies conducted by other research groups when these relevant control data are not reported. It was established in the preliminary experiments detailed here 1) that the apparatus had to be tested repeatedly, 2) that only well trained animals should be used to detect unintended cues, 3) that such cues could not be detected by casual human inspection, and 4) that a crucial element in the design of the apparatus was to ensure that odourised airflow was never permitted to contaminate valve

systems. Other investigators have not systematically examined these factors as far as can be determined from published work. Control experiments have generally not been reported, save in 2 studies where the tasks were either used only on naive animals (Eichenbaum et al, 1980), or only on a small proportion of subjects (Slotnick, 1981). In all published reports describing automated apparatus, it appears that odour delivery systems were constructed such that odourised air flowed through fixed valve systems (Eichenbaum et al, 1986; Staubli et al, 1984; Slotnick and Kaneko, 1981).

I was very kindly given the opportunity to test the apparatus used by Howard Eichenbaum in his recent olfactory experiments, when he visited Edinburgh University in 1989. The test was conducted as follows: Professor Eichenbaum generously agreed to instruct his research assistant in Boston (via electronic mail) to train 4 '*experienced*' rats on an 'identical odour' discrimination, in the manner used in the studies reported here. The research assistant was not informed of the purpose of the experiment. She reported back 48 hours later stating that "the rats' performance was remarkable - they could even discriminate identical odours." At the time, Professor Eichenbaum acknowledged that his apparatus must contain unintended cues to reward in the light of this finding. To Professor Eichenbaum's credit, he immediately returned to Boston from Edinburgh to make changes to his equipment, and it is important to note that at a visit some 6 months later it was clear that his apparatus was operating satisfactorily as a consequence of these changes.

It is likely, however, that previously reported rodent olfactory learning studies are potentially unreliable on this account, and must be reconsidered in the light of these findings. This is not mere speculation based on observations restricted to my own apparatus - at least one other set of apparatus used in published rodent olfactory discrimination studies has been demonstrated, as a direct consequence of this work, to be flawed exactly as predicted.

Learning Sets:

The use of additional control groups also played an important part in the investigation of rodent 'learning set' formation detailed in chapter 6. Drawing on the work of Kamil et al (1977) on 'higher order' learning in blue jays, 2 groups were chosen to control for 'non-specific' elements (as opposed to the acquisition of higher order, abstract strategies) which might account for progressive improvement in serial olfactory discrimination learning. The reversal group (which clearly failed to develop an abstract strategy) and the single discrimination group (which had no opportunity to develop an abstract strategy) performed as well on novel problems as a group of animals previously trained on a series of novel olfactory problems, casting doubt on the much discussed notion that the progressive improvement and rapid learning seen in the novel problem group was a consequence of 'learning set' formation. In addition, the fact that the rats performed at chance on the early trials of novel problems, and did not improve in this respect across a series of problems, further extends the evidence that 'learning set' formation is unlikely to be a feature of rodent olfactory discrimination learning. Despite the fact that these latter measures are conventionally used in 'learning set' studies, they had not previously been reported in relation to rodent olfactory learning. It now seems clear that the question of whether or not rats form 'learning sets' when tested with olfactory cues had not (as

proposed in the introduction to this thesis) been fully evaluated. These findings have important implications for the interpretation of studies which claim to examine the effects of brain lesions on 'higher order' olfactory learning in rats (e.g. Eichenbaum et al, 1986, 1988; Slotnick and Kaneko, 1981).

Overall, it seems reasonable to conclude that there is no evidence that olfactory discrimination learning in rats need be qualitatively distinguished from instrumental learning as studied via other sensory modalities. The experiments reported here do not, however, address the possibility that abstract strategies may be developed with much more extensive training, or via other procedures. This is also true, of course, of visual discrimination learning in analogous rodent 'learning set' experiments - with extended training (perhaps thousands of discrimination problems) rats may eventually form learning sets via any sensory modality. The more modest aim of this thesis was to examine pre-existing claims that rats could form "complete" (Eichenbaum et al, 1986) olfactory learning sets after exposure to only a few discrimination problems. After all, it was this report of *rapid* 'learning set' acquisition which underpinned the claims that rodent olfactory capabilities might prove useful to an animal model of human amnesia. In fact, the performance of the rats trained on serial reversal olfactory problems suggests that rodent olfactory learning is remarkably inflexible and perhaps even 'primitive' when compared with rodent visual learning - precisely the opposite conclusion to that drawn in other studies. Aside from the reversal learning finding, in which rats failed to show progressive improvement across serial reversal problems, it is *not* the case that the findings on which these conclusions are based conflict with results obtained by other research groups. In general, the results reported here replicate those obtained in earlier studies. The additional control groups used in this study permitted the development of alternative (and contrary) interpretations to those offered in earlier

work.

Regarding the modelling of human amnesia, 3 related points can be made on the basis of the above finding. First, from a psychological point of view, there would (now) seem to be no more reason for suspecting olfactory discrimination to be sensitive to hippocampal lesions than any other form of discrimination learning; second, a material analogy between 'higher order olfactory learning' in rats and the spared 'cognitive rule' learning in human amnesics (as proposed by Eichenbaum et al, 1986; Lynch, 1986; and Staubli et al, 1984, 1987a) is difficult to sustain; and third, the notion that the learning of individual olfactory cues by rats is in some way akin to 'explicit' or 'episodic memory' can no longer be supported by the claim that rats rapidly form olfactory learning sets (and must therefore use the outcome of individual trial 'episodes' to determine performance trial by trial).

Lesion/Pharmacological Studies:

Viewed from this perspective, the results of the lesion/pharmacological studies are of particular interest. Neither AP5 nor hippocampal lesions had a significant effect on the acquisition, or 24 hour retention of olfactory information. These findings are broadly consistent with the early studies of Allen (1941), and later work by Slotnick and Kaneko (1981) and Slotnick and Risser (1989), in which limbic olfactory targets were de-afferented by bilateral destruction of the lateral olfactory tract and piriform/entorhinal cortex. The results are, however, inconsistent with the work of Staubli et al (1984), in which rapid forgetting of olfactory information over a period of 1 hour was claimed to occur after hippocampal denervation; and the work of Eichenbaum et al (1988) in which simultaneous 2-odour discrimination performance was reported to be seriously impaired following lesions to the fornix. In weighing

the evidence, it should be noted that these latter 2 studies are not only inconsistent with my own results and those from the other studies mentioned above, but they are also inconsistent with one another. Furthermore, as pointed out in chapter 3 (p.61-62), the findings from Eichenbaum's group have varied from experiment to experiment (Eichenbaum et al, 1986 v. Eichenbaum et al, 1988) despite identical conditions; and their apparatus has been shown, at least for a period, to be potentially unreliable.

The reported effects of lesions to the dorso-medial nucleus of the thalamus on olfactory learning in rats are largely consistent across published studies. Variable impairment has been observed in almost all reports. The argument that this impairment probably represents a perceptual rather than cognitive deficit has already been outlined (see chapter 3 pp. 50-56; and chapter 7, p.145). It should be noted that even if one were to accept the cognitive interpretations of deficits in olfactory learning after DMN damage offered by Slotnick and Kaneko (1981) and Staubli et al (1987) (which *depend* on the claim that rats form olfactory learning sets) then it would have to be concluded that the nature of the impairment described in rats is exactly the opposite to that described in human diencephalic amnesia; while the perceptual interpretation offered in this thesis accords with the (uncontroversial) perceptual olfactory impairment demonstrated in humans with Korsakoff's syndrome.

The suggestion that olfactory learning in the rat is in some way akin to explicit, episodic, data-based learning in humans (Lynch, 1986; Staubli et al, 1987a) is not supported by the findings reported here. Olfactory learning neither results in the development of a "win-stay, lose-shift" strategy, nor is it sensitive to direct, selective hippocampal lesions.

Given that the 2 elements required for the development of an animal model of human amnesia could not be demonstrated in lesioned rats (1: failure in the acquisition, or rapid forgetting, of new material and 2: spared 'cognitive rule learning') it must be concluded that the olfactory system in the rat cannot be considered an "ideal model system for the study of the biology of memory" as claimed by Otto and Eichenbaum (1991).

The Use of Olfactory Cues in the Study of Animal Learning:

There are many technical and theoretical disadvantages to the use of olfactory cues in animal learning experiments. The cues are difficult to prepare and deliver in a controlled and reliable fashion. They must be freshly made as their nature and concentration may vary over time. Odour quality cannot yet be quantified nor varied systematically: while visual cues such as elipses can be made more or less circular, there is no known way of making 'strawberry' a bit more 'minty'. Adding one odour to another may produce a completely different odour rather than some balanced combination (this is how deodorants work!) and they may even react chemically with one another. Human appreciation of odour is poor, making the evaluation and selection of candidate odour cues for learning experiments less than easy - human perception of the hedonic qualities of different odours, for example, may be very

different from that of other species. Matching odours for intensity may prove equally difficult on this account. Similarly, generalisation from one odour to another cannot be easily predicted (for rats by humans) without prior testing, and careful counter-balancing is required (but rarely used in other studies) where multiple cues are used.

It has been suggested that despite the above difficulties, one special advantage is that many different potential odour cues are available, and may be used in much the same way as 'junk' visual objects are in primate learning studies (Staubli, 1987a; Otto and Eichenbaum, 1990). In my experience, however, maintaining a stock different odour pairs which are readily and reliably obtainable and which do not rapidly perish, is a difficult and expensive task. I suspect that this is reflected in the fact that most studies have actually used very few different cues. Eichenbaum and colleagues used only (the same) 3 pairs in recent published studies, while in Staubli et al's studies (e.g. Staubli et al, 1987b) 8 odour problems are reported, but, in fact, only 4 pairs of cues are used, twice in succession.

It should be appreciated that current understanding of the olfactory system is limited, especially when compared with existing knowledge of, for example, visual processing. The precise nature of olfactory stimuli is uncertain, the neural transduction process is not known, and as a consequence, tests of olfactory function in both human and non-human subjects are often crude, arbitrary and poorly standardised.

In considering the use of olfactory cues in animal learning studies, it is important to decide whether potential advantages outweigh the many disadvantages. The study of olfactory processing *per se* obviously requires the use of olfactory cues, but their use in the study of learning generally can only be recommended with caution. While olfactory discrimination in rats may be rapid, the numerous technical difficulties (not least the emergence of unintended contaminatory cues which are difficult to detect), lack of precise control of stimulus dimensions, and the fact that olfactory learning in rats appears little different from other forms of simple discrimination learning, may preclude their routine use.

Further Studies:

While generally concluding that olfactory learning in the rat may not prove as useful a model system for the study of human amnesia as had been hoped, many specific issues remain to be addressed. As noted above, it is not clear whether rats could, with extended training or via other procedures, acquire an olfactory learning set. It would be interesting, though extremely time consuming, to repeat the study outlined in chapter 6 using hundreds of olfactory problems. Only when this had been done could a true comparison with primate learning be made. In a similar vein, it would be of interest to examine rats' capacities in learning olfactory matching tasks. The failure of rats to show progressive improvement across a series of reversal problems was surprising, and it may be of value to explore further the possibility that olfactory cue-reward relationships are relatively inflexible once formed.

No attempt was made here to replicate Staubli et al's (1989) finding that AP5 infusion caused an impairment in olfactory discrimination problem acquisition at long inter-trial intervals if low-intensity odours were used. Preliminary work,

conducted by Robert Beigler and Richard Morris, suggests that AP5 infused rats may actually be *perceptually* impaired when tested with low intensity odours, and show a modest deficit even at short inter-trial intervals (personal communication). My own preliminary experiments examining the effects of increasing the inter-trial interval in olfactory problem acquisition by hippocampally lesioned rats have so far produced negative results. Given the fact that lesions to the lateral olfactory tract (Slotnick and Risser, 1990) and the hippocampus itself produce no effect on rodent olfactory discrimination learning, one cannot help but wonder what the olfactory projections to lateral entorhinal cortex and hippocampus actually 'do'. One possibility, suggested by Mark Good and Richard Morris (Department of Pharmacology, University of Edinburgh), is that they may be necessary for the learning of 'olfactory context'. I await their results with interest.

CONCLUDING SUMMARY

Failure to Demonstrate Learning Set Formation

Forty years after the rhinencephalic concept was first rejected (Brodal, 1947), it has been proposed that olfaction may nonetheless be an ideal stimulus modality to study hippocampal function in rodents and to model certain restricted features of human amnesia. The rationale behind this approach has been couched in terms of rats' "superb" olfactory learning capacities (Eichenbaum et al, 1986) and the "primate-like" abstract learning it was thought could be accomplished in this modality (Slotnick and Katz, 1974). It has also been claimed that, in allegedly forming an olfactory "learning set", rats acquire both "procedural and knowledge memory" in one and the same task (Staubli et al, 1987a, p.757). As studies of human amnesia have pointed to a dissociation between these two types of memory, it has been suggested that rodent olfactory discrimination learning, in apparently displaying both types simultaneously, affords a means of modelling in rats this specific aspect of human amnesia (Staubli et al, 1987a).

The data presented here, however, indicate that there is little reason to distinguish olfactory discrimination learning from simple instrumental learning in other sensory modalities. It is neither faster nor more flexible. The notion that the learning of individual odour-reward and odour-nonreward associations by rats is in some way akin to 'explicit', 'episodic' or 'knowledge' memory (Staubli et al, 1987a) is difficult to reconcile with our finding that learning set formation does not actually occur. It is

important to be precise in the logic here. Had it been the case that rats did rapidly form olfactory learning sets, it would have implied that rats can and do use this form of memory to recall events from trial to trial. The discovery that progressive improvement in rate of learning does not depend on the acquisition of a higher order strategy akin to a cognitive skill indicates that Staubli et al's (1987a) assertion that procedural *and* knowledge memory are both acquired in olfactory learning is unlikely to be correct. This discovery does not, in fact, rule out the possibility that olfactory associations are remembered in an explicit or episodic manner. It does, however, indicate that this intriguing idea must be tested in a different way - such as with delayed non-matching to sample which is less ambiguous a test of event memory than a discrimination task. The possibility that olfactory projections to the hippocampus via the lateral olfactory tract and lateral entorhinal cortex are a potent source of contextual information for assisting in the memory retrieval of other events should also be considered. But these ideas are radically different from supposing that olfactory discrimination learning offers the best route for investigating higher cognitive processes and should be recognised as such.

Inflexibility of Odour Reward Associations

The fact that the proposed characteristics of rodent olfactory learning fail to meet the criteria specified for the study and development of animal analogues of declarative or explicit memory is compounded by evidence that olfaction may, in fact, be a particularly poor modality on which to base rodent investigations in any case. The failure of the group performing the reversal series to develop progressive improvement is of particular interest in this regard. In pilot experiments rats developed what appeared at first sight to be a 'reversal set' after very few reversal

problems. Unfortunately, when tested on the 'discrimination' between identical odours, it became clear that the rats were able to solve the task by using unintended cues to reward which were not under experimental control. When these cues were eliminated by improving the design of our apparatus, further groups of rats no longer showed improvement from reversal to reversal.

As noted earlier, an important point of comparison is with brightness discrimination reversal learning in rats (Mackintosh et al, 1968) which shows remarkably rapid progressive improvement and the development of proactive interference between stimulus-reward and stimulus-nonreward associations, but which, consistent with the analysis presented so far, does not develop by virtue of the acquisition of any abstract higher order strategy. Successive olfactory reversals are characterised by long periods in which the rats persist in selecting the formerly rewarded odour with no build up of proactive interference between odour-reward and odour-nonreward associations across reversals. In so far as satisfactory comparisons can be drawn between these two studies in two different sensory modalities, it seems that olfactory learning is, if anything, *less* flexible than brightness discrimination learning. As "flexibility" is the hallmark of those forms of memory in which facts can be related one to another (Squire, L.R., 1992, Psychol. Rev. 99, 195-231) this inflexibility constitutes a further reason to be suspicious that olfactory learning is a suitable model of, or provides privileged access to, such forms of memory.

The Role of the Hippocampus in Rodent Olfactory Learning

This is the only study to examine the effects of selective, bilateral ibotenic acid hippocampal lesions on olfactory discrimination learning in the rat. No evidence of impaired simultaneous odour discrimination learning or rapid forgetting was found. Lesioned rats failed, however, to learn a task known to be sensitive to hippocampal damage. The insensitivity of olfactory learning to hippocampal lesions is consistent with the formulation outlined above, and entirely inconsistent with the proposal that the study of rodent olfactory learning constitutes an ideal model system for either investigating hippocampal function or developing rodent analogues of human amnesia (Otto and Eichenbaum, 1991).

Implications for Non-Human Models of Amnesia

In light of the above, it is concluded specifically that the rodent olfactory capacity *per se* is not a useful target system for the modelling of human amnesia, both in terms of the kind of cognitive function supported, and the effects of hippocampal damage on olfactory learning.

The more general issue of whether learning set formation provides a key to the examination of analogues of episodic and procedural learning in non-human species remains a subject for debate. Given that learning set formation was not demonstrated in this study, the issue could not be addressed experimentally here. This does not imply that the general approach, in itself, lacks validity. The theoretical notion that learning set formation may be of value (Staubli et al, 1987a; Slotnick and Kaneko,

1981; Eichenbaum et al, 1986; Lynch, 1986) rests on the formulations proposed by Restle (1958) and Levine (1959), supported by the experimental work of Schusterman (1962), Bessemer and Stollnitz (1971), and Kamil (1977) outlined in the introductory chapters. On this view, the principles which underlie the proposal that animals which have acquired a learning set use (in concert) analogues of procedural and episodic memory to solve further discrimination problems in a single trial, are generally in accord with the widely and successfully used primate recognition memory tests which employ a match or non-match rule with trial unique cues, in the sense that a rule or procedure must be learned which operates on material which changes across trials. The fact that learning set performance eventually deteriorates even in normal subjects when the intertrial interval is sufficiently long (Bessemer and Stollnitz, 1971) supports the contention that information acquired in the course of discrimination learning following learning set acquisition is processed in a different manner than that which occurs in simple discrimination learning (which is resistant to ITI effects). This difference in the characteristics of discrimination learning dependant on whether or not a learning set has been acquired underpins the rationale for comparing the performance of animals trained on a single problem continuously, with the performance of animals trained on a series of novel problems (as outlined in the experiments detailed in chapter 6). Were it to have been the case that learning set formation had occurred as claimed (Eichenbaum et al, 1986; Slotnick and Kaneko, 1981; Staubli et al, 1987a; Lynch, 1986) the question would then arise as to whether the established psychological differences in discrimination learning following learning set formation *versus* simple discrimination learning would be mirrored by a biological as well as psychological distinction. Assuming successful learning set formation in the species of interest, two predictions concerning the effects of limbic damage might be be made: first,

subjects with limbic damage would only exhibit successful performance at shorter inter-trial intervals than controls, indicating that while the 'win-stay, lose shift' rule could be successfully applied by lesioned animals (spared procedural learning), the individual 'episodes' guiding performance (the events and outcomes of individual trials) would be ineffective (impaired episodic memory); and second, that lesioned subjects would be unable to acquire a learning set at the longest intertrial intervals sustaining control performance. As far as I am aware, these hypotheses remain untested, even in primates.

Appendix

The following tables summarise the rodent olfactory learning literature, with particular reference to the use of control task procedures. Note first that such procedures are only rarely recorded, and second that in the studies by Eichenbaum's group the *serial* reversal learning findings (which were most affected by control task procedures in this study) were not examined. Nonetheless, it is difficult to be confident about the reliability of any study conducted without the benefit of a control procedure to ensure that cues are under experimental control. For example, the effects of fornix lesions reported by this research group change between 1986 (no effect on odour discrimination learning) and 1988 (facilitation of odour discrimination learning). The study to which the control findings are most relevant, however, is that of Slotnick and Kaneko (1981) in which apparent reversal set formation is described.

Author	Task	Lesion	Outcome	Control Tasks
Swann, 1934	T-maze odour trail learning	Bilateral temporal lobe excision	No effect of lesion	None recorded
Allen, 1941	Conditioned foreleg response (dogs) Meat detection (dogs)	Bilateral extirpation pyriform-amygdaloid areas, surrounding neocortex + hippocampus	No effect on detection, discrimination disrupted (subjects unable to withold responding - see text)	None recorded
Jennings and Keefer, 1965	Simultaneous 2-odour discrimination	None	Interproblem improvement	'Naive' control group run in apparatus without odour cues present
Slotnick and Katz, 1974	Go/No-go 2-odour discrimination	None	Interproblem improvement	4/12 subjects tested either without airflow, or with identical odours
Eichenbaum et al, 1980	Go/No-go 2-odour discrimination	DMN RS MW	Discrimination impaired Discrimination impaired No effect of lesion	'Naive' subjects trained without odours
Slotnick and Kaneko, 1981	Go/No-go 2-odour discrimination	DMN LOT	No effect on initial discrimination - reversal learning impaired No effect of lesion	None recorded
Staubli et al, 1984	Simultaneous 2-odour discrimination Reversal	Entorhinal cortex	Discrimination impaired at long (>3 min ITI), unaffected at short Reversal facilitated over 24 hour interval	None recorded

Author	Task	Lesion	Outcome	Control Tasks
Eichenbaum et al, 1985 /86	Go/No-go 2-odour discrimination	Fornix	No effect of lesion	None recorded
	Reversal		Reversal facilitation	
Staubli et al. 1987b	Simultaneous 2 or 3-odour discrimination	DMN Pyriform cortex	Learning impaired on early post-operative problems, improving with training	None recorded
Eichenbaum et al, 1988	Go/No-go 2-odour discrimination	Fornix	Rate of learning facilitated	None recorded
	Simultaneous 2-odour discrimination		Learning impaired (at ITI of 10 secs max)	
Staubli et al, 1989	Simultaneous 2-odour discrimination	AP5 infusion	Learning impaired with 'weak' odours at long (>3 min) interval	None recorded
	Reversal		Reversal unaffected	
Slotnick and Risser, 1990	8 odour pair 'memory' task	DMN	1 2-odour problem, and reversal problem impaired	None recorded
	Go/no-go 2-odour discrimination		'Memory' task unimpaired	
	Reversal discrimination	LOT	No effect of lesion	
		Combined DMN/LOT	8-odour pair memory test, some 2-odour problems, and reversal impaired	

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