Perirhinal cortex and the neural basis of object memory in the rat

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Abstract: Perirhinal Cortex and the neural basis of object memory in the rat.
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This thesis aimed to investigate the role of the perirhinal cortex in object memory in the rat. The first experiment tested the hypothesis that the perirhinal cortex is critical to memory for relationships between objects by testing postoperative learning of novel visual-visual stimulus associations following lesions of the perirhinal cortex. The hypothesis was not supported: postoperative performance was not impaired. Experiments 3.1-3.4 tested the hypothesis that perirhinal cortex is crucial to the integration of multiple features into a representation of an object using spontaneous object recognition with either reconfigured objects or multiple objects. The hypothesis was supported: perirhinal lesions caused disproportionate impairment on tasks involving feature ambiguity. Experiments 4.1-4.7 investigated the effects of, perirhinal, postrhinal or fornix lesions on aspects of memory for object-context associations. The hypothesis that postrhinal and fornix lesioned animals would be more impaired than perirhinal animals was confirmed. Postrhinal lesions impaired memory for object-context associations, as, less severely, did fornix lesions; perirhinal lesions impaired memory when another object was used as the context. Experiment 5.1 used a novel model for episodic-like memory and tested the hypothesis that postrhinal or fornix, but not perirhinal lesions would cause impairment. One of these predictions was supported: fornix but not postrhinal or perirhinal lesions caused severe impairment of episodic-like memory. The fornix impairment was not due to an impairment of memory for object-place associations (experiment 5.2). Finally, experiments 6.1-6.7 investigated the possible function of L-type calcium channels in perirhinal cortex. The dihydropyridine nimodipine was successfully used to reverse the effects of the muscarinic antagonist scopolamine on the spontaneous object recognition task.

It is concluded that perirhinal lesions in the rat result in impairments of memory which involve the processing of objects and the relation of their constituent features to each other. They do not impair memory for the association either of distinct objects or of objects and background contexts or locations. This is contrasted with the impairment of memory for object-in-context which results from postrhinal lesions and the impairment of episodic-like memory which results from fornix lesions. The importance of the cholinergic system in object recognition is confirmed and the importance of L-type calcium channels to such memory is suggested.

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Chapter 1: Introduction

1.1: The Medial Temporal Lobe and Memory.

This thesis will consider the role of medial temporal lobe structures in memory. It will concentrate on the contributions of the perirhinal and postrhinal cortices to aspects of object memory, including the relationship of an object to the wider environment. Where appropriate these contributions will be compared with that of the hippocampal formation. This chapter will outline the anatomy of the structures under consideration and will also examine the development of research into the functions of these structures and some of the aspects of memory to which they may contribute.

The role of the medial temporal lobe in memory became a major focus of research following the use of psychosurgery as a therapeutic tool. Penfield (1958) found that stimulation of the exposed temporal lobe produced vivid recollection of past events in his conscious epileptic patients. Conversely, Bickford, Mulder, Dodge & Svien et al. (1958) found that stimulation of the mid-temporal gyrus could induce transitory amnesia which was both retrograde and anterograde to the stimulation. The employment of bilateral temporal lobe resection for the treatment of psychosis or epilepsy was found to cause severe anterograde amnesia where the hippocampus proper and the hippocampal gyrus constituted part of the tissue removed (Scoville & Milner, 1957). A particularly severe example of this effect was provided by patient HM who had undergone a radical bilateral temporal lobe resection in order to relieve his intractable epilepsy. The notes of the operating surgeon indicate the removal of the anterior two thirds of the hippocampus proper, the amygdala, the rhinal and parahippocampal cortices and the hippocampal gyrus. Subsequent psychological testing of HM showed severe anterograde and partial retrograde amnesias, the latter extending for three years previous to the surgery. With the advent of MRI scanning it has become possible to examine the exact extent of HM's lesion. Corkin, Amaral, Gonzalez & Johnson et al. (1997) found that it does not appear to be as extensive as originally thought, as there is some intact tissue in the hippocampus and the perirhinal and parahippocampal cortices. This would indicate that heavy cell loss in these structures is sufficient to cause severe anterograde amnesia in humans.

Further studies of HM have revealed intact learning and memory for motor and skill based tasks (Corkin, 1984) as opposed to his dense amnesia for episodic experience. Studies on other amnesic patient showed residual ability to form certain types of new memories, (Weiskrantz & Warrington 1979; Brooks & Baddeley 1976; Warrington & Weiskrantz 1970). Such findings suggested that memory could not be considered as a single entity resulting from a single global system with integrated function (Squire, 1992; Tulving, 1983; Weiskrantz 1987). Work using animal models of memory has suggested that both memory and the memory system within the medial temporal lobe are capable of division into largely independent sub-systems. In order to consider this further it is necessary to consider some of the constituent structures of the medial temporal memory system.

The amnesia seen in HM was attributed to the ablation of his hippocampus partly because of previous reports of memory impairments among patients with bilateral hippocampal damage (Von Bechterev; Glees & Griffith, cited in Mumby, 2001) and partly because of the apparent correlation between the extent of hippocampal damage and severity of memory impairment among patients with bilateral medial temporal lobe resections (Scoville & Milner, 1957). However, evidence began to accumulate which cast doubt upon the interpretation of memory deficits as resulting from hippocampal damage. For example, Gol & Faibish (1967) found that there was a greater correlation between the extent of neocortical damage and the severity of amnesia than there was between amnesic severity and the extent of hippocampal damage. Horel (1978) argued against the hippocampus-centred view of memory on several grounds. First, he claimed that the extent of damage to the white matter of the temporal lobe known as the temporal stem (which necessarily increased with the extent of hippocampal resection) was partly responsible for the impairments. Connected to this was his view that the interaction of the temporal lobe and prefrontal areas was essential to memory. His second argument was that the effects of hippocampal or fornix lesions were primarily impairments in the processing of spatial information, rather than impairments of memory, and that it was this that accounted for the observed pattern of impairments in memory tasks. As Gaffan (2001) and Mumby (2001) argue, Horel's views showed remarkable prescience and have subsequently been largely vindicated by research findings.

Meanwhile evidence from animal studies was accumulating which cast doubt on the attribution of such a central role in memory to the hippocampus. As early as 1954 it had been found (Mishkin, 1954) that monkeys with bilateral hippocampal lesions showed only slight impairments on pre-operatively acquired visual discriminations, and, more significantly, no impairment in the learning of new discriminations, even under conditions designed to promote interference. It was in precisely such tasks that human amnesia were severely impaired. Clearly if an animal model of amnesia were to be developed, structures apart from the hippocampus required investigation. The following section will therefore consider the anatomy and some relevant electrophysiology of some of the candidate regions. The perirhinal cortex, as the principal focus of this thesis, is discussed in greater detail than are other structures, with particular attention being given to some of its cytoarchitectural properties which may support object recognition.

1.2: The Anatomy of the Medial Temporal Lobe

Within the medial temporal lobe are a number of structures which form part of the limbic system. The concept of the limbic system derives from the idea of a "limbic lobe" comprised of the phylogenetically primitive three-layered allocortex, organised into gyri which enclose the brain stem. In addition to the parahippocampal, cingulate and subcallosal gyri, the limbic lobe also includes the hippocampal formation comprising the hippocampus proper, the dentate gyrus and the subiculum. In the human the hippocampus and the fornix are closely interconnected with the cingulate and parahippocampal gyri, despite their separation by the corpus callosum.

1.21: The hippocampal formation and the hippocampus proper

In the rat the hippocampal formation extends rostrodorsally in a C-shape from the septal nuclei of the basal forebrain passing over and behind the diencephalon. It is composed of six cytoarchitecturally distinct regions which make up the structure at different septotemporal levels. These are the dentate gyrus, the hippocampus proper, the subiculum, presubiculum and parasubiculum (sometimes grouped together as the subicular formation) and the entorhinal cortex. These structures are included under the label of "hippocampal formation" largely because of the strong and primarily unidirectional projections which link them one to another. Unidirectional projections

are rare among cortico-cortical connections which is why the structures linked in this way are considered as a single formation.

Within the hippocampus proper there are three divisions; CA1, CA2, CA3, which may be functionally as well as structurally discrete. Cytoarchitecturally they are distinguished by the size of the pyramidal cells which are considerably larger in CA2 and CA3 than in CA1. More importantly, the CA3 cells receive mossy fibre input from the dentate gyrus while the CA1 cells do not. In the rat the septal levels of the hippocampus receive more cortically derived afferents than temporal levels. This is suggestive of a functional distinction between these areas (Burwell & Amaral, 1998). The hippocampus has strong connections, via the perforant path, with the rhinal and parahippocampal cortex in both primates (Suzuki & Amaral, 1994) and in rodents (Burwell Witter & Amaral., 1995) (see figures 1.1 and 1.2 for the anatomy of the temporal lobe in, respectively, the macaque monkey and in the rat).

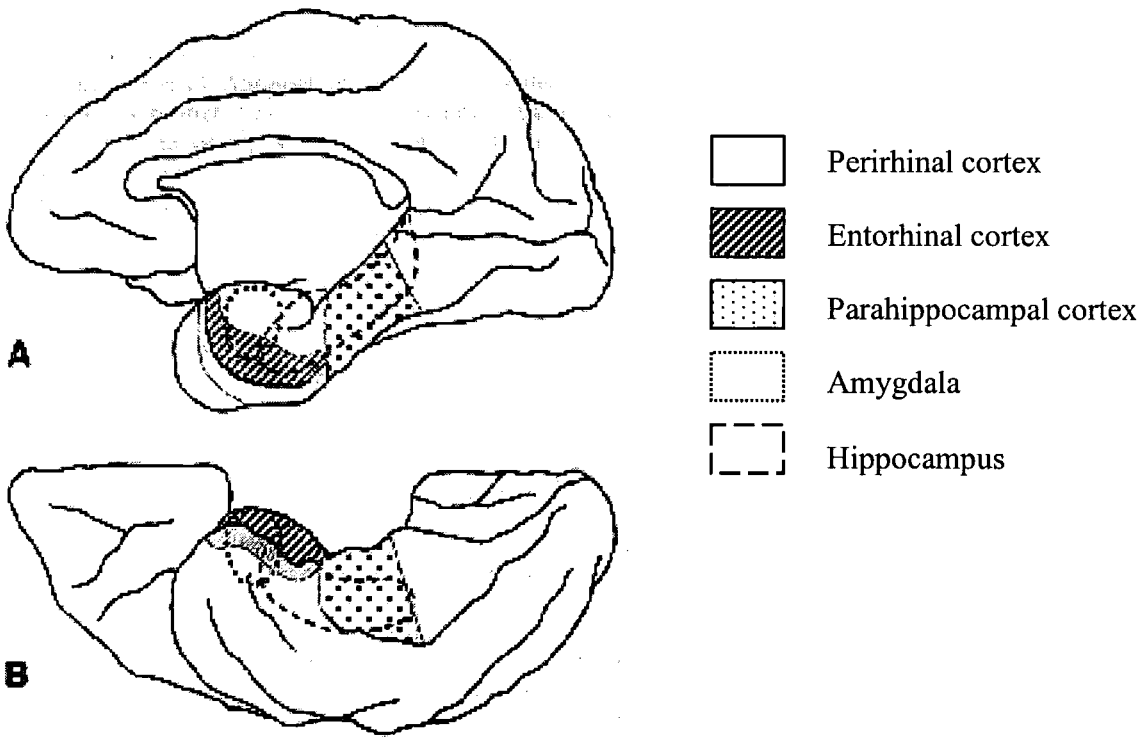


Figure 1.1: Medial (A) and ventral (B) views of the right hemisphere of a macaque brain showing the location and extent of various structures in the medial temporal lobe. The amygdala and hippocampus are buried deep within the medial temporal lobe whereas the perirhinal, entorhinal and parahippocampal cortices are located on the surface of the brain. Taken from Murray, Bussey, Hampton & Saksida (2000).

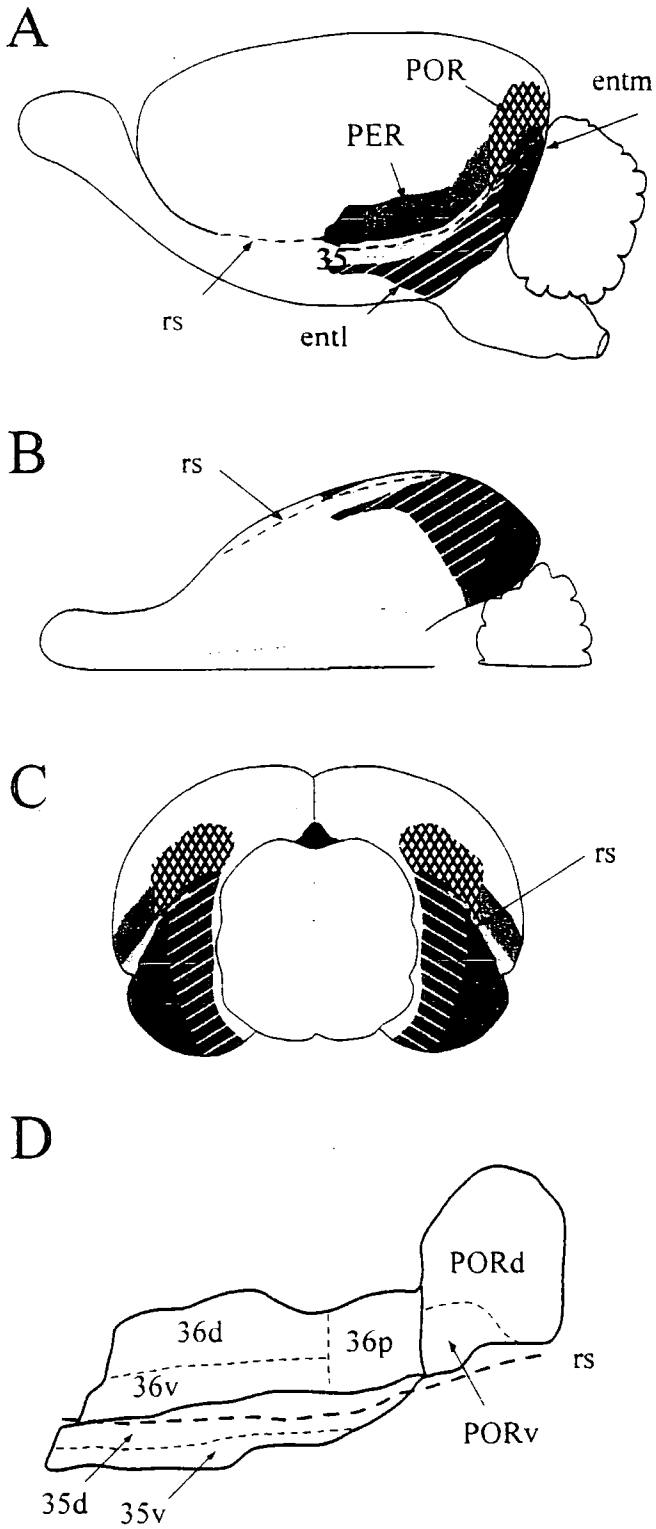


Figure 1.2: Lateral (A), ventral (B) and caudal (C) surface views of the rat brain illustrating the locations of the perirhinal, postrhinal and entorhinal cortices. The perirhinal cortex (PER) is shown in gray, area 36 in dark gray and area 35 in light gray. The postrhinal cortex (POR) is shown with a cross-hatched pattern. The entorhinal cortex is shown in gray with diagonal stripes, dark stripes for the medial entorhinal area (Entm) and light for the lateral entorhinal area (Entl). (D) an unfolded map of the perirhinal and postrhinal cortices showing regional and subdivisional borders. Taken from Burwell (2001).

The fornix is the term applied to the major fibre bundle which consists partly of the fibres which originate in the pyramidal cells of the hippocampus and the subiculum and which innervate the contralateral hippocampus as well as subcortical structures such as the mammillary bodies. Initially the bundle is known as the fimbria before the fibre bundles descend into the forebrain, at which point they form the columns of the fornix. The fornix divides around the anterior commissure to form a rostrally directed component innervating the septal nuclei and a caudally directed component which is directed toward the diencephalon. This postcommisural tract divides in turn to innervate the anterior hypothalamus and the anterior thalamic nuclei. As well as carrying efferent fibres from the hippocampus the fimbria and fornix also carry afferent fibres to the hippocampal formation. It can clearly be seen that the fimbria-fornix is crucial to hippocampal function as part of an integrated memory system including diencephalic structures.

1.22: The rhinal cortices

The rhinal cortices are comprised of the entorhinal cortex (although this is sometimes considered to form part of the hippocampal formation), the perirhinal cortex and the postrhinal cortex. In the macaque monkey the rhinal cortices surround the rhinal sulcus on the ventromedial aspect of the temporal lobe, and are therefore vulnerable to damage during surgery on the hippocampus proper.

Entorhinal Cortex

Although the entorhinal cortex is considered to form part of the hippocampal formation it is not, properly speaking, archicortex and is included primarily because its projectional properties (see above). The major portion of the entorhinal cortex is sited both caudal and ventral to the other structures comprising the hippocampal formation, with its lateral border being defined by the caudal half of the rhinal sulcus. In both the monkey and the rat the entorhinal cortex and the constituent areas of the perirhinal cortex lie adjacent to each other: in the ventrodorsal plane in the rat and the mediolateral plane in the monkey. It is defined by the strong projection from layer II to the molecular level of the dentate gyrus. The entorhinal cortex receives over 95% of cortical input from other polymodal association areas, including the perirhinal and parahippocampal (parahippocampal cortex in monkeys; postrhinal in rats – see

discussion below: p 11) cortices, as well as the orbitofrontal cortex, the retrosplenial cortex and the cortex on the dorsal bank of the superior temporal sulcus. The lateral area of the entorhinal cortex receives strong projections from the piriform cortex and from temporal and frontal regions, while the more medial area receives more afferents from the cingulate cortex and parietal and occipital areas (Burwell & Amaral, 1998). In both rodents and primates the entorhinal cortex provides a strong output to the dentate gyrus via the perforant path. In contrast to the perirhinal and parahippocampal cortices there are no unimodal visual inputs from areas TE, TEO or V4 to the entorhinal cortex. Indeed, around two-thirds of the cortical inputs to the entorhinal cortex originate in the perirhinal and parahippocampal cortices (Insausti, Amaral & Cowan 1987), which has been regarded as an indication that the entorhinal cortex is of a higher order, functionally speaking, than the other rhinal cortices.

Perirhinal Cortex

The perirhinal cortex consists of areas 35 and 36. In monkeys these form a band of cortex situated lateral to the full rostro-caudal extent of the rhinal sulcus. The perirhinal cortex extends anteriorly to include the medial portion of the temporal pole and laterally to include a substantial area of the cortex on the inferotemporal gyrus (Suzuki & Amaral, 1994a; Insausti et al., 1987; Amaral, Insausti & Cowan, 1987).

In rats there has been more controversy over the exact extent of the perirhinal cortex. Since Deacon, Eichenbaum, Roseberg & Eckman's (1983) comprehensive study of the rat perirhinal cortex and the cortical afferents to it there has been a substantial revision of the boundaries of this cortical area. Burwell, Witter & Amaral (1995) found that the perirhinal cortex surrounds the posterior portion of the rhinal sulcus. It is bounded medially by the entorhinal cortex and laterally by temporal association areas. The posterior boundary is formed by the postrhinal cortex which has connectional similarities with the parahippocampal cortex in the monkey. The postrhinal cortex was originally regarded as forming the caudal section of the perirhinal cortex, but has been re-classified as a separate structure due to the pattern of connectivity shown (Burwell et al. 1995).

The perirhinal cortex receives inputs from several areas including areas TE and TEO. Cells in area TE respond to specific features of stimuli, and, for the first time in the

ventral stream of visual processing, are organised into columns of cells which respond to similar stimulus properties (Tanaka, 1996) ; and both of these areas are known to be important in the visual identification of visual objects. The inputs to the perirhinal cortex from areas TE and TEO are extremely robust in the monkey (Suzuki & Amaral 1994a), suggesting that visual object identification is an important part of the function of the perirhinal cortex. The view that this function is object identification *per se* is supported by the fact that perirhinal cortex also receives inputs from somatosensory association areas of the insular cortex, and from an area of the superior temporal gyrus in which cells respond to both auditory and visual stimuli (Suzuki & Amaral 1994a). Whilst there are substantial feedback projections from the monkey perirhinal cortex to sensory cortical areas these efferents are much weaker in the rat (Burwell & Amaral, 1998). The perirhinal cortex also receives robust connections from the orbitofrontal cortex and the dorsal bank of the temporal sulcus, both of which are polymodal association areas (Suzuki & Amaral, 1994, Suzuki, 1996). The perirhinal cortex also has interconnections with the amygdaloid complex, particularly the lateral nucleus of the amygdala, although there are also minor reciprocal connections with the accessory basal nucleus (Amaral, Price & Pitkanen et al., 1992; Suzuki, 1996). While such detailed studies have yet to be carried out in the rat, studies by Burwell and colleagues indicate a similar pattern of connectivity (Burwell et al., 1995; Burwell, Witter & Amaral, 1998). Taken together this pattern of connections is consonant with the perirhinal cortex having a substantial involvement in the identification of individual objects on the basis of information which may be integrated from a number of different modalities, although in the monkey the information may be primarily visual.

As will be discussed later, the perirhinal cortex is important in many aspects of object memory. The mechanisms by which such memories may be formed or consolidated and maintained are therefore of particular importance. In addition to its connectional characteristics, the perirhinal cortex appears to have certain cytoarchitectonic and neurochemical properties which may render it uniquely suited to stimulus recognition. Perirhinal cortex contains neurons with some unusual properties (e.g. McGann, Moyer & Brown, 2001; Beggs, Moyer, McGann & Brown, 2000). The existence of certain cell types with firing patterns which are, so far, unique, and the demonstration of several types of long term synaptic plasticity both point to a system with the

capacity for differential response to novel and familiar stimulus events. Taken in conjunction with the evidence of cell firing patterns in response to novel objects this could be the basis of an object recognition system.

In particular, layer VI of perirhinal cortex contains a predominance of late-spiking (LS) neurons (McGann et al., 2001). These are neurons which delay the onset of their spiking trains by several seconds relative to the onset of a depolarising current step and then sustain firing for the duration of the step. These neurons have only previously been found in very small numbers in a limited number of cortical areas. Layers II and III of the perirhinal cortex also contain substantial numbers of LS neurons which have been found to show persistent delayed firing in response to a train of excitatory synaptic inputs (Beggs et al., 2000). The second most common type of cell in layer VI are single-spiking neurons which issue only a single action potential even in response to extreme depolarisation (McGann et al., 2001). These have not been documented elsewhere in neocortex, and have previously been found only in the amygdala. This layer of perirhinal cortex contained almost no regular spiking neurons, making it unique among cortical areas so far studied. This unique pattern of neuronal composition is suggestive of a role which requires very particular neuronal properties, and, in particular, the property of limited response to repeated stimulus exposure.

Layers III and V of the perirhinal cortex have been found to contain a substantial proportion of neurons whose membrane potential oscillations are at theta-frequency (Bilkey & Heinemann, 1999). When these neurons were depolarised past spike threshold they tended to fire in clusters. The within-cluster inter-spike interval was close to the peak-to-peak activity of the membrane potential oscillation. These intrinsic properties may be important to the synchronisation of a proportion of perirhinal neurons with hippocampal theta activity and may therefore enhance communication between the two structures. This might potentially support perirhinal involvement in processing an object-in-context scenario such as those discussed in chapter 4.

Finally, layers V and VI of the perirhinal cortex, in common with layers II and III of the entorhinal cortex, show a large number of cells which express the messenger RNA

brain derived neurotrophic factor (Hashimoto, Okuno, Tokuyama & Li et al., 2000). The laminar distribution of this messenger RNA, and of neurotrophin 3, which is found predominantly in layers II and III of entorhinal cortex suggests that they are primarily expressed in projection neurons. This may be indicative of a particular role in regulating feed-forward and feed-back projections from the rhinal cortex, and in the regulation of functional reorganisation underlying long-term memory formation and maintenance.

The connectional and cytoarchitectural characteristics outlined above, together with the variety of synaptic plasticity found in the perirhinal cortex (see chapter 6 pp193 for a detailed discussion) would seem to support its position as the principal candidate for the neural basis of a system which is involved in the high-level processing of, and in memory for, objects.

Postrhinal Cortex

Postrhinal cortex in the rat is considered to be the homologue of parahippocampal cortex in monkeys, because of the connectional similarities between the two structures (Burwell & Amaral, 1998). Whilst the equivalence is not perfect it is sufficiently useful for work on the monkey parahippocampal cortex to have relevance to a study of the postrhinal cortex in the rat. In monkeys the parahippocampal cortex lies caudal to the entorhinal and perirhinal cortices, consisting of areas TH and TF. These areas, unlike the constituent areas of the perirhinal cortex, cannot be treated as a single entity since they receive different inputs. Area TF receives projections from the areas of the posterior parietal cortex concerned with visuo-spatial processing (Andersen, Anasuma & Essick & Siegal, 1990; Burwell & Amaral, 1998). It also receives inputs from other polymodal areas such as the dorsolateral prefrontal and retrosplenial cortices and the dorsal bank of the superior temporal cortex, which are in turn interconnected with the posterior parietal cortex. Area TH receives similar polymodal inputs, but also receives unimodal auditory information from area TPT of the superior temporal gyrus. By comparison with the perirhinal cortex the parahippocampal cortex receives very little input from area TE, although it does receive unimodal visual input from VT1 and V4, which is concentrated in projections to area TF.

The boundaries of the postrhinal cortex in the rat have provided considerable demarcation difficulties based purely on cytoarchitectonic criteria, as, to a lesser extent, have those of the perirhinal cortex. A clearer picture of the boundaries emerges only when chemoarchitectonic and connectional data are also considered. Taking these factors into account Burwell et al. (1995) define the postrhinal cortex as being composed of the caudal levels of area 35 and the caudal levels of area 36, the medial border being with the entorhinal cortex. The dorsal border of postrhinal cortex is with association cortex, but it is unclear whether this is polymodal association cortex or visual association cortex.

Connections between the rhinal cortices

Suzuki & Amaral (1994b) used anterograde and retrograde tracers to investigate the topographic and laminar organisation of reciprocal projections between the entorhinal and the perirhinal and postrhinal cortices in the monkey. Both the perirhinal and parahippocampal cortices have distinct but partially overlapping interconnections with the entorhinal cortex. The perirhinal cortex connects reciprocally with the rostral two-thirds of the entorhinal cortex, whilst the parahippocampal cortex connects reciprocally with the caudal two-thirds of the entorhinal cortex. All regions of the perirhinal cortex project in a highly convergent manner on to both rostral and lateral portions of the entorhinal cortex. In contrast, the outputs of the parahippocampal cortex have a topographic organisation such that the medial parahippocampal cortex projects to the caudomedial entorhinal cortex, while the lateral parahippocampal cortex projects to the caudolateral entorhinal cortex. In the rat anterograde tracing has shown that the perirhinal cortex preferentially projects to the lateral entorhinal cortex, whereas the postrhinal cortex mainly sends fibres to the medial entorhinal cortex (Naber, CalleroBleda, JottismaByham & Witter, 1997).

The perirhinal and parahippocampal cortices, which receive input from both unimodal and polymodal association cortices of the temporal, frontal and parietal lobes and also output to these cortices, form 60% of the input to the entorhinal cortex (Insausti et al, 1987). The degree of reciprocity of the projections between the rhinal cortices varies. All the connections between the entorhinal and parahippocampal cortices have a high degree of reciprocity. The degree of reciprocity of connections between the perirhinal and entorhinal cortices varies depending on the mediolateral position of the

projection's origin within the perirhinal cortex. The medial perirhinal cortex gives rise to projections which have a higher degree of reciprocity than does the lateral perirhinal cortex. Projections from the perirhinal and parahippocampal cortices to the entorhinal cortex resemble a feed-forward projection, whilst those from the entorhinal cortex to these cortices resemble a feed-back projection (Suzuki & Amaral, 1994). This is supportive of the view that the entorhinal cortex may, in functional terms, be higher order than the other rhinal cortices. As with the projections from the perirhinal to the sensory cortices, the feedback projections from the entorhinal cortex to the perirhinal cortex are considerably weaker in the rat than in the monkey (Burwell & Amaral, 1998). As we have seen the entorhinal cortex outputs to the rest of the hippocampal formation via the perforant path.

1.3: Memory

Research into memory has primarily focused on episodic memory; the explicit memory for events which is lost in amnesia. Implicit learning which can be tested by tasks such as priming is spared in amnesia (Weiskrantz & Warrington 1979; Brooks & Baddeley 1976; Warrington & Weiskrantz 1970; Hamann & Squire, 1997). Human amnesia appears to result from damage to the Delay-Brion (Delay & Brion, 1969) system, which involves structures in the medial temporal lobe, the diencephalon and the basal forebrain. It consists of the hippocampus, the entorhinal and perirhinal cortices, the mammillary bodies, the mammillo-thalamic tract and the anterior thalamic nucleus (Kopelman, 1995). There is growing evidence that damage to one of these regions impacts on the function of other structures in the system. For example, amnesic patients with differing aetiologies and damage to disparate anatomical structures have all been found to show bilateral metabolic depression in the frontal basal cortex, the hippocampal region, the thalamus and the cingulate gyrus (Fazio, Perani, Gilardi & Cappa et al., 1992), while the differential c-fos expression in the perirhinal cortex and area TE following exposure to novel and familiar objects is abolished by lesions of the medial thalamus (Dix, Wan & Aggleton & Brown, 1998). Despite the evidence that lesions to one area can impair the function of other structures, research continues the attempt to isolate the contribution each of these structures is making to the function of the memory system.

Lesion studies in animals largely stemmed from the dramatic evidence outlined earlier that damage to specific brain regions causes profound impairments in humans (Scoville & Milner, 1957). However there was considerable difficulty in producing an animal model of the global memory deficits found in classic cases of anterograde amnesia. It became apparent that monkeys with severe hippocampal damage were unimpaired on standard tests of visual memory such as the object discrimination test (Orbach, Milner & Rasmussen, 1960), despite having lesions of the hippocampus similar to those received by HM (Scoville & Milner, 1957; Corkin et al., 1997). Hippocampal damage in non-human primates appeared to produce primarily spatial deficits (Morris, Garrud, Rawlins & O'Keefe, 1982; Rasmussen, Barnes & McNaughton, 1989; Riedel, Micheau & Lam et al., 1999), in contrast to the patients with hippocampal lesions who were severely impaired on all tasks that required episodic memory, even when damage was known to be restricted to area CA1 of the hippocampus (Zola-Morgan, Squire & Amaral, 1986). HM was profoundly impaired on tasks such as delayed pair comparison (Milner, Corkin & Teuber, 1968) and delayed matching to sample (Sidman, Stoddart & Mohr, 1968). However animals with hippocampal lesions performed normally on such tasks (Orbach et al., 1960). A primarily spatial model of hippocampal function, with the hippocampus as an allocentric spatial mapping system emerged (O'Keefe & Nadel, 1978) and has been largely supported by subsequent work. However, the problem of reconciling human and animal data remained, even allowing for the uncircumscribed nature of the majority of the lesions found in humans.

It gradually became apparent that the problem of modelling amnesia in animals might lie in the precise nature of the task used. Freed & Corkin (1988) found that even HM was able to remember certain things for long periods if the task were structured in the right way. When the standard object recognition tasks used to test primates were re-examined it was realised that they were not necessarily engaging episodic memory, or indeed a form of memory analogous to episodic memory. When an animal version of a human episodic memory test is developed it is often difficult to determine whether explicit rather than implicit memory is required for task completion. Only with the development of tasks for primates in which success required the engagement of aspects of episodic memory, such as the object-in-scene test (Gaffan & Harrison, 1989), has it become possible to demonstrate deficits analagous to those found in

human amnesia. A similar study in rats found that fornix transection reduced sensitivity to object-scene combinations (Simpson, Gaffan & Eacott, 1998).

Demonstrating impaired object memory in monkeys as a result of hippocampal damage requires relatively sophisticated tasks. The development of such tasks and the demonstration of a primate analogue to the impairment of episodic rather than specifically spatial memory found in human amnesic patients (Gaffan, 1994), is a breakthrough in the understanding of hippocampal function. However, for a full understanding of the impairments produced it is necessary to look at the neural bases of both spatial and object memory. The scope of this thesis necessarily requires that a considerably more detailed discussion be devoted to object memory than to spatial memory

1.31: Spatial memory

Numerous studies have clearly indicated that the hippocampus plays a central role in the processing of allocentric spatial information. Performance on tasks which require such spatial processing such as delayed non-matching-to-place (DNMTP), radial maze, and Morris water maze is severely impaired by hippocampal or fornix lesion.

While the involvement of the hippocampus is clear (Morris et al., 1982; Rasmussen et al., 1989; Riedel et al., 1999), the evidence for a role for entorhinal cortex in spatial memory is equivocal. A large number of studies using aspiration lesions in rodents have shown deficits on spatial tasks including the Morris maze (e.g. Schenk & Morris, 1985) the radial maze (e.g. Olton, Walker & Gage 1978) and DNMTP on a T-maze (Loesche & Steward, 1977). The findings from studies using excitotoxic lesions, which spare the fibres of passage to the hippocampus (Cho, Berracochea & Jaffard, 1993; Cho & Jaffard, 1994; Holscher & Schmidt 1994; Pouzet, Welzl, Gubler & Broersen et al., 1999) suggested milder impairments of spatial reference memory, while Bouffard & Jarrard (1988) found no impairments. Bannerman, Yee, Lemaire & Wilbrecht et al., (2001) found that entorhinal cortex lesions impaired DNMTP, but did not affect performance on a spatial reference memory task in the Morris maze. The authors suggest that the bulk of the evidence supports a role for the entorhinal cortex in supporting some aspects of spatial memory which only the more sensitive behavioural tests detect. The probability seems to be that the entorhinal cortex is

involved in supporting spatial memory in normal animals (Vann, Brown & Erichsen & Aggleton, 2000) but is not required for performance to be maintained in lesioned animals.

The role of the postrhinal cortex in memory is not clear. This area of cortex has been included in some of the studies that have used large lesions of the medial temporal area, but there have been very few studies that have employed circumscribed lesions of the postrhinal cortex in rats or the parahippocampal gyrus/cortex in monkeys. In particular the postrhinal area was not included in the lesions used by Wiig & Bilkey (1994) or Phillips & LeDoux (1995).

It seems possible that the postrhinal cortex is involved in spatial memory. It does not appear to be involved in object memory (Murray & Gaffan, 1993; Ramus, Zola-Morgan & Squire, 1994), although an FMRI study (Gabrielli, Brewer, Desmond & Glover, 1997) suggested that parahippocampal cortex in humans is involved in encoding complex object-position configurations. A study of patients with lesions which affected the perirhinal or parahippocampal cortex found that those with parahippocampal damage were impaired on a memory-guided eye movement task contralateral to the lesioned side, whereas those with perirhinal damage were not affected (Ploner, Gaymard, Rivaud Pechoux & Baulac et al., 2000), and another lesion study in humans supports a role for the parahippocampal cortex (Bohbot, Allen & Nadel, 2000). Rolls & O'Mara (1995) found evidence of cells in parahippocampal cortex which respond to differences in visual fixation. Wan, Aggleton & Brown (1999) found that postrhinal cortical cells, like those in hippocampal area CA1 responded differentially to novel arrangements of familiar objects but, unlike cells in the perirhinal cortex, did not respond differentially to novel objects. This result is consonant with the lack of input from area TE of the inferotemporal cortex compared to the projections received by the perirhinal cortex (Suzuki & Amaral, 1990) and the robust projections it receives from the posterior parietal cortex which is heavily implicated in spatial processing (Andersen, et al., 1990). Behavioural studies in primates would appear to support the involvement of the parahippocampal cortex in aspects of spatial memory. Lesions of the area cause impairments in object-place associations (Malkova & Mishkin, 1997), spatial reversal tasks (Teng, Squire & Zola 1997) and spatial orientation tasks (Habib & Sirigu, 1987).

However, there is some evidence that postrhinal lesions do not cause comparable behavioural impairments in rodents. Performance on allocentric spatial memory tasks such as the Morris maze and radial arm maze is unimpaired by combined perirhinal and postrhinal cortex lesions (Bussey, Muir & Aggleton, 1999). Myhrer (2000) found that postrhinal as well as perirhinal lesions impaired acquisition of a three-choice visual discrimination task, but that postrhinal lesions did not affect retention, whereas perirhinal lesions did. This would suggest that the postrhinal cortex is not necessary for spatial memory processing in rodents and may be more involved in object memory. This may imply that functionally, if not anatomically, there may be little equivalence between the parahippocampal cortex in the monkey and postrhinal cortex in the rat.

1.32: Object Memory

The debate about the role of the hippocampus in memory was clarified by the realisation that large temporal lobe lesions, which did impair visual recognition memory in monkeys, included damage to the areas of rhinal cortex overlying the hippocampal formation as well as to the hippocampus and amygdala. Impairments which had previously been attributed to combined hippocampal and amygdalar damage in both monkeys (Mishkin, 1978) and humans were in fact due to the rhinal cortex damage that occurred with these combined lesions of the hippocampus and amygdala. This was clearly demonstrated by the use of circumscribed excitotoxic lesions of both the amygdala and hippocampus which did not impair object recognition memory as measured by performance on a delayed non-matching to sample (DNMS) task (Murray & Mishkin 1998). This finding contrasted with the classic H+A+ lesion (involving hippocampus, amygdala and surrounding cortex) which caused severe mnemonic impairments (Mishkin, 1978). This difference in behavioural impairments appeared to be due to the extensive damage to the rhinal cortices which was caused by the single H+A+ lesion, but not by the separate excitotoxic lesions. These excitotoxic lesions did not impair performance even when there was near total cell loss in both structures. Alvarez, Zola-Morgan & Squire (1995) did find that hippocampal animals were impaired on DNMS at very long delays, but this is a single result which is at odds with the bulk of the evidence: Murray & Mishkin (1998) failed to confirm this result in their study when they

replicated the forty minute delay used by Alvarez et al. (1995). Much more typically it is found that hippocampal lesions fail to affect memory of object discriminations, even at very long delays, contrasting sharply with the impairments on spatial tasks at all delays (Mumby, Astur, Weisend & Sutherland, 1999). It is possible that the impairment observed by Alvarez et al. (1995) may represent deficient identification of context rather than deficient stimulus recognition per se. Also, this study used radiofrequency lesions which may have damaged fibres of passage from the rhinal cortex. It is possible that disruption of this connection would be sufficient to disrupt object memory. The difficulty in identifying such damage as the cause of an impairment, and the ease with which conventional hippocampal lesions can damage the rhinal cortices can be seen from the discussion of the anatomy of the medial temporal lobe (pp4 above).

It therefore seems likely that some of the severe impairments of explicit object memory found in human amnesic patients are at least partially due to damage to the perirhinal cortex as well as to hippocampal damage. The precise role of the rhinal cortices in memory has been investigated extensively in the monkey. The deficits in stimulus memory observed in monkeys with rhinal cortex lesions appear similar to those found in humans who have sustained damage to the ventromedial temporal cortex (Kapur, Ellison, Parkin & Hunkin et al., 1994). Gaffan & Murray (1992) found that rhinal cortex lesions in monkeys produced an impairment equivalent to the traditional H+A+ lesion, in contrast to intact performance following circumscribed amygdalar and hippocampal lesions. Other studies have supported the theory that circumscribed lesions of the rhinal cortices are both necessary and sufficient to cause the severe deficits on tasks that required visual recognition memory, which had previously been attributed to combined damage to the hippocampal and amygdalar structures (Zola-Morgan, Squire, Amaral & Suzuki, 1989; Gaffan & Murray, 1992; Meunier, Bachevalier & Mishkin & Murray, 1993; Suzuki, Zola-Morgan, Squire & Amaral, 1993).

Gaffan & Murray (1992) found that rhinal cortex ablations produced impairment in re-acquisition of pre-operatively learnt concurrent discrimination problems using 24 hour inter-trial intervals, but no impairment in the post-operative acquisition of new discriminations. Area TE was also damaged during surgery, and such damage is

known to impair object recognition memory (e.g. Gaffan & Weiskrantz, 1980), but it also impairs learning of concurrent discriminations (Phillips, Malamut, Bachevalier, & Mishkin 1988). As no global visual impairment was observed, it is unlikely that the observed memory impairment was due to incidental damage to area TE rather than to the rhinal cortex lesions. This interpretation is supported by Buffalo, Ramus, Clark & Teng et al.'s (1999) study which found that monkeys with perirhinal lesions displayed a different pattern of impairments to monkeys with lesions of area TE. This pattern suggested that whilst the perirhinal cortex was involved in multi-modal mnemonic processing, area TE was primarily involved in visual perceptual processing.

In monkeys the perirhinal cortex has been found to be the most important of the rhinal cortices for visual recognition, when the volume of the lesion is considered relative to the magnitude of the impairment. Although combined lesions of the entorhinal and perirhinal cortices produced an impairment greater than lesions to either structure alone (Meunier et al., 1993; Leonard, Amaral, Squire & Zola-Morgan, 1995) the differential was slight compared to the difference in lesion size. Lesioning the parahippocampal cortex alone produced little or no impairment on object memory (Ramus et al., 1994), raising again the question of the nature of this structure's mnemonic function.

The view that the entorhinal cortex does in fact make a contribution to object memory is supported by work on humans. Owen, Milner, Petrides & Evans (1996) carried out a PET study on encoding and retrieval of object memories and found that, when subjects were retrieving the objects from memory, there was an increase in the regional cerebral blood flow in the region of the right hippocampal gyrus corresponding to the entorhinal cortex. Whilst the lateralisation effect would be predicted to be restricted to humans, the involvement of the entorhinal cortex is interesting, given that lesion work (Meunier et al., 1993; Leonard et al., 1995) suggested a minimal role for the entorhinal cortex. It is possible that the discrepancy between the two sets of evidence could be due to a distinction between anatomical and functional damage. It is plausible that the entorhinal cortex, which receives a substantial proportion of its cortical afferents from the perirhinal cortex, is metabolically affected by perirhinal lesions although the histology shows it to be

untouched by the lesion. Therefore, a considerable amount of the memory impairment which has been attributed to perirhinal damage could, in fact, be due to indirect damage to entorhinal cortex function.

The role of the perirhinal cortex itself in object memory is supported not only by the pattern of its connections with other cortical areas but also by various neurophysiological studies. There is also evidence from electrophysiological studies that cells in the perirhinal cortex have differential responses to novel and familiar stimuli (Brown, Wilson & Riches, 1987; Fahy, Riches & Brown 1993; Li, Miller & Desimone, 1993; Riches, Wilson & Brown, 1991; Zhu, Brown, McCabe & Aggleton 1995). Neurons in monkey perirhinal cortex respond to visual, somatosensory, auditory or a combination of these stimulus modalities (Desimone & Gross, 1979). In the rat both odour-responsive and visually-responsive neurons have been documented (Zhu, Brown & Aggleton, 1995). Unlike hippocampal neurons, the firing patterns of perirhinal neuron have not been found to have stable spatial correlates (Burwell et al., 1998). Perirhinal neurons which respond to visual stimuli are, like those in the adjacent unimodal area, TE, stimulus selective (e.g. Miller, Li & Desimone., 1993). Their responses, also like those of neurons in TE, are invariant for changes in size or location (Lueschow, Miller & Desimone, 1994).

Comparison of the time courses of perceptual and memory retrieval signals show that the perceptual signal reaches area TE before perirhinal cortex; while the reverse is true of the memory retrieval signal (Naya, Yoshida & Miyashita, 2001). Xiang and Brown (1999) used a task in which the geometrical arrangement of three shapes determined the correctness of a behavioural response. They found that 50% of neurons in perirhinal cortex, entorhinal cortex and area TE responded differentially to different types of trial, while only 13% of hippocampal neurons did so. The differential responses that would allow tasks to be solved using a conditional rule were twice as common as were those which indicated a spatial rule. The differential latencies for the responses allowing a conditional response were also considerably shorter. However, the level of hippocampal responsiveness was such that, while animals may adopt conditional strategies, spatial solutions are open to them as an alternative. C-fos studies have supported the electrophysiological findings (Brown, 1996) that perirhinal neurons respond differentially to novel objects (Wan et al., 1999)

or pictures (Zhu, Brown, McCabe & Aggleton, 1995)., but not to novel arrangements of familiar objects (Zhu, McCabe, Aggleton & Brown, 1997). Tassoni, Lorenzini, Balsi & Sacchetti et al. (1999) used a reversible inactivation by tetrodotoxin to demonstrate that perirhinal cortex was involved in retrieval of a passive avoidance response, and in the late consolidation of the memory, but not in the early stages of memory acquisition. This supports the behavioural findings that rats (Machin & Eacott, 1999) and monkeys (Thornton, Rothblat & Murray, 1997) with perirhinal ablation showed a deficit in reacquisition of pre-operatively learned object discriminations but no deficit in the acquisition of new object discriminations.

It is clear from the evidence outlined above that the perirhinal cortex is important to memory for objects. The role of the perirhinal cortex in visual memory should not, however, be regarded as simple or stand-alone object memory. Monkeys with bilateral lesions of the perirhinal cortex are impaired on concurrent discrimination learning tasks, either when the number of objects involved is a large (Eacott, Gaffan & Murray 1994) or when the number is small but the objects are presented in a number of different orientations (Buckley & Gaffan 1998a). Such animals are also impaired in the visual identification of familiar objects which are presented in novel orientations or which are presented embedded in a scene (Buckley & Gaffan, 1998b). Monkeys with rhinal or perirhinal lesions are impaired on visual discrimination learning (Buckley & Gaffan, 1997; Buckley & Gaffan, 1998a), visual paired associate tasks (Buckley & Gaffan 1998b; Murray, Gaffan & Mishkin, 1993; Parker & Gaffan 1998) and visual discrimination reversal (Eacott et al., 1994; Murray, Baxter & Gaffan, 1998) while rats with such lesions show poor performance on visual discrimination tasks (Eacott, 1998) and on retention of that learning (Kornecook Anzarat & Pinel, 1999; Kornecook, Lui, Duva & Anzarat et al., 1995; Machin & Eacott, 1999; Wiig, Cooper & Bear, 1996) as well as on stimulus-stimulus association learning (Bunsey and Eichenbaum, 1993; Herzog & Otto, 1998).

It has been argued (Eichenbaum, Otto & Cohen, 1994, 1996) that the parahippocampal region (i.e. the rhinal cortices) has the mnemonic function of retaining representations of individual items for a more extended period of time than the neocortical areas. This theory is reminiscent of the temporal discontinuity theory of hippocampal function proposed by Rawlins (1985), although it is not entirely based

on the role of the hippocampus as a medium term memory store. They argue that, since the hippocampus does not maintain single item representations but only the relations between items, the integrity of the parahippocampal region is required for normal hippocampal function, but not vice-versa. If this theory were correct then it would be impossible to obtain a double dissociation of the effects of hippocampal and rhinal cortex lesions.

There is, however, very considerable evidence for just such a double dissociation. A study by Gaffan (1994b) demonstrated that the effects of fornix lesion and perirhinal lesion could be doubly dissociated in monkeys. Using rats, Ennaceur, Neave & Aggleton (1996) found that, whilst fornix lesions impaired performance on spatial tasks but not on object recognition, the converse was the case when perirhinal lesions were employed. Contrary to Eichenbaum et al's (1994, 1996) view, both Gaffan (1994b) and Ennaceur et al. (1996) found that lesions to the parahippocampal region – and specifically to the perirhinal cortex - impaired object recognition without affecting performance on spatial tasks. It now seems clear that, despite the anatomical connections which exist between the rhinal cortices and the hippocampus, lesions of the former do not functionally disconnect the latter. This is despite the fact that studies using c-fos imaging (Vann et al., 2000) support the involvement in allocentric spatial processing of the entorhinal, postrhinal and retrosplenial, though not the perirhinal cortices.

It is also possible to argue that Ennaceur et al (1996)'s study is not definitive, and that if complete rhinal lesions, which included the entorhinal and postrhinal cortices as well as the perirhinal cortex, had been employed then the rhinal lesions would have disrupted hippocampal function. It is true that the evidence is somewhat contradictory on this point. Although Ennaceur et al's (1996) finding that rhinal lesions produced no impairment on T-maze spatial alternation is supported by other studies (Aggleton, Keen, Warburton & Bussey, 1997; Bussey, 1997) there is evidence that animals with rhinal lesions do experience problems on other spatial tasks. These include the radial arm maze (Liu & Bilkey 1999; Nagahara, Otto & Gallagher 1995) and the Morris water maze (Otto, Wolf & Walsh, 1997). However, Glenn & Mumby (1998) found no impairment even at long delays in the water maze, whilst Machin

Vann, Muir & Aggleton (2002) found no impairment on any version of either task at substantial delays.

Conversely, fornix-transected monkeys have been found to be impaired on non-spatial reversal learning problems (Gaffan & Harrison, 1984), although even neonatal hippocampal lesions have only extremely mild effects on object recognition (Bachevalier, Beauregard & Alvarado, 1999). A meta-analysis of studies using a delayed nonmatching to sample paradigm with monkeys given either hippocampal or rhinal cortex lesions (Baxter & Murray, 2001) found reliable differences in the magnitude of deficits. Hippocampally lesioned monkeys were consistently less impaired than those with rhinal lesions. Some studies such as Buffalo et al. (1998) and Teng et al. (2000) have concluded that impaired performance on concurrent learning, apparently resulting from hippocampal lesions, is in fact due to damage to inferotemporal cortex or to the tail of the caudate nucleus. Despite the fact that rats with perirhinal lesions do experience some mild impairments on some spatial tasks it seems reasonable to attribute these to the processing of non-spatial information by the perirhinal cortex, which is useful but non-essential to successful performance of the spatial task (Mumby & Glenn, 2000).

It is possible to argue that fornix transection is not equivalent to hippocampal ablation, and indeed there would seem to be some support for this view from the studies of human patients with bilateral fornix transection. Patients with fornix transection following the removal of colloid cysts are not densely amnesic, although they do have some memory impairments (Aggleton, McMackin, Carpenter & Hornak et al., 2000; Gaffan, Gaffan & Hodges, 1991; Hodges & Carpenter, 1991; McMackin, Cockburn, Anslow & Gaffan, 1995). In particular, they are unimpaired on the Warrington Recognition Memory Test (Warrington, 1984), developed specifically for the detection of amnesia. It should be noted that some patients with bilateral hippocampal cell loss as a result of ischemic incidents show similar patterns of memory loss, rather than the dense amnesia that might have been predicted (Rempel-Clower, Zola, Squire & Amaral 1996). This may be indicative of the fact that "hippocampal" lesions in humans are rarely circumscribed, while cases such as that of the dense amnesic with damage limited to CA1 (Zola-Morgan et al., 1986) are

extremely rare, with amnesic patients customarily having damage to structures other than the hippocampus proper.

There is strong evidence that the effects on measurable performance of hippocampal ablation and fornix transection in rodents are identical. On a simple spatial task such as delayed non-matching to position (DNMTP) in an operant chamber, fornix-transected and hippocampal animals are indistinguishable (Aggleton, Keith, Rawlins & Hunt et al., 1992). In fact fornix lesions may be argued to represent a more circumscribed removal of hippocampal function than a hippocampal lesion itself. Although fornix transection severs outputs from the subiculum and the perirhinal and entorhinal cortices (Aggleton, Desimone & Mishkin, 1986), aspiration lesions of the hippocampus actually remove the underlying parahippocampal cortex and part of the posterior entorhinal cortex (Murray, 1996). More recent work (Aggleton & Brown, 1999) emphasises the importance of hippocampal efferents to the diencephalon via the fornix in an extended hippocampal system comprising the hippocampus, the fornix, the mammillary bodies and the anterior thalamic nuclei. This suggests the reverse: that a fornix transection may be a more complete as well as a circumscribed removal of hippocampal function.

However, the nature of the memory process which permits the recognition of an object or of knowledge about an object is less clear. There are several processes which are involved in the recognition of an object as familiar, not least the integration by which several features are resolved into a whole or "gestalt" which can be treated as a single entity. Other related processes are those by which the relationship of objects to each other (or their integration into a single construct) and the context in which objects are viewed. Ultimately object memory must by its very nature form part of an episodic memory which may be conceived of as "what" (object); "where" (place) and "when" (timing) (e.g. Clayton & Dickinson, 1998). It is the role of medial temporal lobe structures in these aspects of memory which this thesis seeks to examine and, if possible, to differentiate.

1.33: Associative memory

The rhinal cortex is also implicated in associative memory performance (Buckley & Gaffan, 1998c). Although some studies had suggested that the amygdala is critical for

cross-modal stimulus-stimulus associations (Murray & Mishkin, 1985, Gaffan & Harrison, 1987) it now appears that amygdalar lesions produce an equal impairment of intra-modal associative learning. Additionally, Murray et al., (1993) compared the effects of rhinal cortex lesions, lesions to the amygdala and the underlying cortex, lesions to the hippocampus and the underlying cortex and lesions to both hippocampus and amygdala together with the underlying cortex. Murray et al. (1993) found that monkeys with rhinal cortex lesions were as severely impaired on an associative memory task as monkeys with the combined lesions of the hippocampus and amygdala which included much of the underlying cortex. By contrast, removal of the hippocampus and underlying cortex or amygdala and underlying cortex produced no impairment. This demonstrates that the rhinal cortex, but not the amygdala or hippocampus, is crucial to the acquisition of stimulus-stimulus associations. The view that the amygdala is not of paramount importance is given additional support by the fact monkeys with neurotoxic amygdalar lesions can still learn to associate two stimuli with each other, although learning about the strength of the reinforcement is affected (Malkova, Gaffan & Murray, 1997). This would indicate that the primary amygdalar function is concerned with the reward value of stimuli, not the ability to learn about stimuli *per se.* Baxter, Hadfield & Murray (1999) argue that aspiration, but not neurotoxic, lesions of the amygdala cause severe damage to stimulus-stimulus learning because of the disruption of connections with other areas including the rhinal cortices. It now seems probable that the observed deficits in such studies were attributable to damage to the rostral section of the rhinal cortex. This is confirmed by Buckley and Gaffan (1998c) who found that monkeys with circumscribed lesions to the perirhinal cortex were impaired on visual paired-associate learning. In this form of the task subjects had to learn which of two possible stimuli was associated with a cue stimulus in order to gain a food reward.

This has been found to be the case for cross-modal stimulus-stimulus associations, including auditory-visual associations (Gaffan & Eacott, 1995; Nicholson & Freeman, 2000) and flavour-visual associations (Parker & Gaffan, 1998), as well as visual-visual stimulus associations (Murray et al., 1993). Electrophysiological studies (Erickson & Desimone, 1999; Messinger, Squire, Zola & Albright, 2001; Sakai & Miyashita, 1991) have found changes in responses of perirhinal neurons in monkeys to frequently paired stimuli, as compared to responses to infrequent pairings, that

were indicative of linking of representations of the frequently paired stimuli. These patterns of neuronal activity are consonant with memory for visual paired associates. The inferotemporal cortex, which is involved in discriminative learning, also contains neurons that potentially code for visual paired associate learning. Lesions of the entorhinal and perirhinal cortex (which send and receive large projections to and from the inferotemporal cortex) prevent such responses to paired associates being formed in the inferotemporal cortex (Higuchi & Miyashita, 1996), suggesting that it is the rhinal cortical areas that are crucial for this form of learning. There is also evidence that experience can cause the clustering of neurons with similar properties in adult macaque perirhinal cortex (Erickson, Jagadeesh & Desimone, 2000).

Little work using rats has been carried out on the role of perirhinal cortex in the formation and maintenance of stimulus-stimulus associations. The available literature does however indicate that its role in rats may be broadly similar to that found in monkeys. Otto & Eichenbaum (1992) found that while orbitofrontal cortex lesions impaired acquisition of odour-guided non-matching to sample they did not impair performance (except when interference was introduced); combined perirhinal and entorhinal lesions did not impair acquisition but did impair performance at delay and fornix lesions impaired neither acquisition nor performance in any condition. The experiment described in chapter 2 of this thesis examines whether visual-visual stimulus associations are dependent upon perirhinal cortex in the rat.

1.34: Configural Processing and Memory

The weight of the evidence discussed so far suggests that the perirhinal cortex is important not just for recognition *per se*, but for knowledge about objects. It is involved in knowledge about objects such as the associations between objects which appear together either simultaneously or sequentially. In particular, the view is that the perception and representation of an object as a whole rather than as the sum of its component parts requires an intact perirhinal cortex. This view is supported by connectionist modelling which locates the relationships between features in the more rostral regions of the ventral visual stream (Bussey & Saksida, 2002). If indeed the perirhinal cortex is required for what has been described as a " 'gestalt' representation of a complete stimulus" (Murray et al, 1998 p146) then two clear predictions arise. The first of these is that not only visual recognition memory, but also performance on

any task requiring the use of more than one feature of the object would be impaired by perirhinal lesions. The second and more specific prediction is that any task in which recognition of an object requires a configural strategy would be impaired in animals with perirhinal lesions. The greater the reliance which must be placed upon a configural approach, the more severe the predicted impairment.

The question arises of when an object representation results from the perception of a number of features. For example, if two objects are always seen in absolute proximity to each other, will a single object representation result? Is it possible to differentiate such a configuration from an association between objects? Another issue that needs to be addressed is at what point a group of features, which are always seen together, cease to be processed as a single object, and are instead processed as an object in a particular context comprised of other features.

The hippocampus has been implicated in configural learning, with hippocampal lesions impairing learning of negative patterning tasks (Rudy & Sutherland, 1989). However, subsequent studies have not replicated this result (Davidson, McKernan & Jarrard 1993; Deacon & Rawlins, 1996). Hippocampal lesions have also been found not to impair other forms of configural learning such as biconditional discrimination tasks (Murphy, McDonald & Baker, 1998). Indeed hippocampal lesions which spare rhinal cortices may actually facilitate configural learning (Bussey, Warburton, Aggleton & Muir, 1998). Ennaceur & Aggleton (1994) failed to find a fornix impairment on a version of the object recognition task which used a reconfigured version of the familiar object and tested discrimination between this and the original or the novel object. It has been found (Cho & Kesner, 1995; Han, Gallagher & Holland, 1998) that rats with hippocampal lesions which spare the rhinal cortices are actually facilitated on configural learning tasks. This suggests that, without the information about relations between objects normally supplied by the hippocampus, the encoding of object attributes is no longer divided up into information about individual objects, but is fused into a configural representation. Under such an interpretation the role of the hippocampus is the provision of support for cortically based configural representations (Rudy & Sutherland, 1995). This supports the findings of Parker & Gaffan (1995) that it is information flow between the rhinal

cortices and the hippocampus which is required for the identification of an individual object in a context of other objects.

It seems possible that the perirhinal cortex codes not for objects, but rather for groups of properties. The role of the perirhinal cortex in the formation of associations *between* stimuli has already been discussed However, the definition of a stimulus can in some senses be regarded as the arbitrary decision of the experimenter (particularly when two stimuli always appear in conjunction). In such circumstances the association of properties which are defined as belonging to the same stimulus would seem to be a closely related function. It is not, therefore, implausible that the processing of a stimulus' associative properties might be closely linked to the processing of the object's features as constituting a single entity.

The evidence for hippocampal or rhinal cortical involvement in configural memory processing is conflicting. Ennaceur et al. (1996) reported a pattern of results which suggest that, at least at short delays, perirhinal lesions might actually facilitate configural processing. Bussey, Dias, Redhead & Pearce et al. (2000) found intact negative patterning in rats with combined perirhinal and postrhinal lesions as well as in those with fornix lesions. However, as the feature ambiguity inherent in the task increases so does the severity of the impairment in monkeys with perirhinal lesions (Bussey, Saksida & Murray, 2002). Eacott, Machin & Gaffan (2001) also found that rats with perirhinal cortex lesions were impaired when processing of more than one overlapping feature was required to identify a two dimensional stimulus uniquely, but not when identification could be made on the basis of one feature, even when the figures involved were complex. Buckley & Gaffan (1998c) report similar findings of impairment following perirhinal lesions in primates. Using a naturalistic paradigm Ennaceur et al. (1996) found a complex picture which seemed to indicate that perirhinal cortex might facilitate recognition using configural processing under certain circumstances, whilst impairing it in others.

Overall the picture of perirhinal cortex involvement in the processing of feature ambiguity is unclear. Some of the literature would appear to indicate that an intact perirhinal cortex is crucial to processing of the feature ambiguity inherent in a configural paradigm, whilst other studies appear to show no effect or even to suggest

that perirhinal lesions may actually facilitate such processing. The experiments in chapter 3 therefore represent a systematic examination of the effects of different levels of feature ambiguity on animals with perirhinal lesions.

1.35: Context and Memory

There is a considerable body of evidence for a clear role for the hippocampus in memory for context. Many of these studies use paradigms such as place conditioning which cannot strictly speaking be related to aspects of object memory (for a brief review of such studies see chapter 4). However, Mumby, Gaskin, Glenn & Schramek et al. (2002) found that hippocampal lesions not only impaired performance on the object in place task, but also impaired performance on an object in context task. It could be argued that Gaffan's (1994) object-in-place task is actually a test of memory for context. Certainly the deficits following hippocampal lesions in monkeys reported by Ridley, Hardy, Maclean & Baker (2001) of object choice which was conditional on the background to the object would seem to be a test of object-in-context. Bucci, Phillips & Burwell (2000) investigated the role of the perirhinal and postrhinal cortices in such contextual memory processing, and found that lesions to either structure produced impairments in fear conditioning to context, but not to an auditory stimulus, suggesting that both structures are involved in contextual learning.

The object-in-place task, which Gaffan (1994b) used to demonstrate a primate analogue of human amnesia and the involvement of the hippocampus in object-memory, has also been used to demonstrate the contributions of both the hippocampus and the rhinal cortex to such relatively complex mnemonic exercises. Gaffan & Parker (1996) carried out a unilateral perirhinal ablation combined with contralateral fornix section, also sectioning the body and splenum of the corpus callosum, the anterior commissure, and carrying out a partial forebrain commissurotomy. The surgery was carried out in two stages. After either a unilateral fornix section or a unilateral ablation of the perirhinal cortex animals exhibited mild impairment on the object-in-place task. After surgery was completed the impairment was severe, and comparable to that found after bilateral fornix section (Gaffan, 1994a) , bilateral mammillary body lesions (Parker & Gaffan, 1997a), or bilateral lesions of the anterior thalamic nuclei (Parker & Gaffan, (1997b). The flow of visual information from the perirhinal cortex through the fornix to the mammillary bodies and the anterior

thalamus was disrupted by the complete surgery, and object-in-place memory is clearly dependent on this flow. This task requires the hippocampus (accessed via the fornix) for memory of the spatial arrangement of the scene, but not for memory of multiple individual objects. It also requires the perirhinal cortex for the complement of this function - memory for multiple individual objects but not the spatial relations between them.

Easton & Gaffan (2000) found that, while the perirhinal cortex was critical to the learning or retrieval of scene problems, it was not necessary for the retrieval of pre-operatively learned object-reward relationships. This contrasted with crossed lesions of the medial forebrain bundle and inferior temporal cortex, which resulted in impairment of all new learning but the preservation of previously learnt information. This suggests that isolation of inferior temporal cortex from its basal forebrain and midbrain afferents results in dense anterograde amnesia, whereas the involvement of the perirhinal cortex is dependent on task difficulty.

Parker & Gaffan (1995) argue that this information flow represents the co-operation between the predominantly spatial fornix-mammillary memory system and the object-based memory system of the rhinal cortices. Alternative views suggest that there is functional specialisation within a single memory system. However this debate seems to resolve on the issue of what constitutes a memory system. Parker & Gaffan (1995, 1997a, 1997b) established the functional unity of the fornix-mammillary system, in that by lesioning one part the mnemonic function of the whole was impaired. However it is possible to regard a memory system as involving parallel as well as serial processing, with the possibility of double dissociation of function on some but not all tasks. The definition that is chosen determines the answer to the question of whether there is co-operation between or within memory systems, but what is clear is that co-operation between functionally specialised anatomically distinct structures is required for intact performance on this type of task. Even if we regard this as co-operation between two distinct systems, it would be advisable to bear in mind the contributions made by structures other than that which has been lesioned.

The use of the term "object-in-place" to describe the task used by Gaffan and colleagues is potentially confusing. It is not clear whether the results found reflect the

processing of an object in place, taken literally as a spatial location which is allocentrically determinable, or whether they reflect the processing of an object in the context of a particular background pattern. It is also not clear whether the rhinal cortices, as well as the hippocampus, are involved in processing the object in context in rodents where fear conditioning is not involved. Bussey, Dias, Amin & Muir et al. (2001) found that perirhinal lesions impaired learning of what would appear to be a pure object-place task. This used a Y-maze, but the question still arises of whether arms within a Y-maze can be considered purely as places, or whether the locations are so large as to constitute contexts.

1.36: Episodic Memory Revisited

The issue that is perhaps central to the nature of memory systems in the medial temporal lobe is the question of whether episodic memory is more than the sum of its parts? Answering this question effectively will tell us whether episodic memory is effectively additive: memory for what combined with memory for where and when; or whether it is functionally dissociable from its component parts. As discussed in relation to the role of the hippocampus, finding an animal model of episodic memory has been a matter of considerable difficulty. Tulving (1985) defined episodic memory as

"receiving and storing information about temporally dated episodes or events and temporal-spatial relations among these events." (Tulving 1983)

Episodic memory therefore provides information about the 'what' and 'when' of events ('temporally dated experiences') and about 'where' they happened ('temporal-spatial relations'). Most tests of animal memory tend to examine, at most, two elements of the three that constitute episodic memory, with the "when" element being omitted. However, as well as the concise and relatively undemanding definition above, Tulving also emphasised the autonoetic nature of episodic memory, and such aspects of human episodic memory have been regarded as precluding the development of a true animal model of episodic memory.

However, an elegant paradigm which takes the "what, where and when" criteria as the basis of a potential model has been developed by Clayton, Griffiths, Emery &

Dickinson (2001). This model, which exploits the storage habits of scrub jays and the differential decay rate of the foodstuffs stored, includes all three parameters in its mnemonic requirements and has been described as modelling episodic-like memory. However, this has not yet been used to examine the effects of lesions to the hippocampus or other structures, although it is known that lesions to the hippocampus can impair retrieval of stored food in black-capped chickadees (Clayton & Dickinson., 1998; Clayton et al., 2001). Even if episodic memory were so complex as to be confined to humans (and this would seem to be debatable), an adequate model for some of its fundamental parameters can nonetheless be developed. Indeed it is arguable that some of the higher level requirements provided by some definitions of episodic memory can be more accurately considered as describing autobiographical memory (Conway, 2001, Review).

1.4: Conclusions.

The studies reviewed here suggest that the hippocampus and the rhinal cortices all contribute to a memory system which in humans supports episodic memory. However, it is clear that the specific contributions of these structures are non-identical, and, in some cases, capable of being doubly dissociated. In particular it is clear that the contributions of the hippocampus and of the perirhinal cortex are distinct. The roles of the entorhinal cortex and the postrhinal cortex are less clearly distinguished from those of these, as yet more extensively investigated structures. It does, however, seem probable that the postrhinal cortex is involved in supporting memory for the positions objects occupy, while the entorhinal cortex may play a similar role.

The extent to which the disparate functions of the structures of the medial temporal lobe are integrated into a single memory system which is responsible for the integrated representations involved in episodic memory is open to question. Indeed Gaffan (2001) questions the usefulness of such a "system" concept at all, arguing that memory formation may simply be a very vulnerable process and that damage to areas which contribute to processing of aspects of memory, such as spatial location, will, of necessity, damage memory. Under such a view the various structures implicated in memory should be seen more as processors of a particular type of information, for which memory formation is incidental to function rather than its *raison d'être.*

This thesis has a broad aim of further investigating the mnemonic functions of the perirhinal cortex, and comparing it with the roles of the postrhinal cortex and of the hippocampus. Bearing in mind the possibility that memory may indeed be incidental to their function, there is considerable evidence; behavioural, electrophysiological and neuroanatomical, that they are involved in supporting aspects of memory in rats. The perirhinal cortex very clearly has a considerable role in object memory. It seems clear that it is involved, not merely in simple object recognition but in aspects of learning and memory such as associations between stimuli (Buckley & Gaffan, 1998c) and associations between aspects of stimuli (Bussey et al., 2002; Eacott et al., 2001). The postrhinal cortex appears to be involved in aspects of spatial memory, but its relationship with hippocampal function is unclear.

The specific aims of this thesis include the further elucidation of the role of the perirhinal cortex in the binding together of features into objects and the limits of such binding. The experiment in chapter 2 was designed to determine the importance of the perirhinal cortex to a visual stimulus-stimulus association task where there were no overlapping features. Following from this, the experiments in chapter 3 investigated the role of the perirhinal cortex in the formation of a single gestalt representation as opposed to the formation of an association between two such distinct and separate representations.

The experiments in chapter 4 examined the point at which information, rather than being processed as either of the associative relationships outlined above, is processed as background or context to an object. This is examined in respect of the contributions of the perirhinal and postrhinal cortices and the hippocampus. Chapter 5 was a logical extension of this, with experiments investigating a potential rodent model of episodic-like memory and the possible role of the rhinal cortices and the hippocampus in supporting such memory.

Finally, the experiments in chapter 6 examined the role of intracellular calcium and the cholinergic system in the perirhinal cortex in object recognition with a view to expanding an understanding of the neurochemical basis of object memory. An

extensive discussion of the background to this work can be found in the introduction to chapter 6, as it is somewhat tangential to the work which is introduced above.

Chapter 2: Perirhinal cortex and stimulus-stimulus association learning

2.1: Introduction

As outlined in chapter 1, damage to the perirhinal cortex has been implicated in a wide range of learning and memory impairments in both monkeys and rats. These impairments include visual discrimination learning (Buckley & Gaffan, 1997; 1998a; Eacott, 1998) and retention (Kornecook et al., 1999; Machin & Eacott, 1999; Wiig et al, 1996), delayed match (and nonmatch) to sample (Gaffan & Murray, 1992; Eacott et al, 1994; Otto & Eichenbaum, 1992; Zola-Morgan et al, 1989) and stimulus associative learning (Buckley & Gaffan, 1998b; Bunsey & Eichenbaum, 1993; Eacott et al., 2001; Herzog & Otto, 1998; Murray et al., 1993; Parker & Gaffan, 1998). These impairments have been interpreted by some as revealing a deficit in learning about the characteristics of discrete objects (Eacott & Heywood, 1995; Gaffan, 1994; Murray, 1996; Murray & Bussey, 1999).

One important characteristic of an object may be its association with other stimuli. Indeed, studies have suggested that neurons within monkey perirhinal cortex code the association between two visual stimuli (Higuchi & Miyashita, 1996; Sakai & Miyashita, 1991; 1994). Moreover, lesion studies have supported the suggestion that rhinal or perirhinal cortex plays an important role in learning to associate two stimuli, whether they be both visual (Murray et al., 1993), two odours (Bunsey & Eichenbaum, 1993) flavour-visual (Parker & Gaffan, 1998) or tactile-visual associations (Goulet & Murray, 2001).

There are extensive reciprocal connections between the perirhinal cortex and the amygdala (Aggleton, Burton & Passingham, 1980; Stefanacci, Suzuki & Amaral, 1996), and perirhinal cortex would therefore seem well placed to be involved in associating a stimulus and reward. However behavioural studies have not supported such a role and instead suggest that, despite the extensive anatomical connections, the perirhinal cortex is not involved in associating objects directly with a reward value (Thornton, Malkova & Murray, 1998), although it may have a role in associating visual stimuli with motivational significance (Lui, Murray & Richmond, 2000). It would appear that, as long as a visual stimulus can be efficiently discriminated, associating it with a reward value is not impaired by lesions to the perirhinal cortex.

What the literature does suggest is that the ability to associate a stimulus with another stimulus, as opposed to directly with reward, is impaired by perirhinal lesions.

Baxter et al. (1999) investigated the ability of monkeys with rhinal or perirhinal cortex lesions to learn visual discriminations for an auditory secondary reinforcer. This task requires that the monkey use an association between an auditory stimulus which has previously been associated with reward and the visual discriminanda to perform the task. Correct choices are reinforced by the auditory reinforcer, an initially neutral stimulus which has gained reinforcing properties though its association with primary food reward. While there were early reports that lesions of the amygdala impaired ability to learn this task (Gaffan and Harrison, 1987), it was subsequently shown that they did so only because of collateral damage to efferent fibres from rhinal and perirhinal cortex (Malkova, Gaffan & Murray, 1997). Baxter et al. (1999) therefore proposed the view that damage to the rhinal or perirhinal cortex might be sufficient to result in an impairment in such learning. In fact, they found that rhinal or perirhinal lesions had only a mild effect on learning such tasks, far milder than the effects of the amygdala lesions of Gaffan and Harrison (1987). They therefore concluded that

"perirhinal cortex is not required for associating neutral visual stimuli with reinforcement, regardless of modality and whether the reinforcement is primary or secondary" (Baxter et al. p251).

However, learning for secondary reinforcement may take place in at least two ways (Gaffan and Harrison, 1987). First, there may be a direct association between the discriminandum and primary reward. In Baxter et al.'s (1999) study, this would mean that the visual discriminanda became associated with reward or nonreward *via* the association with the secondary reinforcer, even though the discriminanda themselves had never been directly paired with reward or nonreward. Alternatively, the visual discriminanda may become associated with the presence or absence of the secondary reinforcer which is itself associated with the primary reinforcer. The difference between these two explanations lies in the nature of the association which is formed in learning new discriminations using an existing secondary reinforcer. In the first case, learning a new discrimination involves making a new stimulus-reward association (*via*

an existing stimulus-reward association), whereas in the second case, it involves making a new stimulus-stimulus association.

In practice, of course, it is likely that learning for a secondary reinforcer involves learning both types of associations to some extent. Perirhinal cortex lesions do not generally impair stimulus-reward associations, as simple visual discrimination learning for food reward may proceed normally in animals with perirhinal lesions (Astur, Mumby & Sutherland, 1995; Machin & Eacott, 1999; Thornton et al., 1998). Perirhinal lesions would not, therefore, be predicted to impair this aspect of learning for secondary reinforcement. However, perirhinal cortex lesions do affect stimulus-stimulus associative learning (Buckley & Gaffan, 1998b; Bunsey & Eichenbaum, 1993; Herzog & Otto, 1998; Eacott, Machin & Gaffan, 2001; Murray et al, 1993; Parker & Gaffan, 1998).

A plausible interpretation of the mild impairment in learning for an auditory secondary reinforcer reported by Baxter et al.(1999) would be that it resulted from the disruption of stimulus-stimulus associative learning mechanism which would normally operate in conjunction with the still intact stimulus-reward associative mechanism. In such a situation, the observed effect might be only a mild impairment, although neither mechanism is itself only mildly impaired. In light of this argument, it is possible that Baxter et al's conclusion that the perirhinal cortex is not significantly involved in the formation of stimulus-reward associations, even when reinforcement is secondarily associated with the stimulus, should be modified. The degree to which learning in any individual secondary reinforcer study is dependent on stimulus-reward associations or stimulus-stimulus associations, and thus its dependence on perirhinal cortex, is likely to vary with the particular conditions used in that study.

Using visual-visual associations, as opposed to cross-modal associations gives rise to the issue of whether stimuli are perceived as separate but associated (paired) or as elements of a single stimulus (configured). The possibility of a configuration of the representations of the stimuli being formed is an important consideration, and every effort was made to prevent this occurring in the present study. If such a configuration were formed it would no longer be possible to regard the problem as requiring stimulus-stimulus association: it would become one which required configural rather

than elemental processing. Given that there is already some persuasive evidence that the perirhinal cortex is implicated in configural processing (Bussey et al., 2002) this would be likely to give rise to artefactual results in an experiment designed to investigate stimulus-stimulus association.

Therefore, in order to further investigate this interpretation of the impairment reported by Baxter et al. (1999), this study examined visual learning for a secondary reinforcer in the rat following bilateral lesions to perirhinal cortex. The task was designed so as to emphasise those aspects of the task which have been hypothesised to encourage stimulus-stimulus learning over direct stimulus-reward associations. For example, it has been shown using normal animals in secondary reinforcer paradigms that stimulus-stimulus associations are most easily made when the stimulus and reinforcer are in the same modality and are spatially close together (Rescorla, 1980; 1985). Conversely, they are harder to associate together when they are in different modalities and are spatially separated (Rescorla, 1980; 1985). Temporal contiguity has also been shown to be important in determining the nature of the association formed.

In support of the view that learning for similar and disparate secondary reinforcers differ, Gaffan and colleagues have found that visual learning for a visual, but not an auditory, secondary reinforcer can recover from the effects of bilateral amygdalectomy (Gaffan, Gaffan & Harrison, 1989; Gaffan & Eacott, 1995), suggesting the availability of an alternative learning strategy in the former, but not the latter. According to these principles, the study of Baxter et al., (1999) would have favoured direct stimulus-reward associations over stimulus-stimulus associations, as the discriminanda and the secondary reinforcer differed in modality and spatial location. In the present study, therefore, we used a secondary reinforcer which was both in the same modality and in the same spatial location as the visual discriminanda. In this way, we hoped to encourage the learning of the task by formation of stimulus-stimulus associations over stimulus-reward associations. Thus we predicted that perirhinal lesions would have a deleterious effect on learning.

2.2: Methods and Materials

Subjects

Twenty male Dark Agouti rats (Bantin & Kingman, Hull, UK) were used in this experiment. Of these eleven reached the preoperative training criteria for surgery and were operated. Three operated animals died due to respiratory infection in the postoperative period. Animals were housed in pairs in diurnal conditions (12h light/ 12h dark cycle) and all testing was carried out during the light phase. Throughout the study animals had free access to water except when in the testing apparatus. During training and testing they were fed on a restricted diet to maintain body weight at approximately 85% of normal weight. They were weighed regularly to ensure that weight was maintained within this limit. Immediately before and following surgery they were given *ad libitum* access to food. At the start of testing they were aged approximately 3 months. They were tested five or six days a week.

Apparatus

All training and testing was carried out in a computer controlled testing apparatus (Gaffan & Eacott, 1995). It consisted of a computer controlled Y-maze (Figures 2.1 and 2.2). The maze was composed of three identical arms. The end of each arm was composed of 2 angled monitors on which computer generated forms could be displayed. The floor of the maze was made of wood cut to fit against the edges of the monitors. The centre of each monitor screen was situated 46.5cm from the centre of the maze. The sides of the maze were made from aluminium painted black and were 22cm high. The maze was covered by a Perspex lid in four sections. The central section was removed to place rats into the maze.

The maze was housed in a room lit by a partially covered 40W bulb at a height of 2m above the floor. A constant level of background noise was provided by white noise during all testing. This also served to mask any extraneous noise. Activity within the maze was viewed in an adjacent room via a video camera suspended 160cm above the floor of the maze. The computer controlling the maze was also situated in the adjacent room. This computer used a program which monitored the animals' reaction times to stimuli onset and reward collection, the number of trials and errors made in each session and the length of each session.

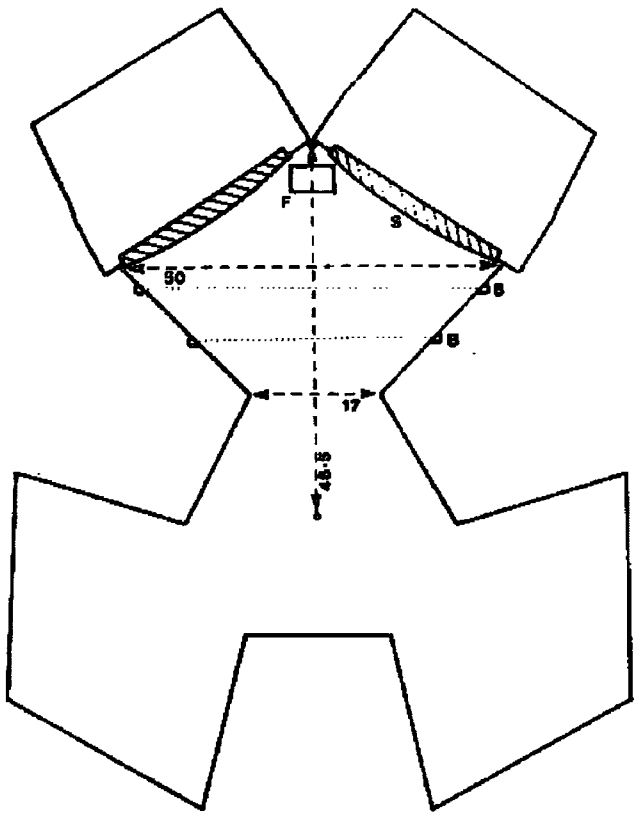


Figure 2.1: Schematic aerial view of the computer controlled Y-maze

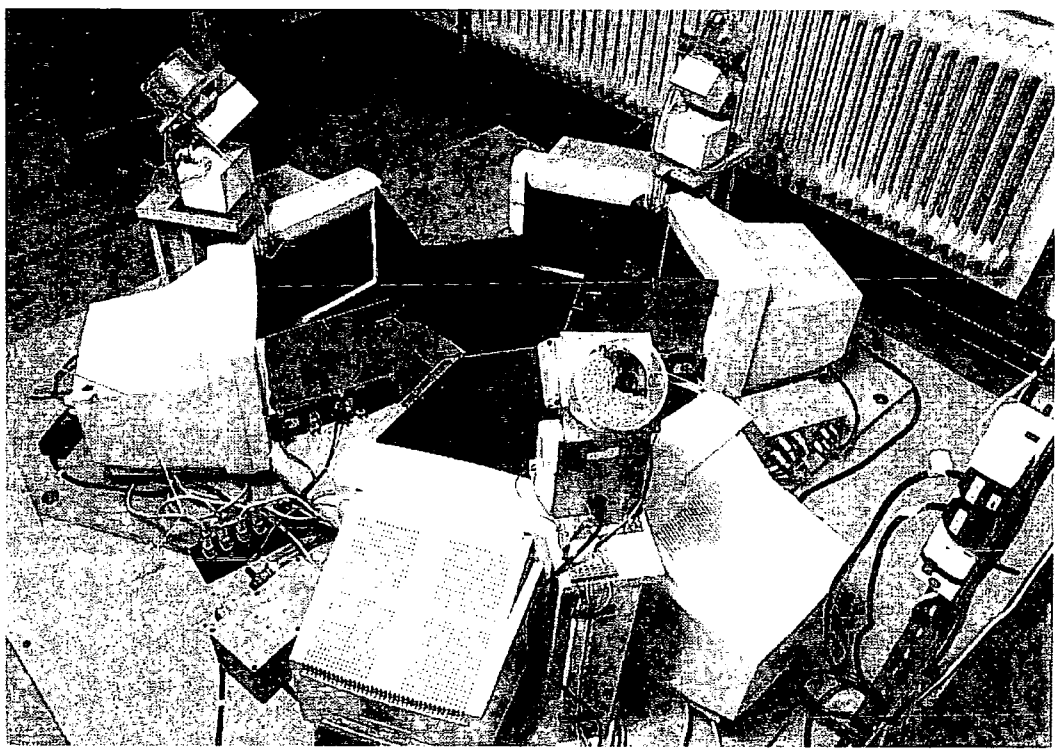


Figure 2.2: Photographic illustration of the computer controlled Y-maze.

The monitors at the ends of each arm had maximum image areas of 18.5cm by 23cm. The monitors in each arm were separated by an automated food well 7cm wide. Each well had a Perspex door covering a food tray which animals had to push open to collect 45mg food pellets which were delivered as a reward by a dispenser situated above the food well. The dispenser released pellets into the food tray via a plastic tube encased in black aluminium. Delivery of a food pellet triggered the illumination of the food tray by a 3W, 24V bulb. The opening and closing of the door to the food well was sensed by a microswitch that signalled that the pellet had been collected and switched the bulb off.

The animal's position in the Y-maze was determined by photodetector beams which crossed the arms of the maze at 23cm and 30cm from the monitors and were situated 3cm above the floor. An approach to the end of an arm in which the correct stimulus was displayed on the screens could be automatically rewarded by delivery of reward pellet(s) to the dispenser between the two monitors in that arm.

The computer controlling the maze was a 486 DX 66 HZ Viglen computer with 4 MB ram, 2 x 325 MB hard disks and 8 full length expansion slots. A locally built interface operating at 24 V DC fed information to the computer on the status of foodtray flaps and photodetector beams and sent signals to the traylights and feeders in response to this information.

The monitors were 6 black and white Viglen monitors driven by 3 dual VGA plus cards each with 2 x 512 Kbytes video RAM. Graphics were sent to the maze monitors and a text display for the experimenter was provided on a standard Brother monitor. All experimental programs were written in-house in turbo Pascal V 6.0.

Surgery

Surgery was performed on all eleven animals which attained the preoperative criterion in training. Each rat was anaesthetised using halothane, its head was shaved and it was positioned in a stereotaxic headholder. 0.5ml of the analgesic vetagesic was administered subcutaneously. An incision of the scalp was made along the midline and bregma was measured at an angle of 12^{o}. The top of the skull was then measured at three points: 3.0, 4.0 and 5.0mm posterior to bregma and 5.1mm lateral to bregma.

Using a dental drill, an area of the skull overlying the rhinal sulcus was removed. The dura was cut to allow the insertion of an electrode into the brain. Lesions were made using an RFG4-A RF lesion generator (Radionics Inc). The electrode, (0.3mm tip length, 0.25mm diameter) was lowered at an angle of 12° at three points: 3.0, 4.0 and 5.0mm posterior to bregma and lateral to bregma by 5.1mm (measured at an angle of 12°). At each point the electrode was lowered to 6.6mm below the top of the skull and current was passed such that a temperature of approximately 75°C was achieved for one minute. Bregma was then measured at an angle of −12° and the procedure performed contralaterally. The scalp was then closed using wound clips and antibacterial wound powder was applied. Each animal received 5ml warmed saline and 0.3ml of the respiratory stimulant milophyline subcutaneously.

Perfusion

For histological purposes, at the end of testing operated animals were perfused intracardially with a 5% formal saline solution. Their brains were removed, embedded in wax and coronally sectioned into 10μ slices. Every 10th section was stained with cresyl violet (Nissl stain).

Training

Habituation and Pre-Training

All animals received initial training in the maze which habituated them to the testing apparatus. During these sessions they learnt to approach the illuminated food wells and collect rewards and to respond to changes in visual stimuli on the monitor screens. Initial sessions took place with the doors of the food wells fixed open and the wells constantly illuminated. Pellets were placed inside the food trays and were also scattered around the food wells. Over several sessions the doors were gradually closed and the pellets placed only inside the food trays until the animals were opening the doors in order to retrieve the pellets. The mean time to complete this stage of training was 9 sessions (range 8-13 sessions).

During the next stage of training only one food well at a time was illuminated and would dispense pellets when approached. This taught the animals that only illuminated wells would dispense pellets, and also accustomed them to the sound

made by the automated dispenser. At this stage the trial was not ended by an approach to an incorrect arm, allowing rewarded self-correction by the animal. When animals were performing well at this stage visual stimuli were introduced. Up till this point the screens had been switched on but showed only a background grey. At this stage a rewarded stimulus (S+) was introduced. This was a white bar of dimensions 1.5 x 23.5cm, and 7cm above the bottom of the screen on a black background which appeared across the monitors in one arm of the maze. If the animal approached the S+ a reward pellet was dispensed. Collection of this pellet resulted in a new trial beginning following a brief inter-trial interval of 1 minute. If the animal left the arm in which it had been rewarded before this interval had elapsed it had to return to that arm before the next trial would begin. Animals completed up to 50 trials a day at this stage. When they were performing well the task was altered so that an error ended the trial, with the stimulus presentation disappearing and a new trial beginning.

The final stage of training, which started after the animals had attained a performance of 80% correct on the previous task, was to introduce a non-rewarded stimulus (S-). This was a display of small white dots across the screen with an identical level of luminance to the S+. At this stage animals learnt to discriminate between two concurrently presented stimuli on the basis of reward. Again they were required to reach a performance level of 80% before progressing.

In the final stage of training a number of different rewarded stimuli were gradually introduced in order to ensure that animals responded to stimuli in any part of the screen. The stimuli were white bars 1.5cm deep but of varied length and presented at different horizontal positions on the monitor screens. These were presented in opposition to the original S-. Initial training was complete when animals achieved 80% correct on this phase of the training.

Training: Experiment 2

Following the initial training described above, animals were trained in a discrimination between two stimuli (Figure 2.3). An approach to the correct stimulus (S+) was followed by the secondary reinforcer, which was a white bar, appearing over the S+ and remaining there for a period of 5 seconds (Figure 2.4). Ultimately primary reinforcement to be delivered on an FR5 schedule (five 45mg pellets delivered only

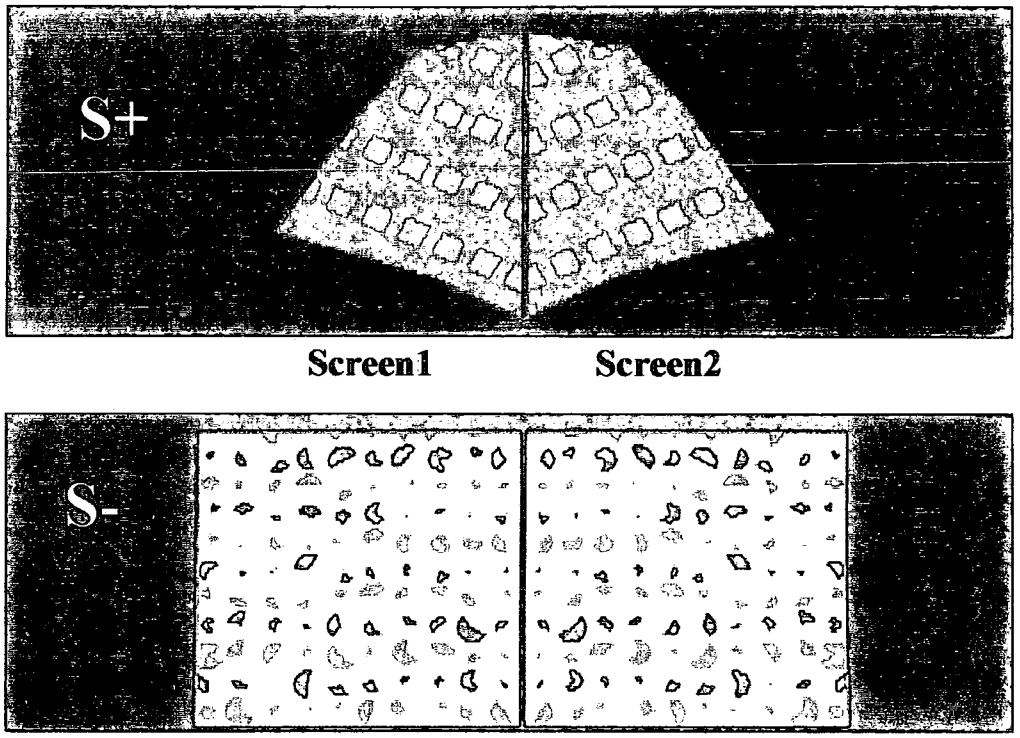


Figure 2.3: Examples of initial stimulus choice

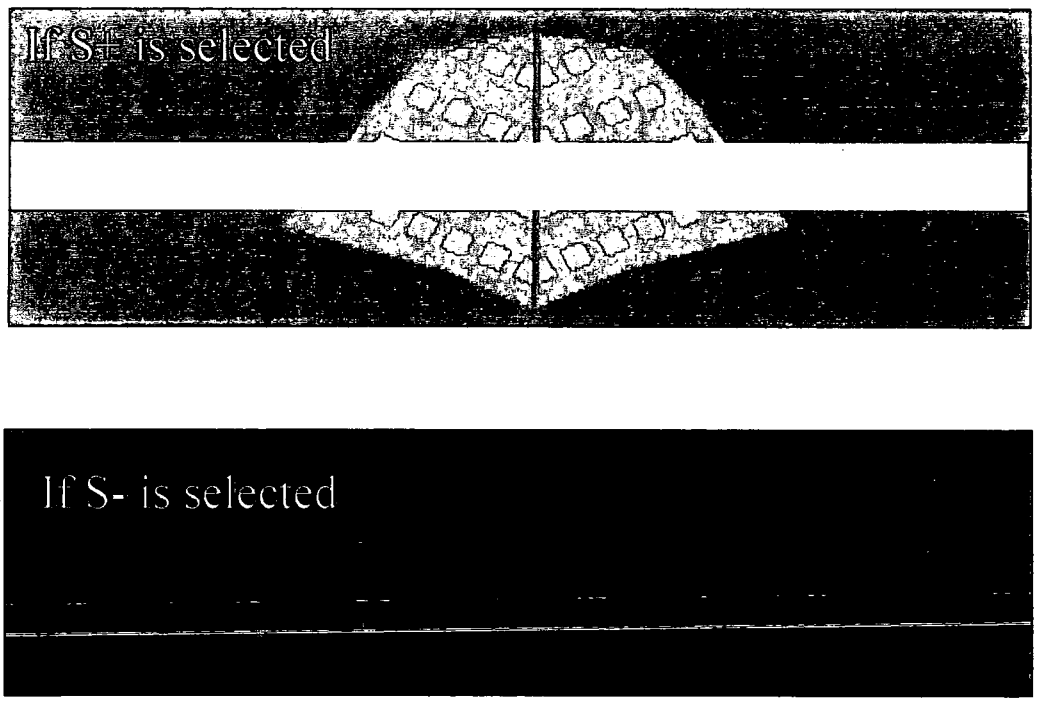


Figure 2.4: Displays following choice of stimulus

after there had been five correct responses). Because this was a difficult task training took place in several stages.

Animals initially learnt a series of discriminations between stimuli on a reward schedule of FR1. This meant that all correct choices were followed by a primary reward of one 45mg food pellet. However the secondary reinforcer always appeared immediately after a correct response and only after a delay of 3 seconds was primary reinforcement delivered. When animals reached a criterion of 5 errors or fewer in 40 trials, the number of correct choices before primary reward was delivered was gradually increased through 2, 3 and 4 until criterion was maintained at FR5.

At this point a "probe" session was carried out. In this the animal was presented with a different pair of objects on each of 100 trials. The stimulus which was the S+ appeared with a white bar identical to the secondary reinforcer across it. Every correct choice was rewarded. The aim of this session was to determine whether the animals had formed an association between the secondary reinforcer and reward. Results of this session indicated that there was significant learning of this association. The number of errors ranged from 14 – 44 (mean = 26.95).

At this point a new discrimination was introduced each day, regardless of daily performance, with an initial FR of 2. If 8 or fewer errors were made during the final 40 trials (a performance of 80% correct) then the problem was considered to have been solved. When an animal solved 7 out of 10 consecutive problems then the FR was increased. After 18 sessions of training in this phase those animals which had not yet reached FR3 were reassessed. Those rats which had not achieved an average of 70% over the final 20 trials in each problem were given additional training in 3 novel problems which were repeated across training sessions until a strict criterion of 87.5% correct over 40 trials was achieved. Following this extra training they were returned to the original schedule in which new problems were introduced each day.

After 40 sessions of training an additional criterion for moving on to higher levels of FR was introduced. Rats progressed to FR3 if they had achieved at least 72.5% correct on the final 20 trials of each problem over the previous 10 sessions. Animals which failed to meet this criterion continued to train until they met either this criterion

or the previous one of solving 7 out of 10 problems. This alternative criterion of 72.5% over final 20 trials was also used after animals had completed at least 25 sessions of FR3 and 15 sessions of FR4.

Training was almost complete when animals had reached the stage of learning problems using FR5 to the criterion of 5 or fewer errors over the final 40 trials (80% correct). At this stage the number of trials per problem was gradually decreased from 100 to 40, maintaining an FR of 5. In this final phase of training the stimulus chosen by the animal on the first trial was designated correct (S+) or incorrect (S-) on alternate problems, thereby controlling for initial stimulus preferences. For this reason performance on trial 1 was of necessity at chance regardless of the animal's choice. Therefore, correct choices on the first trial of any problem did not count towards the FR requirement.

Rats performed up to 120 trials a day and thus at the end of training could perform up to 3 problems, each of 40 trials in one day. Rats were tested once a day five or six days a week until they were able to show significant learning before delivery of the first primary reinforcement (i.e. at trial 6). At this point all animals which reached criterion underwent bilateral perirhinal ablation. After surgery and at least 2 weeks recovery the animals were tested on a further 60 problems with secondary reinforcer at FR5.

2.3: Results.

2.31: Histological

Histological analysis indicated that all 8 of the animals which completed testing and survived surgery had perirhinal lesions which were largely as intended. The lesions extended from approximately 3.5 mm to 6.5 mm posterior to Bregma. Figure 2.5 shows representative sections from rat R102 which was judged to be a typical lesion, while Figure 2.6 shows the smallest (R101) and largest (R103) lesions drawn on to standard sections taken from Paxinos & Watson (1998). The estimated range of damage to the perirhinal cortex was 29% - 82% of the total extent of the perirhinal cortex. This was calculated using lesion drawings on four standard sections from Paxinos & Watson (1998) (as shown in figure 2.6) copied on to squared paper to enable the calculation of damage proportional to the total area of the perirhinal cortex.

As can be seen from figure 2.6, there was some unintended hippocampal and entorhinal damage in a number of animals. However, this damage was extremely partial and almost always unilateral, while the perirhinal lesions were extensive and bilateral. In the light of the behavioural results reported below there is no indication that this extra-perirhinal had any behavioural effects. Figure 2.7 shows the pre- and post-operative performance over the first block of trials (before any primary reinforcement could be received) of the animals with the largest and smallest lesions in relation to the group as a whole. As can be seen there is no indication that lesion size affected the postoperative performance.

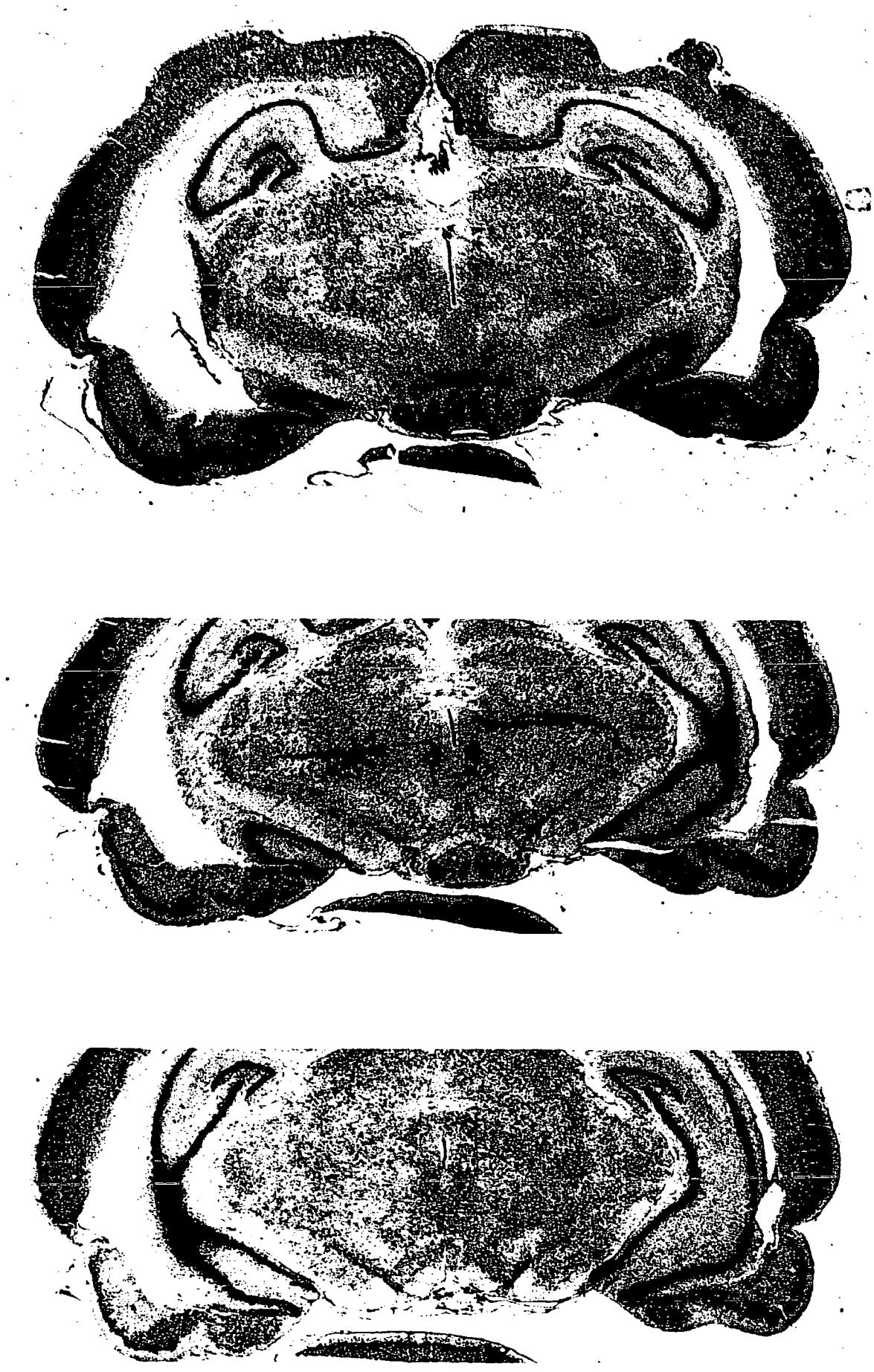


Figure 2.5: Three representative sections of a bilateral perirhinal lesion from R102

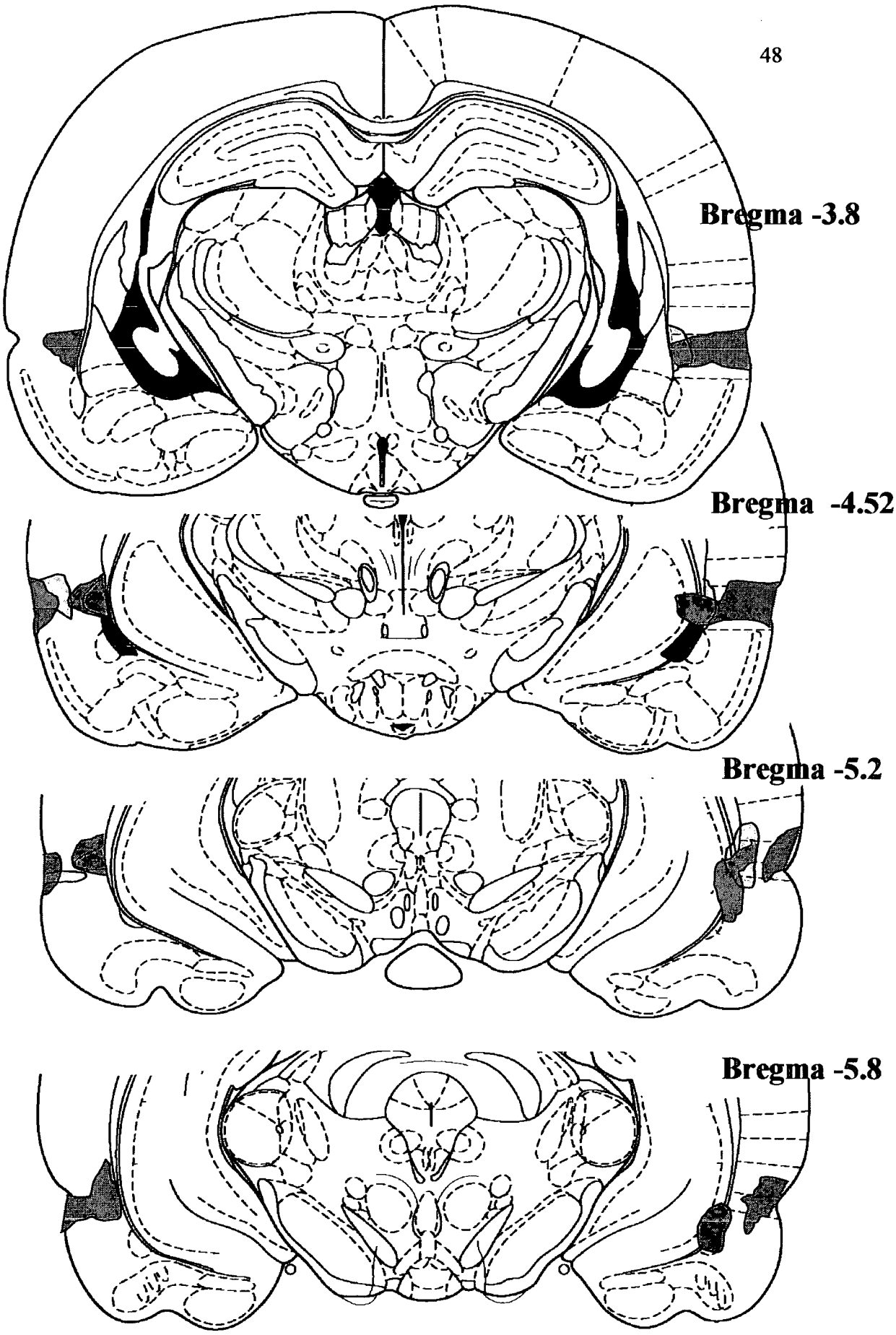


Figure 2.6: The extent of the smallest (R101, semi-transparent grey) and largest (R103, diagonal stripes) lesions drawn on to standard sections taken from Paxinos & Watson(1998).

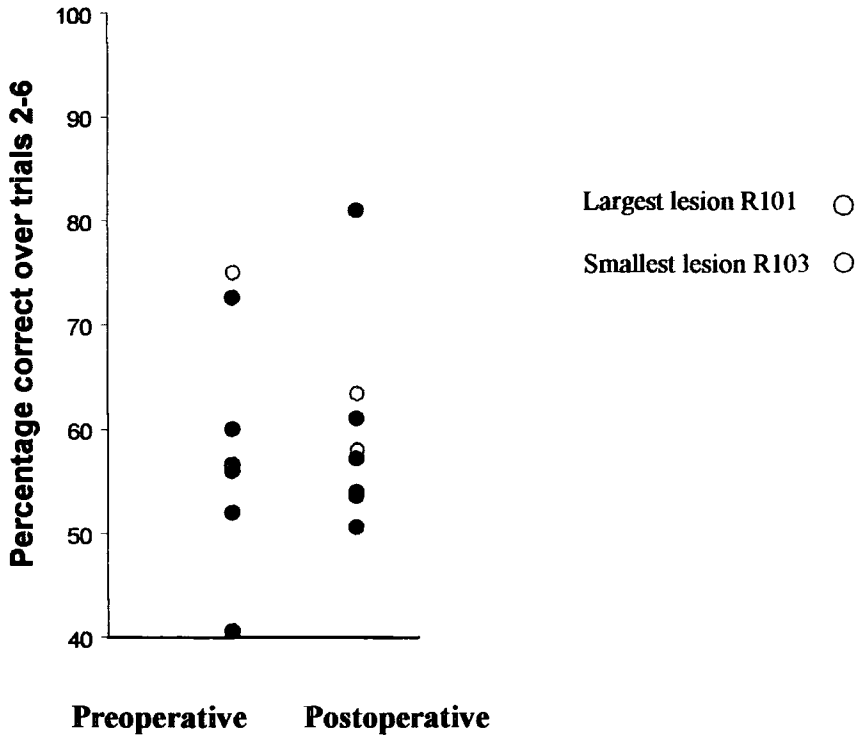


Figure 2.7: Pre- and post-operative performance of animals with largest and smallest lesions in relation to the cohort.

2.32: Behavioural

The task was a hard one to learn. Those rats which reached criterion took an average of 111 sessions to reach the criterion for surgery (range 92-132). Furthermore only 11 of the 20 rats who began training in this task reached this level, training of the remainder being abandoned due to lack of sufficient progress at earlier stages (mean number of sessions is not reported here, as training of animals which failed to meet intermediate criteria was discontinued at different points). However the results of the eight animals which completed the preoperative stages of testing and which survived surgery are reported here. The mean number of sessions to reach the surgical criterion for these eight animals was 116 (range 97-132).

In the final stages of preoperative training and in all postoperative training, the animals were learning discriminations using an FR5 reward schedule (in which the first trial of any problem did not count towards the FR ratio). For this reason, even an animal whose performance was 100% correct (but see below for trial 1) could not receive primary reinforcement until after trial 6 had been completed. A pure measure of

learning using the secondary reinforcer is therefore performance over the first 6 trials (i.e. before any primary reinforcement has been delivered). In these final stages, the discriminandum chosen on the first trial of any problem was designated correct or incorrect on alternate problems, controlling for initial stimulus preferences. However, analysis of performance at trial 6, before any primary reinforcement had been received, showed that performance in problems in which the initial choice was designated correct did not differ from those in which initial choice was designated incorrect, either preoperatively (mean when initial choice was correct = 63.8%, when initially incorrect = 62.6%; $t < 1$, $df = 7$, $p > 0.05$) or postoperatively (mean when initial choice was correct = 63.4%, when initially incorrect = 60.6%; $t < 1$, $df = 7$, $p > 0.05$). In view of this, problems in which the animal's initial choice was designated correct and incorrect were pooled and are considered together for the purposes of all further analysis.

Figure 2.8 shows the performance of the rats over trials 1-6 over the final 30 preoperative problems and the 60 postoperative trials. Performance over these trials shows learning on the basis of secondary reinforcement alone, as no primary reinforcement could have been received before this point. From this figure it can be seen that both pre and post-operatively the animals were able to learn for secondary reinforcement. When performance averaged over the block of trials 2-6 was tested the animals were above chance on both the first and second sets of 30 post-operative problems (first set: $t = 2.60$, $df = 7$, $p < 0.05$; second set: $t = 3.21$, $df = 7$, $p < 0.05$) although this was not the case pre-operatively ($t = 2.07$, $df = 7$, $p > 0.05$). Performance was significantly above chance on trial 6, before first delivery of primary reinforcement, both preoperatively ($t = 3.57$, $df = 7$, $p < 0.01$) and for the second set of 30 postoperative problems ($t = 2.87$, $df = 7$, $p < 0.05$). For the first set of post-operative problems performance was not above chance ($t = 1.96$, $df = 7$, $p = 0.091$). When the two sets of post-operative problems were combined performance was again above chance ($t = 2.87$, $df = 7$, $p < 0.05$). The measure of performance averaged over trials 2-6 is less susceptible to variability and might be expected to show less evidence of performance above chance, including as it does data from trials earlier than trial 6. The balance of the evidence is therefore that, both pre and postoperatively, the rats were capable of performing at above chance levels before the first delivery of primary reinforcement.

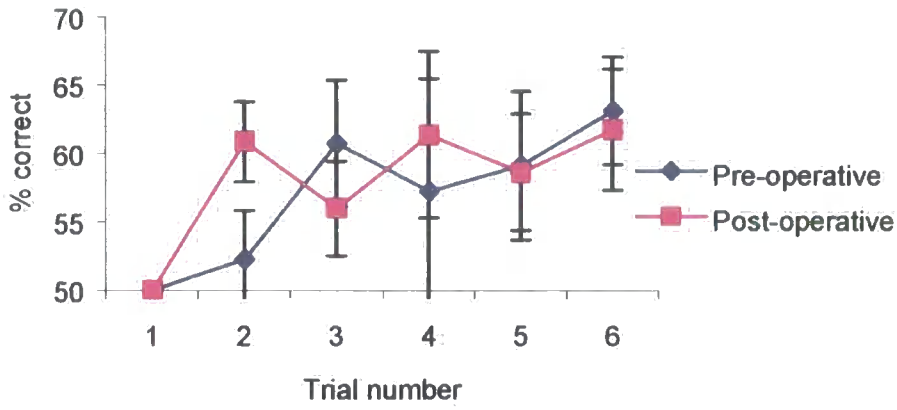


Figure 2.8: Performance over the first 6 trials (before any primary reinforcement has been received) for the last 30 pre-operative problems and the first 60 postoperative problems.

The lack of a postoperative impairment is further emphasised by comparison of pre- and postoperative performance on trial 6 alone, before primary reinforcement, which reveals no significant difference between the pre and post operative performance levels either when analysed using a three (operated condition) x 1 (trial) repeated measure ANOVA ($F < 1$, $df = 2,14$, $p > 0.05$) or when the two sets of postoperative problems were combined ($t < 1$, $df = 7$, $p > 0.05$) (see figure 2.9).

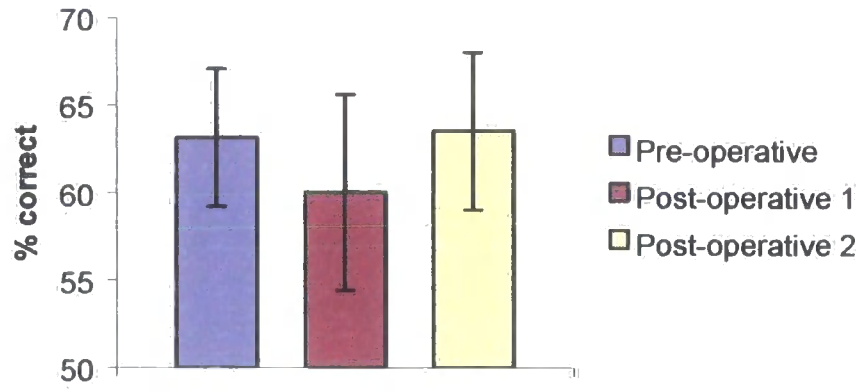


Figure 2.9: Pre- and post-operative performance on trial 6, the final trial before primary reinforcement could be received.

Furthermore, comparison of the mean performance over the entire block (trials 2-6) also reveals no evidence of a difference between the groups. These trials were initially analysed using a 3 (operated status) x 1 (trial block) repeated measures ANOVA, which contrasted the final 30 pre-operative problems with the first 30 post-operative problems and the second 30 post-operative problems. This found no effect of operated condition ($F < 1$, $df = 2,14$, $p > 0.05$). When the two blocks of postoperative trials were combined and the data analysed using paired-samples T-test there was no difference between pre- and post-operative problems ($t < 1$, $df = 7$, $p > 0.05$). Indeed, using this measure the animals' performance was marginally better on postoperative than on preoperative problems (mean preoperative = 58.6%; postoperative = 59.9%) (see figure 2.10). There is therefore no evidence that lesions of the perirhinal cortex disrupted the ability to learn two choice discriminations for secondary reinforcement before receiving any primary reinforcement.

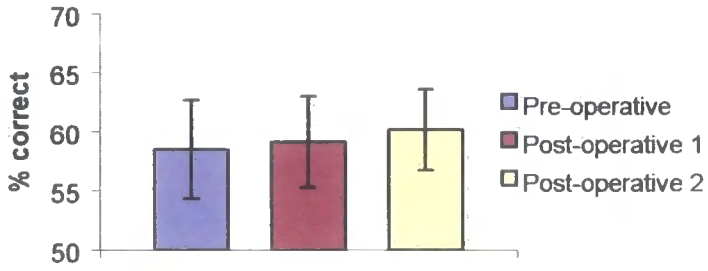


Figure 2.10: Pre- and post-operative performance averaged over the first block of trials 2-6, before any primary reinforcement was received.

Although a rat which, following the initial trial made all choices correctly, effectively performing at 100% accuracy, could obtain primary reinforcement following trial 6, in practice, this would be only rarely achieved (as discussed above, the mean performance was approximately 60% on postoperative trials). In fact the mean number of trials before reinforcement was received was 8.4. Therefore, when choosing between discriminanda on trial 11, for example, a rat has received a maximum of only one episode of primary reinforcement (following the fifth correct response after trial 1), yet up to 10 episodes of secondary reinforcement. Equally, performance on trial 16 follows a maximum of 2 primary reinforcement episodes, yet up to 15 secondary reinforcement episodes. Therefore, at any point after trial 6, even though primary reinforcement may have been received, an animal learning purely on the basis of information received through primary reinforcement would have been significantly disadvantaged over one which was able to learn using secondary reinforcement as an additional, if not as the sole learning strategy. The performance of the animals was therefore also examined over 30 trials for each problem. Figure 2.11 shows the performance of the rats averaged over blocks of 5 trials (excluding trial 1, as before). As before the data were initially analysed using a 3 (operated status) x 6 (trial blocks) repeated measure ANOVA. This showed no effect of surgical condition ($F = 1.15$ $df = 2,14$, $p > 0.05$) although as was expected there was an effect of trial block ($F = 5.84$, $df = 5,35$, $p = 0.01$) as performance improved over the course of a problem. There was no interaction between surgical condition and trial block ($F < 1$, $df = 5,35$, $p > 0.05$). As there was no difference between the first and second sets of post-operative problems these were collapsed. A 2 (surgical condition) x 6 (trial blocks) repeated measure ANOVA also reveals no effect of surgical condition ($F < 1$, $df = 1,7$, $p > 0.05$). Again, as expected, there was a main effect of block ($F = 4.85$, $df = 5, 35$, p

< 0.01), as the animals' performance improved over the blocks as they learned each problem. However, importantly, there was no interaction of surgical condition and trial block ($F = 1.25$, $df = 5, 35$, $p > 0.05$), revealing that this learning did not differ between pre and postoperative problems. There is no evidence that perirhinal lesions disrupted at any point the animals' ability to learn two-choice discrimination for secondary reinforcement.

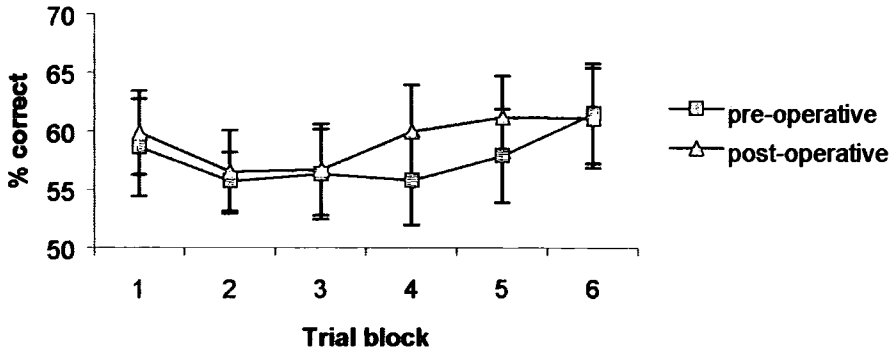


Figure 2.11: Pre- and post-operative performance over the full problem (30 trials in 6 blocks of 5, excluding trial 1).

In summary, over a number of measures of learning, either solely measuring secondary reinforcement or allowing primary reinforcement aided by secondary reinforcement, there was no evidence of an effect of perirhinal lesions.

2.4: Discussion

The results of this experiment showed that the ability of rats to learn visual discriminations for visual secondary reinforcement was not affected by bilateral perirhinal lesions. This result was found over a number of measures despite the fact that the task had been designed to maximise the chances of finding such a deficit, had one existed. The only exception to this pattern of results was that the first set of post-operative problems did not show performance above chance on trial 6. The significance of this result is, however, substantially reduced by the fact that performance over the block of trials 2-6 was above chance for both sets of post-operative problems, whereas this was not the case pre-operatively. On neither measure did performance on this set of immediate post-operative problems differ either from

preoperative performance or from later post-operative performance. This further suggests that the failure to find performance significantly above chance on this particular trial does not reflect a genuine impairment.

The discriminanda and the secondary reinforcer were in the same modality and spatial location, both of which have been found to encourage indirect stimulus-stimulus associations (Rescorla, 1980; 1985) hypothesised to be impaired in perirhinal lesioned animals. This study therefore supports the conclusions of Baxter et al. (1999) that the perirhinal cortex does not appear to play a critical role in learning for visual secondary reinforcement. In addition this conclusion can be extended to include perirhinal function in rats as well as monkeys. The rat perirhinal cortex receives a considerably smaller proportion of its afferents from visual areas than does the same area in the monkey, and provides substantially weaker feedback to sensory areas (Burwell & Amaral, 1998). In the light of this it would have been a slightly anomalous finding had the perirhinal cortex proved to be more essential for visual secondary reinforcement in the rat than in the monkey.

The finding that perirhinal lesions caused no impairment in the learning of the discrimination is surprising, as it had been predicted that there would be a deficit as a result of a stimulus-stimulus associative learning impairment, which would at least slow learning in this task. The only sign of a mild impairment was the fact that the animals were not significantly above chance on trial 6 in the first set of post-operative problems. However, given that their performance over the first block of trials as a whole was above chance, and that this measure included earlier trials on which poorer performance would be expected, the importance of this result should not be over-estimated. This is particularly the case as no difference between performance was found when the different sets of problems (pre-operative and first and second post-operative) were compared. A stimulus-stimulus associative impairment after lesions to the rhinal region has been seen in monkeys (Buckley & Gaffan, 1998; Goulet & Murray, 2001; Murray et al., 1993; Parker & Gaffan 1998) and in rats (Bunsey & Eichenbaum 1993). However, it is possible that, despite the design of the current study which was intended to favour stimulus-stimulus solutions to this task, the rats learned to use the alternative strategy of using the indirect stimulus-reward

association, discussed earlier, and thus the intact discrimination learning seen did not involve any stimulus-stimulus learning at all.

Although this explanation of the results cannot be ruled out, it would appear to be improbable for two reasons. First, the design of the task is one that favours stimulus-stimulus associations, and, second, the initial acquisition of the task took place before surgery, when there would be neither a necessity nor an advantage in using stimulus-reward associations as opposed stimulus-stimulus associations. Following surgery, learning to use direct stimulus-reward associations would have been an advantageous strategy, but the study found no deficit over 60 postoperative problems. In particular, there was little evidence of a postoperative deficit using learning over the first 6 trials (before the delivery of any primary reinforcement) as a measure. As discussed above, the value of the evidence that in the first set of post-operative problems animals did not differ from chance on trial 6 is limited by the fact that they were above chance on the block of trials 2-6. This was clearly a difficult task even for intact rats to learn and thus the suggestion that they may have acquired an alternative learning strategy after surgery in under 60 problems appears implausible, particularly given the fact that there was no difference in performance between the first 30 problems after surgery and the subsequent 30 problems, either on the trials before any primary reinforcement was received or on the problem as a whole.

It is, of course, possible, if not probable, that both strategies were initially used, but that when, during postoperative training only the strategy of stimulus-reward association was available (Thornton et al., 1998), this was sufficient to maintain performance at the preoperative level. However, a stimulus reward association cannot explain the above chance discriminations observed across trials 2-6 before any primary reinforcement was available. Therefore, whilst this is a possible explanation for the lack of impairment across entire problems, it cannot explain the spared performance over the first five trials. Additionally, it does not appear to be the most likely given the ratio of primary : secondary reinforcement involved, and therefore the relative reliance likely to have been placed on each strategy during preoperative training. It is, however, impossible to dismiss the possibility that both strategies were employed during the later stages of each problem: the difficulties inherent in designing a task in which only one of the two strategies is possible (as opposed to probable) are

considerable, given the motivational requirement for some primary reinforcement. This is particularly the case when rodents rather than monkeys are used. Whether or not preoperative performance involved some element of stimulus-reward association, there is clearly reason to believe that the intact performance was based on an intact ability to form stimulus-stimulus associations following perirhinal cortex lesions.

This result may initially appear to be paradoxical in suggesting that the perirhinal lesioned animals had no problem forming stimulus-stimulus associations in order to learn for secondary reinforcers, despite evidence that in other tasks perirhinal lesions impair such stimulus-stimulus learning. One possible solution to this quandary is to suggest that perirhinal cortex is not involved in stimulus-stimulus associations *per se*, but in knowledge about objects. This knowledge about objects has been described as

"the precise specification of objects by associating together the various visual features inherent in particular objects" (Gaffan, 1994, p421),
or as providing

"a 'gestalt' representation of a complete stimulus." (Murray et al., 1998 p146).

It is therefore possible that perirhinal cortex may be involved in associating together elements which form a single meaningful whole stimulus, but not for associating together different discrete stimuli. Of course, defining whether two associated stimuli should be viewed as a single entity, or merely as two associated entities will depend on a great many factors. Amongst these factors may be the extent to which the occurrence of the associated stimuli is correlated in time and space but also whether each stimulus has any independent meaning. For example, in the currently reported task, the secondary reinforcer was seen in every problem and had a clear meaning in signalling the availability of primary reinforcement which was independent of any other discriminanda. The fact that animals learned to respond for this secondary reinforcer demonstrates that they had learned this meaning. In this way, the secondary reinforcer may have been processed as an object in its own right, rather as a part of any other discriminandum. Thus although efficient learning in this task relied upon forming an association between the correct discriminandum and the secondary reinforcer, they did not themselves form a "a 'gestalt' representation of a complete

stimulus." (Murray et al., 1998 p146), but rather an association between two separate entities.

This contrasts with the situation in a recent study (Eacott et al., 2001) in which perirhinal lesioned rats were impaired in a biconditional configural learning task for primary reinforcement. In that study, the authors argued that rats were impaired at learning to associate two visual elements of a stimulus to form a configural stimulus. As in the current study, the association was between two visual stimuli which appeared in the same spatial location as each other. However, unlike the current study, none of the visual elements had a consistent reward value independent of its configural associate. With such complete feature ambiguity, efficient learning relied upon associating the two elements to form a meaningful whole, a task which was impaired by perirhinal lesions. On this basis this thesis proposes that perirhinal cortex is not crucial for forming stimulus-stimulus associations *per se*, but is crucial for combining the representations of visual elements so as to produce a single representation of an object.

This interpretation can also be examined in other tasks in which deficits in stimulus-stimulus associative tasks have been seen after lesions in this region in monkeys (Buckley & Gaffan 1998c, Goulet & Murray 2001, Murray et al., 1993, Parker & Gaffan 1998) and in rats (Bunsey & Eichenbaum 1993). All the tasks given to monkeys were similar in having a one-to-one correspondence between a stimulus and its paired associate. In each case, given a stimulus cue, the animals were trained to choose the associated stimulus over an unassociated stimulus. The study which used rats (Bunsey & Eichenbaum, 1993) differed slightly in that rats were presented with two stimuli which either formed an (arbitrarily designated) pair or were a mismatched pair, while reward was earned by distinguishing pairs from mismatches. However, all these tasks are similar in that no stimulus had an independent association with reward in the absence of its associate and thus had meaning only when associated with another stimulus in its correct pair. In this way the pair could be regarded as forming a single meaningful entity, with the seemingly independent stimuli in fact serving as no more than elements which only carried significance in the context of the whole. This common property is shared by all these tasks which are impaired by lesions to the perirhinal or rhinal region. In contrast, in the current task, the secondary reinforcer

had an independent association with reward, and could therefore be regarded as a truly independent entity. Since the formation of associations between this stimulus with others was not impaired by perirhinal lesions it would seem possible that it is the meaning of the stimuli involved in associative learning, and in particular the question of whether they possess independent significance, rather than their appearance or modality, which is critical in determining perirhinal involvement.

In summary, this study supports the conclusion of Baxter et al. (1999) that the perirhinal cortex is not required for associating neutral visual stimuli with a secondary reinforcer even when both are in the same modality. In addition this conclusion can be argued to include rats as well as monkeys. Finally, from contrasting this result with other positive findings of a perirhinal or rhinal impairment, we suggest that the role of perirhinal cortex is in "within-object" associations between elements, and that perirhinal cortex plays a much lesser role in stimulus-stimulus associations between discrete objects. This theory is explored extensively by the experiments described in chapters 3 and 4, which examine the effect of perirhinal lesions on object recognition for which a configural as opposed to a primarily elemental representation of an object is required for successful discrimination. These experiments will also explore the possible role of the perirhinal cortex in processing context, which is often considered intrinsically to involve configural processing of multiple elements.

Chapter 3: The Perirhinal Cortex and Configural Processing

3.1: Introduction

As outlined in earlier chapters, the weight of the evidence suggests that the perirhinal cortex is important not just for recognition *per se*, but for knowledge about objects. The experiments in this chapter aim to examine more precisely the role of the perirhinal cortex in object identification, or, perhaps more accurately, in object specification. The distinction is important: it has been clear for some considerable period of time that lesions of the perirhinal cortex cause severe impairments in object recognition. What has not been clear, and which the experiments discussed here aim to elucidate, is the *nature* of the perirhinal contribution to object recognition.

As discussed in chapter 2, the perirhinal cortex is involved in knowledge about objects, such as the associations between objects which appear together either simultaneously or sequentially. In particular, the idea has been put forward that the perirhinal cortex is necessary for the identification of complex objects which have visual features in common (Eacott & Heywood, 1995). A more general statement would be that the perception and representation of an object as a whole rather than as the sum of its component parts requires an intact perirhinal cortex. If the perirhinal cortex codes for groups of properties rather than objects, then its associative properties for relations between stimuli (see chapter 2) could also be applicable to the summing of features to form an object representation. This line of argument leads to the idea that configural processing, rather than being necessarily a function of the hippocampus (Rudy & Sutherland, 1989, 1995), may in fact be dependent on the perirhinal cortex. The evidence which indicates that hippocampal involvement in configural processing is probably incidental to the processing of spatial information has been discussed already (see chapter 1 pp27), but the evidence which implicates the perirhinal cortex in configural processing bears further elaboration.

It should be noted that the experiments in this chapter do not attempt to examine the role of the perirhinal cortex in processing configuration of any sort, but purely the particular question of whether processing the configuration of features within an object are dependent on perirhinal function. The issue of the relationship between objects and the context in which they occur, whether background or other objects is explored in the experiments in chapter 4, where an appropriate discussion of the

possible role of configural processing in perception and memory of context can be found.

The argument for perirhinal involvement in configural processing is supported by connectionist modelling which locates the relationships between features in the more rostral regions of the ventral visual stream (Saksida, 1999; Bussey & Saksida, 2002), and particularly, though not exclusively, in the perirhinal cortex. This perceptual-mnemonic feature conjunction (PMFC) model postulates a rostral-caudal stream of increasing levels of feature integration in the ventral visual stream. This visual stream is of course considered to be concerned with the "what" aspects of visual processing (Ungerleider & Mishkin, 1982). This PMFC model has been shown to account for a considerable amount of data on the effects of perirhinal lesion.

If indeed the perirhinal cortex is required for what has been described as a '"gestalt" representation of a complete stimulus" (Murray & Bussey, 1999) then two clear predictions arise. The first of these is that not only visual recognition memory, but also performance on any task requiring the use of more than one feature of the object, would be impaired by perirhinal lesions. The second and more specific prediction is that any task in which recognition of an object requires or is aided by a configural strategy would be impaired in animals with perirhinal lesions. The greater the reliance which must be placed upon a configural approach the more severe the predicted impairment. Such a theory predicts that not only mnemonic tasks which contain configural demands, but also concurrent discriminations which do so, will produce impairments in animals with perirhinal lesions.

This prediction was supported by a task which used increasing levels of feature ambiguity. Bussey et al. (2002) found that the level of perirhinal impairment in lesioned monkeys increased with the level of feature ambiguity in the stimuli used. The lesioned monkeys were unimpaired in the minimum ambiguity condition where there were no explicitly ambiguous features, mildly impaired in the condition in which half of the features were explicitly ambiguous, and severely impaired in the maximum ambiguity condition in which all features were explicitly ambiguous. This finding is supported by Eacott et al. (2001) who found that rats with perirhinal lesions were impaired when processing of more than one overlapping feature was required to

identify a two dimensional stimulus uniquely, but not when identification could be made on the basis of one feature, even when the figures involved were complex. Buckley & Gaffan (1998c) provide further support, reporting similar findings of impairment following perirhinal lesions in primates. These findings should be contrasted with those which show intact performance on negative patterning tasks in rats with combined perirhinal and postrhinal lesions as well as those with fornix lesions (Bussey et al., 2000).

In addition to the deficits reported on tasks which explicitly involve feature ambiguity, there have been findings of deficits on concurrent discrimination when large numbers of objects are used as stimuli, but not when only a small number are used (Buckley & Gaffan, 1997; Eacott et al., 1994). A possible explanation for these findings emerges when they are considered together with those of the studies when configural processing was required in order to perform the discrimination. The PMFC connectionist model accounted for deficits when there was explicit feature ambiguity in terms of a hierarchically structured system within the ventral visual stream in which the more rostral regions represented feature conjunctions and resolved feature ambiguity. The model accurately predicted that it would not be the number of objects in a stimuli set *per se* which is responsible for deficits in perirhinally lesioned animals' performance, but the level of feature ambiguity. However, it is clear that the level of feature ambiguity will tend to increase *de facto* with the number of objects in a stimulus set as it becomes more likely that some objects will share distinctive features. This is, of course, not the only reason that task difficulty increases if a larger number of objects are employed in a stimulus set, but the nature of the stimuli used in such studies makes it a plausible explanation in this instance.

As is indicated by the forgoing discussion, there is a distinction between object identification and object recognition. The former is dependent upon retrieval of a specific memory, whilst the latter may occur merely as a result of a feeling of familiarity. It is probable that most object recognition in the standard paradigms does in fact result from this second form of less specific memory, and that this is particularly the case with the object recognition test developed by Ennaceur & Delacour (1988). This one-trial test of object memory relies on the preference for novelty of the normal rat, which, given a free choice, will explore a novel object for

longer than an identical copy of a familiar object (copies are employed so as to eliminate the effect of odour marking). However, where there is mnemonic failure the animal will explore both objects equally. It has been shown that perirhinal lesions prevent discrimination between novel and familiar objects in this task both alone (Ennaceur et al, 1996) and when combined with fornix lesions (Ennaceur & Aggleton, 1997).

By introduction of the need for multiple feature identification and for identification of spatial relationships between features the task of object recognition can be converted into one which requires object specification. This has previously been attempted by Ennaceur & Aggleton (1994) who used objects constructed from plastic and metal to examine the effects of object reconfiguration on sham-operated and fornix-transected rats. They found that fornix lesions did not impair the ability of the animals to distinguish between a familiar object (A) and that object reconfigured (A*) or between A* and a novel object (N). This contributed to the mounting evidence that an account of configural memory based on hippocampal function could not be sustained. This was confirmed by the authors' subsequent work which examined the effects of reconfiguring objects on animals with either fornix or perirhinal lesions (Ennaceur et al., 1996). This study produced results which did not provide a clear pattern of impairment: all groups performed equally in discriminating between A* and A, whilst the perirhinal group produced counter-intuitive results on the A*/N discrimination, showing preference for A* at a 1 minute delay, but preference for N at a 15 minute delay, in contrast to the same animals' performance on an A/N discrimination, where they did not discriminate between the objects.

Whilst these results are indicative of a heightened sensitivity to stimulus configuration by animals with perirhinal lesions, at least at short delays, there is clearly a degree of confusion about the effects of perirhinal lesions on recognition of objects once feature ambiguity is introduced. In particular it is unclear precisely what is remembered or results in a feeling of familiarity, and what the time course of such memories is. In particular the experiments discussed above are suggestive of a non-linear interaction between memory for the whole of an object (the "gestalt") and memory for certain features.

The experiments discussed here attempt to examine in detail the effects of reconfiguring stimuli on object recognition in animals with perirhinal lesions, as compared to sham-operated controls. Initially an experiment was designed which used the automated Y-maze to teach rats discriminations for which configural processing was required. However, despite extensive training over a period of months the animals failed to learn the initial stages of the task which used partial feature ambiguity. A different approach was therefore taken, and experiments using naturalistic objects were designed. Rather than the specifically constructed objects used in the studies discussed above, these experiments employed objects assembled from plastic construction blocks (Duplo^{TM}). This had the advantage of permitting the rapid construction of a large number of unique objects, which were ideally suited to reconfiguration without the introduction of novel features. This allowed the use of a wide range of delays in each experiment (between 1 and 15 minutes in all experiments, between 1 minute and 24 hours in some experiments) and permitted easy repetition of experiments.

There is a possibility that stimuli which permit reconfiguration are in some way intrinsically different to standard objects such as bottles and jars which are hard or impossible to reconfigure. Because of this great care was taken, first of all to establish that performance by the animals used in this study was similar to that on previous studies which employed the standard version of this task (discrimination between familiar (A) and novel (N). Secondly an attempt was made to ensure that the Duplo objects employed to allow reconfiguration also produced such expected results. An attempt was then made to replicate the results of Ennaceur et al. (1996) on a discrimination between (A*) and (A).

The next experiment exploited the fact that with small stimulus sets feature ambiguity may increase only marginally with stimuli number, although the mnemonic task difficulty will increase for other reasons. The use of explicit feature ambiguity is therefore contrasted with the use of a still small, but nonetheless larger than usual stimulus set from which one object is then used as (A) in a discrimination between (A) and (N). Caution should always be employed when stating that a task does not require configural strategies for successful discriminations between stimuli - as Healey & Gaffan (2001) demonstrated, learning of configural strategies may occur

without training which requires such learning. However, this task used a total of only five objects in its stimulus set, as compared to the usual two, and the objects were selected to be as different from each other as possible. It is therefore extremely likely that the level of feature ambiguity remained low, whilst the level of difficulty compared with the baseline task (A/N) was increased.

The final experiment sought to establish the pattern of preference between the familiar object reconfigured (A*) and N over increasing delay, and, if possible to elucidate the complex pattern of results found by Ennaceur et al. (1996). The authors of this study used only two delays, and it is unclear what pattern would be predicted for delays lying intermediate between 1 minute and 15 minutes. In particular it is hoped that the conflict between a lack of discrimination on the standard (A/N) task and the apparent discrimination between (A*) and (N) (though not between (A*) and (A)) can be resolved. In particular, the ability to discriminate between A* and N and the preference at the 1 minute delay for A* (an object which was only partially novel) over N (an object which was wholly novel) requires clarification.

3.2: Methods and Materials

Subjects

Twenty Dark Agouti rats (Bantin & Kingman, Hull, UK) were used in this experiment. They had previously undergone extensive training in the automated Y-maze (see chapter 2 for a description) as part of an experiment which proved to be impractical (see above). They were housed in pairs in diurnal conditions (12h light/ 12h dark cycle) and all testing was carried out during the light phase. Throughout the study animals had *ad libitum* access to both food and water. At the time of surgery animals were approximately three months old. One animal died due to post-operative complications. Nineteen animals (10 perirhinal and 9 sham-operated) took part in the experiments presented here.

Apparatus

All testing was carried out in a maze made of wood of base dimensions $1m^2$ and height 48 cm. The base was painted matt black and the walls matt white. The objects were placed into the maze equidistant from the sides of the maze. Standard objects used included bottles, jars, tubs and bowls. Other objects were constructed in house

from Duplo™, manufactured by the LEGO Group, the base blocks of which were weighted with wax (figure 3.1 – pictures of objects). These weighted blocks were only ever used to form the bases of objects. Testing took place in a room lit by a 40W anglepoise bulb positioned so that light was directed away from the maze, resulting in low-level lighting. Noise was kept to a minimum in the region of the testing room. A number of sessions were videotaped to allow tests experimenter consistency and rel

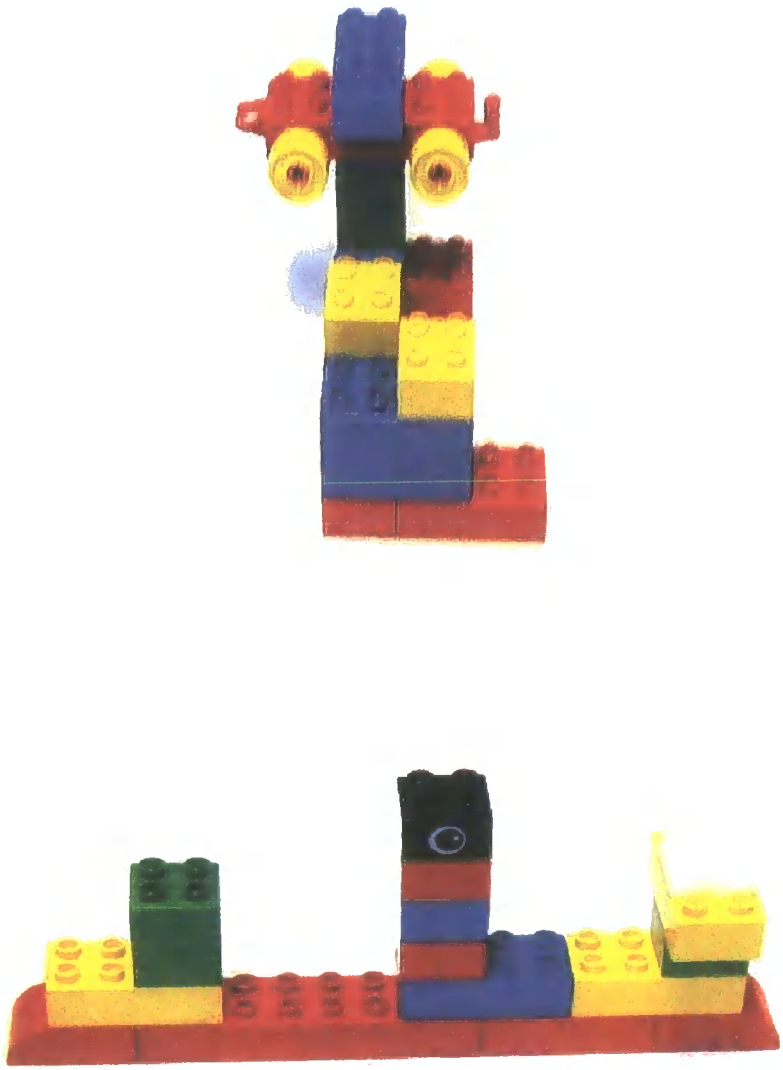


Figure 3.1: Typical objects made of Duplo

Surgery

Surgery was performed on all animals. 10 animals received bilateral lesions of the perirhinal cortex and 9 animals were sham-operated. Surgery was identical to the procedure described in chapter 2. Procedure for the sham animals was identical except that the electrode was not lowered into the brain.

Perfusion

Procedure was identical to that described in chapter 2.

Task

Habituation

Prior to the start of testing animals received three habituation sessions in which they were allowed to explore the maze. The first of these took place in cage-mate pairs and lasted for 10 minutes. The two subsequent sessions took place individually and each lasted 5 minutes. For each habituation session a different novel object was placed in the centre of the maze.

Establishment of a baseline

Baseline performance for the two groups at different delays was established by replicating the results of Ennaceur et al. (1996) on a standard object recognition task. Animals were placed individually in the maze which contained two identical copies of a novel object, placed equidistant from the sides. They were allowed to explore freely until they had spent a total of 30 seconds exploring the objects. If they failed to explore for 30 seconds they were removed after 5 minutes, returned to their home cage, and the time spent exploring was noted. After a delay of 1 minute, 3 minutes, 5 minutes, 10 minutes, 15 minutes, 1 hour, 4 hours or 24 hours they were returned to the maze which now contained a new copy of the object they had previously explored and a novel object, placed equidistant from the sides of the maze (figure 3.2). They were allowed to explore for 3 minutes and the time spent exploring each object was recorded for each of three 1 minute periods. Different pairs of objects were used for each test. Intrinsic interest of objects was controlled for by varying the designated familiar and novel objects between rats. Effects of place were controlled for by alternating the position of the novel and the familiar objects across both animals and

delay points. The experiment was repeated twice at each delay, each time using different objects.

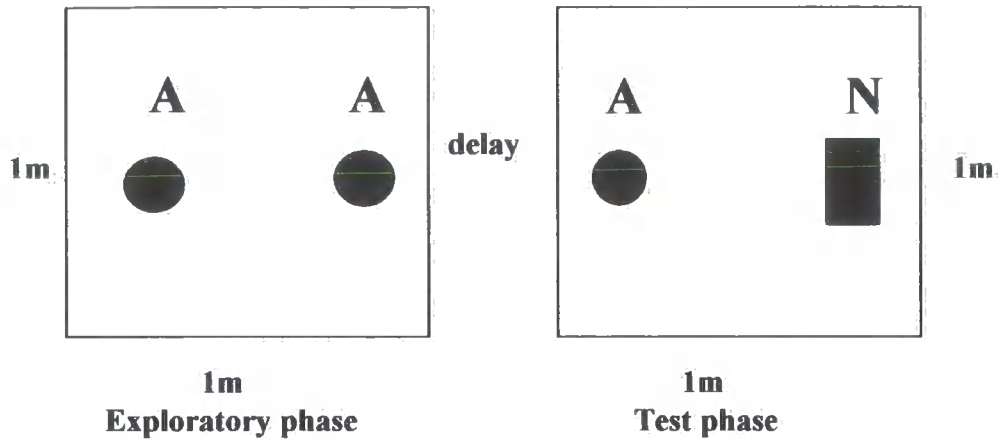


Figure 3.2: Exploratory and test phases for the establishment of a baseline.

Experiment 3.1: A vs. N (Duplo)

Experiment 3.1 exactly repeated the procedure used to establish a baseline using objects constructed out of Duplo (figure 3.3) at the same delays. It was repeated twice at each delay using different objects for each repetition

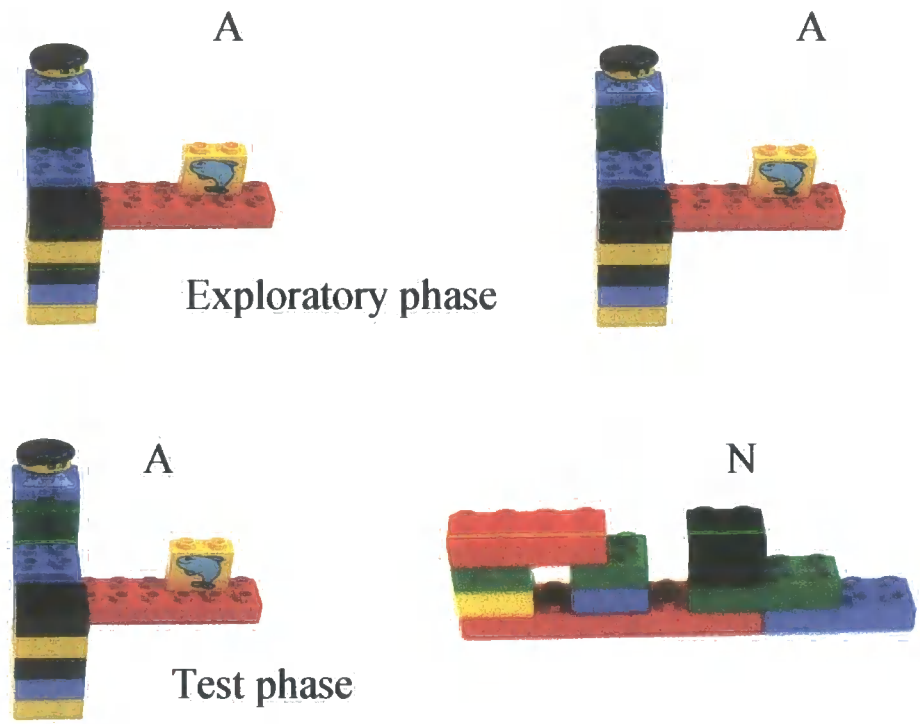


Figure 3.3: Exploratory and test phases for Duplo A vs. N

Experiment 3.2: A vs. A*

Experiment 3.2 also used objects constructed out of Duplo. The exposure phase was identical to that previously described, with two identical copies of a Duplo object placed in the maze. In the test phase the maze contained a new copy of the familiar object and a reconfigured version of this object (figure 3.4). Care was taken to ensure that the reconfigured object did not contain novel features not found in the original object. Object position and the configuration which was experienced as novel were controlled for between animals. Because performance of the sham animals fell to chance at a delay of 15 minutes, longer delays were not used in this experiment. It was repeated twice at each delay used.

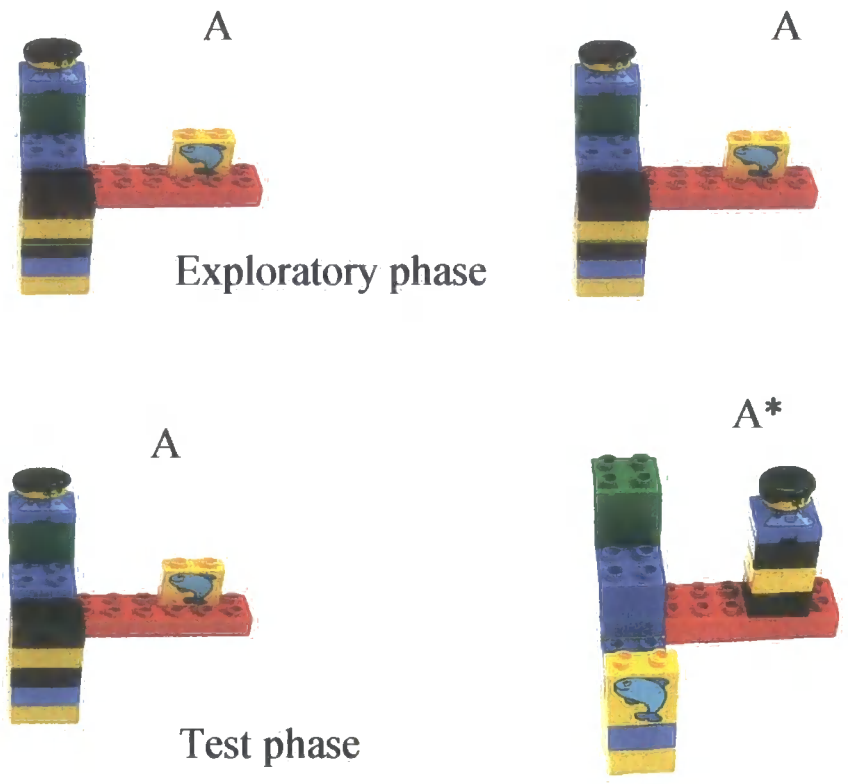


Figure 3.4: Exploratory and test phases for A* vs. A

Experiment 3.3: Multiple objects

Experiment 3.3 was designed as a control for the effects of discrimination difficulty as opposed to the effects of a discrimination requiring a configural strategy. In the initial exploration phase the maze contained four different objects placed equidistant from the corners of the maze. During the test phase the maze contained a different copy of

one of the four objects explored during the test phase and a novel object placed equidistant from the sides of the maze (figure 3.5). Animals were placed in the maze and allowed to explore until they had explored the object pre-designated as the familiar object in the test phase for 15 seconds. All animals remained in the maze for a minimum of 2 minutes, even if they completed this 15 seconds of exploration in a shorter time. If they failed to explore the designated object for 15 seconds they were removed from the maze after 5 minutes and the time spent exploring the object was noted. After a delay of 1, 3, 5, 10 or 15 minutes the animal was returned to the maze for 3 minutes and allowed to explore the novel and the familiar object. Time spent exploring each object was recorded for each of three 1 minute periods. The position of the 4 objects in the exploration phase remained constant but intrinsic interest was controlled for between rats by varying the object which was used for the test phase and the object which was experienced as novel. The position of the objects in the test phase was controlled for by alternation between rats. The experiment was repeated twice using a different set of objects each time.

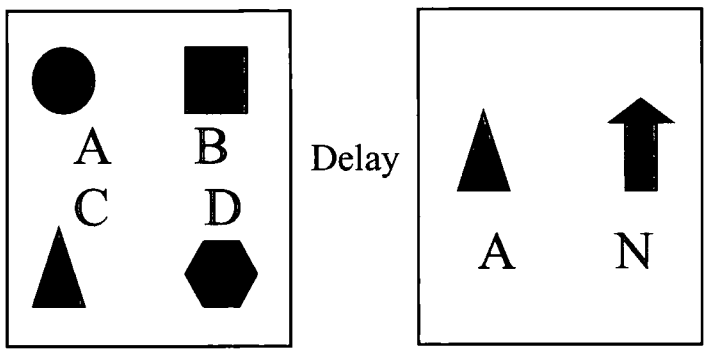


Figure 3.5: Exploratory and test phases for multiple objects

Experiment 3.4: A* vs. N

Experiment 3.4 used objects constructed out of Duplo. The exposure phase was identical to that previously described for experiments 3.1 and 3.2, with two identical copies of a Duplo object placed in the maze. For the test phase the maze contained a novel object and a reconfigured version of the familiar object. The object was reconfigured by altering the position of one or more features within the object (figure 3.6). The positions of the objects and the object which was experienced as novel were

controlled for between animals. The experiment was repeated twice at each delay, using different objects.

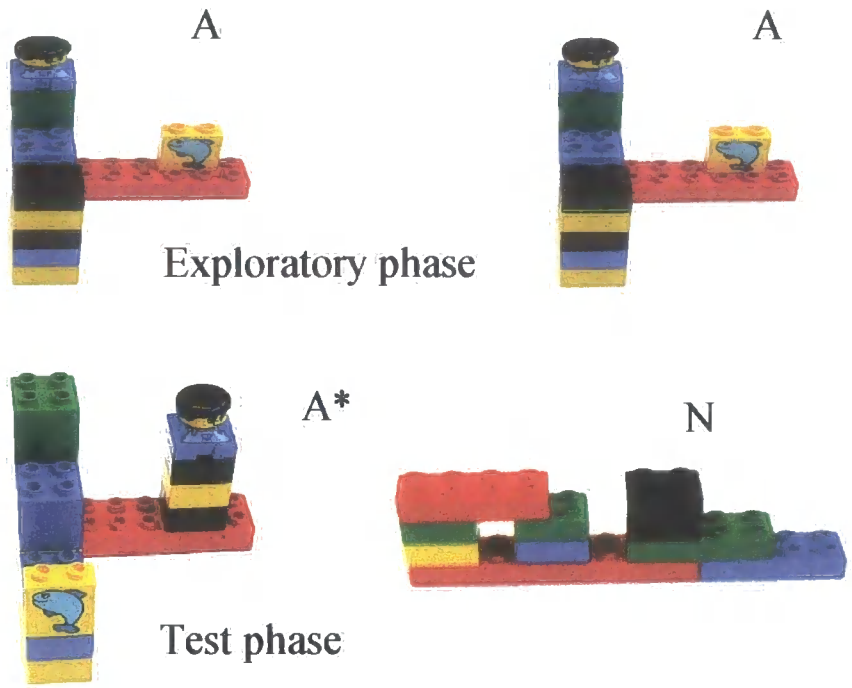


Figure 3.6: Exploratory and test phases for A* vs. N

Data analysis

Following Ennaceur & Delacour (1988), the difference in exploration of the objects in seconds at a given time-point was calculated (D1). Secondly, the difference between the time spent exploring the objects was calculated as a proportion of the total time spent exploring both objects (D2). The data from the baseline and experiments 3.1 and 3.3 were analysed according to the calculation below:

D1 = Total exploration of N – Total exploration of A

$$D2 = \frac{\text{Total exploration of N} - \text{Total exploration of A}}{\text{Total exploration of N} + \text{Total exploration of A}}$$

In experiment 3.2 the reconfigured object (A*) was designated as "N" for the purposes of calculating these variables, so:

D1 = Total exploration of A* – Total exploration of A

while

$$D2 = \frac{\text{Total exploration of A* – Total exploration of A}}{\text{Total exploration of A* + Total exploration of A}}$$

In experiment 3.4 the reconfigured object (A*) was designated as "A" for the purposes of calculating these variables, so:

D1 = Total exploration of N – Total exploration of A*

while

$$D2 = \frac{\text{Total exploration of N – Total exploration of A*}}{\text{Total exploration of N + Total exploration of A*}}$$

Repeated measures ANOVA's were carried out on each of these measures, followed by post-hoc Tukey's test. Simple effects were also calculated where appropriate.

A number of sessions were scored on videotape by both the experimenter and another person experienced in object recognition testing. These scores were compared with the original scoring by the experimenter using one-way ANOVA's. The experimenter also re-scored the tapes blind after an interval of approximately 18 months and compared this with the original scoring of the videotape using paired-sample t-tests.

3.3: Results

3.31: Histological

One animal (R207) died before perfusion could take place. Histological analysis revealed that in all other cases the perirhinal lesions were essentially as intended, extending approximately 3.5 mm – 6.5 mm posterior to Bregma, although they were not complete lesions. The estimated damage to the perirhinal cortex ranged from 26% - 67% of the total extent of perirhinal cortex. However, the smallest lesion shown (R215) was atypical and was considerably smaller than the other lesions. Figure 3.7 shows representative sections from animal 202. Figure 3.8 shows the extent of the smallest (R215, semi-transparent grey) and largest lesions (R210, diagonal stripes) drawn on to standard sections taken from Paxinos & Watson (1998). As can be seen from the behavioural results described below it is unlikely that the size of the lesions affected the behavioural impairments, as these were both extensive and in accordance with the majority of the extant literature. In addition the performance of the perirhinal

animals on each experiment at the 15 minute delay was plotted and neither animal with the largest nor the smallest lesion was atypical (see figure 3.9). The performance of the animal which died before perfusion could be performed (R207) is also shown. As can be seen from figure 3.9, this animal shows no indication of deviating from the performance of the cohort.

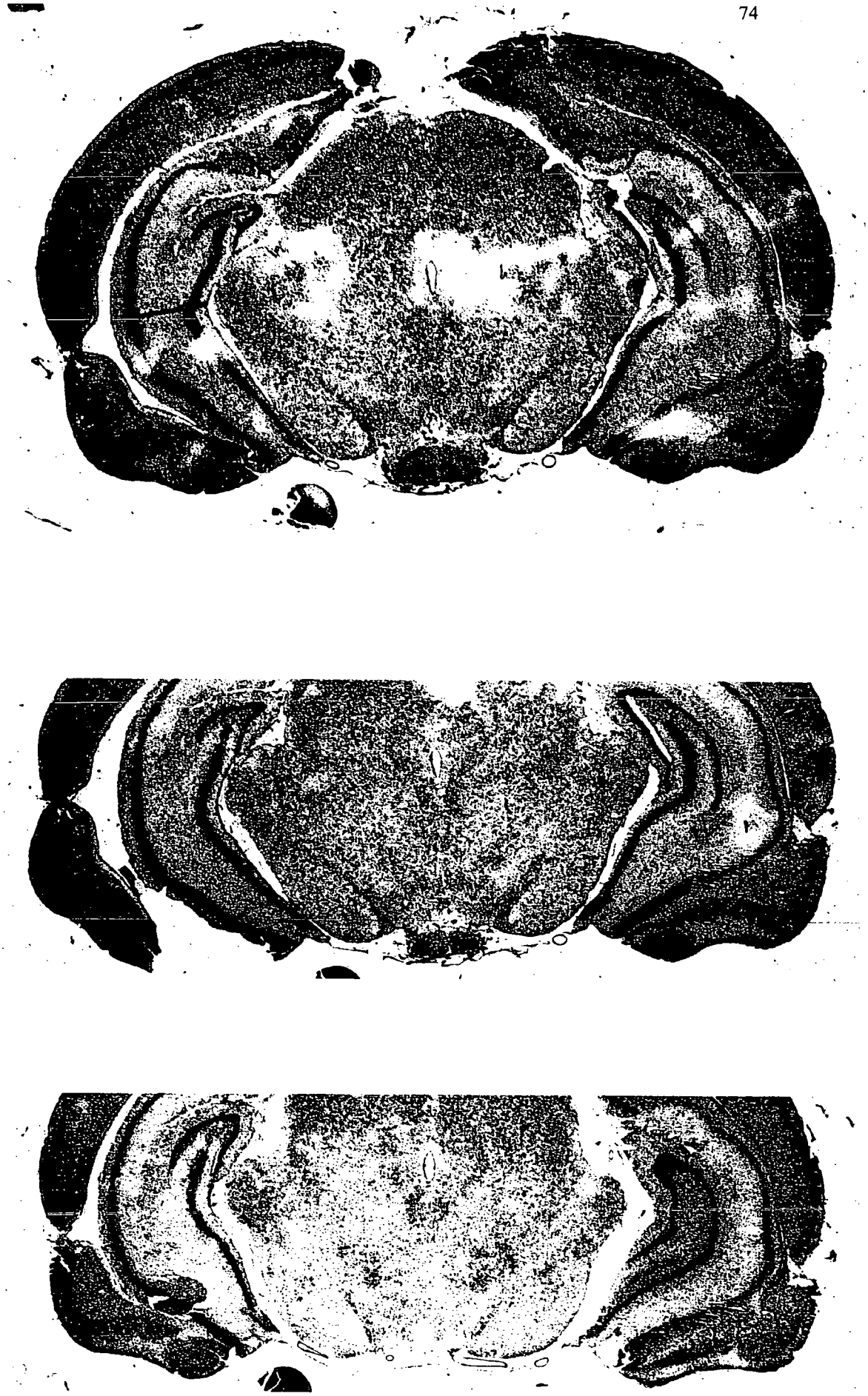


Figure 3.7: Three representative sections of a bilateral perirhinal lesion from animal R202

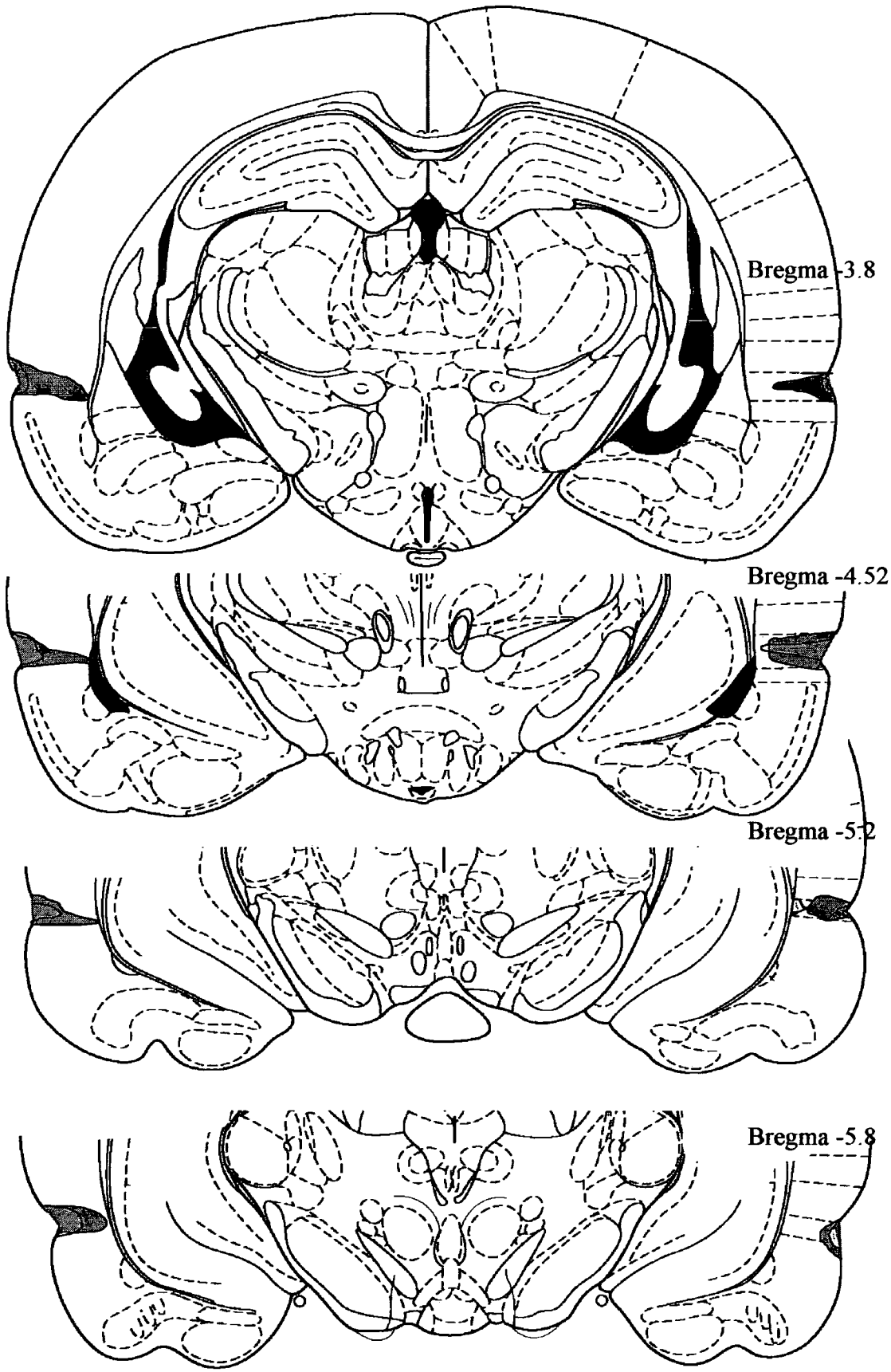


Figure 3.8: The extent of the smallest (R215, semi-transparent grey) and largest (R210, diagonal stripes) lesions drawn on to standard sections taken from Paxinos & Watson(1998).

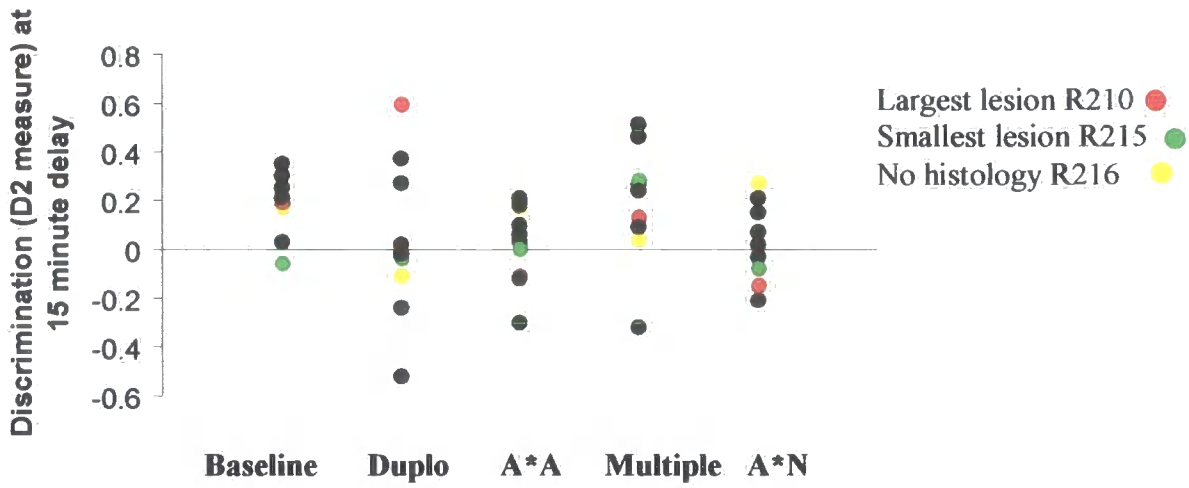


Figure 3.9: Performance of animals with largest, smallest and typical lesions on each experiment at a delay of 15 minutes. Animal R216 for which no histology is available is also shown

3.32: Behavioural

The results of each experiment were analysed separately on both the D1 and the D2 measures using repeated measures ANOVA's with simple effects analysis. The absolute, as opposed to the relative performance of each group at each delay was also analysed using one-sample T-tests to establish whether discrimination was above chance.

Three experiments involved discrimination between a familiar object and an object which had not previously been presented. These were the baseline task, in which the unfamiliar stimulus was an object; experiment 3.1, in which the unfamiliar stimulus was a novel duplo construction and experiment 3.2 in which the unfamiliar stimulus was a reconfigured version of the familiar. Performance on experiments 3.1 and 3.2 is analysed in comparison with baseline performance established on a standard version of the object recognition task (see below) in order to establish whether tasks requiring configural processing are differentially harder for animals with perirhinal lesions than for sham animals. The effect of difficulty is controlled for by the inclusion of a task (experiment 3.3, multiple objects) which is more difficult than the baseline task but in which discrimination of the two objects does not require configural processing. The experiment in which discrimination is between a novel object (N) and a novel configuration of familiar elements (A*) is also analysed in comparison with baseline

performance to establish whether this task, requiring configural processing, is differentially more difficult for the perirhinal animals.

Inter-experimenter Reliability

The results of video scoring by the experimenter and another experienced experimenter were compared using a 2 (observer) x 3 (task scored) repeated measures ANOVA on the D1 measure. As expected there was a main effect of task scored ($F = 7.46$, $df = 2,70$, $p < 0.01$). There was no main effect of observer ($F < 1$, $df = 2,70$, $p < 0.01$) and no interaction between task and observer ($F < 1$, $df = 1,36$, $p > 0.05$), indicating that the experimenters' scores agreed on each task.

Intra-experimenter Reliability

The results of on-line scoring by the experimenter and video scoring by the experimenter were compared using a 2 (viewing) x 3 (task scored) repeated measures ANOVA on the D1 measure. As expected this showed an effect of task ($F = 3.87$, $df = 2,36$, $p < 0.05$). There was also an effect of viewing ($F = 3.74$, $df = 2,18$, $p < 0.05$) but no interaction between viewing and task ($F < 1$, $df = 2,36$, $p > 0.05$), with the video scores lower than the on-line score. It seemed probable that this was due to the greater difficulty in detecting very short periods of exploration on video. To test this the original scoring of the videos by the experimenter at the time of testing was compared with a second scoring carried out approximately two years later by the same experimenter. A repeated measures ANOVA showed that there was no difference between the two scores for each task ($F < 1$, $df = 1,18$, $p > 0.05$). As expected there was an effect of task ($F = 3.87$, $df = 2,36$, $p < 0.05$), but there was no interaction between score and task ($F < 1$, $df = 2,36$, $p > 0.05$). This suggests that scoring was stable across different tests, and that any differences found were due to the medium used (on-line or video).

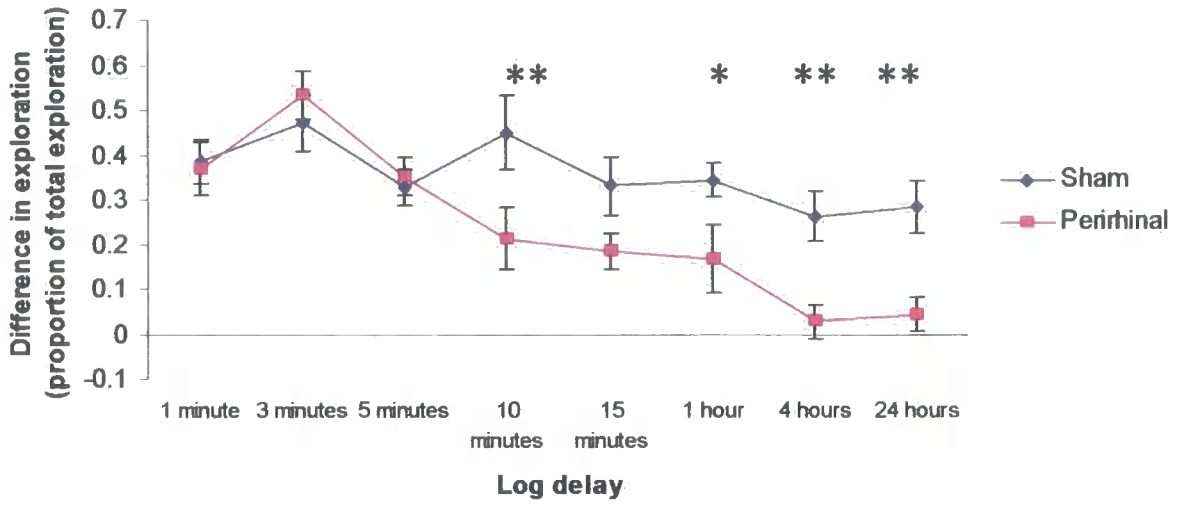
Standard Objects: Baseline

This experiment replicated the results of previous studies by showing clear perirhinal impairments at the longer delays on both the D2 ($F = 29.267$, $df = 1,17$, $p < 0.001$) and the D1 ($F = 17.739$, $df = 1,17$, $p = 0.001$) measures. Results using the two measures differ slightly, and so are presented separately.

D2

The perirhinal animals were impaired compared to the shams on the D2 measure as is shown by the main effect of lesion ($F = 29.267$, $df = 1,17$, $p < 0.001$). There was also a main effect of delay ($F = 9.175$, $df = 7, 119$, $p < 0.001$) and an interaction between delay and lesion ($F = 2.632$, $df = 7,119$, $p < 0.05$) reflecting the fact that perirhinal performance declined with increasing delay, as can be seen in figure 3.10a. Analysis of this interaction using simple effects showed that the perirhinal animals were impaired compared with sham performance at delays of 10 minutes ($F = 9.65$, $df = 1,134$, $p < 0.01$), and delays of 1 hour and above (1 hour: $F = 5.50$, $df = 1,134$, $p < 0.05$; 4 hours: $F = 9.76$, $df = 1,134$, $p < 0.01$; 24 hours: $F = 9.92$, $df = 1,134$, $p < 0.01$). The lack of an impairment at the 15 minute delay may be due to increased variance in the performance of the sham animals (see figure 3.10a).

Analysis of the animals' absolute performance showed that the perirhinal animals did not discriminate between the objects at the two longest delays: one sample T-tests showed that the perirhinal animals' performance did not differ from zero at 4 hours or 24 hours, whilst sham animals performed above chance at all delays (see table 3.1).



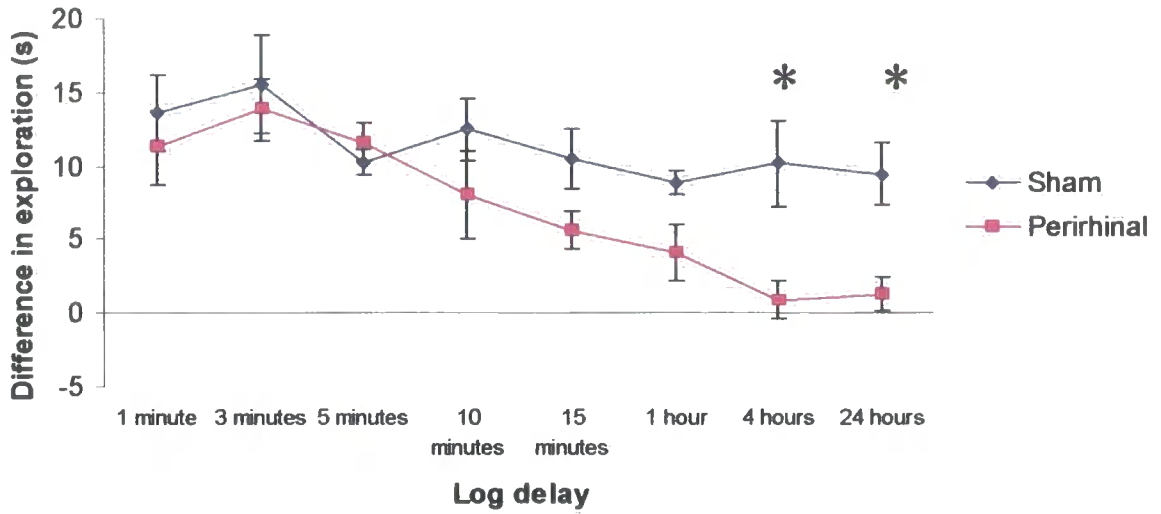


Figure 3.10: Discrimination between novel (N) and familiar (A) objects at increasing delays. (a) D2 measure. (b) D1 measure * indicates $p < 0.05$ ** indicates $p<0.01$.

	Sham (DF = 8)			Perirhinal (DF = 9)		
Delay	T	P	Differs from chance	T	P	Differs from chance
1 minute	8.422	<0.001	Yes	6.425	<0.001	Yes
3 minutes	7.754	<0.001	Yes	10.372	<0.001	Yes
5 minutes	8.549	<0.001	Yes	8.477	<0.001	Yes
10 minutes	5.677	<0.001	Yes	3.317	0.009	Yes
15 minutes	5.355	0.001	Yes	4.811	0.001	Yes
1 hour	9.185	<0.001	Yes	2.346	0.044	Yes
4 hours	4.916	0.001	Yes	0.727	0.486*	No
24 hours	5.212	0.001	Yes	1.181	0.268*	No

Table 3.1: One-sample T-tests on performance of each group at increasing delays on standard object discrimination of N and A: D2 measure. * indicates performance was at chance

D1

As with the D2 measure, the perirhinal animals were impaired compared to the shams ($F = 17.739$, $df = 1,17$, $p = 0.001$). There was also a main effect of delay ($F = 6.216$, $df = 17,119$, $p < 0.001$) as both groups performed more poorly with increasing delay (see figure 3.10b). Although there was no interaction between delay and lesion ($F = 1.553$, $df = 7,119$, $p > 0.05$), the delay-dependent nature of the impairment of the perirhinal animals' impairment can be seen in figure 3.10b, and is also partially confirmed by one-sample T-tests. Analysis of the animals' performance at each delay using simple effects with a Bonferroni correction for 8 multiple comparisons showed that the perirhinal animals were impaired at delays of 4 hours ($F = 10.253$, $df = 1,134$, $p < 0.05$) and 24 hours ($F = 13.917$, $df = 1,134$, $p < 0.05$).

As with the D2 measure, analysis of the animals' absolute performance confirmed that the perirhinal impairment in discrimination between the objects was not uniform across delay. One sample T-tests showed that the perirhinal animals' performance did not differ from zero at 4 hours or 24 hours, while that of the sham animals was greater than zero at all delays (see table 3.2).

	Sham (DF = 8)			Perirhinal (DF = 9)		
	T	P	Differs from chance	T	P	Differs from chance
1 minute	5.605	0.001	Yes	4.742	0.001	Yes
3 minutes	4.995	0.001	Yes	7.013	<0.001	Yes
5 minutes	12.992	<0.001	Yes	9.600	<0.001	Yes
10 minutes	6.283	<0.001	Yes	2.886	0.018	Yes
15 minutes	6.618	<0.001	Yes	4.579	0.001	Yes
1 hour	11.430	<0.001	Yes	2.340	0.044	Yes
4 hours	3.742	0.006	Yes	0.714	0.493*	No
24 hours	4.835	0.001	Yes	1.181	0.268*	No

Table 3.2: One-sample T-tests on performance of each group at increasing delays on standard object discrimination of N and A: D1 measure. * indicates performance was at chance

Exploration

There was no difference between the groups in the total amount of time spent exploring in the test phase ($F < 1$, $df = 1,17$, $p > 0.05$). There was a main effect of delay ($F = 3.983$, $df = 1,7$, $p = 0.001$) but there was no interaction between delay and lesion ($F = 1.616$, $df = 1,7$, $p > 0.05$). The effect of delay would appear to result from non-linear variation in the exploration of both groups, which was probably due to the varying intrinsic interest of the objects selected at each delay.

Summary

Overall the perirhinal animals were impaired compared to the shams. Performance of both groups fell with increasing delay and this was particularly the case for the perirhinal animals, whose performance was at chance at the two longest delays used. Total exploration did not differ between the groups

Experiment 3.1: Duplo A vs. N

This experiment aimed to replicate the results of the baseline by showing that both perirhinal and sham operated animals discriminated novel and familiar Duplo objects in the same way that they discriminated between standard objects such as those used by other researchers. However, whilst the perirhinal animals were impaired compared to the shams on both the D2 ($F = 22.003$, $df = 1,17$, $p < 0.001$) and the D1 ($F = 8.602$, $df = 1,17$, $p < 0.01$) measures, the pattern of impairment differed somewhat from that

with standard objects, with the task seemingly being more difficult for both groups. The results of the two measures also differed somewhat and so are presented separately

D2

The perirhinal animals were impaired compared with the shams ($F = 22.003$, $df = 1,17$, $p < 0.001$). There was also a main effect of delay ($F= 3.217$, $df = 7, 119$, $p < 0.01$), although there was no interaction between lesion and delay ($F < 1$, $df =1,7$, $p >0.05$). However, the impairment was not uniform across all delays as is shown in figure 3.11a, and, as can be seen from one-sample T-tests (table 3.3), absolute performance certainly differed across delays.

Analysis of the effects of increasing delay using simple effects with a Bonferroni correction for 8 multiple comparisons showed that the perirhinal animals were impaired compared to the sham animals at a delay of 5 minutes ($F = 10.416$, $df = 1,136$, $p < 0.05$). The failure to find a difference between the groups at longer delays would seem to be due to poorer performance by the shams at these delays, rather than to any decrease in the perirhinal impairment (see figure 3.11a).

This interpretation of the pattern of impairment is supported by analysis of the absolute performance of the two groups at each delay: one sample T-tests showed that perirhinal animals were performing at chance at delays of 5 minutes and longer (see table 3.3) whereas the sham animals performed above chance at all delays.

	Sham			Perirhinal		
	T	P	Differs from chance	T	P	Differs from chance
1 minute	6.372	<0.001	Yes	4.424	0.002	Yes
3 minutes	3.495	0.008	Yes	3.885	0.004	Yes
5 minutes	5.436	0.001	Yes	1.730	0.118*	No
10 minutes	3.745	0.006	Yes	0.633	0.542*	No
15 minutes	2.855	0.021	Yes	0.281	0.785*	No
1 hour	2.393	0.044	Yes	0.112	0.914*	No
4 hours	3.618	0.007	Yes	0.246	0.811*	No
24 hours	3.310	0.011	Yes	0.182	0.860*	No

Table 3.3: One-sample T-tests on performance of each group at increasing delays on Duplo object discrimination of N and A: D2 measure. * indicates performance was at chance

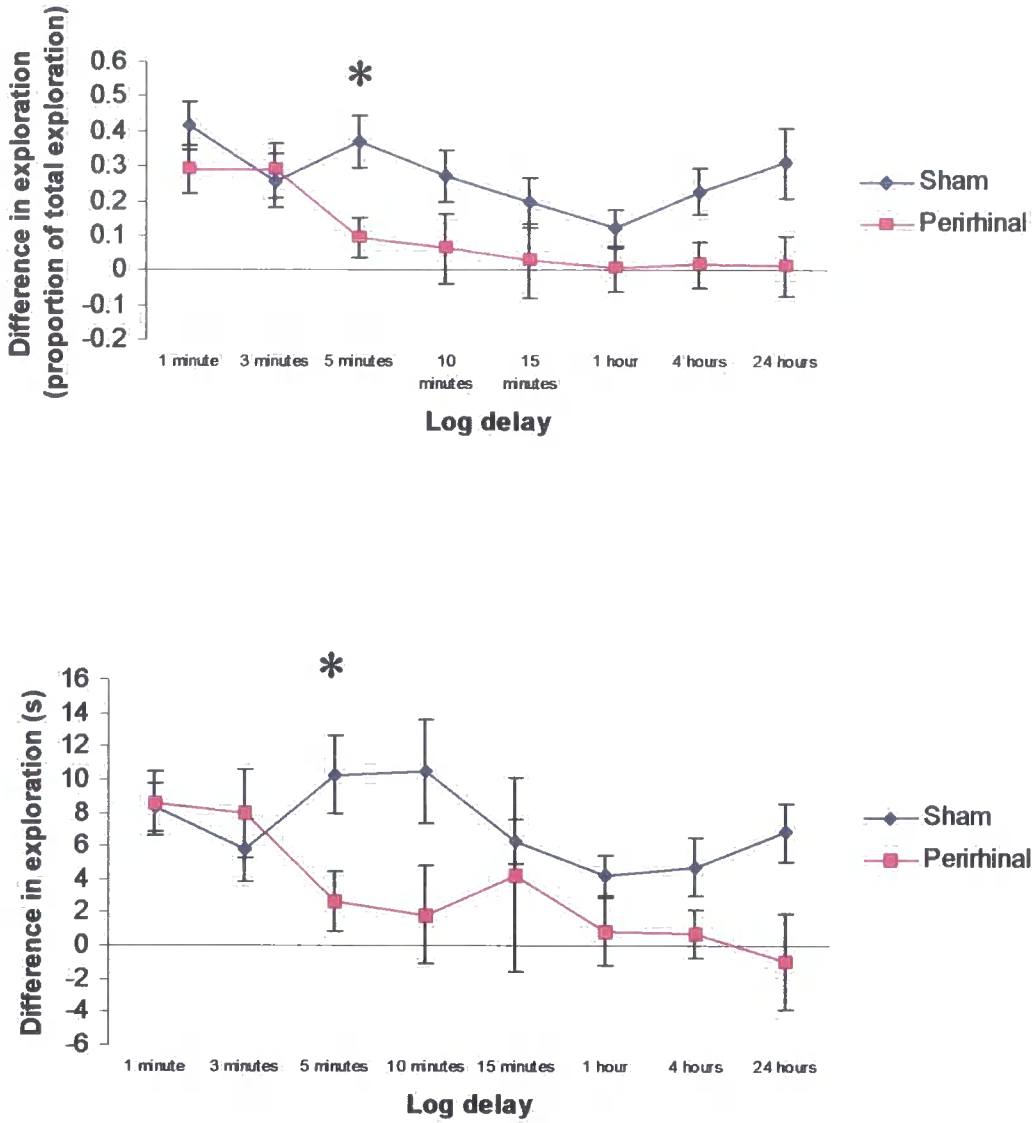


Figure 3.11: Discrimination between novel (N) and familiar (A) Duplo objects at increasing delays. (a) D2 measure. (b) D1 measure.* indicates $p < 0.05$.

D1

As with the D2 measure, the perirhinal animals were impaired relative to the shams ($F = 8.602$, $df = 1,17$, $p < 0.01$). Although there was no effect of delay on this measure ($F = 1.675$, $df = 7,119$, $p > 0.05$) the impairment was not uniform across all delays as can be seen from figure 3.11b and analysis of absolute performance (table 3.4). Analysis using simple effects with a Bonferroni correction for 8 multiple comparisons showed that the perirhinal animals were impaired compared with the shams at a delay of 5 minutes ($F = 7.593$, $df = 1,135$, $p < 0.01$). As with the D2 measure it seems probable that, as can be seen from figure 3.11b, the lack of impairment at longer delays was, with the exception of anomalous performance at the delay of 15 minutes, due to a reduced discrimination by the sham animals, rather than to a bimodal distribution of the impairment of the perirhinal animals.

This is supported by analysis of absolute performance: one-sample T-tests showed that perirhinal animals were performing at chance at delays of 5 minutes and longer (see table 3.4) whereas the sham animals performed above chance at all delays.

	Sham			Perirhinal		
	T	P	Differs from chance	T	P	Differs from chance
1 minute	6.097	<0.001	Yes	4.660	0.001	Yes
3 minutes	3.288	0.011	Yes	3.186	0.011	Yes
5 minutes	4.658	0.002	Yes	1.540	0.158*	No
10 minutes	3.560	0.007	Yes	0.664	0.542*	No
15 minutes	4.913	0.001	Yes	0.771	0.461*	No
1 hour	3.714	0.006	Yes	0.441	0.670*	No
4 hours	2.888	0.020	Yes	0.555	0.593*	No
24 hours	4.212	0.003	Yes	-0.353	0.732*	No

Table 3.4: One-sample T-tests on performance of each group at increasing delays on Duplo object discrimination of N and A: D1 measure. * indicates performance was at chance.

Exploration

There was a main effect of lesion ($F = 4.717$, $df = 1,17$, $p < 0.05$) as the perirhinal animals explored the objects for longer than the shams and a main effect of delay ($F = 7.884$, $df = 7,119$, $p < 0.001$), but no interaction between delay and lesion ($F = 1.636$, $df = 7,119$, $p > 0.05$). The main effect of delay would seem to be due to non-linear

variation in exploration by both groups, probably resulting from differences in the intrinsic interest of the objects used. As before, where there is a difference in exploration between the groups the D2 measure is more reliable than the D1 measure.

Summary

Overall the perirhinal animals were impaired compared with the sham animals. The poor performance by the perirhinal animals persisted at all delays of 5 minutes and longer, as they performed at chance, whilst the sham animals were above chance at all delays. The perirhinal animals showed more total exploration than the shams.

Experiment 3.2: Duplo A* vs. A

This experiment explicitly introduced the necessity for configural processing in order to discriminate between the two objects presented, by requiring the animals to discriminate between a familiar object (A) and the same familiar object reconfigured (A*).

On this task perirhinal animals were impaired compared to sham animals on both the D2 ($F = 33.672$, $df = 1,17$, $p < 0.001$) and the D1 measures ($F = 32.366$, $df = 1,17$, $p < 0.001$). Unlike the previous experiments, there was no effect of delay (D2: $F = 1.646$, $df = 4,68$; D1: $F = 1.623$, $df = 4,68$, $p > 0.05$). There was also no interaction between delay and lesion (D2: $F < 1$, $df = 4,68$, $p > 0.05$; D1: $F < 1$, $df = 4,68$, $p > 0.05$) with the perirhinal animals showing a uniform impairment at all delays. This absence of an effect of delay can be clearly seen in figures 3.12a and 3.12b. Although there was no effect of delay, analysis of the main effect of lesion using simple effects with a Bonferroni correction for 8 multiple comparisons showed that the perirhinal animals were only impaired relative to the sham animals at the 3 minute delay (D2: $F = 14.346$, $p < 0.01$; D1: $F = 18.692$, $p < 0.01$) and at the 5 minute delay (D2: $F = 8.275$, $p < 0.05$; D1: $F = 9.675$, $p < 0.05$). This was clearly due to the poor performance of the sham animals at the longer delays (figures 3.12a and 3.12b).

The difficulty of the task became evident during testing, and analysis of absolute performance showed that the perirhinal animals were performing at chance at all delays used on both the D1 and the D2 measures (tables 3.5 and 3.6), while the sham animals performed at chance at the 10 minute delay on both measures, with marginal

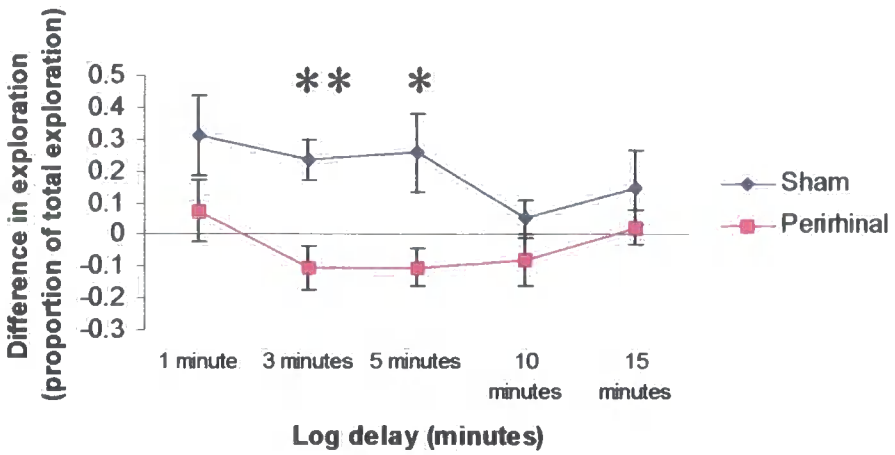
performance at 5 minutes on the D2 measure and chance performance at 1 minute on the D1 measure. In view of the uniformly poor performance of the perirhinal animals and the decline in the sham animals' performance, testing at delays longer than 15 minutes was considered to be unnecessary.

	Sham (DF = 8)			Perirhinal (DF = 9)		
	T	P	Differs from chance	T	P	Differs from chance
1 minute	2.618	0.031	Yes	0.788	0.451*	No
3 minutes	3.906	0.005	Yes	-1.590	0.146*	No
5 minutes	2.241	0.055*	No	-1.738	0.116*	No
10 minutes	0.885	0.402*	No	-1.005	0.341*	No
15 minutes	2.528	0.035	Yes	0.547	0.598*	No

Table 3.5: One-sample T-tests on performance of each group at increasing delays on Duplo object discrimination of A* and A: D2 measure. * indicates performance was at chance

	Sham (DF = 8)			Perirhinal (DF = 9)		
	T	P	Differs from chance	T	P	Differs from chance
1 minute	1.906	0.093*	No	0.177	0.863*	No
3 minutes	5.183	0.001	Yes	-1.299	0.226*	No
5 minutes	2.547	0.034	Yes	-1.718	0.120*	No
10 minutes	0.783	0.456*	No	-0.956	0.364*	No
15 minutes	2.347	0.044	Yes	0.348	0.737*	No

Table 3.6: One-sample T-tests on performance of each group at increasing delays on Duplo object discrimination of A* and A: D1 measure. * indicates performance was at chance



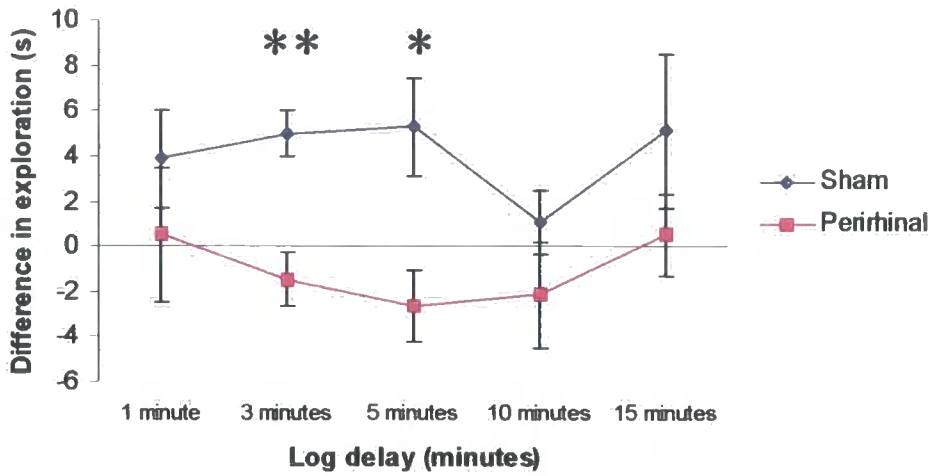


Figure 3.12: Discrimination between familiar reconfigured (A*) and familiar (A) Duplo objects at increasing delays. (a) D2 measure. (b) D1 measure. * indicates $p < 0.05$; ** indicates $p < 0.01$.

Exploration

There was a main effect of lesion ($F = 7.946$, $df = 1,17$, $p < 0.05$) as the perirhinal animals spent more time exploring the objects than the sham animals did, and a main effect of delay ($F = 8.613$, $df = 4,68$, $p < 0.001$) but no interaction between delay and lesion ($F < 1$, $df = 4,68$, $p > 0.05$). The main effect of delay resulted from an increased amount of exploration at the longer delays. It is therefore possible that both the lesion and the delay effects result from more exploration occurring where the familiarity of the familiar object is reduced. As previously stated, the D1 measure is sensitive to exploration duration, and is therefore less reliable when the groups differ on this variable; the D2 measure is, however, unaffected.

Summary

This task was extremely difficult for both groups. The perirhinal animals were impaired compared with the shams and performed at chance at all delays. The sham animals did not discriminate between the objects at delays of 5 and 10 minutes, whereas on previous experiments they discriminated at delays of 24 hours. The perirhinal animals explored the objects more than the sham animals and both groups explored more at longer delays.

Comparison of Standard Objects Baseline, Duplo A vs. N and Duplo A vs. A*

This is a comparison of the three sets of data which compare a familiar object (A) with a novel (N) or novel configuration of standard object (A*) object in order to examine the effects of increasing the difficulty of discrimination or of increasing the configural processing required in order to discriminate between the objects. Because the A* vs. A task did not use delays of longer than 15 minutes, the results of longer delays in the earlier experiments are not included in this analysis.

Only the D2 measure, the more reliable of the two is used in the comparison of the data sets. This is because of the inevitable problem of disparity in levels of exploration between tasks which use different types of object, particularly when, as is the case with the A* vs. A task, there is a much smaller total level novelty in the objects explored. As previously discussed the D1 measure is liable to be unreliable where there are differences in the levels of exploration, whilst the D2 measure is designed such that it does not reflect these differences.

The data were analysed using a 3 (task) x 5 (delay) x 2 (group) repeated measures ANOVA. The perirhinal animals were clearly impaired compared to the shams ($F = 33.323$, $df = 1,1$, $p < 0.001$). There was also a main effect of delay ($F = 5.391$, $df = 1,4$ $p = 0.001$) but no interaction between delay and lesion ($F < 1$, $df = 1,4$, $p > 0.05$) showing that while the tasks became more difficult with increasing delay this was not particularly the case for the perirhinal animals. There was a main effect of task ($F = 36.022$, $df = 1,2$, $p < 0.001$) as successive tasks increased in difficulty and, more importantly, an interaction between task and lesion ($F = 4.341$, $df = 1,2$, $p < 0.05$) (see figures 3.13a and 3.14a). Analysis of this interaction using simple effects showed that the perirhinal animals' performance was impaired relative to the sham animals' on the Duplo A vs. N ($F = 6.773$, $df = 2,20$, $p < 0.01$) and on the A vs. A* ($F = 33.672$, $df = 2,20$, $p < 0.01$) tasks but not on the baseline task ($F = 3.545$, $df = 2,20$, $p > 0.05$). The lack of a perirhinal impairment on the baseline task, when analysis of this data alone showed such an impairment (see p67 above), is due to the exclusion of the three longest delays here in order to produce a balanced analysis. However, there was no interaction between delay and task ($F = 1.777$, $df = 1,8$, $p = 0.087$) and no three-way interaction between delay, task and lesion ($F = 1.507$, $df =, 1,8$, $p > 0.05$). The lack of such interactions between the main effect of delay and the other variables would seem to indicate that, whilst the other tasks were more difficult, and particularly more difficult for the perirhinal animals, they did not become differentially more difficult with increasing delay, but rather the effect of delay in increasing difficulty remained constant across the tasks for each lesion group.

Summary

The first two experiments discussed here were both more difficult than the baseline tasks, and this was especially the case for the A vs. A*. It is possible to interpret this result as representing the introduction of a demand for configural processing of the tasks. This is implicit in the case of the Duplo A vs. N where objects have features which resemble one another and, despite the attempt to avoid it, may have features in common, and explicit in the case of the A* vs. A, where identification of a configural structure is essential to the discrimination. In order to rule out the possibility that this result represents not the effects of configurality, but simply of difficulty, the next

experiment attempted to increase the difficulty of the task but without the introduction of configural processing.

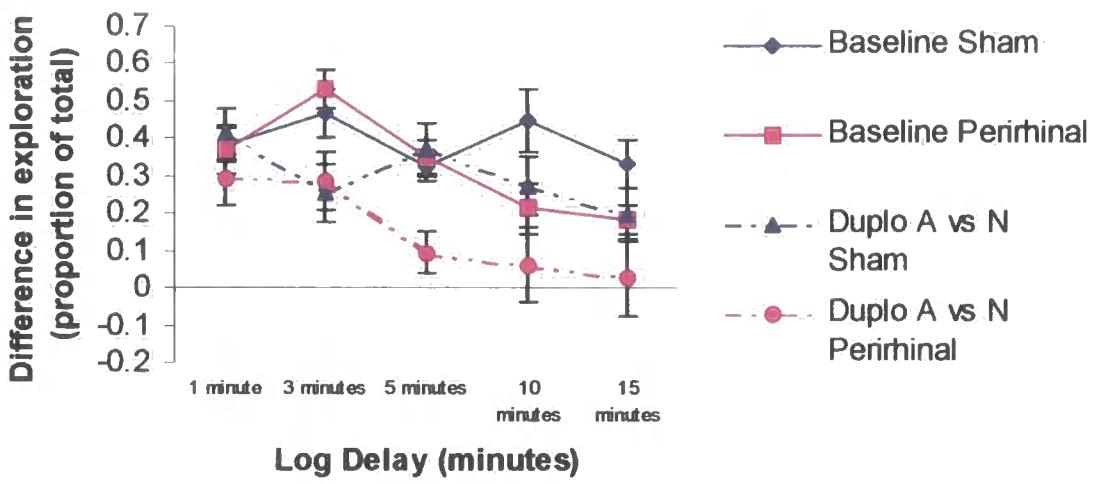


Figure 3.13: Duplo A vs. N compared with baseline. D2 measure

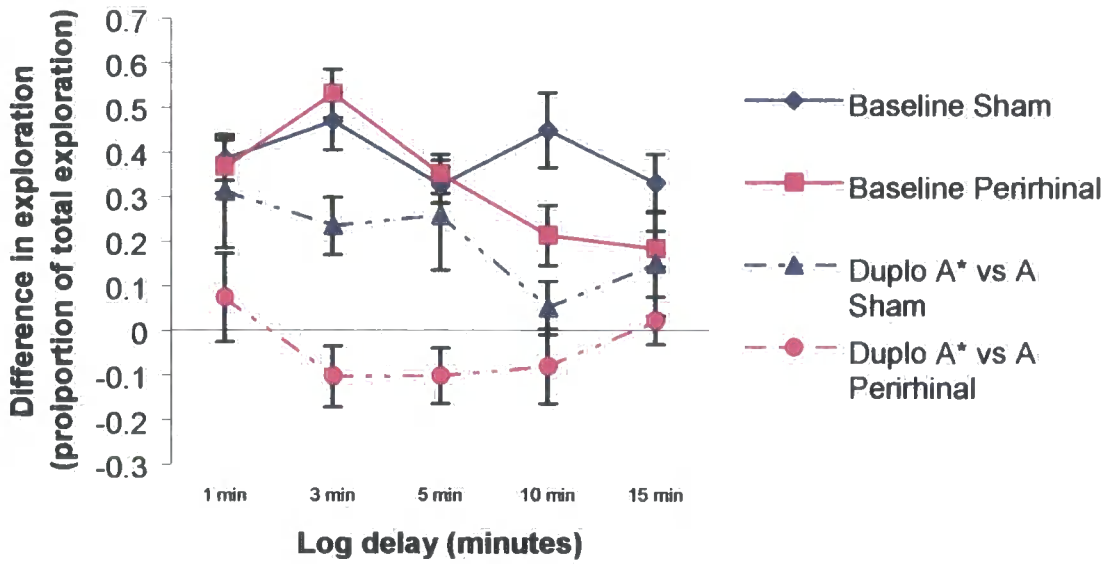


Figure 3.14: Duplo A vs. A* compared with baseline. D2 measure

Experiment 3.3: Multiple objects

This experiment was designed as a control for the increased difficulty, compared to baseline, of experiments 3.1 and 3.2 discussed above, as opposed to the increased requirement for configural processing. In order for the experiment to be a successful control for difficulty it must have represented a more difficult discrimination than the baseline. The 2 tasks were therefore compared for difficulty using a 2 (tasks) x 5 (delays) x 2 (groups) repeated measures ANOVA, before the results of the multiple objects experiment were analysed.

Comparison of baseline and multiple objects tasks.

D2

Analysis of the 2 tasks using the D2 measure found that, as can be seen from figure 3.15 the animals performed significantly more poorly on the multiple objects than on the baseline task ($F = 20.901$, $df = 1,17$, $p < 0.001$). The perirhinal animals were impaired compared with the shams ($F = 4.773$, $df = 1,17$, $p < 0.05$), but this effect was delay-dependent as was shown by the interaction between delay and lesion ($F = 3.194$, $df = 4,17$, $p < 0.05$). Importantly there was no interaction between task and lesion ($F < 1$, $df = 1,17$, $p > 0.05$), showing that both groups found the multiple objects task equally hard compared with the baseline task.

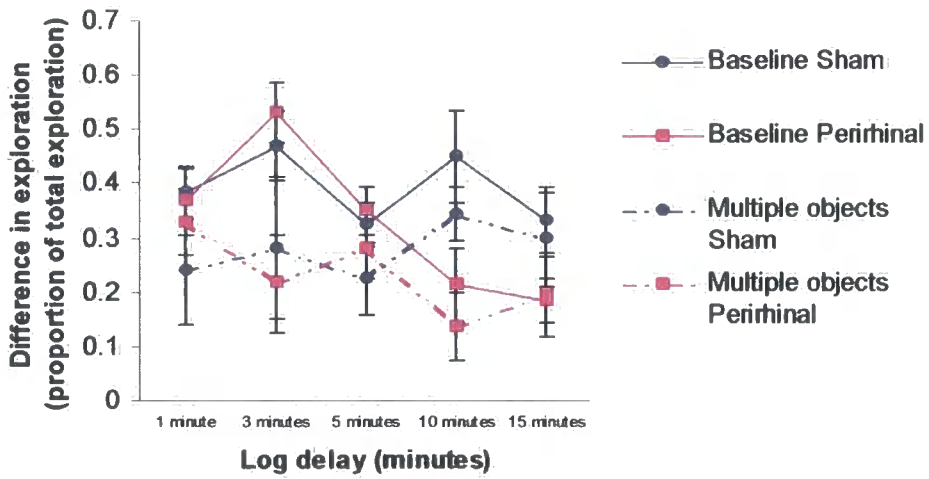


Figure 3.15: Multiple standard objects compared with baseline. D2 measure

Analysis of multiple objects data

D2

The multiple objects data were analysed using a 5 (delay) x 2 (group) repeated measures ANOVA. There was no main effect of lesion ($F = 1.686$, $df = 1,17$, $p > 0.05$) or of delay ($F < 1$, $df = 4,68$, $p > 0.05$) and no interaction between delay and lesion ($F = 1.151$, $df = 4,68$, $p > 0.05$) on the D2 measure.

Analysis of absolute performance using one sample T-tests showed that both groups were above chance at all delays (see table 3.7). Although the marginal significance of the sham's performance at the 3 minute delay should be noted, in the light of the above chance performance at all longer delays it would seem to represent a statistical anomaly.

	Sham (DF = 8)			Perirhinal (DF = 9)		
	T	P	Differs from chance	T	P	Differs from chance
1 minute	2.586	0.032	Yes	5.609	<0.001	Yes
3 minutes	2.292	0.051*	No	2.529	0.032	Yes
5 minutes	3.539	0.008	Yes	5.369	0.000	Yes
10 minutes	7.423	<0.001	Yes	2.332	0.045	Yes
15 minutes	3.659	0.006	Yes	2.627	0.027	Yes

Table 3.7: One-sample T-tests on performance of each group at increasing delays on standard object discrimination using multiple objects: D2 measure. * indicates performance was at chance

D1

There was no main effect of lesion ($F = 1.547$, $df = 1,17$, $p > 0.05$) or of delay ($F < 1$, $df = 4,68$, $p > 0.05$) and no interaction between delay and lesion ($F = 1.306$, $df = 4,68$, $p > 0.05$) on the D1 measure.

Analysis of absolute performance using one-sample T-tests indicates considerable variability on the D1 measure (see table 3.8). The sham animals performed at chance at the 3 minute delay, while the perirhinal animals were at chance at the 10 and 15 minute delays. However, both groups showed consistent performance of on the D2 measure. This, together with the lack of any effect of lesion would seem to indicate that this task - although known to be harder than the standard task - is not

differentially harder for the perirhinal animals. In this respect the task clearly differs from experiment 3.2 which requires configural processing, and perhaps also, as will be discussed later, from experiments 3.1 and 3.4.

	Sham			Perirhinal		
	T	P	Differs from chance	T	P	Differs from chance
1 minute	2.446	0.040	Yes	6.745	<0.001	Yes
3 minutes	1.309	0.227*	No	2.452	0.037	Yes
5 minutes	3.759	0.006	Yes	5.491	<0.001	Yes
10 minutes	4.962	0.001	Yes	1.953	0.083*	No
15 minutes	3.183	0.013	Yes	1.535	0.159*	No

Table 3.8: One-sample T-tests on performance of each group at increasing delays on standard object discrimination using multiple objects: D1 measure. * indicates performance was at chance

Exploration

There was no effect of lesion ($F < 1$, $df = 1,17$, $p > 0.05$). There was an effect of delay ($F = 2.813$, $df = 4, 68$, $p < 0.05$), but there was no interaction between delay and lesion ($F < 1$, $df = 4,68$, $p > 0.05$). The effect of delay would seem to be due to non-linear variation in both groups' exploration, probably resulting from the differing intrinsic interest of the objects used at each delay.

Summary

The groups did not differ from each other and showed no decrease in discrimination with increasing delay. They discriminated between the objects at all the delays tested (see comments above on anomalous result of shams at 3 minute delay). Despite this strong performance by both groups the task was more difficult than the baseline task, with both groups performing more poorly on this task.

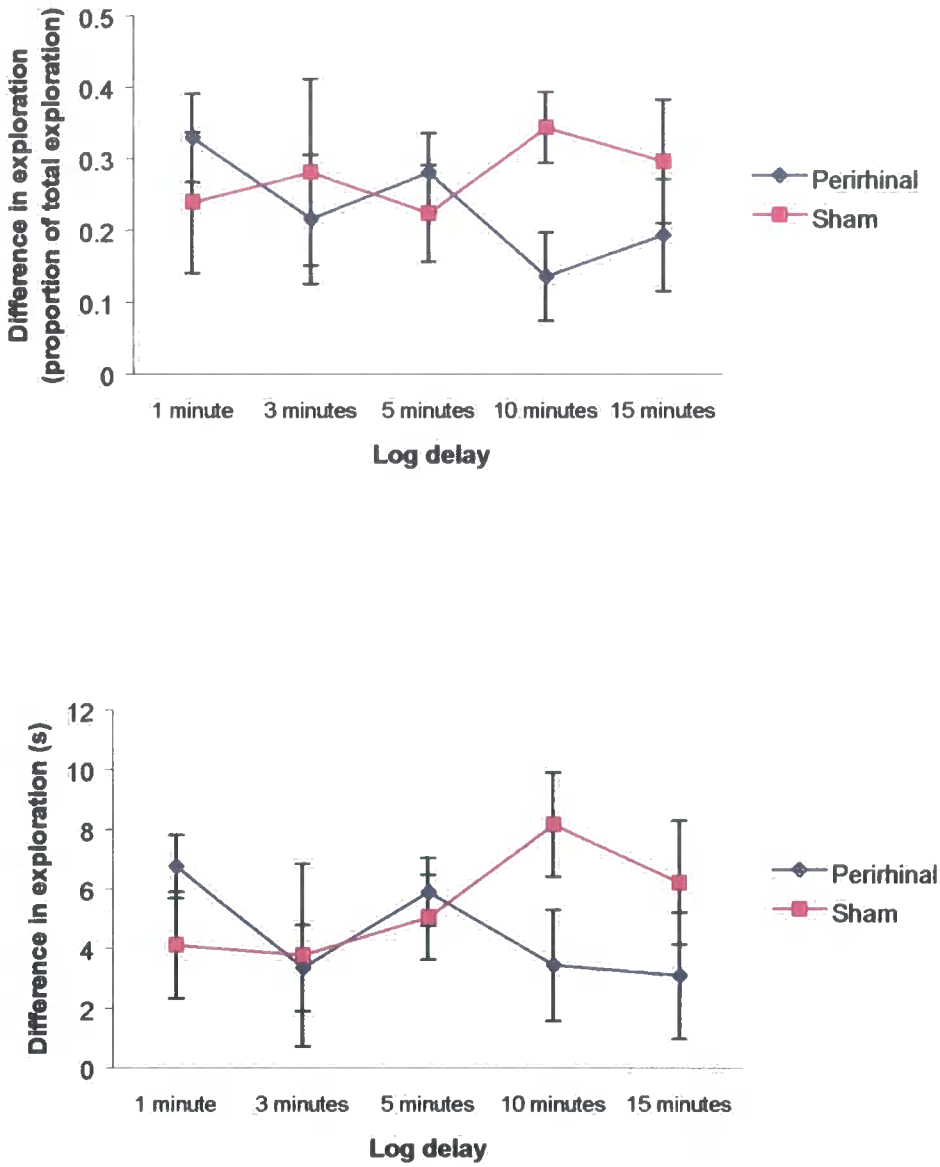


Figure 3.16: Discrimination between novel (N) and familiar (A) standard objects at increasing delays, when multiple objects are seen at exposure. (a) D2 measure. (b) D1 measure

Experiment 3.4: Duplo A* vs. N

This experiment differs somewhat from the others presented here in that animals are not asked to discriminate between a novel object (or a novel configuration of a familiar object) and a familiar object: instead they are asked to discriminate between a totally novel object (N) and a novel configuration of a familiar object (A*). It attempts to further examine the effects of introducing into the object recognition task a benefit of employing configural processing in order to discriminate between two objects. Data were analysed using an 8 (delay) x 2 (group) repeated measures ANOVA for both the D1 and the D2 measures. In this experiment perirhinal animals were impaired compared to sham animals on both the D2 ($F = 51.202$, $df = 1,17$, $p < 0.001$) and the D1 measures ($F = 21.475$, $df = 1,17$, $p < 0.001$). As the results of the two measures differed somewhat, they are presented separately.

D2

As with the previous experiments, the perirhinal impairment relative to the sham animals ($F = 51.202$, $df = 1,17$, $p < 0.001$) was not uniform across all delays (see figure 3.17a). There was also a main effect of delay ($F = 3.119$, $df = 7,119$, $p < 0.05$). Although there was no interaction between delay and lesion ($F = 1.805$, $df = 7,119$, $p > 0.05$), further analysis of the main effect of lesion using simple effects with a Bonferroni correction for 8 multiple comparisons found that the perirhinal animals were impaired at delays of 3 minutes ($F = 20.275$, $df = 1, 136$, $p < 0.01$), 5 minutes ($F = 14.197$, $df = 1, 136$, $p < 0.01$), and 15 minutes ($F = 7.515$, $df = 1, 136$, $p < 0.05$). The failure to find a perirhinal impairment at delays of 10 minutes or longer than 15 minutes was almost certainly due to the decline in the performance of the sham animals rather than an improvement in that of the perirhinal group (see figure 3.17a). This interpretation, and the difficulty of the task were confirmed by analysis of the absolute performance of the two groups. One-sample T-tests showed that perirhinal animals' performance did not differ from chance at delays of 3 minutes and longer, whilst sham animals' performance did not differ from chance at delays of 4 hours or 24 hours (see table 3.9)

	Sham (DF = 8)			Perirhinal (DF = 9)		
	T	P	Differs from chance	T	P	Differs from chance
1 minute	4.232	0.003	Yes	4.942	0.001	Yes
3 minutes	4.662	0.002	Yes	-1.925	0.086*	No
5 minutes	7.177	<0.001	Yes	0.745	0.475*	No
10 minutes	4.859	0.001	Yes	0.529	0.610*	No
15 minutes	4.608	0.002	Yes	0.945	0.370*	No
1 hour	3.153	0.014	Yes	-0.691	0.507*	No
4 hours	0.870	0.410*	No	-0.425	0.681*	No
24 hours	1.742	0.120*	No	0.661	0.525*	No

Table 3.9: One-sample T-tests on performance of each group at increasing delays on Duplo object discrimination of N and A*: D2 measure. * indicates performance was at chance

D1

As with the D2 measure, the perirhinal animals were impaired compared with the sham animals ($F = 21.475$, $df = 1,17$, $p < 0.001$). There was no main effect of delay ($F = 1.956$, $df = 7,119$, $p > 0.05$) and no interaction between delay and lesion ($F < 1$, $df = 7,119$, $p > 0.05$). However, analysis using simple effects with a Bonferroni correction for 8 multiple comparisons showed that the perirhinal animals were impaired at delays of 3 minutes ($F = 19.618$, $df = 1,130$, $p < 0.01$), 5 minutes ($F = 12.272$, $df = 1,130$, $p < 0.01$), 10 minutes ($F = 7.897$, $df = 1,130$, $p < 0.05$) and 15 minutes ($F = 13.873$, $df = 1,130$, $p < 0.01$). As with the D2 measure, and as can be seen from figure 3.17b, the failure to find an impairment at the longer delays was clearly due to the decline in the performance of the sham animals at these long delays of an hour or more, rather than to an improvement in the performance if the perirhinal animals.

As with the D2 measure, this interpretation, and the overall difficulty of the task, was confirmed by the analysis of the absolute performance of both groups. One-sample T-tests showed that perirhinal animals' performance did not differ from chance at delays of 3 minutes and longer, whilst sham animals' performance did not differ from chance at delays of 1 hour and longer (see table 3.10).

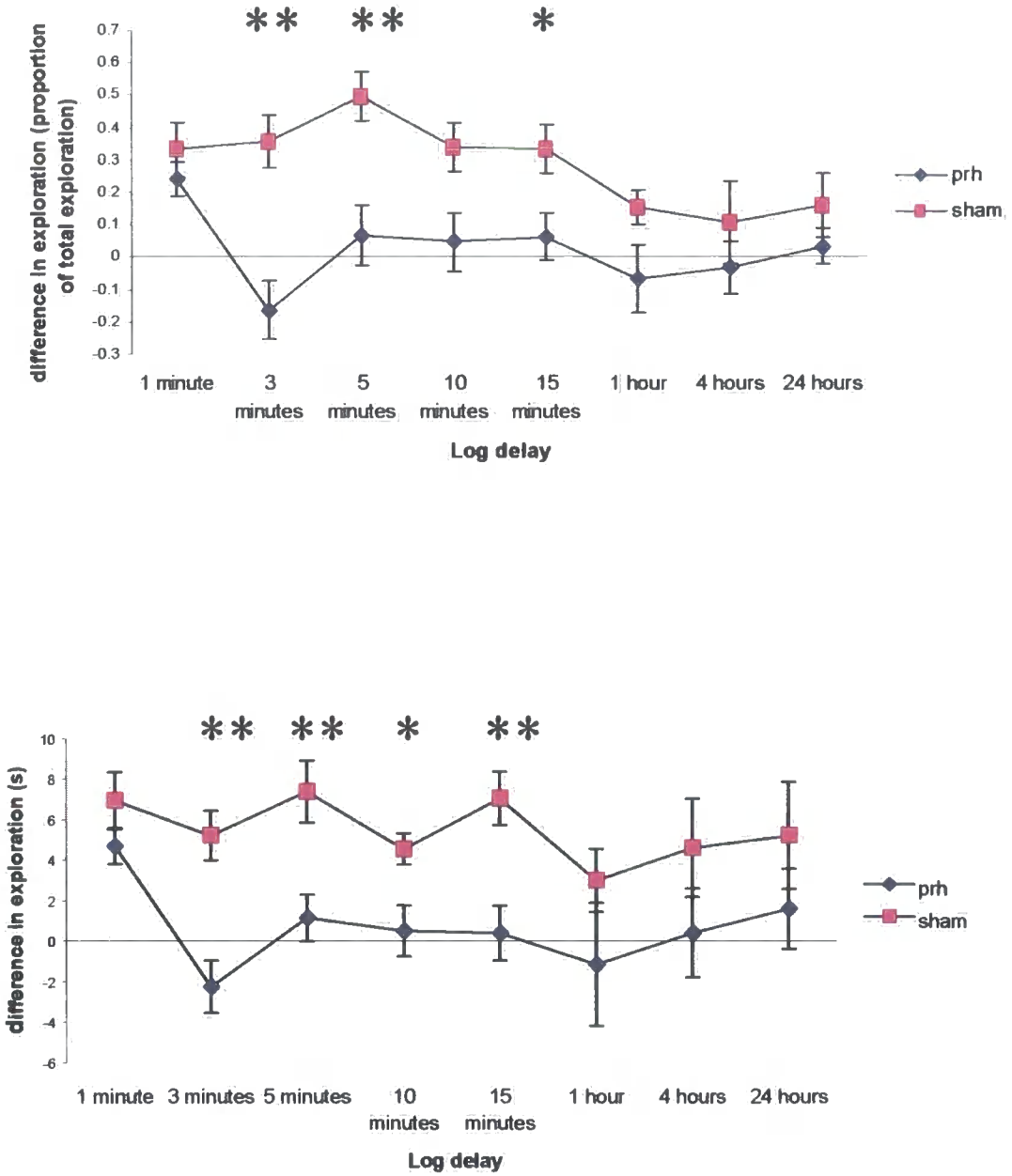


Figure 3.17: Discrimination between novel (N) and reconfigured familiar (A*) Duplo objects at increasing delays. (a) D2 measure. (b) D1 measure * indicates $p < 0.05$; ** indicates $p < 0.01$.

	Sham (DF = 8)			Perirhinal (DF = 9)		
	T	P	Differs from chance	T	P	Differs from chance
1 minute	5.245	0.001	Yes	5.569	<0.001	Yes
3 minutes	4.533	0.002	Yes	-1.847	0.098*	No
5 minutes	5.138	0.001	Yes	1.057	0.318*	No
10 minutes	6.212	<0.001	Yes	0.418	0.686*	No
15 minutes	5.715	<0.001	Yes	0.313	0.762*	No
1 hour	2.038	0.076*	No	-0.398	0.700*	No
4 hours	2.010	0.079*	No	0.192	0.852*	No
24 hours	2.091	0.070*	No	0.848	0.418*	No

Table 3.10: One-sample T-tests on performance of each group at increasing delays on Duplo object discrimination of N and A*: D1 measure. * indicates performance was at chance

Exploration

There was a main effect of lesion ($F = 4.818$, $df = 1,17$, $p < 0.05$) due to the perirhinal animals spending longer exploring the objects than the shams, and a main effect of delay ($F = 14.198$, $df = 7,119$, $p < 0.001$) due to both groups spending more time exploring at the longer delays, but there was no interaction between lesion and delay ($F = 1.389$, $df = 7,119$, $p > 0.05$). It is possible that when the familiar object is less clearly remembered (because of a perirhinal lesion or because of a long delay) then more overall exploration results, although such exploration is not concentrated on either object. Where there are such differences in exploration time the D1 measure should be treated with caution; the reliability of the D2 measure, however, is unaffected.

Summary

The perirhinal animals were impaired compared with the shams. They performed at chance at all delays except that of 1 minute, whereas the shams discriminated between the objects at all delays less than an hour. Performance declined with increasing delay for both groups with the groups differing at delays of between 3 and 15 minutes. The perirhinal animals explored the objects for longer than the sham animals and both groups explored for longer at the longer delays.

Comparison of Baseline with A* vs. N

This analysis compares the results of discrimination between a novel (N) and a novel configuration of a familiar object (A*) object with the baseline of A vs. N using standard objects in an attempt to explore the relative difficulty of the tasks for the two groups of animals. Unlike the other comparative analyses reported, this involves eight delays up to a maximum of 24 hours. As with previous post hoc comparative analyses only the D2 measure, the more reliable of the two measures is used. This is because of the inevitable problem of disparity in levels of exploration between tasks which use different types of object, even when, as was not the case previously, there remains a strong element of novelty in the objects explored. As previously discussed the D1 measure is liable to be unreliable where there are differences in the levels of exploration, whilst the D2 measure is designed such that it does not reflect these differences.

The data were analysed using a 2 (task) x 8 (delay) x 2 (group) repeated measures ANOVA. The perirhinal animals were impaired compared to the sham animals as is shown by the main effect of lesion ($F = 86.281$, $df = 1,17$, $p < 0.001$). The tasks became more difficult with increasing delay, ($F = 7.336$, $df = 1,7$, $p < 0.001$), but this was not particularly the case for the perirhinal animals as there was no interaction between delay and lesion ($F < 1$, $df = 1,7$, $p > 0.05$). The A* vs. N task was more difficult than the baseline task ($F = 40.589$, $df = 1,1$, $p < 0.001$) and this was differentially the case for the perirhinal group, as is shown by the interaction between task and lesion ($F = 10.125$, $df = 1,1$, $p < 0.01$) (see figure 3.18). The A* vs. N task became differentially difficult at increasing delays as is shown by the interaction between delay and task ($F = 2.875$, $df = 1,7$, $p < 0.01$), and this effect was significantly greater for the perirhinal animals than for the shams, as is demonstrated by the three-way interaction between delay, task and lesion ($F = 3.225$, $df = 1,7$, $p < 0.01$).

Summary

This second explicitly configural experiment was clearly much more difficult for the perirhinal animals than the baseline task. It was a more difficult task for the shams too, but it was disproportionately difficult for the perirhinal animals, and became more so with increasing delay.

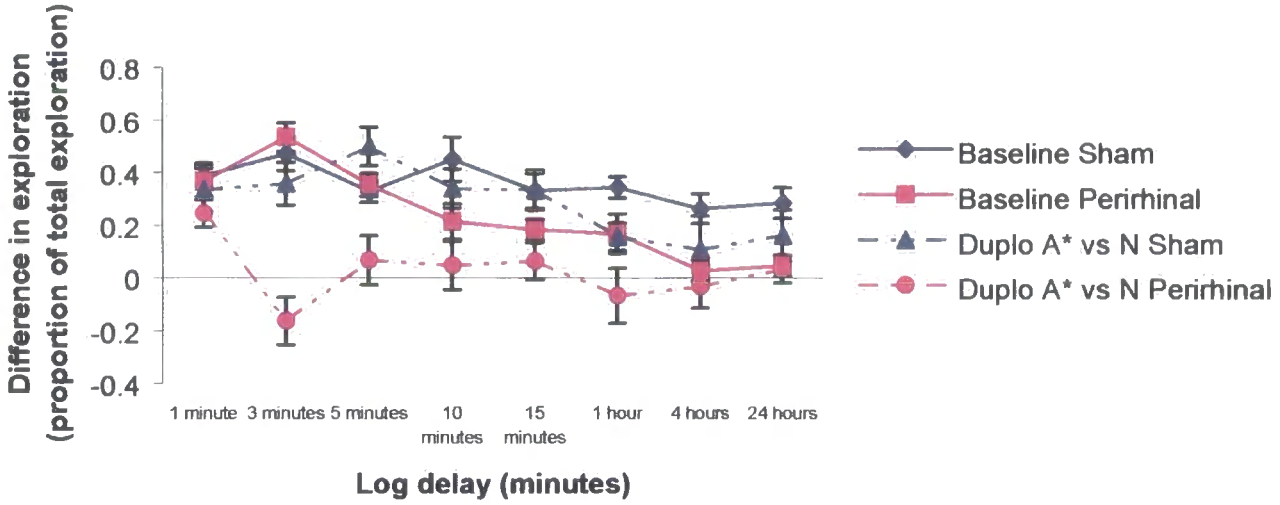


Figure 3.18: Duplo A* vs N compared with baseline. D2 measure

Results Summary

As table 3.11 shows, the absolute performance of each group on each task differs considerably, with disproportionate difficulty occurring for the perirhinal animals on the three experiments which used Duplo and hence some element of configural processing whether implicit (Duplo A vs. N) or explicit (Duplo A* vs. A; Duplo A* vs. N). This contrasted with the intact discrimination of this group on the multiple objects task, which was nevertheless shown to be clearly more difficult than the baseline task.

	Baseline		Duplo A/N		Duplo A*/N		Duplo A*/A		Multiple objects	
Delay	Sham	Prh	Sham	Prh	Sham	Prh	Sham	Prh	Sham	Prh
1 min	Yes	Yes	Yes	Yes	Yes	Yes	Yes	**No**	Yes	Yes
3 min	Yes	Yes	Yes	Yes	Yes	**No**	Yes	**No**	Yes	Yes
5 min	Yes	Yes	Yes	**No**	Yes	**No**	**No**	**No**	Yes	Yes
10 min	Yes	Yes	Yes	**No**	Yes	**No**	**No**	**No**	Yes	Yes
15 min	Yes	Yes	Yes	**No**	Yes	**No**	Yes	**No**	Yes	Yes
1 hour	Yes	Yes	Yes	**No**	Yes	**No**	-	-	-	-
4 hour	Yes	**No**	**No**	**No**	Yes	**No**	-	-	-	-
24 hour	Yes	**No**	**No**	**No**	Yes	**No**	-	-	-	-

Table 3.11: Summary table of both groups' discrimination at each delay in each task. D2 measure. "Yes" indicates above chance performance on a one-sample t-test; "No" indicates performance at chance; - indicates no testing in that task at that delay.

3.4: Discussion

The baseline task reported here demonstrated the impairment of spontaneous object recognition with perirhinal lesions that has been repeatedly demonstrated by other researchers (Ennaceur et al., 1996; Ennaceur & Aggleton, 1997). Having established that there was a perirhinal impairment in object recognition in a standard (Ennaceur & Delacour, 1988) paradigm, it was possible to examine the effects of introducing elements of configural processing into the task.

Unexpectedly it was found that a novel/familiar object discrimination using objects made out of Duplo (experiment 3.1) did not replicate the results of the baseline which employed standard objects. Animals with perirhinal lesions failed to discriminate between the objects at a shorter delay than on the baseline task, although the sham animals performed as well as they did on the baseline task. This failure to discriminate at shorter delays was marked, with perirhinal animals performing at chance at a delays of 5 minutes or longer, as opposed to delays of 4 hours or longer on

the baseline task. This finding indicates a substantial increase in the difficulty of the discrimination for the perirhinal animals, whilst the task difficulty did not increase for the shams.

Given that the only difference between experiment 3.1 and the baseline was the nature of the objects – constructed from Duplo rather than everyday objects such as bottles or jars, this must be the origin of the earlier mnemonic failure of perirhinal animals. As with the standard objects, the two objects used in each test were deliberately selected or constructed so as to differ from each other as much as possible. In the light of this fact there are two possible accounts for the fact that the Duplo version of the discrimination was more difficult for the perirhinal animals. The first of these is that two objects made out of Duplo are inherently more similar to each other than are standard objects, simply because both of them are necessarily constructed from the same material. The task having been made more difficult through this introduction of greater similarity, the animals with a weaker memory for objects naturally find the discrimination more difficult. The second possible explanation is, not that the material of the objects is more similar, but that the form of the objects, through their being constructed from the same building blocks, is more similar. This is because, at some level, and despite the best efforts of the experimenter, the objects contain similar constituent elements (such as long base blocks or towers). These are arranged in very different formations, but nevertheless require processing as part of a configuration in order for the objects to be distinguished. Whilst such implicit feature ambiguity is a possibility, it is also possible that similar results would be found if standard objects were used but similarity were increased by, for example, using only objects made of glass, as opposed to the mix of glass, plastic and metal employed here. This exploration of pure similarity is a control which should be carried out before definite conclusions can be drawn as to the cause of the difference reported here.

Explicit configurality and a high level of feature ambiguity was introduced with the task which required the animals to discriminate the familiar object reconfigured (A*) from the familiar object (A) (experiment 3.2). The perirhinal animals' complete failure to discriminate even at a delay of 1 minute suggested that, where discrimination was heavily dependent on a configural processing of the objects, they

regarded A* as being as familiar as A. The alternative explanation would be that they failed to recognise A and treated both objects as equally novel rather than as equally familiar. This explanation is, however, rendered unlikely by the same animals' clear recognition of a familiar Duplo object in the previous experiment which compared A with an entirely novel object N. It is possible that, if it had been possible to carry out a test in a "no delay" condition, the perirhinal animals would have shown no impairment in the A/A* discrimination. However, given that this experiment in fact involved a condition of maximum feature ambiguity, a condition in which Bussey et al. (2002) found a perirhinal impairment on a concurrent discrimination task, it is plausible to argue that the failure to discriminate between the objects at a 1 minute delay results not from a specifically mnemonic impairment, but rather from a processing failure. This would place the results reported here in accordance with the view of the perirhinal cortex as involved in feature integration both concurrently and retrospectively (Bussey et al., 2002).

However, it is probably necessary to proceed with caution before interpreting this result as firm support for the PMFC model of perirhinal cortex function. The argument that configural processing requirements were responsible for the perirhinal impairment on the Duplo version of the normal task and the explicitly configural comparison of A* and A depends for its validity on exclusion of the possibility that these tasks were merely increasingly difficult. If perirhinal animals simply had weakened memory for objects then it would be predicted that any task that was more difficult than the baseline task would affect disproportionately their ability to discriminate between two objects on the basis of familiarity. It was to exclude the simple explanation of mere difficulty that the task difficulty was increased in a way which did not explicitly introduce a requirement for configural processing.

Although both sham-operated and perirhinal animals performed more poorly on the task involving multiple objects (experiment 3.3) than on the baseline task, this was not especially the case for the perirhinal animals. This supports the contention that it is feature ambiguity, involving a requirement for configural processing, rather than simple difficulty or memory load which increases the difficulty of the discrimination for the perirhinal animals.

As discussed earlier, the possibility of increased feature ambiguity resulting from the increased number of objects was considered. It is possible to argue that our use of multiple (four) objects at the initial exposure stage could have increased the similarity of all the objects to one another, but great care was taken to select objects which appeared (to the experimenter, of necessity) conspicuously different to one another and which, as far as was possible, lacked shared features. It is much more likely that, as was intended, the mnemonic load was increased by the use of multiple objects, all of which will have been separately encoded. The difficulty of the task was also incidentally increased by the requirement that the animals explore the object designated for use as (A) for at least 15 seconds, as opposed to the minimum of 30 required for the baseline task. The reason for this was purely practical, there was only one copy of each object as opposed to the two employed in the standard task and, with four objects in the maze, the exploration period would have become impracticably long had the 30 second requirement been maintained.

Experiment 3.4 involved a different discrimination to the other experiments in this chapter, in that the discrimination was between a novel object and a familiar object reconfigured, rather than between a familiar object and another object (novel or reconfigured). In this experiment the perirhinal animals clearly preferred the novel object (N) at the 1 minute delay (see figures 3.17a and 3.17b). However, at 3 minutes they showed a non-significant preference for A*. As can be seen from figures 8a and 8b, performance at longer delays showed no evidence of discrimination between the objects. It would be reasonable to interpret this finding as indicating that the perirhinal animals discriminated between the objects at the 1 minute delay but not at delays of 3 minutes or longer. The results of this study do not provide any support for an interpretation of a true preference for A* as might have been predicted if reconfiguration were itself of intrinsic interest. Rather, they suggest that an intermediate level of feature ambiguity is responsible for a perirhinal impairment at delays intermediate between that produced by no minimal feature ambiguity (baseline and experiment 3.1) and total feature ambiguity (experiment 3.2).

There is only one extant study to which the present study is directly comparable, and this requires some comparative discussion. Ennaceur et al., (1996) used objects constructed of metal and plastic to test rats at delays of 1 or 15 minutes on versions of

the object recognition test (Ennaceur & Delacour, 1988) which required an A/N, an A*/A or an A*/N discrimination. Their results are summarised briefly. On the A/N discrimination they report a perirhinal deficit at the 15 minutes but not at the 1 minute delay. On the A*/A discrimination they report that neither the perirhinal nor the shams discriminated at the 15 minute delay though both did so at the 1 minute delay. Finally they report that on the A*/N discrimination the perirhinal animals preferred A* at the 1 minute delay, with this preference being reversed at the 15 minute delay.

It is, however, difficult to compare their findings with those of the present study for several reasons. The first of these is that Ennaceur et al. only tested at delays of 1 or 15 minutes. This means that where there was an impairment only at the longer delay as was the case on both the A/N and the A*/A discriminations, it is impossible to ascertain whether their study would have found a perirhinal deficit at intermediate delays, or whether 15 minutes represented the genuine time point for the onset of an impairment.

A second difficulty arises from the fact that Ennaceur et al's study used only one discrimination for each paradigm whereas the present study used two. It is also the case that a greater number of experiments, each with multiple delays, were carried out in the present study, so that over a long period of time the *total* stimulus set was much greater. It has been shown that monkeys (Buckley & Gaffan, 1997; Eacott et al., 1994) with perirhinal lesions are impaired on stimulus-discrimination learning tasks where a large stimulus set is employed, but are not impaired when the set is restricted to a small number of stimuli. It is therefore possible that the larger number of stimuli encountered by the animals in the present study could itself be responsible for a perirhinal deficit. However, if this is a *possible* explanation it does not appear a *likely* one for the simple reason that the perirhinal animals would seem much less likely than the shams to be affected by objects seen several days or weeks previously when they did not discriminate between objects at delays counted in minutes. In addition, if this were part of the reason for the deficits observed, one would have predicted that the performance of the perirhinal animals would have become worse with increasing numbers of experiments. The animals' performance on successive experiments clearly demonstrated that this was not the case.

In the absence of another explanation for the difference in performance between the studies on the A*/A discrimination it seems probable that the different nature of the objects in the two studies allows an explanation in terms of a difficulty differential, resulting perhaps from different spatial properties of the objects in the Ennaceur et al. (1996) study. Indeed it is possible that, unlike the objects in this study, those in their study permitted the formation of unique features as a result of reconfiguration (although care was taken not to introduce novel features), meaning that there was not complete feature ambiguity in their experiment.

The results of the A*/N discrimination reported here are in direct contrast to those reported by Ennaceur et al. (1996). That study found that at the 1 minute delay the perirhinal animals explored the reconfigured object (A*) more than they explored the novel object (N). The authors interpreted this as being indicative of a particular interest in the rearrangement of the object A's features. However, in view of the fact that neither the control nor the fornix lesioned groups in that study discriminated between the objects at the 1 minute delay, it would seem unreasonable to attach too much weight to the finding. This should be contrasted with the finding that the sham animals in this study showed a strong preference for N over A*, a preference which persisted up to a delay of an hour. It is probable, though by no means certain in the absence of data at intermediate delays in the Ennaceur et al. study, that the nature of their objects rendered them easier to discriminate in the partial feature ambiguity paradigm presented by A*/N than were the Duplo objects employed here. However, whilst this would adequately explain their finding of a preference for N at the 15 minute delay it leaves the explanation of the data reported at the 1 minute delay as a statistical anomaly. This however, would seem to be the most probable explanation for their apparently paradoxical results; that it was a statistical anomaly, perhaps generated at least in part by the poor performance of all groups. Discrimination by animals at delays as short as 1 minute is sometimes lower than would be predicted given their performance at longer delays. This is probably due to the anxiogenic effects of being returned to the home cage and then removed again almost immediately, and it provides a possible explanation for anomalous performance at delays of 1 minute. Such an explanation is lent weight by the fact that by 15 minutes the perirhinal animals in the Ennaceur et al. study showed a preference for N, as did the control animals in the study.

A final possibility which must be considered is that the differences result in part from differences in lesion technique. The present study employed electrolytic lesions whilst that of Ennaceur et al. used NMDA lesions. This leaves open the possibility that the electrolytic lesions used here caused damage to fibres of passage and therefore disrupted hippocampal function to a greater extent than would be found with NMDA lesions. If this were the case then it is possible that the combined functional damage to the two structures would result in more severe deficits such as the lack of discrimination at shorter delays seen here. However, given the failure of other studies to implicate the hippocampus in processing of object recognition (including the performance of Ennaceur et al.'s fornix lesioned group) this explanation seems inherently unlikely as an account of the different patterns of results between their study and the present one.

It will be remembered that the explanation advanced for the failure of the perirhinal animals to discriminate between A* and A (experiment 3.2) was that, due to the maximum feature ambiguity of the objects, they did not recognise the novelty of A*, and therefore behaved as if it were A. However, this explanation becomes problematic when considered alongside the results of the discrimination between A* and N (experiment 3.4). In this task the perirhinal animals did not discriminate between the objects at delays of 3 minutes or longer, whereas the sham animals discriminated at delays of up to 1 hour. This failure to discriminate would suggest that at a delay of 3 minutes the perirhinal animals treated A* as essentially novel, just as they did N. Taken in conjunction with their failure to discriminate between A* and A at the same delay this presents a paradox. At a delay of 3 minutes perirhinal animals regard A* as essentially familiar because they do not distinguish it from A, but they also regard it as essentially novel because they do not distinguish it from N. There is therefore a situation which, represented mathematically, is absurd:

If A* = A

and A* = N

$\therefore$ A = N

This representation of the familiarity relationship cannot possibly be accurate because if A = N were true then perirhinal animals at this delay would not discriminate

between A and N, which the baseline and experiment 3.1 have clearly demonstrated that they did, whether the objects were standard ones or constructed from Duplo.

A possible explanation for the results found in the series of experiments reported here would be in terms of increasing feature ambiguity. The baseline established using standard objects represented a condition of minimum feature ambiguity, effectively permitting purely elemental discriminations. The perirhinal animals were able to discriminate in these conditions at delays up to 1 hour. The much shorter delay of 5 minutes at which perirhinal animals failed on the Duplo version of the same A/N discrimination had not been predicted, but, on consideration, probably represents the implicit introduction of a degree of feature ambiguity simply because the same type of blocks were used to construct each object. When a condition of total feature ambiguity was used with the A*/A discrimination the perirhinal animals showed a complete and non delay-dependent impairment, as would be predicted by the PMFC model (Bussey & Saksida, 2002). This model would also predict that a condition of explicit partial feature ambiguity would result in a milder impairment than the maximum feature ambiguity condition, but a more severe one than the minimum feature ambiguity achievable with Duplo objects (the A/N discrimination). When animals were tested in such a condition by the use of the A*/N paradigm this was exactly what we found: in contrast to the counter-intuitive and slightly complex results of Ennaceur et al. (1996) the results of this study showed a clear effect where the perirhinal animals showed a total impairment at a delay of 3 minutes, intermediate between that of the Duplo A/N discrimination (5 minutes) and that of the A*/A discrimination (1 minute). This was in clear contrast to the results of the multiple objects paradigm where, although both groups performed more poorly than they did on the baseline task, and although the perirhinal animals performed more poorly than the shams, (as they had on all tasks), this perirhinal decrement was not disproportionate. In contrast, task analysis revealed that the perirhinal animals *did* perform disproportionately more poorly than the shams on each of the tasks in which Duplo objects had been employed. However, the issue of the extent to which the perirhinal animals do in fact perceive A* to be novel remains unanswered.

While the results reported here are necessarily discussed primarily in the context of previous findings where the same paradigms were employed, they should also be

considered in the context of other studies where feature ambiguity has been employed (Buckley & Gaffan, 1998c; Bussey et al., 2002; Eacott et al., 2001). These studies have used an explicit motivational element which is not present in the object recognition test. However, the assumption in this study that animals process objects configurally in the absence of any explicit training, is supported by the finding of Healey & Gaffan (2001) that rats spontaneously process concurrent features as a configuration. The present study used 3-dimensional objects, in contrast to most other studies which used 2-dimensional images. This is unlikely to have affected the results though, as perirhinal lesions have not been found to affect complex discriminations in monkeys (Buckley, Gaffan & Murray, 1997).

The literature tends to support the theory that perirhinal cortex function can be explained in terms of the processing of feature ambiguity, and memory which is dependent upon that processing (Bussey & Saksida, 2002). Bussey et al., (2002) showed that increasing feature ambiguity increased the severity of the impairment shown by monkeys with perirhinal lesions. Our results are also in accordance with those of Eacott et al. (2001) who found that perirhinal rats were impaired on a biconditional learning task, markedly more so than on a visual discrimination task of increased difficulty, but with only a partial configural element. The findings of this study also agree with those of Buckley & Gaffan (1998c) who found deficits in configural processing following perirhinal lesions in monkeys. However, Bussey et al. (2000) report intact negative patterning in monkeys with combined perirhinal and postrhinal lesion, a finding which may be explicable in terms of the PMFC model.

The experiments discussed in this chapter show that animals with perirhinal lesions are impaired in tasks which require configural processing. The delay required to cause this impairment is shorter the more reliant the task is on configural processing. Where there is complete feature ambiguity the perirhinal animals are impaired at even the shortest delay. This study therefore supports the findings of Bussey et al. (2002) and suggests that in rats the perirhinal cortex is as critical to the processing of feature ambiguity as it is in monkeys. This finding concerns objects and the processing of the relationship between the features of a single object. However, much of the literature which is concerned with configural processing is concerned with contexts rather than with objects. The hippocampus has traditionally been considered to be critical to

contextual memory, and the work of Gaffan and colleagues (Gaffan, 1994a, 1998; Gaffan & Harrison, 1989; EA Gaffan, Bannerman, Warburton & Aggleton, 2001) has confirmed that there is hippocampal involvement in the processing of an object within a scene. There is, however, some indication that the perirhinal cortex, and also the postrhinal cortex, are also involved in contextual processing (Bucci, Saddoris & Burwell, 2002, Buckley & Gaffan, 1998b, Corodimas & LeDoux, 1995). The experiments described in chapter 4 therefore contrasted the effects of perirhinal, postrhinal or fornix lesions on processing of objects within a context.

Chapter 4: Memory for Context: A comparison of lesions to the Perirhinal Cortex, the Postrhinal Cortex and the Fornix.

4.1: Introduction

The experiments in this chapter examine the role played by the context in which an object appears in object recognition and compare the effects of lesions to the perirhinal cortex with those of postrhinal or fornix lesions. The evidence as to the precise roles of these structures in contextual processing and memory is somewhat unclear. While there is a substantial body of evidence which suggests that the hippocampus is implicated in memory for context it is by no means clear that this contribution is both necessary and sufficient. The probability is that the hippocampal contribution, while substantial, may be neither necessary nor sufficient in tasks which do not depend upon conditioning or learned responses, but which do require recognition of a combination of object and context. Indeed it seems likely that the postrhinal cortex, and, to a lesser extent, the perirhinal cortex may play a role in memory for an object-in-context. The possible involvement of configural processing in such contextual memory is considered along with what this might imply about the involvement of both the hippocampus and the rhinal cortex.

Much of the work on hippocampal involvement in memory for contextual information has concerned aspects of fear conditioning, with studies showing that the disruption of hippocampal function prevents extinction of context-specific freezing to a conditioned stimulus (CS) (Corcoran & Maren, 2001), and that hippocampal lesions disrupt only those processes which require an association between the unconditioned stimulus (US) and context (Frohardt, Guarraci & Bouton, 2000). Such context-specific results have also been found in other Pavlovian paradigms, with reversible inactivation of the hippocampus preventing context-specific latent inhibition, indicating that retrieval of context is dependent on hippocampal function (Maren & Holt, 2000), and hippocampal lesions apparently removing the association between conditional and contextual cues (Winocur, 1997). However, the evidence is not uniform on this point. McNish, Gerwitz & Davis (2000) found that while hippocampal lesions disrupted contextual freezing they did not disrupt contextual blocking. Contextual fear-potentiated startle is also unaffected by hippocampal lesions which disrupted contextual freezing (McNish, Gerwitz & Davis, 1997).

Moving away from conditioning studies, there is a considerable amount of evidence which suggests that the context in which an object is encountered is an aspect of object memory for which hippocampal function is important. Studies examining the role of a stimulus in context have shown a deficit in recognition of a stimulus in a particular context following fornix lesions in both monkeys (Gaffan & Harrison, 1989, Gaffan, 1994a) and in rats (Simpson, Gaffan & Eacott, 1998). Parker & Gaffan (1997a) demonstrated that lesions of the mammillary bodies caused an equivalent impairment to that following fornix lesions, and that combined lesions of the two structures did not result in a more severe impairment. They also showed (Parker & Gaffan, 1997b) that lesions of the anterior thalamic nuclei caused similar impairments. This would suggest that it is the functional unity of a memory system (which includes both the hippocampus and the mammillary bodies as well as other structures such as the anterior thalamic nuclei) rather than the hippocampus itself which is required for object in scene memory.

Work by Good, de Hoz & Morris (1998), found that hippocampal lesions disrupted only incidental acquisition of contextual learning; where such learning was necessary for successful performance of an appetitive biconditional task it was unaffected by the lesions, explaining the intact contextual learning found in conditioning tasks following hippocampal lesions (e.g. Gisquetverrier & Schenk 1994; Hall, Purves & Bonardi, 1996). This provides a possible explanation for some of the results discussed: hippocampal processes are the preferred and automatic method of learning about context, but where they cannot be employed and such learning is required it can be supported by other structures.

Bucci et al. (2002) found that lesions of the fornix, the perirhinal and postrhinal cortices all individually produced deficits in fear conditioning to context, although neither perirhinal nor postrhinal lesions affected fear conditioning to an auditory stimulus. However, Sacchetti, Lorenzini, Baldi & Tassoni et al. (1999) found that temporary inactivation (using tetrodotoxin) of the perirhinal cortex impaired conditioning to both these types of stimuli. The question of whether the perirhinal cortex plays a role in context conditioning at all, let alone a specific role as suggested by Bucci et al. would seem to be open to doubt. Indeed Otto and colleagues (Otto Cousens & Herzog, 2000; Herzog & Otto, 1998) found that rats with lesions of the

anterior perirhinal cortex exhibited an attenuation of fear conditioned to the explicit olfactory cue, but no attenuation of fear conditioning to the training context. This is balanced by the finding of Corodimas & LeDoux (1995), who found that animals with perirhinal lesions showed differential freezing to a conditioned stimulus in a novel and a familiar context, still displaying an impairment relative to controls in the conditioned context, but significantly less so than in the novel context. It seems possible that the perirhinal cortex is more sensitive to context when there are no explicit cues available.

Some studies have found that lesions of the entorhinal cortex produce deficits in contextual fear conditioning equivalent to those produced by fornix or hippocampal lesions (Maren & Fanselow, 1997), although it is possible that this reflects the supporting role which some theories (e.g. Sharp, 1999) propose for the entorhinal cortex. However the role of the entorhinal cortex is not clear either: Phillips & LeDoux (1995) found that even combined lesions of the entorhinal and perirhinal cortex did not affect contextual fear conditioning that was affected by fornix lesions.

The experiments discussed in this chapter attempt to use a naturalistic paradigm in the form of a version of the spontaneous object recognition task (Ennaceur & Delacour, 1988) to investigate the possible contributions of the hippocampus, the perirhinal cortex, and also the postrhinal cortex in memory for three-dimensional objects in context. Dellu, Fauchey, LeMoal & Simon (1997) used such an approach in the Y-maze and found that, in normal animals, context change between acquisition and recognition trials affected object recognition. Dix & Aggleton (1999) also demonstrated this in a version of the spontaneous object recognition paradigm, demonstrating the role of context even in simple object recognition. Moreover, Mumby et al. (2002) found hippocampal impairments on both object-in-place and object-in-context versions of the spontaneous object recognition task. This result is perhaps unsurprising in the light of Good et al.'s (1998) finding that hippocampal animals only acquired contextual learning when success in an appetitive task depended upon it. In the spontaneous object recognition task there is no explicit motivational element and, therefore, while acquisition of context may be possible, there is no indication from the observed behaviour that it in fact occurs. Although there is evidence that processing of object-in-place information is impaired by fornix

lesions but not by perirhinal lesions (Mumby et al., 2002; Gaffan et al., 2001) (although see Bussey et al., 2001 for evidence of integrated function), little work has been carried out on the role of the perirhinal cortex in contextual processing or memory, while the possible role of the postrhinal cortex is uncertain. Bussey, Duck, Muir & Aggleton (2000) found that combined lesions of the perirhinal and postrhinal cortices in monkeys caused impairments on the object-in-place task at least as severe as those caused by fornix lesions.

The postrhinal cortex is known to be involved in some aspects of memory involving memory for context. Experiments using fear conditioning had suggested such a role (e.g. Bucci et al., 2002), but the nature of this role remained unclear. Gabrielli et al.'s (1997) FMRI study suggested that parahippocampal cortex in humans was involved in memory for combinations of objects and places. In particular c-fos studies have shown that while the perirhinal cortex and area TE showed differential activation for novel objects over familiar objects, it is novel arrangements of familiar items which cause increased activation in the postrhinal cortex as well as in area CA1 of the hippocampus (Wan et al., 1999). This would certainly indicate postrhinal involvement in the spatial arrangement of objects, and is suggestive of involvement in contextual processing of objects. The involvement of the postrhinal cortex but not the perirhinal cortex in tasks with a spatial component is supported on the basis of c-fos studies (Aggleton, Vann, Oswald & Good, 2000; Vann et al., 2000) as much as lesion studies, which often show only relatively mild deficits on spatial tasks. For example Bussey et al. (2000) found that combined lesions of the perirhinal and postrhinal cortices did not impair performance on a spatial alternation task on the T-maze, but did impair object recognition (as would be predicted with a perirhinal lesion).

It has been suggested that the hippocampus is involved in memory for context because of its role in configural processing. It is argued that its role lies in the integration of the various environmental features which define a context into a meaningful construct. Findings by Rudy and colleagues (Rudy, Barrientos & O'Reilly, 2002; Rudy & O'Reilly, 1999) found that, in contexts formed from a conjunction of multiple features, animals with hippocampal lesions showed impaired contextual conditioning, while pre-exposure to a context but not to its separate features facilitated both contextual fear conditioning and generalisation of that

conditioning. When long-lasting background cues were employed in a conditioning task in the place of more conventional contexts, hippocampal lesions did not affect contextual conditioning (Rawlins & Tanner, 1998), supporting the view that the hippocampus is important in the integration of multiple contextual elements. This would be in accordance with the revised theory of Rudy & Sutherland (1995) which argues for an extrahippocampal location of configural associations required for contextual learning. However, use of a configural interpretation of context processing in order to explain the findings of hippocampal impairments in contextual tasks (e.g. Fanselow, 2000) may be misguided. Firstly, as Gerlai (2001) argues context can often be identified by largely elemental processes, and secondly, as has been discussed extensively in chapter 3, configural processing itself may not depend on an intact hippocampus at all. Certainly Bucci et al. (2002) demonstrated that fear conditioning to context was particularly impaired by either perirhinal or postrhinal lesions when the context was constructed in such a way as to encourage a configural rather than an elemental identification of context, while Bussey et al.'s (1998) finding of enhanced configural learning following fornix lesions provides a clear challenge to views such as Rudy & Sutherland's.

Such results stand in contrast to the standard findings that fornix lesions do not impair stimulus recognition *per se* (e.g. Ennaceur et al., 1996). They are supported by findings on human amnesic patients with hippocampal damage, who show impaired implicit contextual learning while retaining intact implicit perceptual learning (Chun & Phelps, 1999). However, there is also convincing evidence for the contribution of cortical areas to such learning. Buckley & Gaffan's (1998b) study found perirhinal impairments in recognition of familiar stimuli against novel backgrounds. When Gaffan & Parker (1996) used crossed unilateral lesions of the fornix and the perirhinal cortex (together with a partial forebrain commissurotomy) they found that memory for object in scene was mildly impaired by the first surgery (either unilateral lesion of the fornix or of the perirhinal cortex) but was severely impaired following the completion of all lesions. This is interpreted by the authors as being indicative of the perirhinal cortex providing visual information on the object to the hippocampus and related structures.

This study compared the effects of lesions of the fornix and of the perirhinal or postrhinal cortex on aspects of memory for object-in-context. If context is to be considered as a spatial problem then one would predict that lesions of the fornix and possibly of the postrhinal cortex, but not of the perirhinal cortex would produce impairments on an object-in-context version of the spontaneous object recognition task. It would be predicted that perirhinal lesions would only produce an impairment if the object itself could not be remembered. Therefore, if the delays used were sufficiently brief that perirhinal lesions would not cause a recognition deficit in a standard version of the task it would be predicted that there would be no impairment, and any impairment that was found would be attributable to the contextual aspects of the discrimination. Control tasks were used to ensure that the repetition of exploratory phases and the delays involved did not themselves cause deficits in discrimination. A task which examined the effect of an incongruent context on the test phase of discrimination between a novel (N) and a familiar (A) object was also included, as was a task which used objects rather than conventional contexts as a basis for discrimination. It was hypothesised that performance of these discriminations would be more vulnerable to perirhinal damage than the object-in-context task, given that the perirhinal cortex's primary function would appear to be the processing of, and memory for, objects and aspects of objects (see chapter 3 for an extensive discussion of this view).

Although results indicating that configural processing may be important in memory for context (Rudy et al., 2002; Rudy & O'Reilly, 1999) were considered in the design of the contexts used here, it was felt that examination of context, and the possible configurations involved in context, were a separate issue from the configurations of objects used in chapter 3, and which generated such striking perirhinal deficits. The contexts used in this chapter were extremely simple and involved combinations of walls and flooring in the maze which contained no feature ambiguity. In addition particular care was taken to ensure equal familiarisation with both the contexts used, as there is evidence that a lack of such familiarisation can itself be a cause of impairment in object recognition (Besheer & Bevins, 2000).

Care was taken to ensure that the order in which objects appeared was controlled for, as was the context in which testing occurred as it is known that at delays of 1 hour

animals will discriminate on the basis of recency of exploration (Mitchell & Laiacona, 1998). Although the delays used here were considerably shorter than 1 hour it was considered a possibility that order of presentation might form a basis for discrimination, and in each case a control experiment was therefore employed in addition to the counterbalances described in order to assess this possibility.

4.2: Methods and Materials

Subjects

Fifty Dark Agouti rats (Bantin & Kingman, Hull, UK) were used in this experiment. Four died during surgery or of post-operative complications, so forty-six animals were used in the experiments presented here and in the experiments presented in chapter 5. They were housed in pairs in diurnal conditions (12h light/ 12h dark cycle) and all testing was carried out during the light phase. Throughout the study animals had *ad libitum* access to both food and water. At the time of surgery animals were approximately three months old.

Apparatus

As in the experiments in chapter 2, all testing was carried out in an open field made of wood, of base dimensions 1 m^2 and height 48 cm. The maze floor and walls could be changed to provide two different contexts. In context 1 the base was painted matt black and the walls matt white. In context 2 the base consisted of a 1.5 cm^2 mesh of white plastic-coated wire overlaid on a black base and the walls were natural wood. The objects were placed into the open field equidistant from the sides of the maze. Standard objects used included bottles, jars, tubs and bowls.

Surgery

Surgery was performed on all animals. Three animals died during surgery due to anaesthetic complications, and one animal died from post-operative complications. Of the remainder, 12 animals received bilateral lesions of the perirhinal cortex, 12 animals received bilateral lesions of the postrhinal cortex, 11 animals received bilateral lesions of the fornix and 11 animals were sham-operated.

Perirhinal Lesions

The procedure for this group of animals was identical to that described in chapter 2.

Postrhinal lesions

The initial procedure was the same as that for the perirhinal lesions, with the exception that, before surgery began, ear bar zero was measured. Following a midline incision both bregma and lambda were measured. Three lesions sites were calculated using measurements based on these three landmarks. The calculations were as shown in the following table.

	Site 1			Site 2			Site 3		
	EBZ	Br	Lmd	EBZ	Br	Lmd	EBZ	Br	Lmd
AP	+0.2	-5.8	+2.0	-1.3	-7.1	+0.7	-1.8	-7.6	+0.4
ML	+/-6.1			+/-6.1			+/-6.1		
DV	+2.8	-8.5	-6.6	+3.8	-7.8	-5.8	+4.2	-6.8	-4.8

Table 4.1: Calculation of lesion sites for postrhinal lesions relative to landmarks of ear bar zero (EBZ), bregma (Br) and lambda (Lmd). All measurements are given in mm. Other abbreviations are anterior-posterior (AP), medio-lateral (ML) and dorso-ventral (DV).

Where the calculations based on these sites differed from one another the mean was used, or where two calculations closely agreed and differed from the third, the mean of those in close agreement was taken. The top of the skull was measured at each of the selected sites. The dura was cut to allow the insertion of an electrode into the brain at each of the three sites in turn. Using a dental drill, an area of the skull overlying the rhinal sulcus was removed. The electrode was lowered vertically to a depth calculated using the final row of table 1. Current was then passed such that a temperature of approximately 75° C was achieved for 1 minute. The procedure was then repeated contralaterally. The scalp was then closed with the procedure identical to that for the perirhinal lesions.

Fornix lesions

The initial procedure was the same as for the postrhinal lesions. Once the midline incision had been made bregma was measured, and the first lesion site was calculated as 5.3 mm anterior and 0.7 mm lateral to ear bar zero. The calculation was also made

as 0.4 mm posterior and 0.7 mm lateral to bregma. Where the anterior/posterior positions differed between the two calculations a mean was used. A second injection site was calculated in the same way but using a mediolateral measurement of 1.7 mm lateral to both bregma and ear bar zero. The skull overlying these sites on each side of the midline was removed in a single section using a dental drill. The top of the dura was then measured at each of the previously calculated lesion sites. The dura was then cut at each site and at the first lesion site the electrode was lowered to a depth of 4.5 mm relative to the top of the skull measured at bregma and a depth of 3.7 mm relative to the top of the dura at the lesion site. At the second lesion site the depth was 4.6 mm relative to the top of the skull at bregma and 3.8 mm relative to the top of the dura at the lesion site. Current was then passed as previously described. The procedure was repeated at the contralateral lesion sites. The scalp was then closed with the procedure described for the perirhinal and postrhinal lesions.

Sham surgery

Initial procedure for the sham animals was identical to that for the animals in the lesion group. Four of the animals had the skull removed as if for a perirhinal lesion, four as if for a bilateral postrhinal lesion, and three as if for a bilateral fornix lesion. In each case the procedure was identical to that for the relevant lesion, except that the electrode was not lowered into the brain.

Perfusion

The animals used in these experiments were also used in the experiments in chapter 5. Therefore they were not perfused immediately at the end of these experiments. For histological purposes, when testing was completed, operated animals were perfused intracardially with a 5% formal saline solution. Their brains were removed, embedded in wax and coronally sectioned into 10μ slices. Every 10^{th} section was stained with cresyl violet (Nissl stain).

Testing

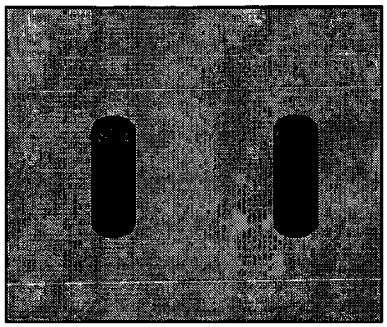
Habituation

Prior to the start of testing animals received six habituation sessions. Three of these took place in the maze configured as context 1 and three with the maze configured as

context 2. The first session in each context took place in cage-mate pairs and lasted for 10 minutes. The two subsequent sessions in each context took place individually and lasted 5 minutes. For each habituation session a different novel object was placed in the centre of the maze. All objects used were standard, as described in chapter 2, with examples being bottles, candlesticks and bowls. This type of object was used for all the experiments in this chapter.

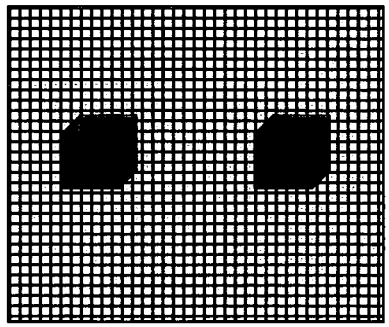
Experiment 4.1: AB in incongruent context

Experiment 4.1 was designed to measure the effect of appearing in an appropriate context on an object's familiarity. There were two exposure phases in the experiment followed by a test phase (see figure 4.1). For the first exposure phase the maze was configured as context 1 and contained 2 identical copies of an object (A), placed equidistant to the sides of the maze. The animal was placed in the maze and allowed to explore until 30 seconds had been spent exploring the objects. The rat was then removed from the maze and returned to its home cage. If animals failed to explore for 30 seconds they were removed after 5 minutes, returned to their home cage, and the time spent exploring was noted. The maze was reconfigured as context 2 and two identical copies of a different object (B) were placed in the maze as before. After an interval of 2 minutes from the end of the first exposure phase the animal was returned to the maze and allowed to explore as before. After removal from the maze after the second exposure phase there was an interval of either 2 or 5 minutes before the test phase. In the test phase the maze was configured as context 1 and contained a copy of each of the congruent object (A) and the non-congruent object (B). The animal was returned to the maze after the appropriate interval and allowed to explore freely. The time spent exploring the two objects was recorded for each of three 1 minute time bins. The order in which the contexts and objects were initially explored was controlled for between animals (for half the animals in each group context 2 was seen first), as was the pairing of the objects and contexts (for half the animals object (B) was seen in context 1). The left/right position of the objects in the test phase was also controlled between animals. There were four repetitions of the experiment at each of the 2 delays used. In 2 of these the context used in the test phase was reversed, with the context seen in the *second* exploration phase being used, controlling for recency effects, and also the effects of interspersing of a different context. Different objects were used for each repetition.



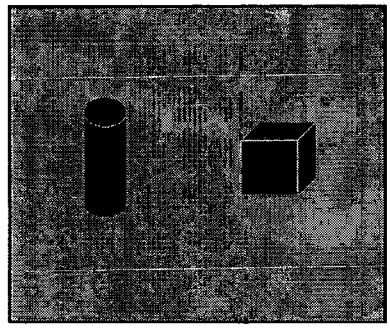
AA in Context 1

1st Exposure Phase



BB in Context 2

2nd Exposure Phase



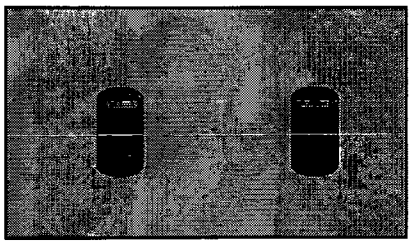
AB in Context 1

Test Phase

Figure 4.1: Exploratory and test phases for AB in incongruent context

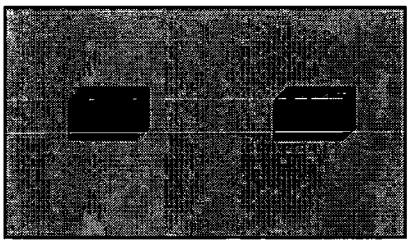
Experiment 4.2: AB in congruent context

Experiment 4.2 was a full control for experiment 4.1. It took an identical form, except that each animal only ever explored in one context (see figure 2). In the first exposure phase the maze was configured as context 1 and contained 2 copies of object (A). In the second phase the maze remained configured as context 1 and contained 2 copies of object (B). In the test phase the maze remained as context 1 and contained one copy each of A and B. Exploration by animals was as previously described for each phase. The positions of objects and the order in which they were explored were controlled for as described above (experiment 4.1). The context used was also controlled for between animals, with half the animals in each group exploring in context 1 and half in context 2. There were 2 repetitions of this experiment at each of the 2 delays used (2 and 5 minutes) and different objects were used in each.



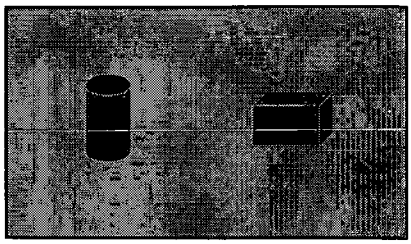
AA in Context 1

1st Exposure Phase



BB in Context 1

2nd Exposure Phase



AB in Context 1

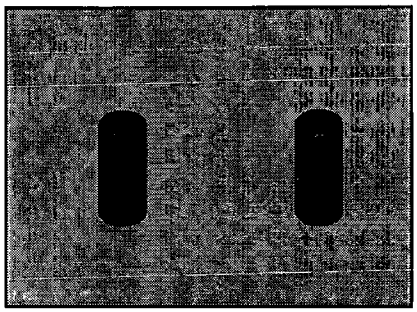
Test Phase

Test Phase

Figure 4.2: Exploratory and test phases for AB in congruent context.

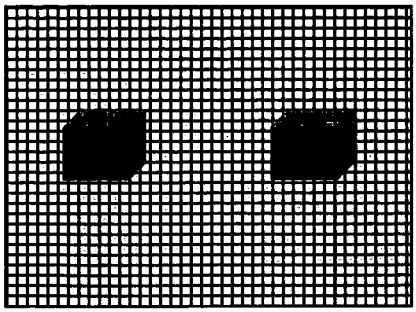
Experiment 4.3: AN in incongruent context

Experiment 4.3 was designed to test whether a comparison between a novel and a familiar object would be affected by the familiar object appearing in a non-congruent context. It was identical to experiment 4.1 with the exception that in the test phase the object in the maze consisted of a familiar object, seen in one of the exposure phases, but now seen in a different context, and a novel object. In the first exposure phase the maze was configured as context 1 and contained 2 identical copies of object (A). In the second exposure phase the maze was configured as context 2 and contained 2 identical copies of object (B). In the test phase the maze was configured as context 1 and contained a copy of (B) and a novel object (N) (see figure 4.3). Exploration was as previously described in each phase. The positions of objects, the order of contexts and the context/object combinations were controlled for between animals as previously described (experiment 4.1). The object which was novel in the test phase was also controlled for between animals, with, for example half the animals who saw objects (A) and (N) in the test phase experiencing (N) as novel, and half experiencing (A) as novel. The context used in the test phase was controlled for between repetitions of the experiment, of which there were four for each of the 2 and 5 minute delays, each using different objects.



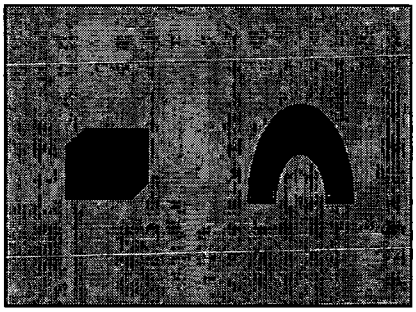
AA in Context 1

1st Exposure Phase



BB in Context 2

2nd Exposure Phase



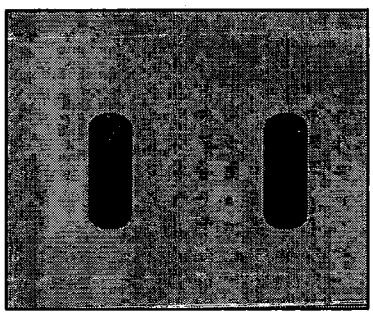
AN in Context 1

Test Phase

Figure 4.3: Exploratory and test phases for AN in incongruent context.

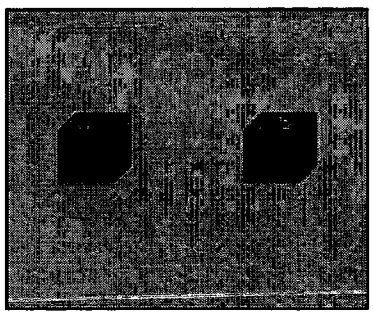
Experiment 4.4: AN in congruent context

Experiment 4.4 was a control for experiment 4.3, and was identical except that context remained invariant for each rat throughout the experiment. In the first exposure the maze was configured as context 1 and contained 2 identical copies of object (A). In the second exposure phase the maze was configured as context 1 and contained 2 identical copies of object (B). In the test phase the maze remained as context 1 and contained a copy of (A) and a novel object (N) (see figure 4.4). The context used and the order of objects used were controlled for between animals, as was the designated novel object. There were 2 repetitions of the experiment at each of the 2 delays used (2 and 5 minutes as above), each using different objects.



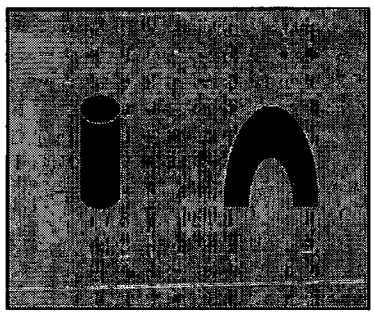
AA in Context 1

1st Exposure Phase



BB in Context 1

2nd Exposure Phase



AN in Context 1

Test Phase

Figure 4.4: Exploratory and test phases for AN in congruent context.

Experiment 4.5: Object-as-context

Experiment 4.5 was designed to explore the limits of context by using small objects such as plates or ashtrays as the context within which standard objects were seen. Objects designated with capital letters (A) are (for example) candlesticks, jars or bottles; objects designated with lower case letters (a) are (for example) saucers, soup plates or ashtrays. The maze was configured as context 1 for the whole experiment. In the first exposure phase the maze contained two copies of an object (A) standing on or in another object (a). In the second exposure phase the maze contained two copies of an object (B) standing on or in an object (b). In the test phase the maze contained one copy of (A) standing on (a) and one copy of (A) standing on (b) (see figure 4.5). Exploration in each of the phases was as previously described. The order in which objects were seen, the position of objects and the combinations of objects used were all controlled for between animals. The experiment was repeated twice at each of the two delays (2 and 5 minutes) used.

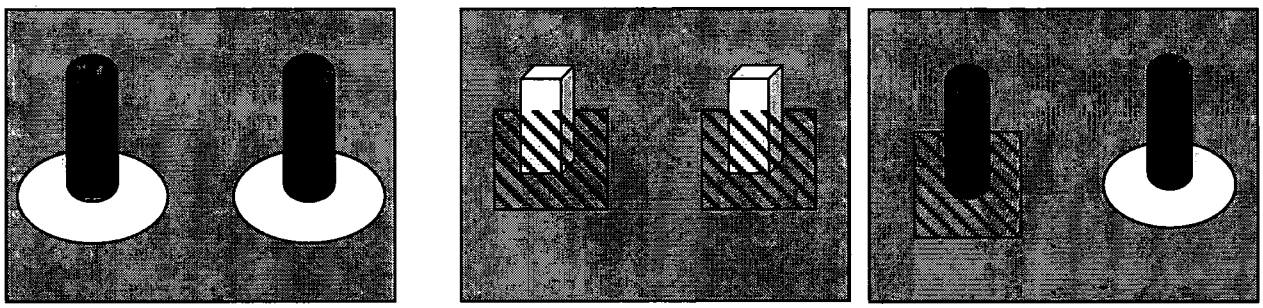


Figure 4.5: Exploratory and test phases for object-as-context.

Experiment 4.6: Simple control

Experiment 4.6 was designed as a control to check that animals were capable of performing a simple discrimination of novel and familiar objects. Because of the design used for the context experiments, with two exposures being employed, the maximum time between an object being initially explored and being encountered at test was 10 minutes. In view of evidence that deficits in object recognition can be found at such delays experiment 4.6 was a simple object recognition experiment in

which animals explored two identical copies of an object (A) in the maze until they had spent 30 seconds exploring them. They were then returned to their home cage. After a delay of 2, 5 or 10 minutes they were returned to the maze which now contained a new copy of the familiar object (A) and a novel object (N) and allowed to explore for 3 minutes. The time spent exploring each object was recorded for each of three 1 minute periods.

Data analysis

Following Ennaceur & Delacour (1988), the difference in exploration of the objects in seconds at a given time-point was calculated (D1). Secondly, the difference between the time spent exploring the objects was calculated as a proportion of the total time spent exploring both objects (D2). In experiment 4.1 the object in the incongruent context was designated as "N" and the object in the congruent context was designated as "A". The data from experiment 4.2 were analysed with the first object encountered arbitrarily designated as "N" and the second designated as "A". In experiments 4.3, 4.4 and 4.6 the objects were familiar (A) and novel (N) and were analysed appropriately. In experiment 4.5 the object in the context of the incongruent object was designated as "N" and the object in the context of the congruent object was designated as "A". Repeated measures ANOVA's were carried out on each of these measures, followed by post-hoc Tukey's test. The performance of each group was also analysed for difference from chance at each delay using one-sample T-tests.

Experiment 4.7: Locomotor activity

This experiment was designed to detect any change in locomotor activity associated with the lesions, which may have had an impact on exploration. Apparatus were Perspex boxes with removable lids, of base dimensions 42 x 46 cm^2 and height 25 cm equipped with infra-red beams which detected movement, and when broken consecutively, detected ambulatory activity which was the measure used to assess activity. Animals were placed in the boxes for a 10 minute habituation period. The following day they were placed in the box again and activity was recorded for two consecutive 5 minute periods. Data were analysed using a one-way ANOVA.

4.3: Results

4.31: Histological Results

The histological analysis reported below is of the animals which completed the experiments described in chapter 5 as well as those reported here.

Perirhinal group

One animal (R311) died before perfusion could take place. Histological analysis revealed that in all other cases the perirhinal lesions were essentially as intended, extending approximately 3.0 mm – 6.5 mm posterior to Bregma, although they were not complete lesions. The estimated damage to the perirhinal cortex ranged from 46% (R316) to 80% (R304) of the total extent of perirhinal cortex. Figure 4.6 shows the extent of the smallest (R316, semi-transparent grey) and largest lesions (R304, diagonal stripes) drawn on to standard sections taken from Paxinos & Watson (1998). As can be seen from the behavioural results described below it is unlikely that the size of the lesions affected the behavioural impairments, as these were both extensive and in accordance with the majority of the extant literature. In addition the performance of the perirhinal animals on each experiment at the 15 minute delay was plotted and neither animal with the largest nor the smallest lesion was atypical (see figure 4.7). The performance of the animal which died before perfusion could be performed (R311) is also shown. As can be seen from figure 4.7, this animal shows no indication of deviating from the performance of the cohort.

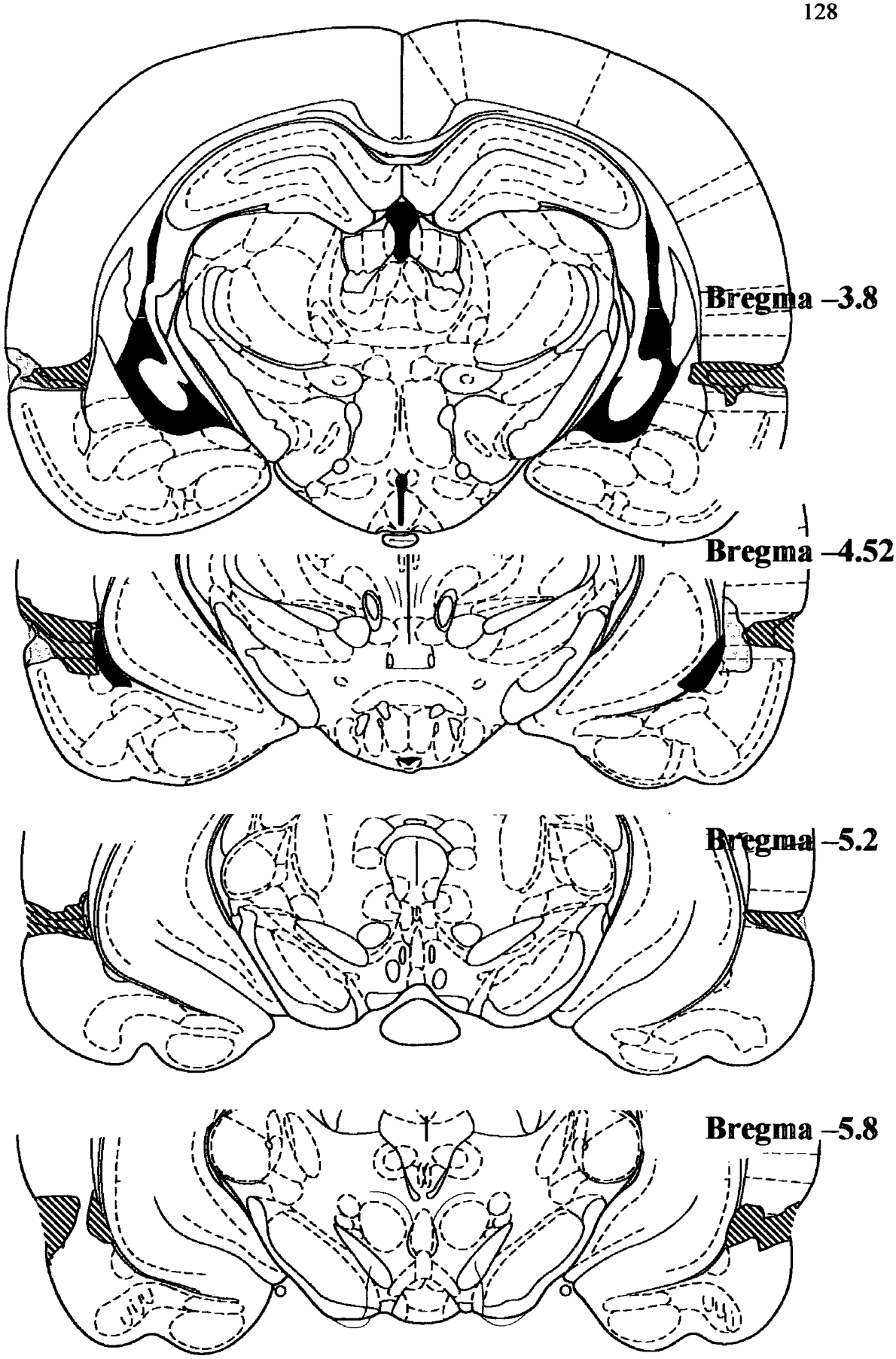


Figure 4.6: The extent of the smallest (R316, semi-transparent grey) and largest (R304, diagonal stripes) perirhinal lesions drawn on to standard sections taken from Paxinos & Watson (1998).

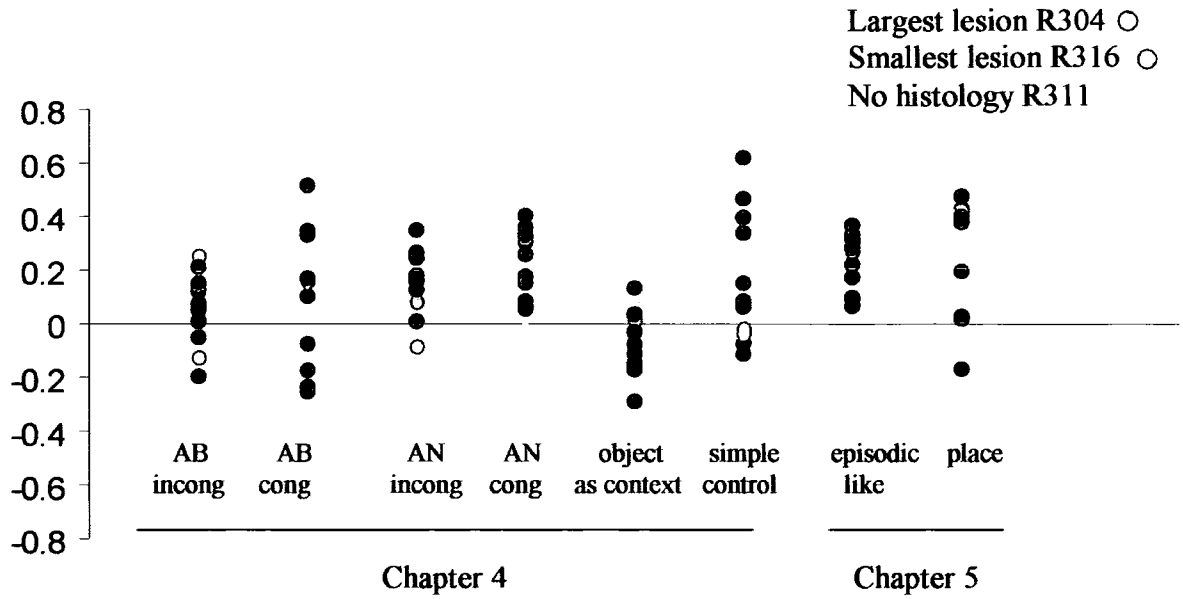


Figure 4.7: Performance at the 5 minute delay of perirhinal animals with the largest and smallest lesions on the experiments described here and in chapter 5 at a delay of 5 minutes (averaged over both contexts where appropriate). The performance of animal R311 which died before perfusion is also shown.

Postrhinal

As intended the lesions were all posterior to those found in the animals with perirhinal lesions. There was considerable variation in the extent of the lesions, as can be seen from figure 4.8 which shows the extent of the largest (R306) and the smallest (R326) lesions drawn on to standard sections taken from Paxinos & Watson (1998). However, the extent of the lesions as a percentage of the total extent of postrhinal cortex has not been estimated. This is because, although work by Burwell and colleagues (Burwell et al., 1995; Burwell & Amaral, 1998; Burwell, 2001) has gone a considerable way toward mapping the extent of the postrhinal cortex, this work has not yet reached the stage where a full stereotaxic atlas such as Paxinos & Watson (1998) incorporates the postrhinal cortex on standardised charts. As can be seen from figure 4.9, there was no indication that the size of these lesions affected behavioural performance.

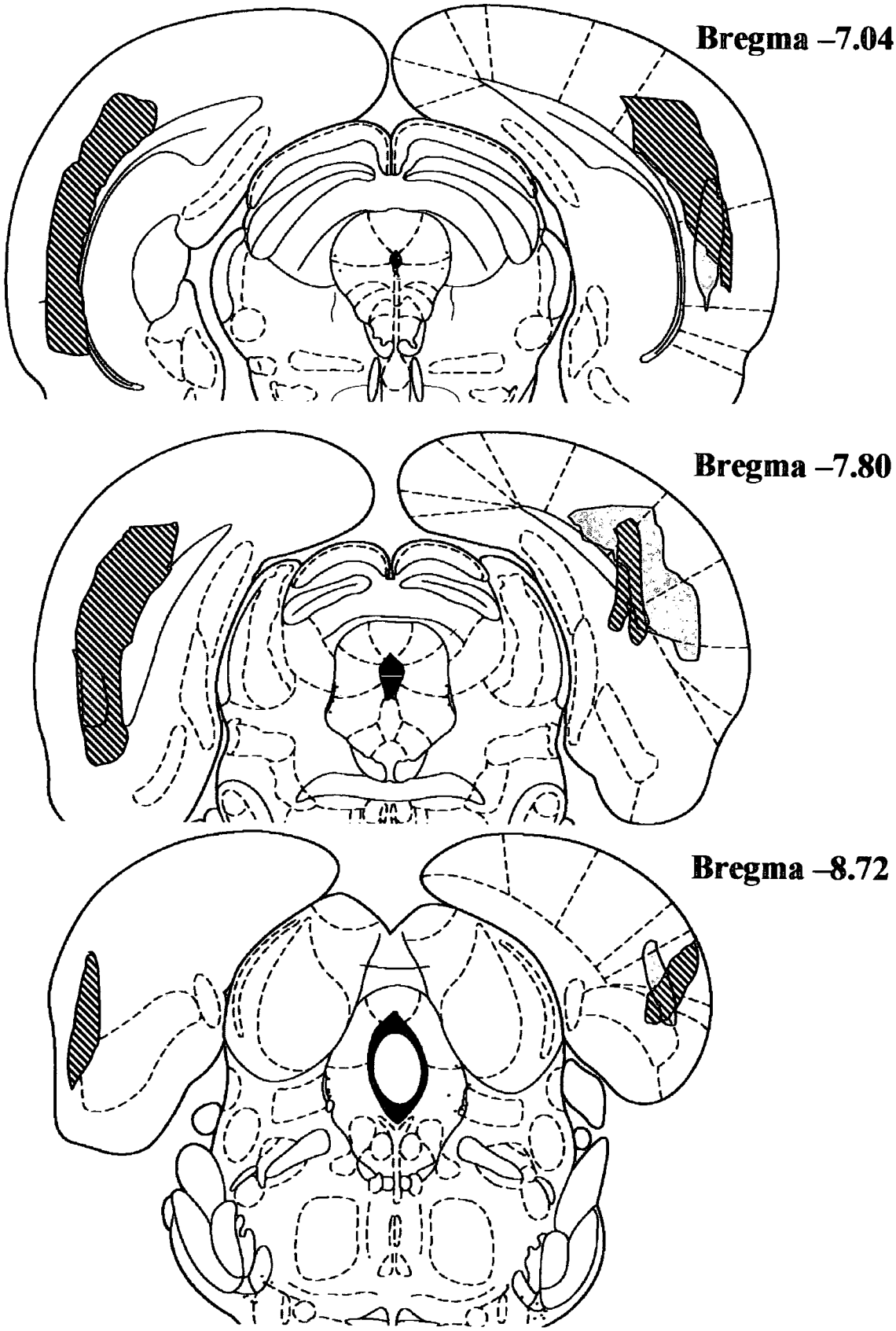


Figure 4.8: The extent of the smallest (R326, semi-transparent grey) and largest (R306, diagonal stripes) postrhinal lesions drawn on to standard sections taken from Paxinos & Watson (1998).

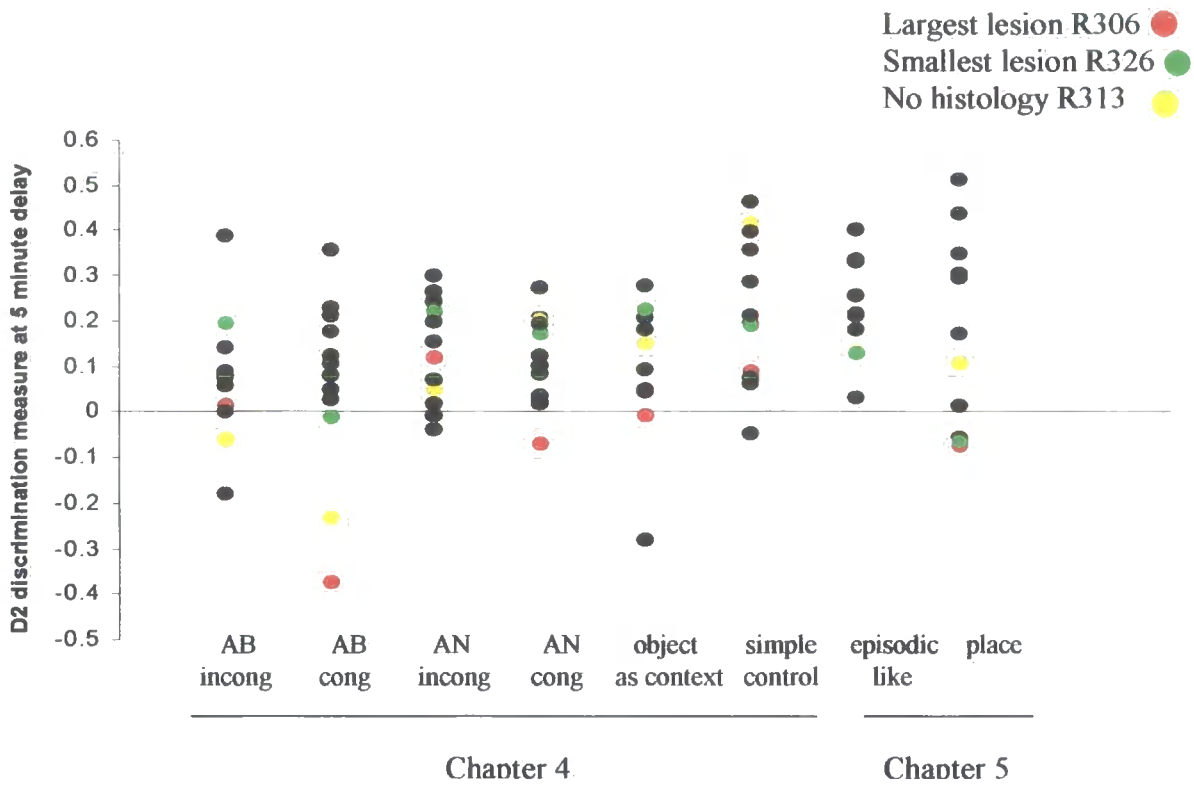


Figure 4.9: Performance at the 5 minute delay of postrhinal animals with the largest and smallest lesions on the experiments described here and in chapter 5 at a delay of 5 minutes (averaged over both contexts where appropriate). The performance of animal R313 which died before perfusion is also shown.

Fornix

All the animals had extensive bilateral damage to the fornix. In all animals the fornix was completely transected at anterior levels, although the anterior-posterior extent of the damage varied. All animals had bilateral damage to the posterior part of the lateral septum in addition to the fornix lesions. There was some hippocampal damage in those animals with more extensive lesions, with two animals showing bilateral damage in this area and three showing unilateral damage. There was no visible damage to the corpus callosum. Figure 4.10 shows the extent of the largest lesion (R308) and the smallest lesion (R337) drawn on to standard sections taken from Paxinos & Watson (1998). There was no evidence that the anterior-posterior extent of the lesion affected behavioural performance, as can be seen from figure 4.11 which shows the performance of the animals with the largest and smallest lesions in relation to the rest of the cohort.

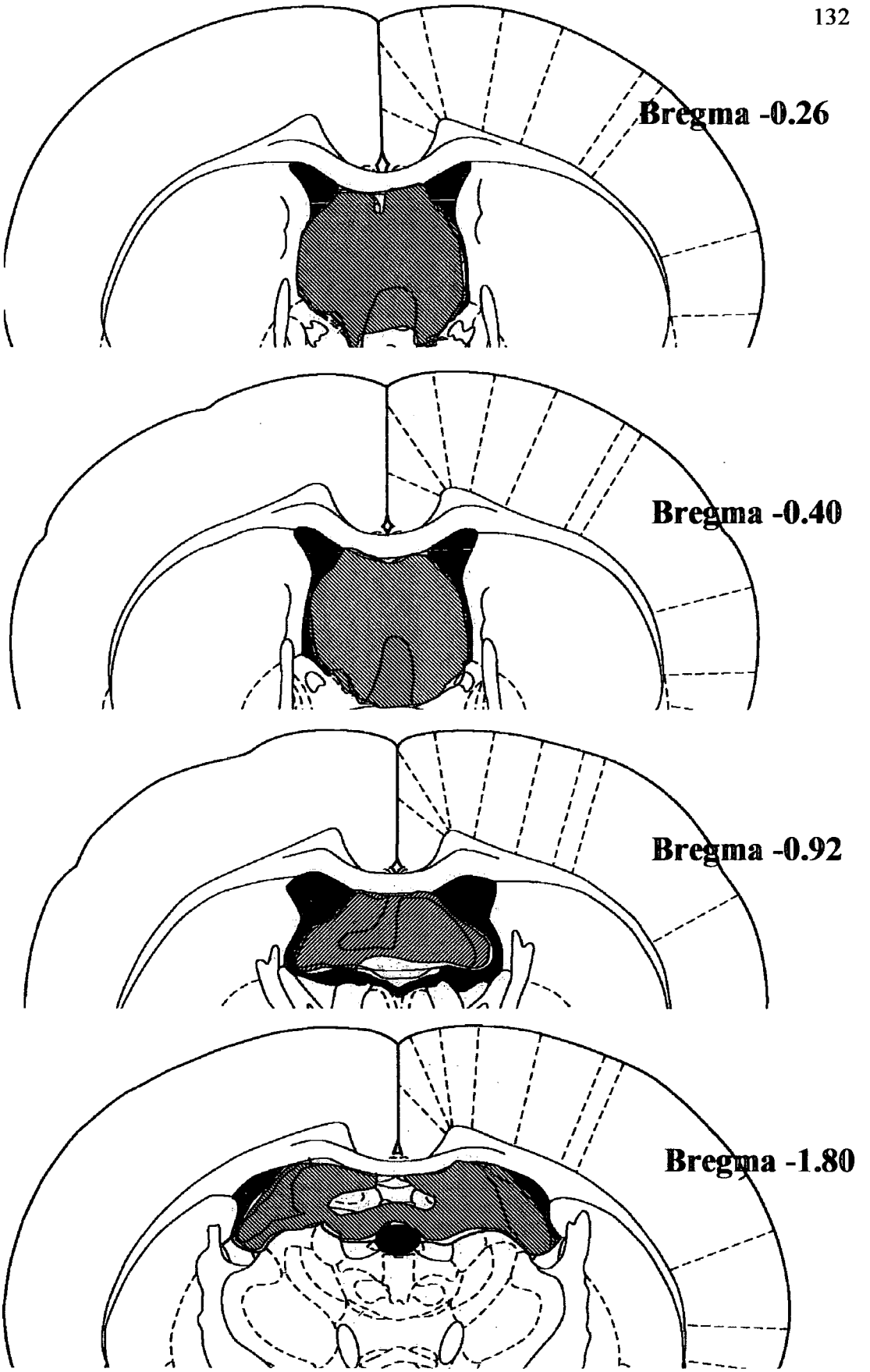


Figure 4.10: The extent of the smallest (R337, semi-transparent grey) and largest fornix lesions drawn on to standard sections taken from Paxinos & Watson (1998).

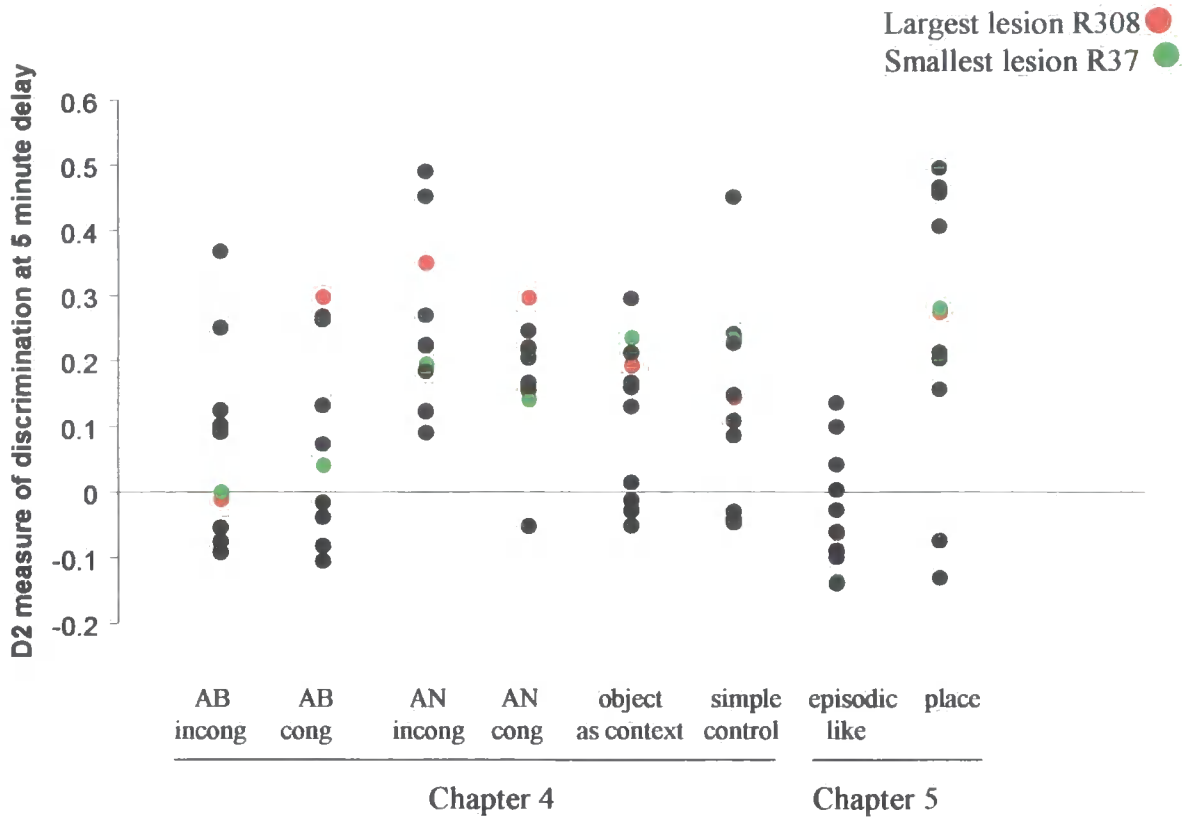


Figure 4.11: Performance at the 5 minute delay of fornix animals with the largest and smallest lesions on the experiments described here and in chapter 5 at a delay of 5 minutes (averaged over both contexts where appropriate).

Behavioural Results

Experiment 4.1. AB in incongruent context

This experiment examined discrimination on the basis of whether a familiar object was encountered in the same context or a different context relative to previous experience of that object. As can be seen from figures 412.a and 4.12b, all lesion groups were impaired compared with sham animals' performance on both the D2 ($F = 8.256$, $df = 3,42$, $p < 0.001$) and the D1 ($F = 12.434$, $df = 3,42$, $p < 0.001$) measures. Although the performance of all the lesion groups is impaired it is not uniformly so, and there is some evidence for a specific postrhinal impairment at the 2 minute delay. Because the results differ slightly between the 2 measures they are presented separately.

D2

The results were analysed using a 2 (delay) x 2 (context) x 4 (lesion) repeated measures ANOVA. Post hoc analysis of the main effect of lesion D2 ($F = 8.256$, $df = 3,42$, $p < 0.001$) showed that all lesion groups were impaired compared with the sham

animals, with the postrhinal animals being the most impaired and the perirhinal animals the least impaired. (Difference between sham and perirhinal animals, $p < 0.05$; difference between sham and postrhinal animals $p < 0.001$; difference between sham and fornix $p < 0.01$). There was no main effect of delay ($F = 1.989$, $df = 1,42$ $p > 0.05$) and no interaction between delay and lesion ($F = 2.619$, $df = 3,42$, $p > 0.05$). There was no effect of context ($F < 1$, $df = 1,42$, $p > 0.05$) and no interaction between context and either delay ($F < 1$, $df = 1,42$, $p > 0.05$) or lesion ($F = 1.545$, $df = 3,42$, $p > 0.05$). Nor was there a three way interaction between delay, context and lesion ($F < 1$, $df = 3,42$, $p > 0.05$). The fact that there was no effect of context and no interaction between context and lesion is surprising. As figure 4.12 shows, at the 2 minute delay the perirhinal and fornix groups appeared to be only impaired relative to the shams in context 1. It must be assumed that the relatively large variance observed prevented this effect from reaching significance.

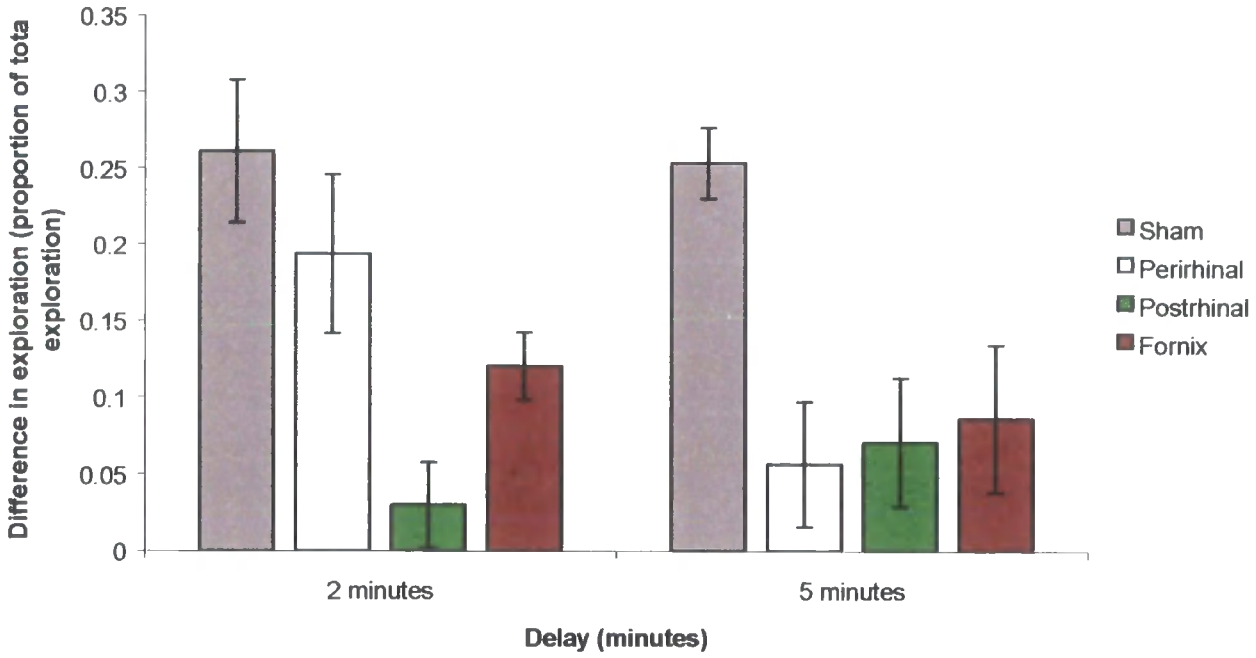
Analysis of absolute performance provides some support for the view that performance differs between the two contexts. One-sample T-tests showed that at the 2 minute delay in context 1 neither the postrhinal nor the fornix groups differed from chance, while in context 2 only the postrhinal group did not differ from chance (table 4.3). When the contexts were combined, again only the postrhinal group did not differ from chance (table 4.3). At the 5 minute delay the postrhinal and fornix groups did not differ from chance in context 1, while in context 2 none of the lesion groups differed from chance. When the contexts were combined none of the lesion groups differed from chance (table 4.2). This suggests that the task is a hard one at 5 minutes, as all lesion groups fail, but that at 2 minutes there is a selective postrhinal impairment (see table 4.3). It also suggests that the fornix animals only fail at 2 minutes when there is a longer delay due to the order of context presentation.

D1

Again, the results were analysed using a 2 (delay) x 2 (context) x 4 (lesion) repeated measures ANOVA. Post hoc analysis of the main effect of lesion ($F = 12.434$, $df = 3,42$, $p < 0.001$) showed that, as can be seen in figure 4.12b, all lesion groups were impaired compared to the sham animals (difference between sham and perirhinal and postrhinal < 0.001; difference between sham and fornix < 0.01). There was also a main effect of delay ($F = 4.317$, $df = 1,42$, $p < 0.05$), although there was no interaction between delay and lesion ($F = 1.525$, $df = 3,42$, $p > 0.05$). There was also no effect of

context ($F = 1.630$, $df = 1,42$, $p > 0.05$) and no interaction between context and either lesion ($F = 2.175$, $df = 3,42$, $p > 0.05$) or delay ($F < 1$, $df = 1,42$, $p > 0.05$). There was also no three-way interaction between delay, context and lesion ($F = 1.437$, $df = 3,42$, $p > 0.05$) The lack of interactions between delay, lesion and context is surprising. From figures 4.12d and 4.12f it is clear that all lesion groups are impaired at both delays compared to sham performance in context 1, whilst in context 2 only the postrhinal animals are impaired at the earlier delay, although all groups are impaired at the 5 minute delay. It seems possible that the failure to find such interactions is due to the relatively large variance in the lesion groups' performance.

Analysis of absolute performance confirmed the relative performance results, showing that while all lesion groups were impaired relative to the shams, the postrhinal animals' performance was particularly poor at the 2 minute delay, although rather surprisingly this was not the case at the 5 minute delay (see table 4.2). One-sample T-tests showed that at 2 minute delay in context 1 neither the postrhinal nor the fornix groups differed from chance, while in context 2 only the postrhinal group did not differ from chance (see table 4.3). This chance performance by the postrhinal animals was also found when the contexts were combined. At the 5 minute delay a different pattern was found. Although, as at 2 minutes, the postrhinal and fornix groups did no differ from chance in context 1, in context 2 it was the perirhinal and fornix groups which did not differ from chance. This perirhinal impairment was also found when the contexts were combined: the perirhinal group did not differ from chance (see table 4.2).



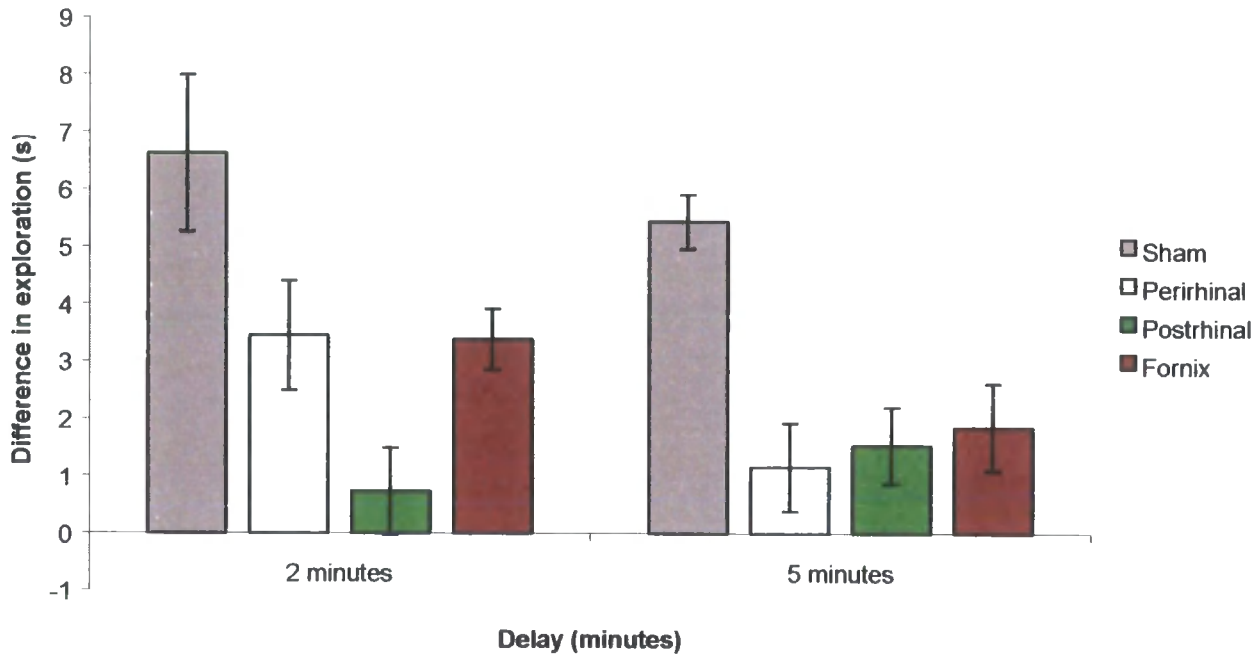


Figure 4.12: Discrimination of A in a congruent context from B in an incongruent context over both contexts. (a) D2 measure (b) D1 measure

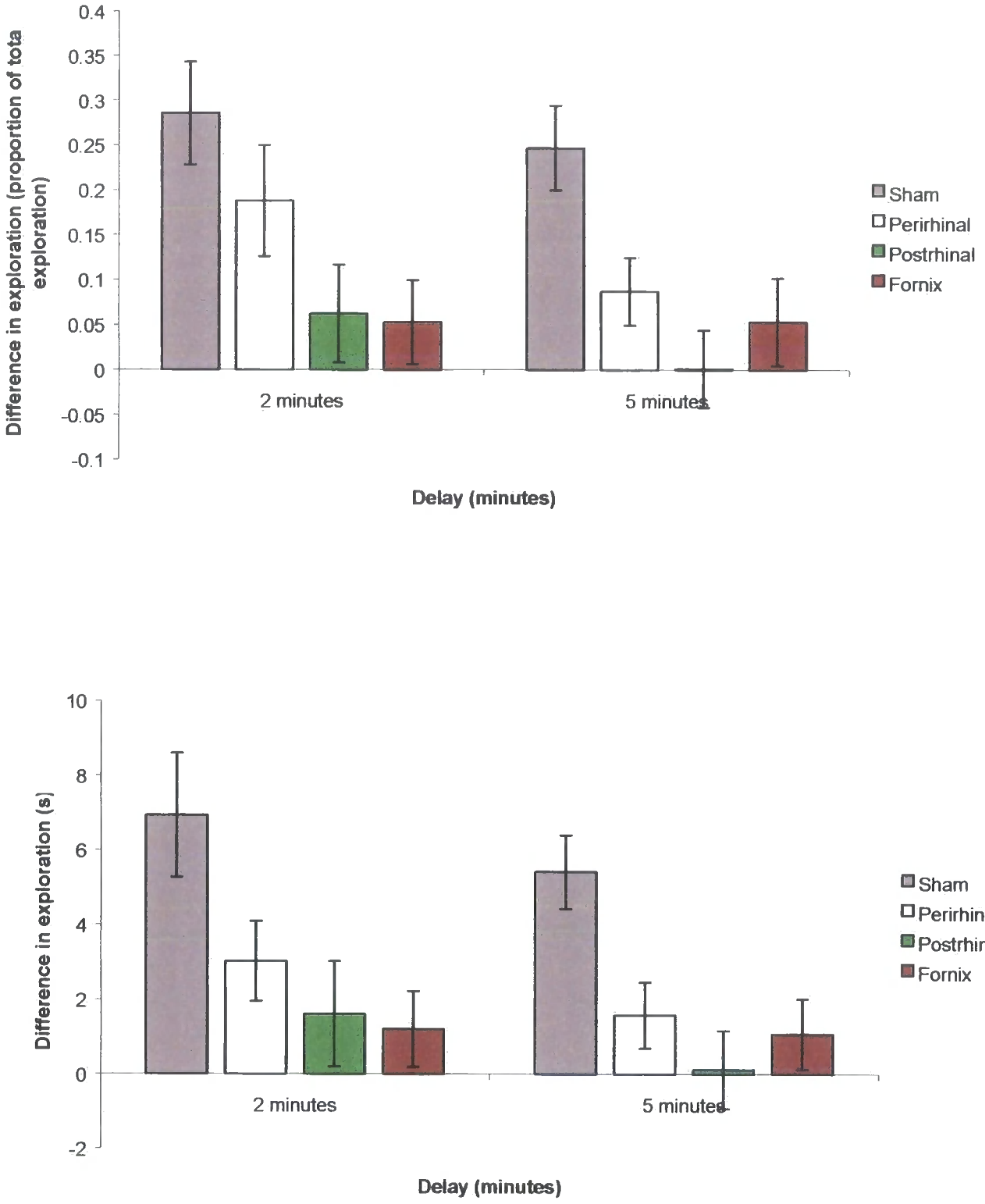


Figure 4.12: Discrimination of A in a congruent context from B in an incongruent context in context 1. (c) D2 measure. (d) D1 measure.

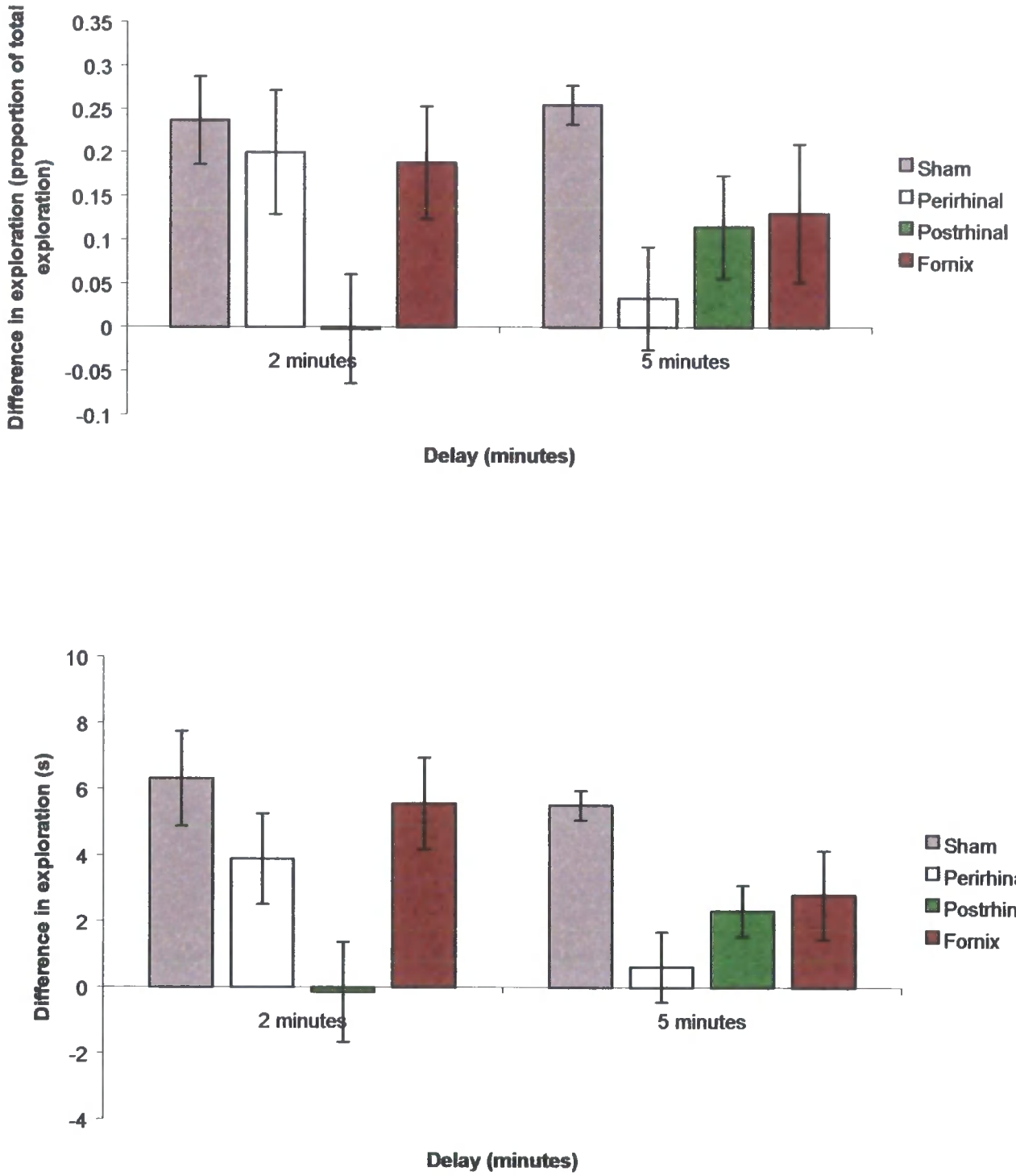


Figure 4.12: Discrimination of A in a congruent context from B in an incongruent context in context 2. (e) D2 measure. (f) D1 measure

D2						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	5.831	<0.001	Yes	11.474	<0.001	Yes
Perirhinal	3.906	0.002	Yes	1.637	0.130	**No**
Postrhinal	1.130	0.282	**No**	1.824	0.095	**No**
Fornix	5.740	<0.001	Yes	1.895	0.087	**No**
D1						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	5.093	<0.001	Yes	12.124	<0.001	Yes
Perirhinal	3.776	0.003	Yes	1.617	0.134	**No**
Postrhinal	1.006	0.336	**No**	2.205	**0.050**	**No**
Fornix	6.691	<0.001	Yes	2.548	0.029	Yes

Table 4.2: One-sample T-tests on each group's performance on an A/B discrimination with context incongruent.

D2						
Testing in Context 1						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	5.231	< 0.001	Yes	6.173	<0.001	Yes
Perirhinal	3.176	0.009	Yes	2.836	0.016	Yes
Postrhinal	1.200	0.256	**No**	0.641	**0.534**	**No**
Fornix	1.186	0.263	**No**	0.983	0.349	**No**
Testing in Context 2						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	4.917	<0.001	Yes	12.07	<0.001	Yes
Perirhinal	2.938	0.014	Yes	0.586	**0.570**	**No**
Postrhinal	0.038	0.970	**No**	2.041	0.066	**No**
Fornix	3.060	0.012	Yes	1.735	0.114	**No**
D1						
Testing in Context 1						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	4.373	0.001	Yes	6.324	<0.001	Yes
Perirhinal	2.948	0.013	Yes	2.227	0.048	Yes
Postrhinal	1.187	0.260	**No**	0.517	0.615	**No**
Fornix	1.235	0.245	**No**	1.081	0.305	**No**
Testing in Context 2						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	4.620	<0.001	Yes	13.030	<0.001	Yes
Perirhinal	2.942	0.013	Yes	0.602	0.560	**No**
Postrhinal	0.097	0.924	**No**	3.105	0.010	Yes
Fornix	4.204	0.008	Yes	2.195	**0.0529**	**No**

Table 4.3: One-sample T-tests on each group's performance in each context on an A/B discrimination with context incongruent.

Exploration

There was a trend towards an effect of lesion ($F = 2.799$, $df = 3,42$, $p = 0.052$) which would appear to be due to the perirhinal animals exploring the objects less than the shams. There was also an effect of delay ($F = 10.130$, $df = 1,42$, $p < 0.01$), but no interaction between delay and lesion ($F < 1$, $df = 3,42$, $p > 0.05$) with all groups exploring less at the 5 minute delay than at the 2 minute delay. There was no effect of

context ($F < 1$, $df = 1,42$, $p > 0.05$) but there was an interaction between delay and context ($F = 8.051$, $df = 1,42$, $p < 0.01$) with the decline in exploration at the 5 minute delay more pronounced in context 2 than in context 1. There was no interaction between context and lesion ($F < 1$, $df = 3,42$, $p > 0.05$) and no three-way interaction between delay, context and lesion ($F < 1$, $df = 3,42$, $p > 0.05$).

Summary

This discrimination was clearly a difficult one which all lesion groups were impaired compared with shams. At 5 minutes all lesion groups performed at chance on some measures. At 2 minutes the picture is more interesting, and it is clear that postrhinal animals are severely impaired and do not differ from chance on any measure, whilst fornix animals fail to discriminate only on some measures and perirhinal animals discriminate between the objects on all measures.

Experiment 4.2: AB in congruent context (control)

This experiment was designed to control for the effect of the order in which objects were experienced in experiment one, and the possibility that animals would experience the object seen most recently as significantly more familiar than the object seen previously. As can be seen from figure 4.13, the results of this experiment confirm that this is not the case, as animals did not discriminate between the object presented first and the object presented second when all presentations were in the same context. A 2 (delay) x 4 (lesion) repeated measures ANOVA was carried out. On neither the D2 ($F < 1$, $df = 3,42$, $p > 0.05$) nor the D1 ($F < 1$, $df = 3,42$, $p > 0.05$) measures were there any differences between the groups. There were also no effects of delay in D2 ($F < 1$, $df = 1,42$, $p > 0.05$) or D1 ($F < 1$, $df = 1,42$, $p > 0.05$). There was no interaction between lesion and delay on either the D2 or the D1 measure (D2: $F < 1$, $df = 3,42$, $p > 0.05$; D1: $F < 1$, $df = 3,42$, $p > 0.05$).

More significantly, when absolute performance was examined using one sample T-tests, on both measures none of the groups differed from chance at either the 2 minute or the 5 minute delays (see table 4.4). This indicates that none of the groups is discriminating between the objects on the only possible basis for discrimination – delay between presentation and test.

2 minutes						
Measure	D2			D1		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	0.122	0.906	No	0.090	0.930	No
Perirhinal	0.232	0.821	No	0.565	0.583	No
Postrhinal	0.913	0.381	No	0.947	0.364	No
Fornix	0.159	0.878	No	0.102	0.921	No
5 minutes						
Measure	D2			D1		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	1.055	0.316	No	1.006	0.338	No
Perirhinal	0.193	0.851	No	0.110	0.914	No
Postrhinal	0.730	0.481	No	1.097	0.296	No
Fornix	1.434	0.182	No	2.024	0.070	No

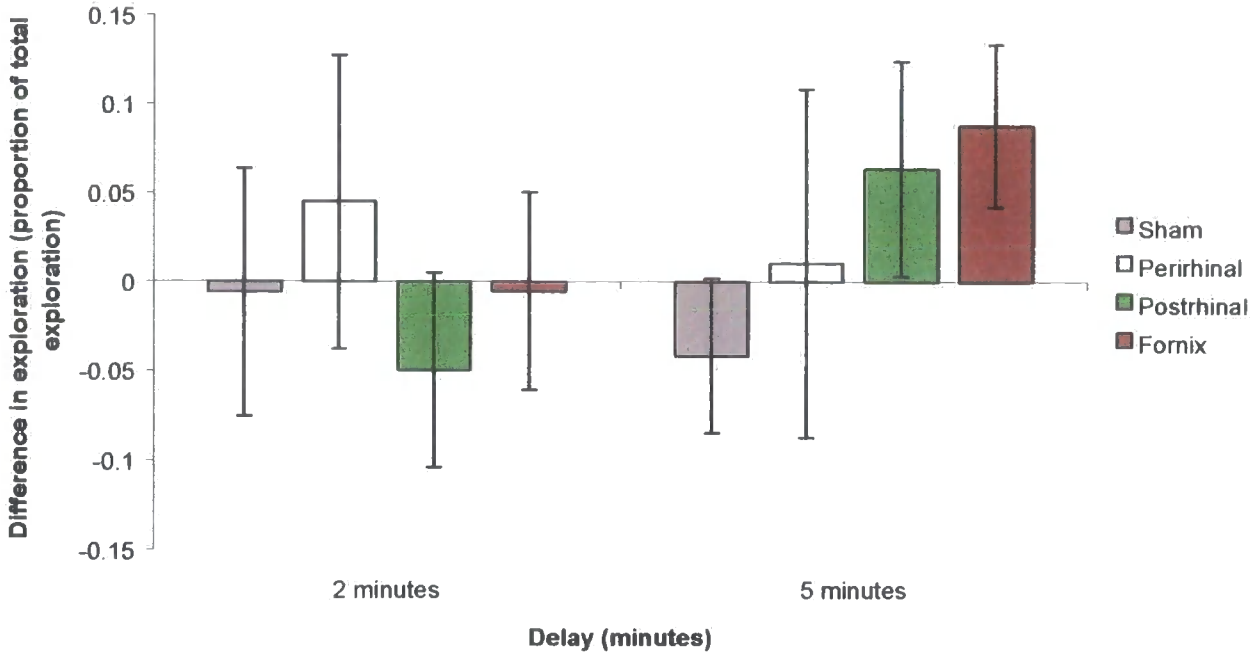
Table 4.4: One-sample T-test for each group at both delays on control A/B condition.

Exploration

There was a main effect of lesion ($F = 4.512$, $df = 3,42$, $p < 0.01$) which post-hoc analysis revealed to be due to the sham animals showing greater exploration than the perirhinal animals ($p < 0.05$). There was also a main effect of delay ($F = 18.409$, $df = 1,42$, $p < 0.001$) and an interaction between delay and lesion ($F = 6.029$, $df = 3,42$, $p < 0.01$). This interaction is due to the sham and fornix groups exploring more at the 5 minute delay than at the 2 minute delay, whilst the perirhinal and postrhinal groups do not differ in their exploration across the delays.

Summary

There was no effect of lesion with all groups failing to discriminate between the objects at all delays, indicating that there was no effect of recency in object preference.



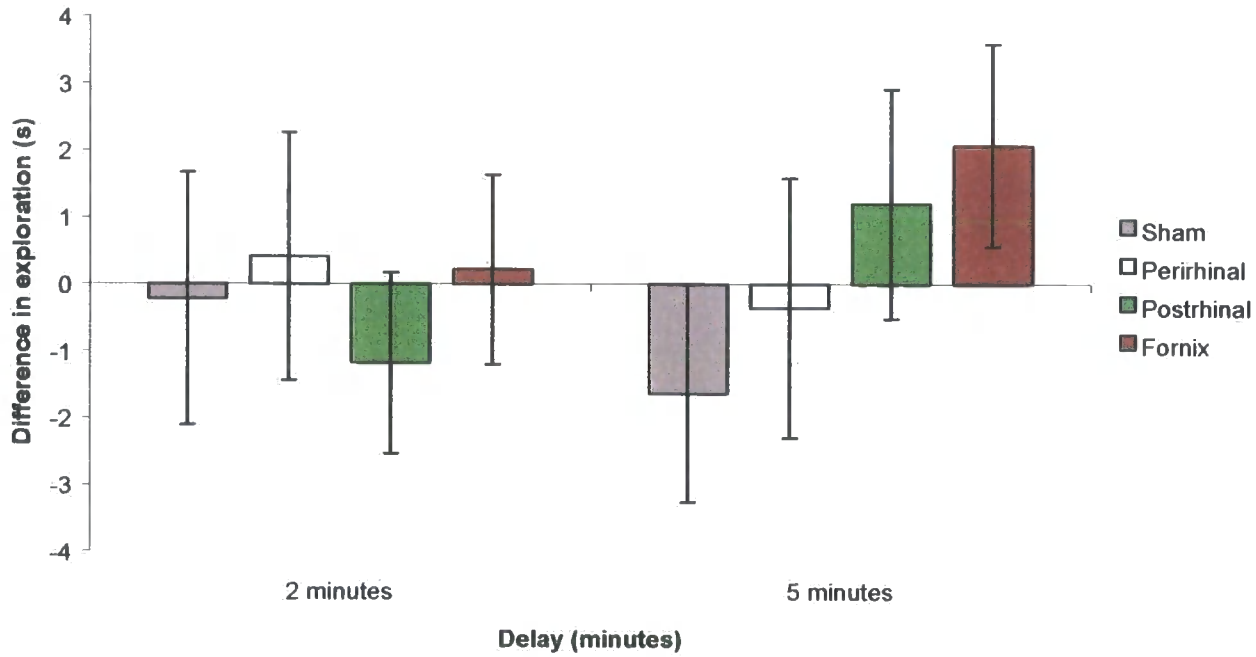


Figure 4.13: Discrimination of A in a congruent context from B in a congruent context. (a) D2 measure. (b) D1 measure

Experiment 4.3 (AN in incongruent context)

A 2 (delay) x 2 (context) x 4 (lesion) repeated measures ANOVA was carried out. All lesion groups performed more poorly than the shams on this task on both a D2 ($F = 15.261$, $df = 3,42$, $p < 0.001$) and a D1 measure of performance ($F = 19.832$, $df = 3,42$ $p < 0.001$). However, as can be seen from figure 4.14a and 4.14b the fornix animals were considerably less impaired than the perirhinal and postrhinal animals on both D1 and D2 measures. Because the results from the two measures differ slightly they will be discussed separately.

D2

Post-hoc analysis of the effect of lesion ($F = 15.261$, $df = 3,42$, $p < 0.001$) following the repeated measures ANOVA confirmed that all other groups were impaired compared with the sham animals, and that the perirhinal and postrhinal groups were more impaired than the fornix group (differences between sham and both perirhinal and postrhinal, $p < 0.001$; between sham and fornix $p < 0.05$). Confirming this differential impairment, perirhinal animals were significantly more impaired than fornix animals ($p < 0.05$). There was no effect of delay ($F < 1$, $df = 1,42$, $p > 0.05$) and no interaction between delay and lesion ($F < 1$, $df = 3,42$, $p > 0.05$). There was no effect of context ($F < 1$, $df = 1,42$, $p > 0.05$) and no interaction either between context and lesion ($F = 1.111$, $df = 3,42$, $p > 0.05$) or between context and delay ($F = 1.075$, $df = 1,42$, $p > 0.05$). There was also no three-way interaction between delay, context and lesion ($F < 1$, $df = 3,42$, $p > 0.05$). Again this failure to find an effect of context is surprising. As can be seen from figures 4.14c and 4.e the main effect of lesion appears to be the result of group differences during testing in context 1, with much less impairment of the lesion groups being observed when testing was in the second context. It seems probable that the failure to find an effect of context, or interactions between context and lesion or delay was due to the large variance observed in some of the groups.

The absolute performance of the animals in the lesion groups is less clear. When actual ability to discriminate is examined by testing performance against chance (zero) using one-sample T-tests the picture is somewhat confused at the 2 minute delay (see table 4.6). At the 2 minute delay the postrhinal animals did not differ from chance during testing in context 1. When animals were tested in the second context the perirhinal and fornix animals did not differ from chance at a delay of 2 minutes. It

seems probable that these results, which at first glance do not seem to be supported by graphical representation of the data (figures 4.14c and 4.14e) reflect the large standard deviations shown by these groups, which may have also contributed to the failure to find effects of context in the analysis of relative performance. This view is supported by the fact that when the data are analysed with both contexts combined all groups differed from chance, with variance being reduced, at least partially by the necessary doubling of the number of repetitions of the test to form this variable (see table 4.5). At a 5 minute delay all groups differed from chance in both contexts and when contexts were combined (see tables 4.5 and 4.6).

D1

Analysis of the D1 measure is largely in agreement with that of D2. As with the D2 measure, post hoc analysis of the main effect of lesion ($F = 19.832$, df 3,42 $p < 0.001$) confirmed that all other groups were impaired compared with the sham animals. However, the perirhinal and postrhinal animals were more impaired than the fornix animals. (Difference between sham animals and both perirhinal and postrhinal animals, $p < 0.001$; difference between sham and fornix animals, $p < 0.01$). The lesser impairment of the fornix animals is confirmed by the fact that they, as well as the shams, also differed from perirhinal and postrhinal animals (difference between fornix animals and perirhinal animals, $p < 0.01$; difference between fornix animals and postrhinal animals, $p < 0.05$). There was no main effect of delay ($F < 1$, df = 1,42, $p > 0.05$) and no interaction between delay and lesion ($F < 1$, df = 3,42, $p > 0.05$). There was also no effect of context ($F < 1$, df = 1,42, $p > 0.05$) and no interactions between context and either lesion ($F = 2.145$, df = 3,42, $p > 0.05$) or delay ($F = 1.046$, df = 1,42, $p > 0.05$). There was also no three-way interaction between lesion, delay and context ($F < 1$, df = 3,42, $p > 0.05$).

When absolute performance is examined the same pattern as with the D2 measure is found (see tables 4.5 and 4.6). One-sample T-tests showed that at a delay of 2 minutes the postrhinal animals did not differ from chance in context 1. In context 2 the perirhinal and fornix animals did not differ from chance. Again, when both contexts were combined all groups differed from chance. In contrast to the D2 measure, at a 5 minute delay in context 1 the postrhinal animals did not differ from

chance. In context 2, and when the contexts were combined the results accorded with those of the D2 measure and all groups differed from chance.

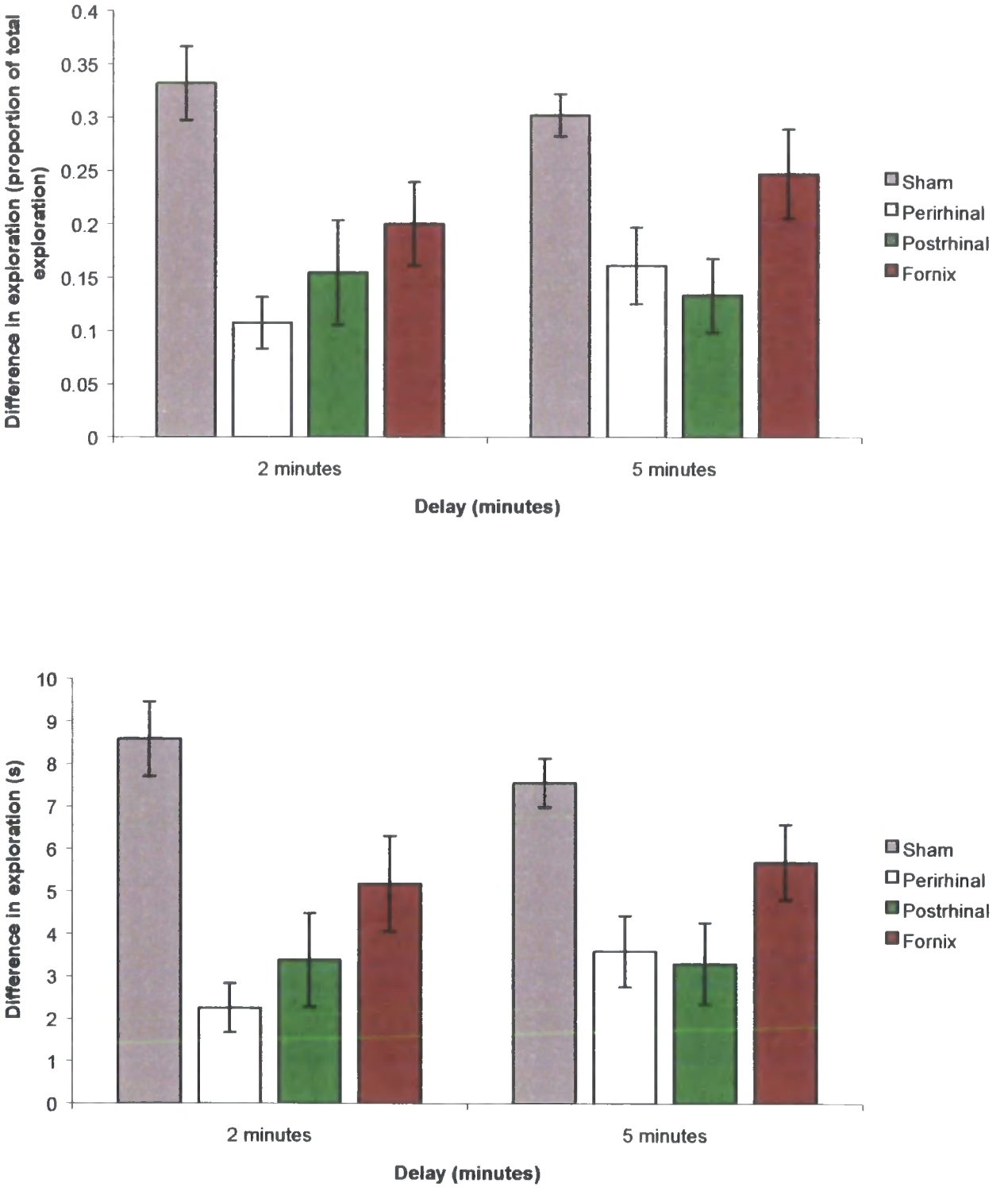


Figure 4.14: Discrimination of A in an incongruent context from N over both contexts. (a) D2 measure. (b) D1 measure

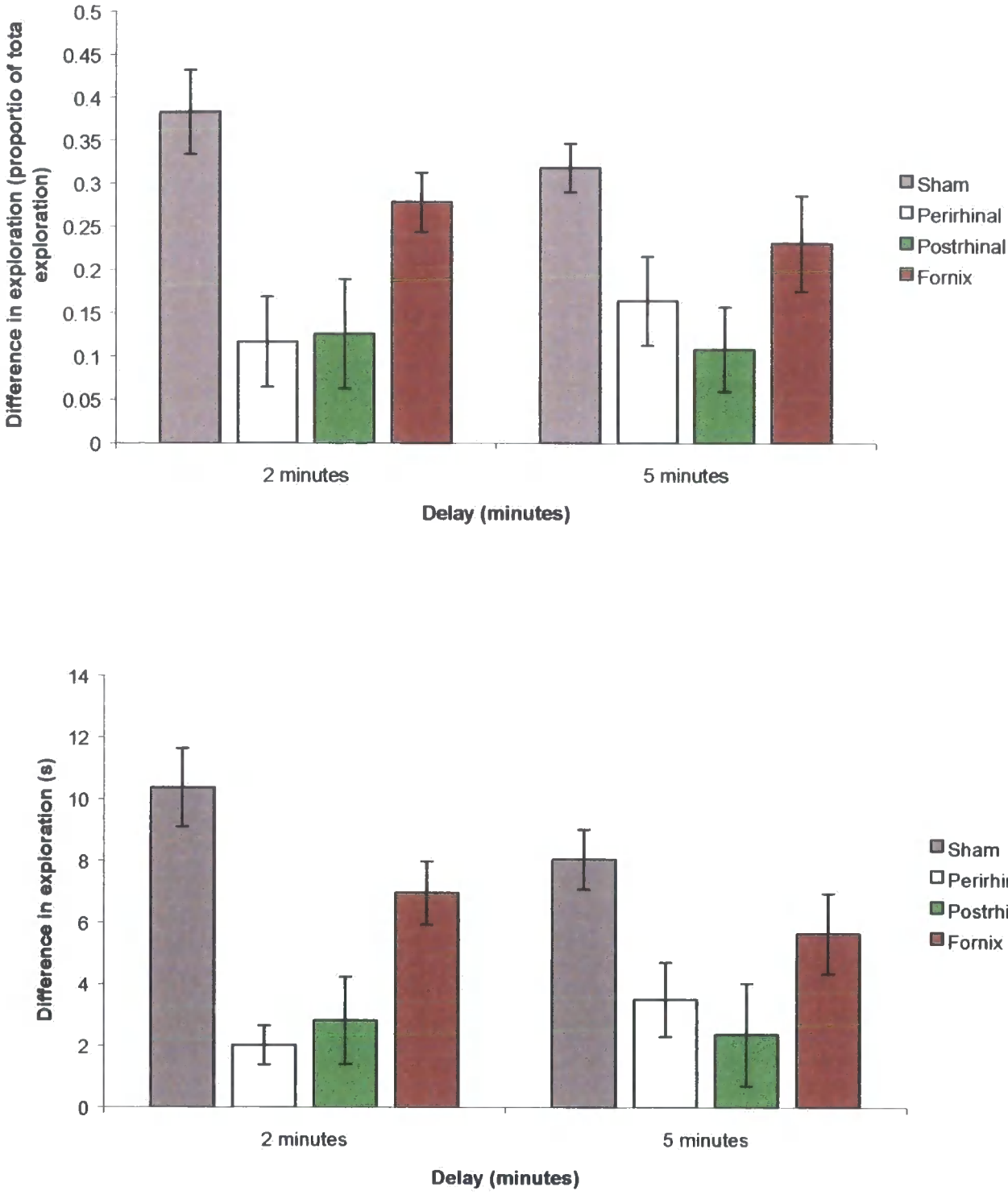
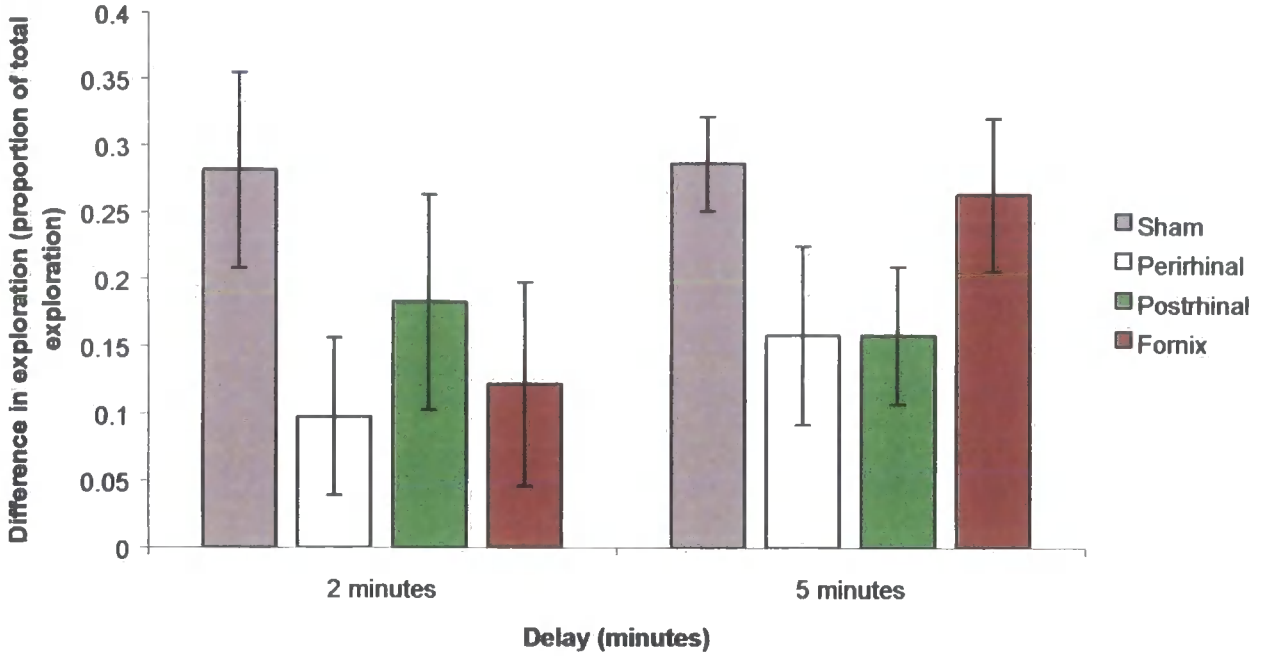


Figure 4.14: Discrimination of A in an incongruent context from N when testing was carried out in context 1. (c) D2 measure. (b) D1 measure.



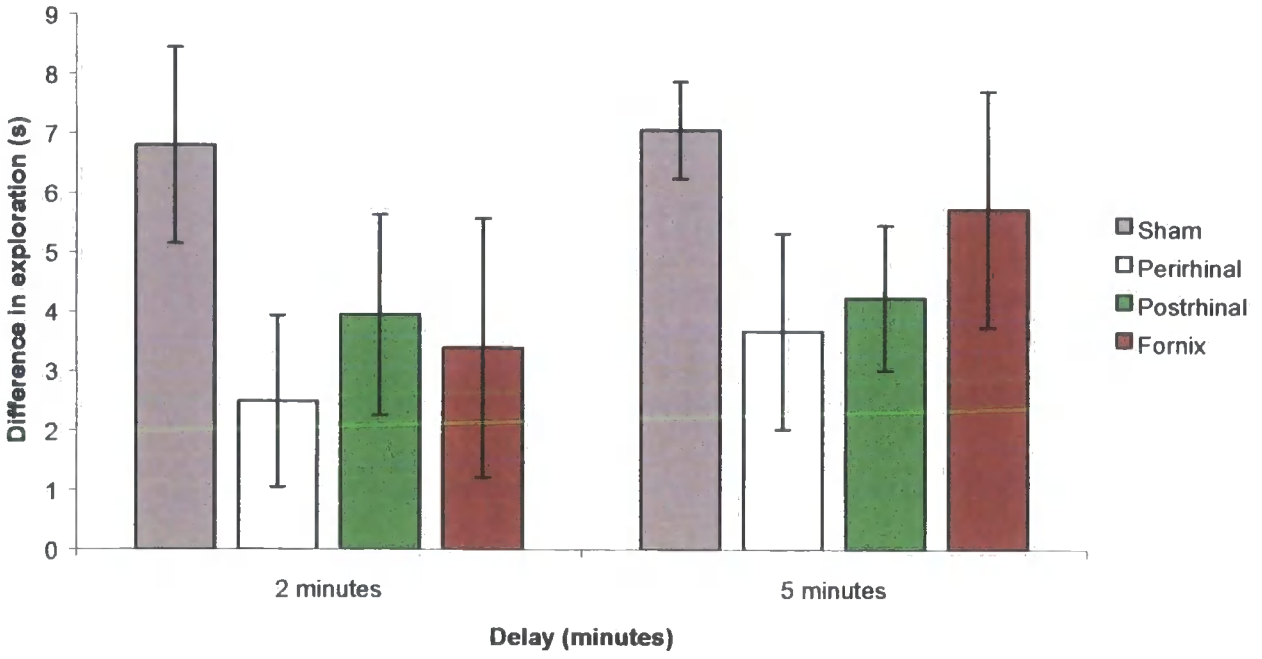


Figure 4.14: Discrimination of A in an incongruent context from N when testing was carried out in context 2. (e) D2 measure. (f) D1 measure

D2						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ	T	P	Differ
Sham	10.05	<0.001	Yes	15.930	<0.001	Yes
Perirhinal	4.628	<0.001	Yes	4.679	<0.001	Yes
Postrhinal	3.284	0.007	Yes	4.041	0.002	Yes
Fornix	5.399	<0.001	Yes	6.209	<0.001	Yes
D1						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ	T	P	Differ
Sham	10.280	<0.001	Yes	12.520	<0.001	Yes
Perirhinal	4.077	0.002	Yes	4.467	0.001	Yes
Postrhinal	3.209	0.008	Yes	3.569	0.004	Yes
Fornix	4.839	<0.001	Yes	6.727	<0.001	Yes

Table 4.5: One-sample T-tests for each of the groups' performance in both contexts at both delays on a discrimination between N and A in the incongruent context.

D2						
Testing in Context 1						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ	T	P	Differ
Sham	8.226	<0.001	Yes	11.92	<0.001	Yes
Perirhinal	2.357	0.038	Yes	3.331	0.007	Yes
Postrhinal	2.096	0.060	**No**	2.325	0.040	Yes
Fornix	8.515	<0.001	**Yes**	4.390	0.001	Yes
Testing in Context 2						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ	T	P	Differ
Sham	4.045	0.002	Yes	8.522	<0.001	Yes
Perirhinal	1.744	0.109	**No**	2.484	0.030	Yes
Postrhinal	2.386	0.036	Yes	3.237	0.008	Yes
Fornix	1.682	0.124	**No**	4.843	0.001	Yes
D1						
Testing in context 1						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ	T	P	Differ
Sham	8.582	<0.001	Yes	8.676	<0.001	Yes
Perirhinal	3.340	0.007	Yes	3.500	0.011	Yes
Postrhinal	2.067	0.063	**No**	1.486	0.165	**No**
Fornix	7.082	<0.001	**Yes**	4.520	0.001	**Yes**
Testing in Context 2						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ	T	P	Differ
Sham	4.326	0.002	Yes	9.095	<0.001	Yes
Perirhinal	1.803	0.099	**No**	2.331	0.040	Yes
Postrhinal	2.446	0.033	Yes	3.623	0.004	Yes
Fornix	1.632	0.134	**No**	5.257	<0.001	Yes

Table 4.6: One-sample T-tests on each group's performance in each context on an A/N discrimination with context incongruent.

Exploration

There was a main effect of lesion ($F = 3.552$, $df = 3,42$, $p < 0.05$). Post hoc analysis revealed that this was due to the perirhinal animals showing less exploration than the sham animals. There was no main effect of delay ($F = 1.000$, $df = 1,42$, $p > 0.05$), but there was an interaction between delay and lesion ($F = 5.702$, $df = 3,42$, $p < 0.01$) as this difference between the shams and the perirhinal animals only occurred at the 2 minute delay. There was also an interaction between delay and context ($F = 9.339$, $df = 1,42$, $p < 0.01$) although there was no main effect of context ($F = 2.465$, $df = 1,42$, $p > 0.05$), as the groups explored more in context 2 than in context 1 at the 2 minute delay, but not at the 5 minute delay. There was no interaction between context and

lesion ($F < 1$, $df = 3,42$, $p > 0.05$) and no three-way interaction between delay, context and lesion ($F < 1$, $df = 3, 42$, $p > 0.05$).

Summary

All lesion groups were impaired compared to the shams on both measures. The perirhinal and postrhinal groups were also more impaired than the fornix group on both measures. However the lesion groups did not consistently fail to discriminate between the objects and there is no clear pattern in the circumstances in which each group failed to discriminate.

Experiment 4.4: AN in congruent context (control)

A 2 (delay) x 2 (context) x 4 (lesion) repeated measures ANOVA was carried out. Although there were differences between the groups on both the D2 ($F = 5.148$, $df = 3,42$, $p < 0.01$) and D1 ($F = 8.951$, $df = 3,42$, $p < 0.001$) measures there were differences in the pattern of results found for each measure, as can be seen from figures 4.15a and 4.15b. They are therefore discussed separately.

D2

Post hoc analysis of the main effect of lesion ($F = 5.148$, $df = 3,42$, $p < 0.01$) showed that the postrhinal animals were impaired compared to the sham animals ($p < 0.01$) and, to a lesser extent that the fornix animals were also impaired ($p < 0.05$). The perirhinal animals were not impaired ($p > 0.05$). There was a significant interaction between delay and context ($F = 9.618$, $df = 1,42$, $p < 0.01$). As can be seen from figures 4.15c and 4.15e this was primarily due to the differences between the contexts in performance by the perirhinal and fornix animals at the 2 minute delay. At this delay both these groups performed much better in context 2 than in context 1, and this difference was absent at the 5 minute delay. This analysis of the context/delay interaction was not contradicted by simple effects analysis which showed that there was no difference between the groups in either context at the 2 minute delay. This may also reflect the large standard deviation of all the lesion groups. At the 5 minute delay the groups differed in context 1 ($F = 5.3$, $df = 1,83$, $p < 0.05$), but not in context 2 ($F = 0.8$, $df = 1,83$, $p > 0.05$).

When absolute performance was assessed the analysis of deficits in performance by the perirhinal and fornix groups as specific to context 1 was supported (see table 4.8).

At the 2 minute delay the postrhinal and fornix groups did not differ from chance in context 1, whilst in context 2 all groups differed from chance (see table 4.7). When the contexts were combined all groups differed from chance, supporting the view that, at least in the case of the perirhinal animals, large standard deviations were partly responsible for the appearance of poor performance. At the 5 minute delay the postrhinal animals did not differ from chance in context 1, while the perirhinal animals did not differ from chance in context 2 (see table 4.8). When the contexts were combined all the groups differed from chance (table 4.7), again supporting the view that when variance is reduced the groups were performing above chance.

D1

As well as the main effect of lesion ($F = 8.951$, $df = 3,42$, $p < 0.001$), there were also significant two-way interactions between context and lesion ($F = 2.865$, $df = 1,42$, $p < 0.05$) and between delay and context ($F = 12.995$, $df = 1,42$, $p = 0.001$) and a three-way interaction between delay, context and lesion ($F = 3.334$, $df = 3,42$, $p < 0.05$). Post hoc analysis of the main effect of lesion showed that all lesion groups were impaired compared with the sham animals. The postrhinal impairment was greatest, followed by the perirhinal impairment, with the fornix animals being the least impaired. (Difference between sham and perirhinal animals $p < 0.01$; between sham and postrhinal animals $p < 0.001$; between sham and fornix animals $p < 0.05$).

Analysis of the interactions between lesion, context and delay shows that, as can be seen from figures 4.15d and 4.15f, at 2 minute delay, but not at 5 minute delay all operated groups, and in particular the perirhinal and fornix groups, performed much better when tested in context 2 than in context 1. In context 1 there was no effect of delay, whilst there was in context 2. The effect of delay in context 2 was to reduce the performance of the perirhinal and the fornix groups, although the postrhinal impairment was unchanged. This analysis is confirmed by simple effects which showed that there was no difference between the groups in either context at the 2 minute delay. Again, in context 1 this would seem to be due to the high level of variance in all groups. At the 5 minute delay the groups differed in context 1 ($F = 6.7$, $df = 1,83$, $p < 0.05$) but not in context 2 ($F = df = 1,83$, $p > 0.05$).

Analysis of the absolute performance of the groups supports this account of the difference between the performance in the different contexts (see table 4.8). At the 2

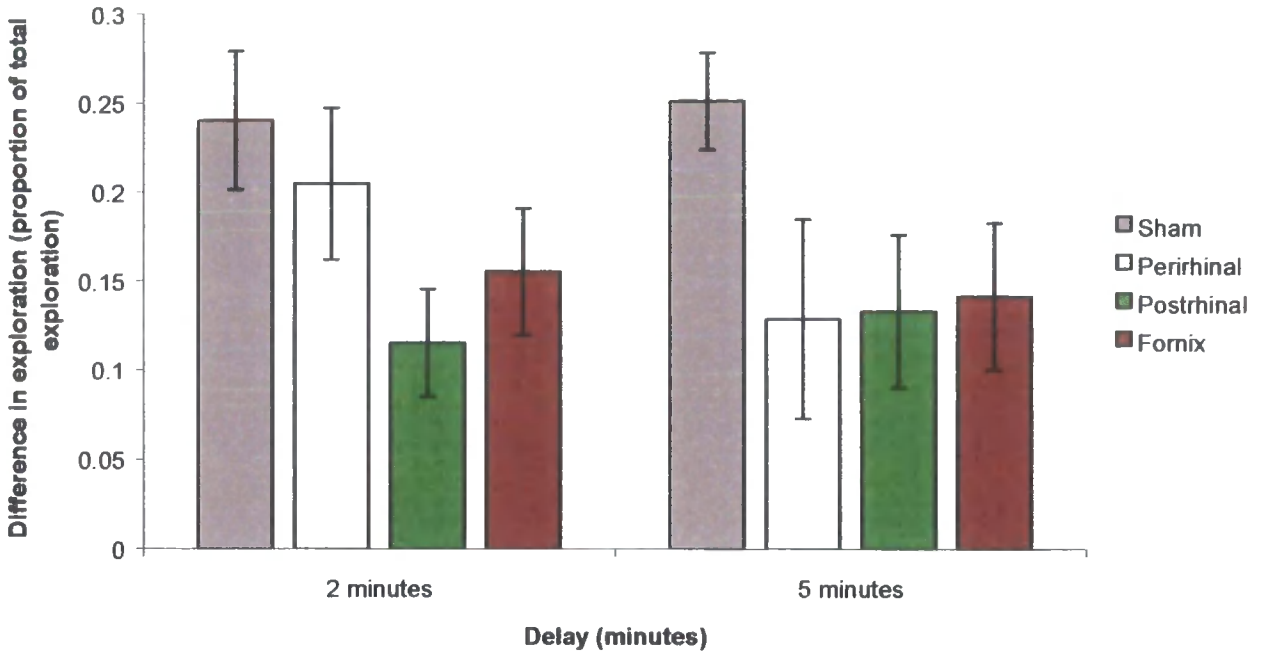
minute delay none of the lesion groups differed from chance in context 1, whilst in context 2 all groups differed from chance. When the contexts were combined all groups differed from chance. Performance at the 5 minute delay is less clear cut. In context 1 the postrhinal animals did not differ from chance, while in context 2 the perirhinal animals did not differ from chance. Again when the contexts were combined all the groups differed from chance.

D2						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	6.470	<0.001	Yes	12.394	<0.001	Yes
Perirhinal	5.014	<0.001	Yes	3.541	0.005	Yes
Postrhinal	3.980	0.002	Yes	4.149	0.002	Yes
Fornix	4.596	0.001	Yes	4.152	0.002	Yes
D1						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	6.916	<0.001	Yes	9.335	<0.001	Yes
Perirhinal	4.779	0.001	Yes	3.328	0.007	Yes
Postrhinal	3.721	0.003	Yes	3.699	0.004	Yes
Fornix	5.739	<0.001	Yes	4.597	0.001	Yes

Table 4.7: One-sample T-tests for each of the groups at both delays on discrimination between N and A in the congruent context.

D2						
Testing in Context 1						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	4.992	0.001	Yes	10.883	<0.001	Yes
Perirhinal	2.435	0.033	Yes	3.179	0.009	Yes
Postrhinal	1.969	0.075	**No**	1.857	0.090	**No**
Fornix	0.871	0.404	No	2.579	0.027	Yes
Testing in Context 2						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	8.049	<0.001	Yes	6.765	<0.001	Yes
Perirhinal	4.551	0.001	Yes	2.020	0.068	No
Postrhinal	4.385	0.001	Yes	3.938	0.002	Yes
Fornix	6.414	0.001	Yes	4.685	0.001	Yes
D1						
Testing in Context 1						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	5.772	<0.001	Yes	7.009	<0.001	Yes
Perirhinal	2.041	0.066	**No**	2.941	0.013	Yes
Postrhinal	1.536	0.153	**No**	1.094	0.297	**No**
Fornix	1.286	0.227	No	2.650	0.024	Yes
Testing in Context 2						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	7.908	<0.001	Yes	5.631	<0.001	Yes
Perirhinal	4.632	0.001	Yes	2.038	0.066	**No**
Postrhinal	4.673	0.001	Yes	5.154	<0.001	Yes
Fornix	7.172	<0.001	Yes	5.314	<0.001	Yes

Table 4.8: One-sample T-tests for each of the groups in each of the contexts at both delays for the A/N in congruent context discrimination.



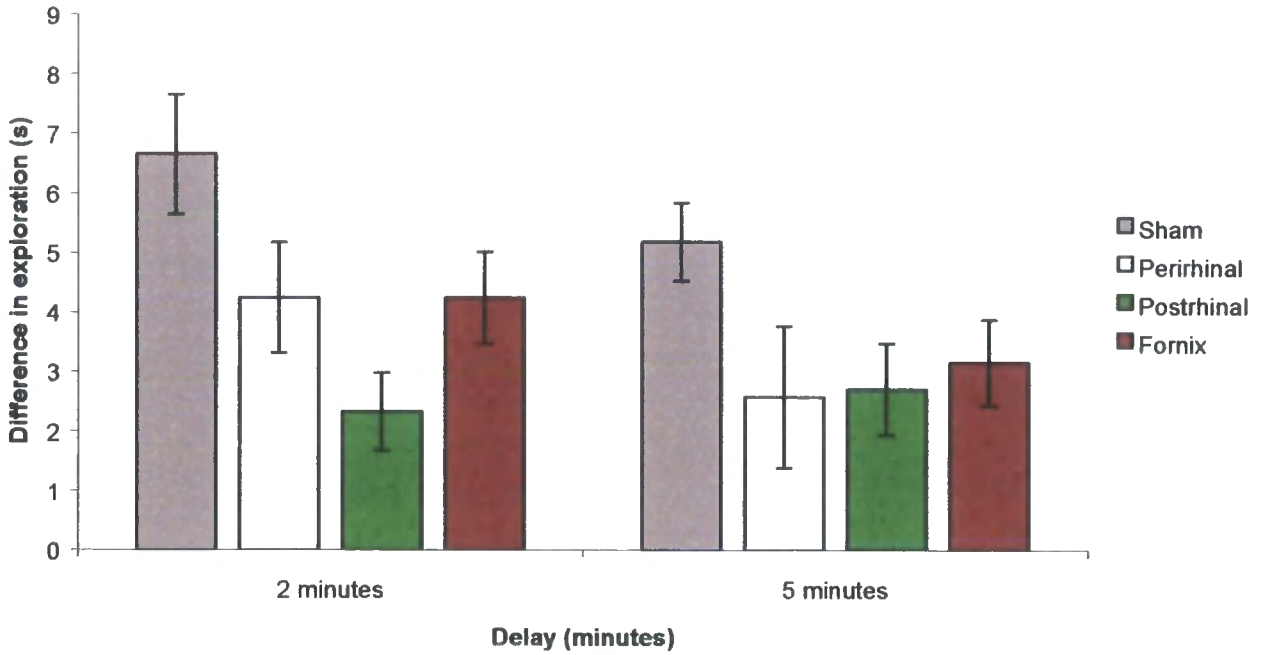
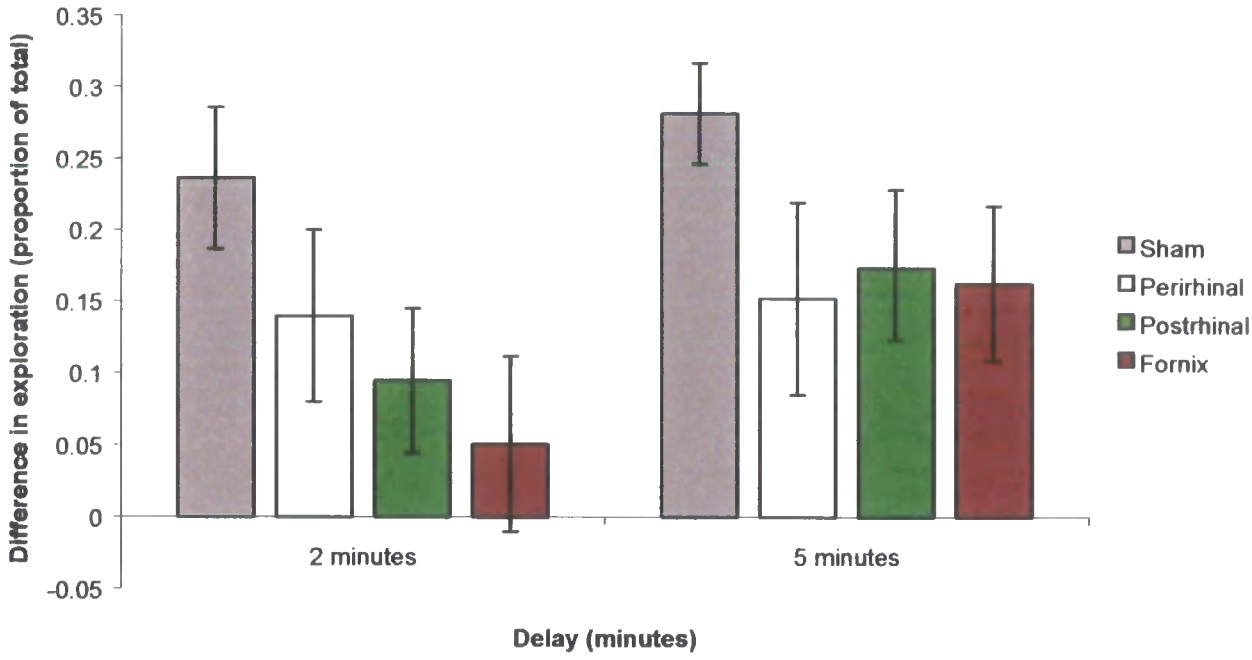


Figure 4.15: Discrimination of A in a congruent context from N over both contexts. (a) D2 measure. (b) D1 measure



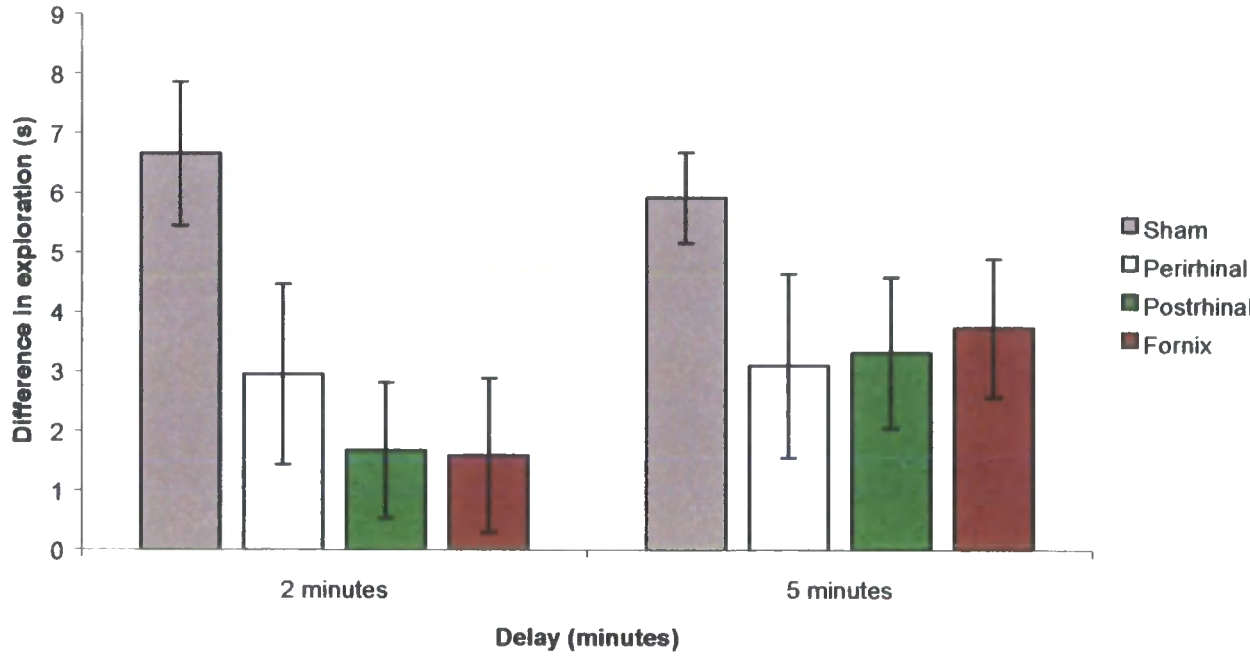
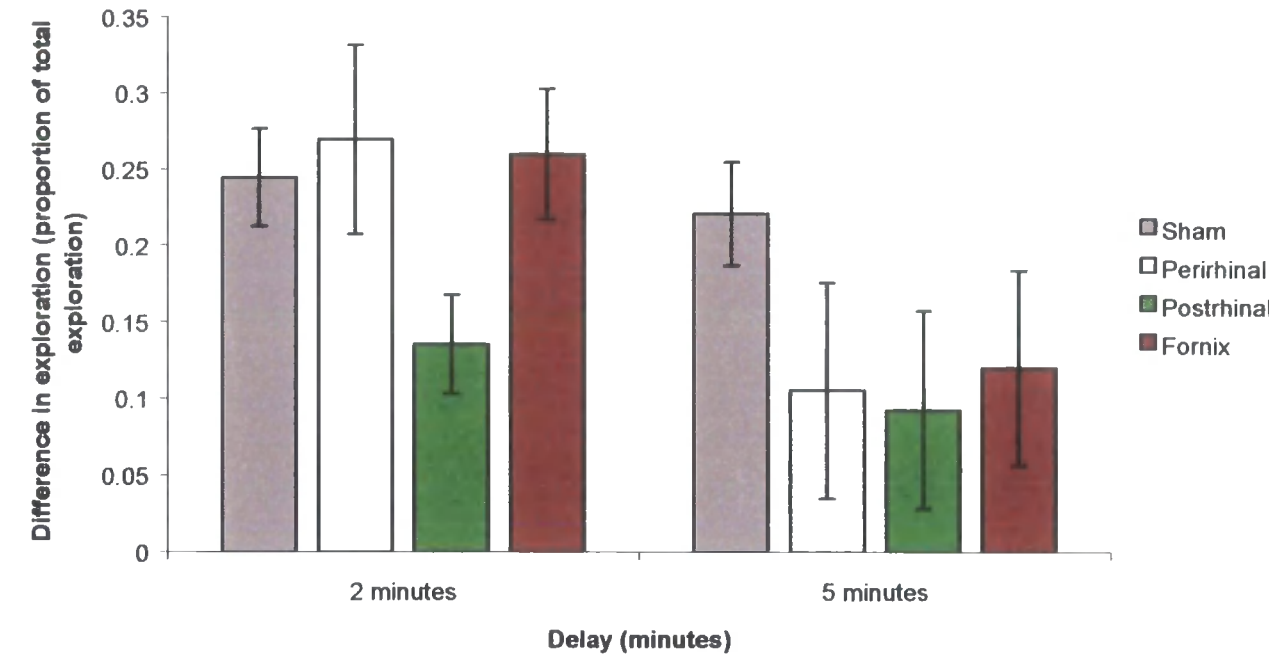


Figure 4.15: Discrimination of A in a congruent context from N in context 1. (c) D2 measure. (d) D1 measure.



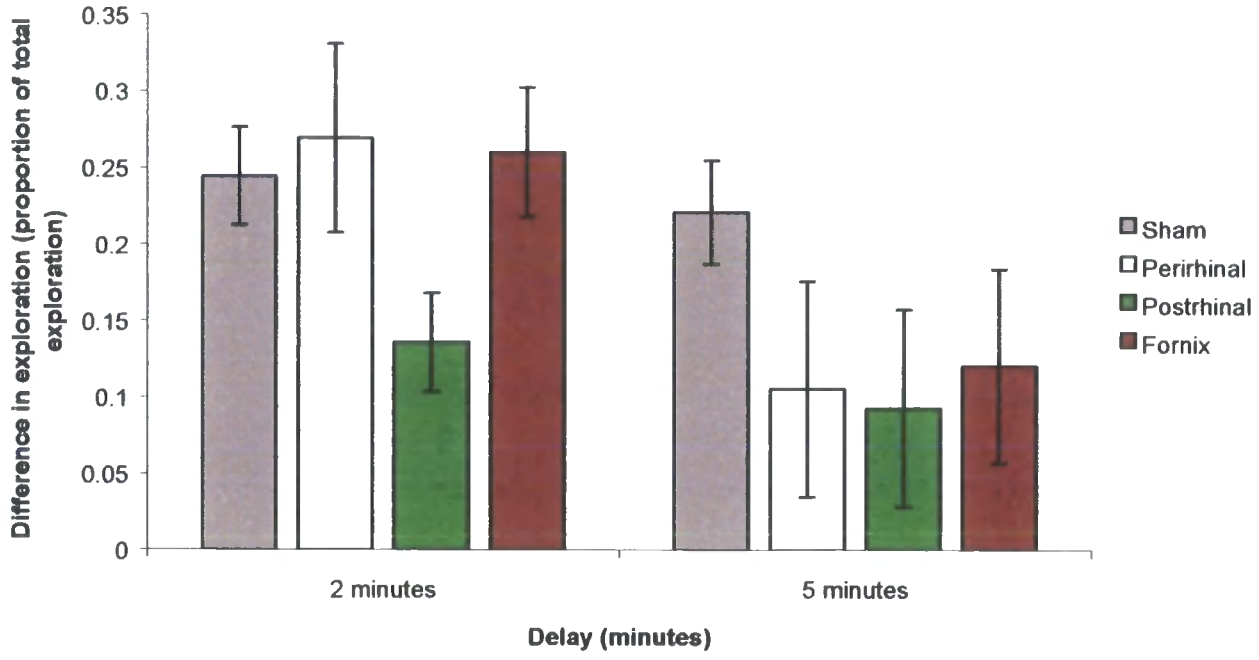


Figure 4.15: Discrimination of A in a congruent context from N in context 2. (e) D2 measure. (f) D1 measure

Exploration

There was a main effect of lesion ($F = 2.888$, $df = 3,42$, $p < 0.05$), but post hoc analysis showed only a trend for the shams to explore more than the postrhinal animals ($p = 0.057$). There was a main effect of delay ($F = 29.257$, $df = 1,42$, $p < 0.001$) with all groups exploring less at the 5 minute delay than at the 2 minute delay, but no interaction between delay and lesion ($F = 1.957$, $df = 3,42$, $p > 0.05$). Although there was no effect of context ($F < 1$, $df = 1,42$, $p > 0.05$), there was an interaction between context and delay ($F = 13.745$, $df = 1,42$, $p = 0.001$) with all groups exploring more in context 2 than context 1 at the 2 minute delay, but displaying the opposite pattern of behaviour at the 5 minute delay. There was no interaction between context and lesion ($F < 1$, $df = 3,42$, $p > 0.05$), and no three-way interaction between context, delay and lesion ($F < 1$, $df = 3,42$, $p > 0.05$).

Summary

The postrhinal animals were the most consistently and the most severely impaired. The fornix animals showed a lesser impairment, while the perirhinal animals were only impaired on the D1 measure, which is the less reliable of the 2 measures, particularly since the groups differed in total exploration time. The impairment was more apparent in context 1 than in context 2. The results indicate that there is an effect of recency on the discrimination of a novel and familiar object when there are two exploratory phases. However, they also indicate that all groups were able to discriminate when the contexts were combined.

Experiment 4.5: Object as context (Aa Bb Ab Aa)

There was a main effect of lesion on both the D2 ($F = 13.488$, $df = 3,42$, $p < 0.001$) and the D1 measure ($F = 13.036$, $df = 3,42$, $p < 0.001$). There was no effect of delay on either measure (D2: $F < 1$, $df = 1,42$, $p > 0.05$; D1: $F < 1$, $df = 1,42$, $p > 0.05$) and no interaction between lesion and delay (D2: $F < 1$, $df = 3,42$, $p > 0.05$; D1: $F < 1$, $df = 3,42$, $p > 0.05$). Post hoc analysis of the main effects showed that, as can be seen from figure 4.16a, on the D2 measure perirhinal animals were impaired compared with the sham animals ($p < 0.001$). They were also impaired compared with the postrhinal and fornix animals ($p < 0.01$ in both cases). There were no other differences between the groups. On the D1 measure post hoc analysis also showed that, as can be seen from figure 4.16b, the perirhinal animals were impaired compared

with the sham animals ($p < 0.001$). They were also impaired compared with the postrhinal ($p < 0.01$) and with the fornix animals ($p < 0.001$), with no other differences between the groups.

The perirhinal impairment at both delays was confirmed by analysis of absolute performance, which showed that that the perirhinal animals did not differ from chance at either the 2 minutes or the 5 minute delay on either the D1 or the D2 measure (table 4.9).

2 minutes						
Measure	**D2**			**D1**		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	4.847	<0.001	Yes	3.420	0.007	Yes
Perirhinal	1.036	0.322	**No**	1.175	0.264	**No**
Postrhinal	2.674	0.022	Yes	2.543	0.027	Yes
Fornix	3.765	0.004	Yes	3.961	0.003	Yes
5 minutes						
Measure	**D2**			**D1**		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	5.013	<0.001	Yes	6.283	<0.001	Yes
Perirhinal	1.704	0.117	**No**	1.613	0.135	**No**
Postrhinal	2.342	0.039	Yes	2.849	0.016	Yes
Fornix	3.328	0.008	Yes	3.075	0.012	Yes

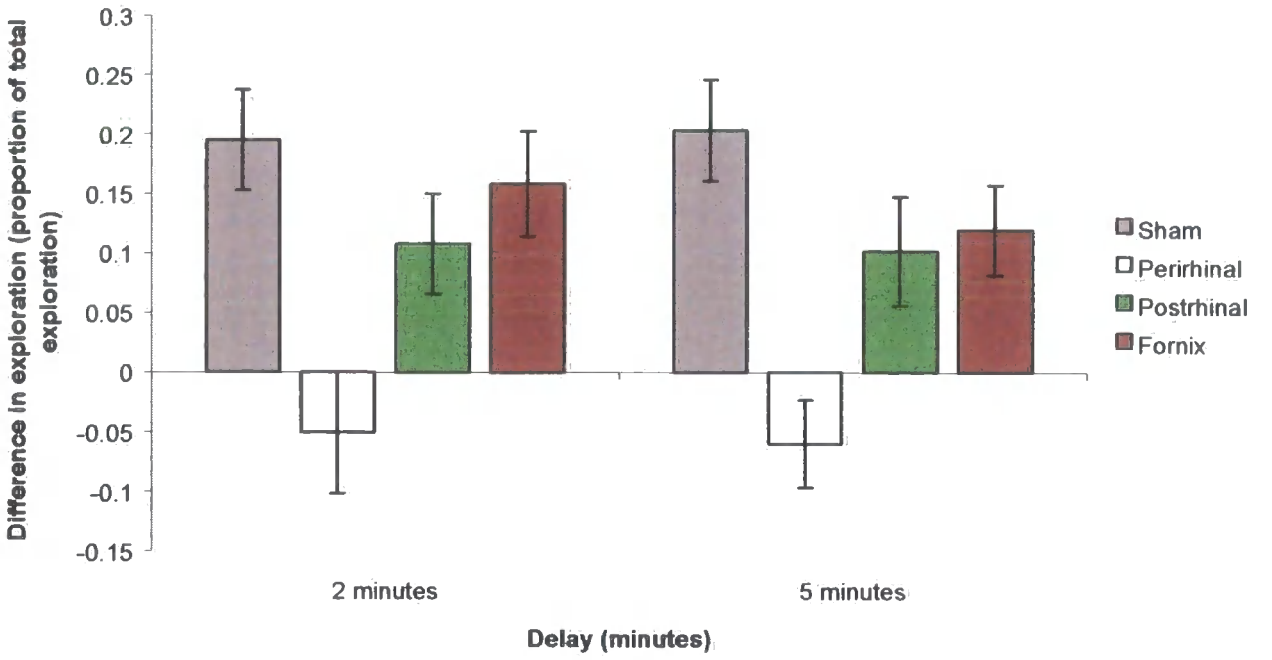
Table 4.9: One-sample T-Tests for each group at both delays on the object-as-context test.

Exploration

There was no effect of lesion ($F < 1$, $df = 3,42$, $p > 0.05$). There was also no effect of delay ($F = 3.224$, $df = 1,42$, $p > 0.05$) and no interaction between delay and lesion ($F = 2.338$, $df = 3,42$, $p > 0.05$).

Summary

There was a clear perirhinal impairment at both delays and in both contexts. The other lesion groups were not impaired.



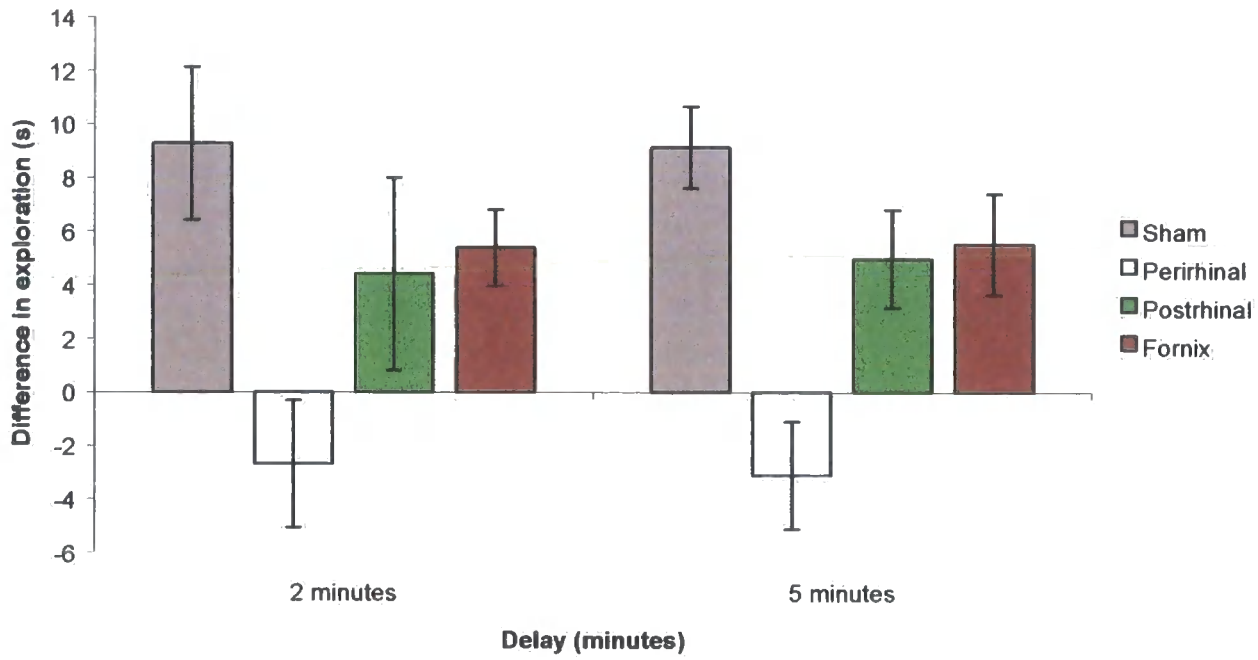
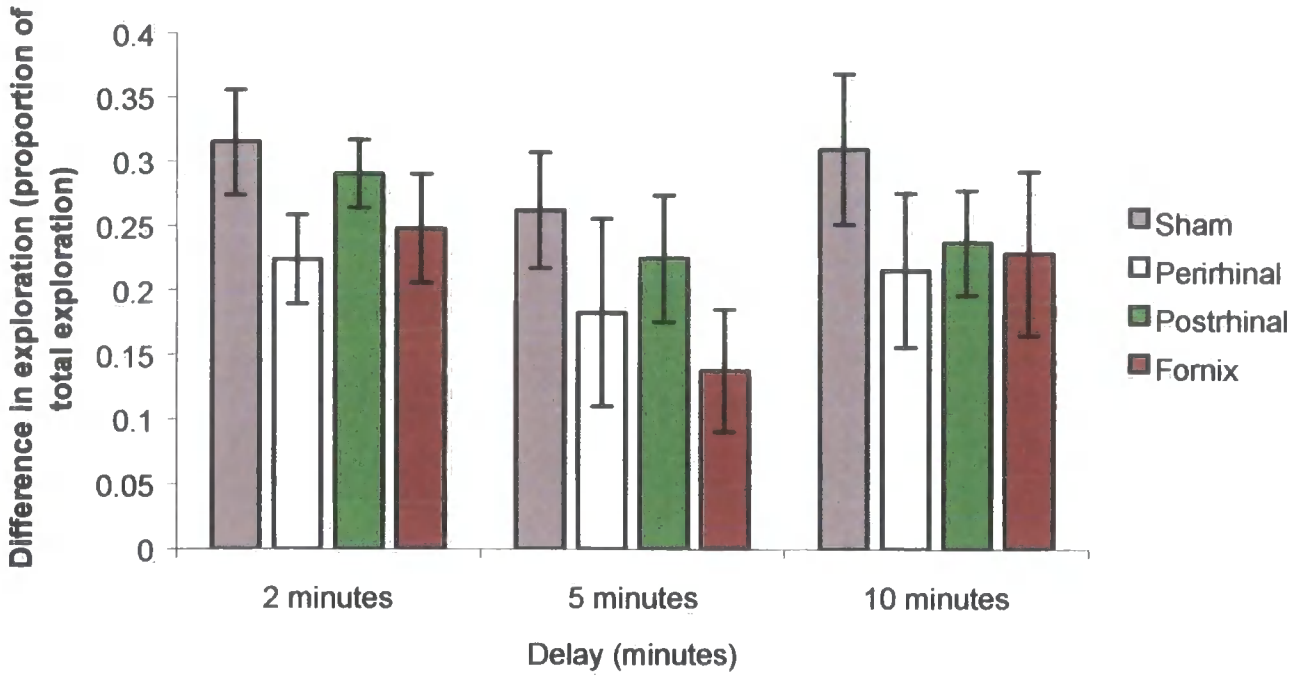


Figure 4.16: Discrimination of novel object combination from familiar object combination (a) D2 measure (b) D1 measure

Experiment 4.6: Simple control

This experiment was designed to check that all groups were able to discriminate a novel object from a familiar object at all the delays used. Because the delay between the first experience of an object and the test was a maximum of 10 minutes in the case of the 5 minute delay, delays of 2,5 and 10 minutes were employed. None of the lesion groups were impaired compared to the sham animals on the D2 measure ($F = 2.3$, $df = 3,42$, $p > 0.05$) (figure 4.17a). However, there was a main effect of lesion on the D1 measure ($F = 3.6$, $df = 3,42$, $p < 0.05$). Post hoc analysis of this effect showed that the perirhinal group were impaired relative to the sham ($p < 0.05$). There was also a main effect of delay, which, as can be seen from figure 4.17b resulted from the reduction in performance of the lesion groups at the 5 minute and 10 minute delays. Since analysis using simple effects showed no difference between the groups at any delay, it seems probable that the perirhinal impairment resulted from a relatively mild decrement at all delays.

Despite this slight impairment of the perirhinal animals (detected only on the less reliable of the 2 measures), assessment of absolute performance with one-sample T-tests confirms that all animals can perform a simple AN discrimination at all the delays encountered in the experiments previously discussed, as all groups were above chance at all three delays (see table 4.10).



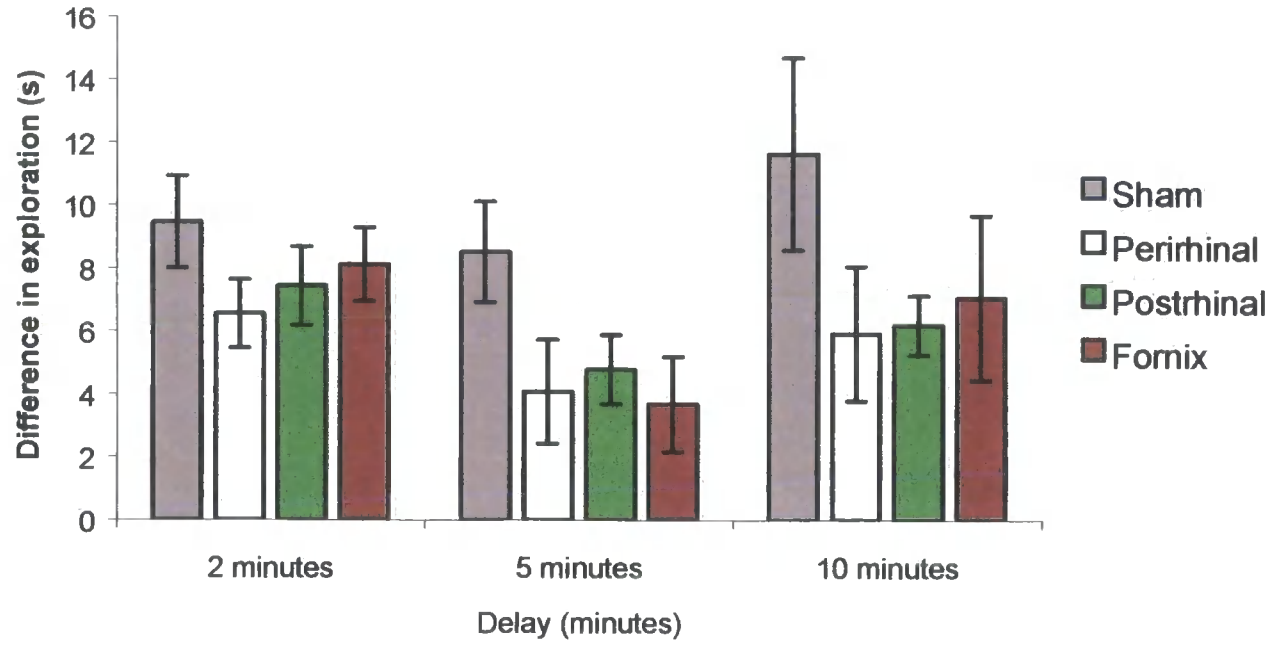


Figure 4.17: Discrimination of novel object (N) from familiar object (A). (a) D2 measure. (b) D1 measure

2 minutes						
Measure	D2			D1		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	8.085	<0.001	Yes	6.762	<0.001	Yes
Perirhinal	6.798	<0.001	Yes	6.248	<0.001	Yes
Postrhinal	11.55	<0.001	Yes	6.237	<0.001	Yes
Fornix	6.183	<0.001	Yes	7.229	<0.001	Yes
5 minutes						
Measure	D2			D1		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	6.174	<0.001	Yes	5.604	<0.001	Yes
Perirhinal	2.644	0.029	Yes	2.578	0.026	Yes
Postrhinal	4.821	<0.001	Yes	4.547	<0.001	Yes
Fornix	3.062	0.012	Yes	2.555	0.029	Yes
10 minutes						
Measure	D2			D1		
Lesion	T	P	Differ from chance?	T	P	Differ from chance?
Sham	5.577	<0.001	Yes	5.604	<0.001	Yes
Perirhinal	3.784	0.003	Yes	2.578	0.026	Yes
Postrhinal	6.163	<0.001	Yes	4.547	<0.001	Yes
Fornix	3.801	0.004	Yes	2.555	0.029	Yes

Table 4.10: One-sample T-Tests for each group at all three delays on a simple control A/N paradigm.

Exploration

There was no effect of lesion ($F = 1.775$, $df = 3,42$, $p > 0.05$). There was a main effect of delay ($F = 17.250$, $df = 2,84$, $p < 0.001$) as all groups explored the objects more at the 10 minute delay than at the 2 minute or 5 minute delay. There was no interaction between delay and lesion ($F = 1.404$, $df = 6,84$, $p > 0.05$).

Summary

The D2 measure - the more reliable of the two measures - indicated that none of the lesion groups was impaired at any delay, while the D1 measure indicated a mild

perirhinal impairment. However, all groups discriminated between the objects at all delays.

As the results presented above are relatively complex, a summary of the results of the memory experiments reported here is given in table 4.11

Experiment	Exposure		Test
	1	2	
1) AB incongruent	AA	BB	AB
2) AB congruent	AA	BB	AB
3) AN incongruent	AA	BB	AN
4) AN congruent	AA	BB	AN
5) Object as context	AaBb	-	AaAb
6) Simple control	AA	-	AN

Table 4.11: Summary of results of experiments 4.1-4.6, showing the paradigm used for testing. Indicates phase took place in context 1, indicates phase took place in context 2. (Order of contexts was controlled for).

Experiment 4.7: Locomotor Activity

The lesion groups did not differ from each other either when the 10 minute test period was considered as a whole ($F < 1$, $df = 3,42$, $p > 0.05$) (see figure 4.12), nor when the two 5 minute time bins were considered separately ($F < 1$, $df = 1,3$, $p > 0.05$).). As can be seen from figure 4.18, there was a difference between the two time bins, with all groups showing less activity in the second time bin than the first time bin when the two bins were included in the analysis separately ($F = 97.9$, $df = 1,1$, $p < 0.001$).

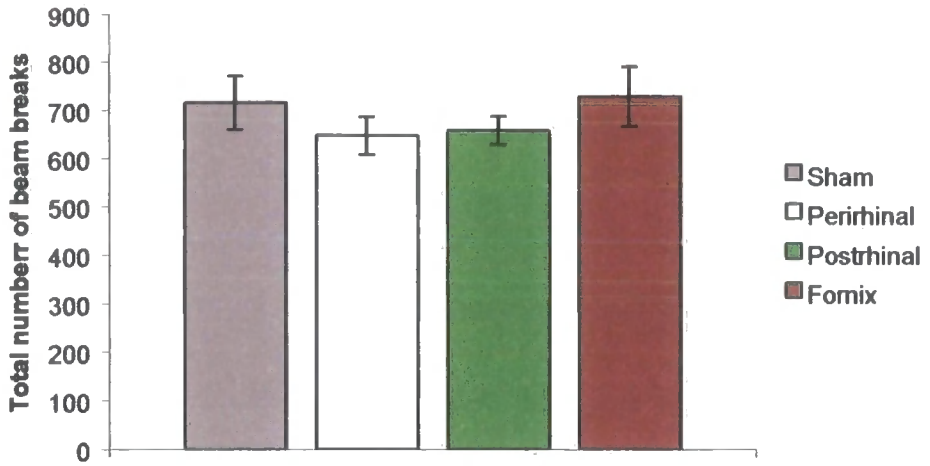


Figure 4.18: Total number of consecutive beam breaks over a 10 minute time bin

4.4: Discussion

The results presented here provide some support for the predictions made, suggesting that the hippocampus and, in particular, the postrhinal cortex are heavily involved in the processing of, and memory for, the context an object is experienced in. In contrast, the results suggest that the perirhinal cortex is only involved in such processing where the context is such that it is possible that it and the designated object are perceived as a single whole. This presents a double dissociation between a postrhinal (and lesser fornix) impairment of memory for object-in-classical context and a perirhinal impairment where the context is formed by another object.

The results of the locomotor activity tests (experiment 4.7) were somewhat surprising. There have been a number of studies which have shown that hippocampal lesions produce hyperactivity (e.g. Jarrard, 1968; Kimble, 1965), while fornix lesions have been found to replicate this (e.g. Mittleman, Bratt & Chase, 1998). The effect is thought to be due to disruption of glutamatergic projections from the subiculum via the fimbria-fornix to the nucleus accumbens (e.g. Kelley & Domesick, 1982). The fact that we did not find such an effect is puzzling, particularly in view of the fact that we obtained deficits in the discrimination of objects-in-contexts in the experiments discussed above (and see below chapter 5 for a discussion of further behavioural impairments). The histology would indicate that all the animals had the fornix

completely transected at the anterior levels (see histological analysis, above pp130), while the behavioural evidence shows clear evidence of mnemonic impairments in this group.

The histological analysis also showed that all animals, in addition to transection of the fornix, had sustained damage to the lateral septum. However, this additional damage is unlikely to have been responsible for a diminution of the hyperactivity normally observed in animals with fornix lesions. Bannerman, Gilmour, Norman & Lemaire et al. (2001) performed a temporary inactivation of the fornix and reported no difference between animals in which the cannulae terminated in the lateral septum and those in which they terminated in the fornix, either on locomotor measures or on a DNMTP task, while Poucet & Buhot (1994) reported similar impairments on a radial maze following inactivation of the medial septum. The septal damage in the animals in the fornix group is unlikely to have altered their performance on the memory tasks discussed in this and the following chapter. There is considerable evidence that lesions of the septum result in mnemonic impairments which parallel those found in animals with hippocampal or fornix damage. Indeed Vann, Brown, Erichsen & Aggleton (2000b) found that fornix lesions themselves caused significant reduction in Fos activity in the lateral septum. It seems probable that the failure to find hyperactivity in the fornix group resulted from insufficient habituation periods rather than from the location of the lesions.

Experiment 4.1 was a test of whether the context an object had been previously encountered in was a determinant of its familiarity as Dix & Aggleton (1999)'s study indicated. It is clear that the sham animals explored an object previously encountered in a different context more than one encountered in the same context. Although all the lesion groups showed less discrimination than the shams, the perirhinal animals discriminated between the objects at the 2 minute delay regardless of whether the test was in the first or second context, whereas the postrhinal animals did not discriminate in either context and the fornix animals only discriminated when tested in the second context. At the 5 minute delay the postrhinal and fornix animals did not discriminate in either context, whereas the perirhinal animals only discriminated in the first context. (This summary concerns the D2 measure, but although the picture is less clear when the D1 measure is considered the ubiquity of the postrhinal impairment is

also found here. As there was a trend towards an effect of lesion in the amount of exploration, the D2 measure should be considered as providing a more reliable account of performance.)

The impairment of the fornix and postrhinal groups is in accordance with the findings of Bucci et al. (2002) who found impaired fear conditioning to context in both these groups as well as in a group with perirhinal lesions. The finding of a mild perirhinal impairment at longer delays is perhaps unsurprising, and would seem to support the contention of Bussey et al. (2000) that the perirhinal cortex and the hippocampus normally function cooperatively. The more pronounced postrhinal deficit is in agreement with C-fos studies which supported the view that the postrhinal cortex was involved in spatial tasks while the perirhinal cortex was not (Aggleton et al., 2000; Vann et al., 2000). Wan et al., (1999) found that novel arrangements of familiar items caused increased fos activity in the postrhinal cortex as well as in hippocampal area CA1, suggesting that it was involved in object-place memory. The evidence of lesion studies (e.g. Bussey et al., 2000) that postrhinal lesions do not cause impairment on a spatial paradigm such as delayed non-matching to place on a T-maze would point towards the postrhinal cortex being involved specifically with the combination of objects and locations, whether the location is a place or a context.

The results of experiment 4.1 would suggest that the postrhinal animals were severely impaired in their memory for which object had been previously encountered in which context. Indeed it is open to question as to whether the deficit is in fact mnemonic or whether it is primarily a processing deficit. A means of reducing the mnemonic component of the test, at least partially, should be found in order to establish whether such a deficit is in fact delay-dependent. Although there would be problems of context-delineation with such an approach, it would be possible to construct a paradigm in which the two objects and contexts are experienced concurrently rather than consecutively, and this would permit testing at a total delay of only 1 minute, considerably reducing the mnemonic element of the discrimination.

It is important to note that even when the animals fail to discriminate between the objects we do not find a simple preference for the object which was first experienced at the longest delay; which might be expected if a simple failure to recognise this

object as familiar were occurring. That failure to discriminate did not result from a failure of memory for the object(s) is made clear both by the results of experiment 4.2 and by those of experiment 4.6. Experiment 4.2 demonstrated clearly that, where context was always congruent for both objects none of the animals, including the sham animals discriminated between the objects, indicating that the effect of delay did not produce a familiarity gradient between the object seen in the first exposure phase and that seen in the second exposure phase. Moreover, experiment 4.6 showed that, even at the longest effective delay encountered between exposure and test, all groups discriminated between a novel and a familiar object, indicating that an object is still recognised as familiar at such delays.

The deficits displayed by the perirhinal and fornix animals present in a more complex fashion than that of the postrhinal animals. It is possible that the results from the 5 minute delay can be explained in terms of the fact that the delay between initial exploration (of the incongruent object) and test depends on which context testing occurs in. This is longer when testing is in the second context (see figure 4.19 below).

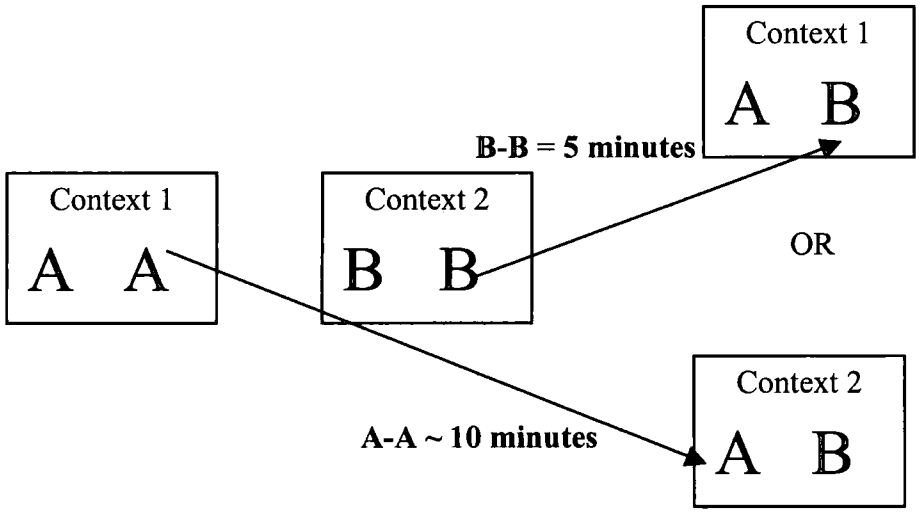


Figure 4.19: Illustration of time between first exploration of object and test exploration in incongruent context at 5 minute delay: 5 minutes when testing in first context; ~ 10 minutes when testing in second context.

If the hippocampus were more involved in memory for context than the perirhinal cortex, as numerous studies (Ennaceur & Aggleton, 1994; Ennaceur et al., 1996; Mumby et al., 2002) would lead one to expect, then animals with fornix lesions might

be predicted to show impaired discrimination at a shorter delay between experiences of the object which requires more processing: the object which is in an incongruent context. This is in fact what we find when the delays imposed by the paradigm are considered (see figure 4.19 above).

However, an alternative explanation for this data might be that animals with marginal memory for context are only impaired when something occurs to disrupt memory for the context in which an object is experienced: namely the interposing between that experience and the test of another exposure phase. This would be a reasonable explanation if the assumption is made that it is memory for the object in the *congruent* context which is crucial. In effect the two explanations assume opposing premises. The argument based on increasing delay presupposes that it is the familiar context of the object which appears congruently at test which must be retrieved and is vulnerable to memory deficits: in effect that it is the comparator for novelty which is important. Conversely, the argument based on the interposing of the second exposure as a disrupter of memory instead presupposes that it is the memory of the previous context of the object which appears incongruently at test which is crucial; that it is the object itself which is crucial to the detection of novelty, not that with which it is contrasted. Whilst both these factors are probably important the issue can be partly resolved by examining the results of experiment 4.3, in which there is no familiar object in a congruent context but only a familiar object in a non-congruent context and a novel object (N).

The results of experiment 4.3 are at first glance difficult to explain in a way that is compatible with the possible explanations advanced for the results of experiments 4.1 and 4.2. Whilst all groups were impaired compared with the sham animals' performance, the postrhinal and perirhinal animals were more impaired than were the fornix animals. This would seem to be problematic if directly compared with the results of experiment 4.1, in which the postrhinal and, to a lesser extent the fornix animals, were more impaired than the perirhinal animals. However the two experiments are fundamentally different. Experiment 4.1 poses a discrimination between two equally familiar objects (see experiment 4.2 for confirmation of this) and allows discrimination to occur purely on the basis of the congruency of the context to each object. The discrimination involved in experiment 4.3, however, provides a

discrimination between (A) and (N) which have a strong familiarity differential and tests whether this differential is reduced by the effects of context. In such a discrimination the prediction would be that animals with perirhinal lesions, which are known from the experiments discussed in chapter 3 to show impairments at shorter delays when a familiarity differential is reduced, would be the animals with the greatest impairments. In fact animals with both perirhinal and postrhinal lesions show clear impairments when tested in either context.

Interestingly the animals with fornix lesions show a greater impairment when tested in the second context. In this context there is both a longer delay and the interposition of an irrelevant set of objects (B) between initial exploration of A and the A/N test. Unfortunately this does not help to elucidate the question of whether it is memory for the congruent or the non-congruent object which is crucial in experiment 4.1. It does, however, suggest that memory for context may be dependent on a hippocampal contribution where there are a sequence of explorations of objects rather than a simple delay between exposure and test which is spent in the home cage. This was confirmed by the results of experiment 4.4, which was designed as a control for experiment 4.3.

Experiment 4.4 repeated experiment 4.3, but with the difference that the test of A/N occurred with A in a context congruent with that of its first exposure. The most interesting aspect of the results of this experiment is that the fornix animals showed a more severe impairment when testing was carried out in the first context, although this was only apparent at the 2 minute delay. In this experiment, because testing was carried out in the congruent context for (A), it is when A/N is tested in the first context that a set of irrelevant objects (B) is interposed. It is also testing in the first context which produces the longest delay between first exploration of (A) and the A/N test. What has become clear from examining the results of experiments 4.3 and 4.4 is that it is the delay between exposure to an object and test and/or the interposing of a set of objects between exploration and test which is crucial in determining the severity of the impairment of animals with fornix lesions. As previously discussed, the results of experiment 4.6 showed that none of the lesion groups was impaired at any of the delays experienced in experiments 4.1 - 4.4. This is quite strongly suggestive of the fact that it is not differences in the absolute delay between exposure

memory for context in which objects appear. Lesions to the perirhinal cortex caused impairment principally when the discrimination was primarily one between novel object and familiar object, and the role of the context in which the object appeared served merely to affect the familiarity of the familiar object.

The results of experiment 4.5 serve to confirm emphatically that the perirhinal cortex is involved in the processing of, and memory for, information about objects. This experiment, which used objects such as ashtrays and soup bowls to provide the context in which another object appeared. In this experiment the perirhinal animals were severely impaired, performing at chance at both the 2 and the 5 minute delays. In contrast both the postrhinal and the fornix groups showed no impairment at any delay. These results form a double dissociation with the results of experiment 4.1 which showed strong postrhinal impairments with milder and more specific fornix impairments. This difference in results would indicate that the objects and contexts in experiment 4.5 were being processed in different ways, with the context provided by the appearance of the whole maze not being paralleled by that of an object on which another object stands.

This result is particularly interesting in view of the fact that explanations for hippocampal involvement in contextual processing have often centred on the configural processing required in order to construct a representation of a context. However, as has been discussed the role of the hippocampus in configural processing has been thrown into doubt by results such as those of Bussey et al. (1998), while the experiments presented in chapter 3 of this thesis suggest that configurations of elements within objects to form a meaningful entity is a process which requires an intact perirhinal cortex. The most parsimonious explanation for the marked difference in the pattern of impairment between the results of experiment 4.5 and those of experiments 4.1, 4.3 and 4.4 is that the object designated as a context in experiment 4.5 was not perceived as such by the rats. It was instead processed as constituting one element of a single whole which was formed by the object and the context. Rather than being perceived as object (A) in context (a) the combination was processed as an object (Aa). Under this interpretation the deficit which was observed exclusively in the perirhinal animals supports the conclusion of chapter 3, namely that the perirhinal cortex is engaged in configural processing which involves feature ambiguity within

to object A and the test in experiments 4.3 and 4.4 which is responsible for differences between testing in the 2 contexts, but rather the interposition of a second exposure phase between exposure and test. This is made particularly clear when the results of experiment 4.4 are compared to those of experiment 4.6. The only difference between the experiments is that in experiment 4.4 some of the delay between exposure to (A) and the A/N test is spent exploring (B) rather than the in the home cage. This would suggest that it is the interference produced by such intermediate exploration which is responsible for a failure of memory particularly in those animals with fornix lesions, but also in those animals with perirhinal lesions. It is noticeable that the animals with postrhinal lesions were impaired in both experiments when tested in either context.

The postrhinal animals were also the most uniformly impaired in the purely contextual discrimination between the familiar objects A/B in experiment 4.1. The postrhinal animals show a clear deficit in all the experiments in which the shams discriminate either between familiar objects in a congruent/incongruent context (experiment 4.1) or between novel and familiar objects with the involvement of context, whether congruent to (A) (experiment 4.4) or not (experiment 4.3). Whilst the fornix group was also impaired on each of these experiments the impairment was more severe where there was an exploration period interposed between exposure to the incongruent but familiar object, or in the case of experiment 4.4, simply the familiar object.

The perirhinal animals were most severely impaired in experiment 4.3 in which the discrimination was effectively between a novel object (N) and a familiar object (A) rendered less familiar by the context in which it appeared. They showed no impairment in experiment 4.4 (A/N in congruent context), indicating that, in contrast to the postrhinal and fornix animals they are able to discriminate between a novel and a familiar object even when there is an irrelevant exploration period of another object (B) between exploration of (A) and test. The perirhinal animals were only impaired on the A/B discrimination in experiment 4.1 at the longest delay when testing was carried out such that there was an exploration period between the first exploration of the object which is incongruent at test and the test. This pattern of results would tend towards the conclusion that the perirhinal cortex is only peripherally involved in

objects. Moreover this experiment would suggest that the perirhinal cortex is not merely necessary for such processing but is also sufficient.

The results of the experiments outlined in this chapter indicate that lesions to the perirhinal cortex, while causing mild impairments in memory for objects-in-context do not prevent discriminations based on such memory. Lesions to the postrhinal cortex, however, do cause serious impairments to such memory and prevent discriminations based on it at even very short delays. Fornix lesions cause impairments which prevent discrimination at longer delays but not at shorter delays. There is, however, a double dissociation between the results of experiment 4.1 (AB in incongruent context) and those of experiment 4.5: (Object-as-context). In experiment 4.1 the postrhinal animals are do not discriminate at any delay whilst the perirhinal animals, although impaired relative to the shams, discriminate at all delays. Conversely, in experiment 4.5 the postrhinal and fornix animals are unimpaired relative to the shams while the perirhinal animals do not discriminate at any delay. This double dissociation clearly suggests that whilst the perirhinal cortex is not critically involved in memory for object-context combinations it *is* critically involved where the designated context is such that it is perceived as forming a single gestalt with the designated object. It is also clear that the postrhinal cortex, by contrast is not critically involved where the context and the object are perceived as forming a single entity, but is critically involved, to a greater extent than the hippocampus, in memory for object-context where the context is not formed by a second object. The perirhinal and postrhinal impairments are both supportive of the roles suggested by previous studies. It has been made clear that the perirhinal cortex is critical to the processing of, and memory for, objects. As is discussed in chapter 3 it is clear that this is particularly the case where an object discrimination contains a degree of feature ambiguity.

The impairments seen in the fornix animals are in accordance with the bulk of the literature which suggests that fornix lesions cause impairment of memory for objects-in-context and scene memory, as well as for object-in-place (Gaffan & Harrison, 1989, Gaffan, 1994a,b). The fornix impairments found are also in accordance with the findings of Mumby et al. (2002), who found a hippocampal impairment on a paradigm similar to experiment 4.1, although the animals in their study were still able

to discriminate between the objects. This more severe impairment following fornix lesions may indicate that it is the hippocampus and its subcortical connections rather than the hippocampus alone which is involved in memory for object-in-context. This view would be supported by the results of Parker & Gaffan (1997a, 1997b) who found that lesions of the mammillary bodies or the anterior thalamic nuclei caused an impairment of object-in-context memory in monkeys equivalent to that produced by fornix lesions. The results of the present study are also in accordance with those of Simpson et al. (1998) who found impairment of object-in-place encoding (which was effectively a test of object-in-context) following fornix transection in rats.

Given that, as with the experiments in chapter 3, there was no appetitive motivation involved in experiment 4.5, it is possible that the perirhinal animals may have shown no impairment if such a motivational component had been used. As has been discussed with reference to the experiments in chapter 3, this is certainly something that would bear investigation using 3-dimensional objects and a system of food rewards. However, the prediction would be that such an experiment would not find intact performance by the perirhinal animals although it might detect some savings which were not apparent in the current paradigm. This prediction is based on two premises. First, that the discrimination is performed easily by intact animals and animals with postrhinal lesions; second, that there is no indication that the perirhinal impairment is delay-dependent. Both of these suggest that, while perirhinal cortex may not be essential to the discrimination, it is certainly the means of choice for the processing and memory involved. Again, Buckley & Gaffan (1998c)'s results indicated that monkeys with perirhinal lesions failed to learn a similarly configural task where there was a substantial motivational component, while Parker & Gaffan's (1997a, 1997b) work indicated a failing in object-in-context memory following lesions to subcortical structures.

The results in this chapter indicate that lesions of the postrhinal cortex and, to a lesser extent, of the fornix cause impairments in the processing of object-in-context. This, however, forms part of a double dissociation with the perirhinal impairment when the context is formed by a second object. There was also a more severe perirhinal impairment when the discrimination was between A in an incongruent context and N, further indicating that object recognition involves perirhinal function. The clear

dissociation of the effects of perirhinal lesions and postrhinal or fornix lesions supports the view that the postrhinal cortex is not primarily involved in object recognition, whilst the perirhinal cortex is only tangentially involved in the processing of object-in-context. The experiments in chapter 5 explore a possible model of episodic-like memory, with the prediction that the postrhinal and fornix groups would show an impairment on such a task, while the perirhinal animals would not.

Chapter 5: A possible model for episodic-like memory in rats

5.1: Introduction

Chapters 2-4 examined memory for aspects of objects and the contexts in which they appear. However, such experiments do not address a common form of memory in humans, namely episodic memory – memory for events. The nature of episodic memory is such that it has often been thought to be exclusive to humans and possibly primates. Tulving himself (Tulving, 1983) emphasised the autonoetic character of episodic memory, which placed a primary emphasis on conscious recollection. In the absence of language it seemed improbable that such recollection could exist in species other than humans, and an impossibility to test its existence. In particular, emphasis was placed on the ability to remember a previously presented item as opposed to merely knowing that it was familiar (Mandler, 1980). This definition, while valuable, places an emphasis on the role of the recollecter, whose explicit "remembered" memory is comprised of multiple episodes, and seems further to preclude the possibility of an animal model of true episodic memory. This is particularly the case when one considers that the great majority of tasks which could be used to explore such memory do not require explicit recognition of past events. That one can only test for implicit memory does not preclude the existence of explicit memory.

A useful model of "episodic-like" memory can be formed if one considers that a primary feature of episodic memory is its power to describe an event in terms of the thing(s) that happened (what); the spatial location it happened at (where) and the temporal location of the happening (when). In episodic-like memory, basic components of episodic memory (what, where and when) are considered without the requirement for active retrieval which is considered to be implicit in the categorisation of a memory as episodic. The approach of investigating "episodic-like" memory is supported by the comment by Tulving himself that:

"The presence or absence of episodic memory is no more an all-or-none matter between species than it is within them." Tulving (1984), cited in Aggleton & Pearce (2001).

That memories which include all three of the "what, where, when" criteria can be formed in animals is clearly demonstrated by the work of Clayton and colleagues

(Clayton & Dickinson, 1998; Clayton et al., 2001). Clayton and colleagues utilised the food storing habits of scrub jays to show that the type of food (what), the location of food (where) and how long ago the storage took place (when) can all be retrieved and used for successful cache retrieval of food which takes account of whether it is highly perishable (worms) or not (nuts). This is an elegant model of a type of memory which further work would imply it may be both possible to implement in rats and dependent upon hippocampal function (N Clayton, personal communication).

Testing of the "when" element of the episodic-like triad has been one of the more problematic aspects of task-development in the attempt to test such memory in animals. This arises from the fact that "when" clearly defines a purely temporal context. However, on consideration, it seems possible that context might also be crucial to episodic or episodic-like memory. Gaffan & Harrison (1989) first demonstrated that memory for the combination of an object and a context was impaired following fornix transection. This was the first indication that aspects of object memory were as dependent on hippocampal function as was episodic memory in humans. Subsequent work has confirmed that scene memory is sensitive to lesions of the hippocampus and its diencephalic projections in both monkeys (Gaffan, 1994a,b; Gaffan & Parker, 1996, Parker & Gaffan, 1997a, 1997b) and humans (Aggleton et al., 2000). E. Gaffan & colleagues (Gaffan & Eacott, 1997; Gaffan et al., 2000; Simpson & Gaffan, 1999; Simpson et al., 1998) have developed scene-learning tasks for rats which parallel those used with primates. The balance of the evidence is that such scene memory in rats is vulnerable to hippocampal damage as is the case in monkeys. However, there are some contradictory results which suggest that hippocampal damage may paradoxically enhance scene-leaning abilities (Gaffan et al. 2001; Simpson et al., 1998) just as fornix transection may facilitate some types of configural learning (Bussey et al., 1998).

In view of this, and of clear evidence from the experiments in chapter 4 that animals discriminate context, even when not specifically rewarded for doing so, a novel paradigm has been developed which is a version of the object recognition test. This version exploits and combines the ability of normal animals to discriminate objects in a novel place (relative to that object's previous position) and objects in a novel context. Experiment 5.1 was designed such that at test animals discriminated between

two objects which they had encountered before, had encountered in that physical location, and had encountered in that context. The objects differed only in that one had not previously been encountered in that location in that context before – the configuration of "what, where and context" was novel. The other object, by contrast had previously been explored in the same position and context as it was encountered at test. If animals possessed the episodic-like memory for this "what, where, context" combination then it would be expected that they would explore the object which was novel in this configuration – though not novel in any of the individual elements.

Based on the evidence available, both from the work reported in previous chapters of this thesis, and from the other studies in this area, one might predict that all three of the lesion groups used in this experiment would be impaired on such a task. However, the results of the experiments on recognition of objects in context in the previous chapter showed that, at the short delays used perirhinal animals were only severely impaired when context was incidental to the task of object recognition. (In that the fundamental discrimination was between a novel (N) and a familiar object (A) where the familiarity of (A) was modified by its being presented in an unfamiliar context.) This would suggest that the perirhinal group would only be mildly impaired on the task outlined here which used short delays. It would, however, be predicted that the animals with postrhinal lesions would be severely impaired on such a task, as it has been demonstrated (chapter 4) that they are severely impaired on tasks involving object-context combinations. Bussey et al's work with combined perirhinal and postrhinal lesions (1999), combined with the evidence that perirhinal lesions alone do not impair object-place memory, would also suggest that postrhinal animals would be impaired on the task because of the object-location element of the "what, where, context" combination. It was also predicted that the animals with fornix lesions would be impaired on the task. There is considerable evidence that the hippocampus, or a neuroanatomical system of which the hippocampus is a part, is required for the processing of episodic or episodic-like memory (Rempel-Clower et al., 1996; Squire, 1992).

5.2: Methods and Materials

Subjects

The forty-six Dark Agouti rats used in these studies were those used in the experiments in chapter 4. Testing began approximately three weeks after the completion of those experiments. As before they were housed in pairs in diurnal conditions (12h light/ 12h dark cycle) and all testing was carried out during the light phase. Throughout the study animals had *ad libitum* access to both food and water. At the start of these experiments animals were approximately 8 months old. There were 12 animals with perirhinal lesions, 12 animals with postrhinal lesions, 11 animals with fornix lesions and 11 animals who had undergone sham surgery.

Apparatus

As in the experiments in chapter 4, all testing was carried out in an open field made of wood, of base dimensions 1 m^2 and height 48 cm. The maze could be configured to provide two different contexts. In context 1 the base was painted matt black and the walls matt white. In context 2 the base consisted of a 1.5 cm^2 mesh of white plastic-coated wire overlaid on a black base and the walls were natural wood. The objects were placed into the open field equidistant from the sides of the maze. Standard objects used included bottles, jars, tubs and bowls.

Perfusion

For histological purposes, at the end of testing operated animals were perfused intracardially with a 5% formal saline solution. Their brains were removed, embedded in wax and coronally sectioned into 10μ slices. Every 10^{th} section was stained with cresyl violet (Nissl stain).

Testing

Habituation

Because the animals had previously been used in the experiments in chapter 4, and had therefore been thoroughly habituated to the maze configured for both contexts, no further habituation sessions were carried out.

Experiment 5.1

Experiment 5.1 was designed to test the effects of a combination of object, place and context on object familiarity. There were two exposure phases followed at a delay of 2 or 5 minutes by a test phase. In the first exposure phase the maze was configured as context 1 and contained a copy of object (A) on the left side of the maze and a copy of a different object (B) on the right side of the maze. The second exposure phase the maze was configured as context 2 and contained a copy of object (B) on the left side of the maze and a copy of object (A) on the right side of the maze. In the test phase the maze was configured as context 1 and contained 2 copies of object (A), one on the left and one on the right (Figure 5.1). These objects were equidistant from the sides of the maze. For each of the two exposure phases the animal was placed into the maze and allowed to explore until they had spent 15 seconds exploring each of the two objects. They remained in the maze for a minimum of 2 minutes. They were then removed from the maze. If they failed to explore the objects for 15 seconds each they were removed after 5 minutes and the time they had spent exploring was recorded. After an interval of 2 minutes the animals were returned to the maze for the second exploration phase. After the second exploration phase there was a delay of either 2 or 5 minutes before the animals were returned to the maze for the test phase. In the test phase the animals were placed in the maze for three minutes, and the time spent exploring each of the 2 copies of object (A) was recorded for each of three 1 minute periods. The side of the maze on which object appeared in each context was controlled for between animals, as was the object used in the test phase. The order in which the contexts were experienced was controlled for between repetitions, of which there were four for each delay. Different objects were used for each repetition.

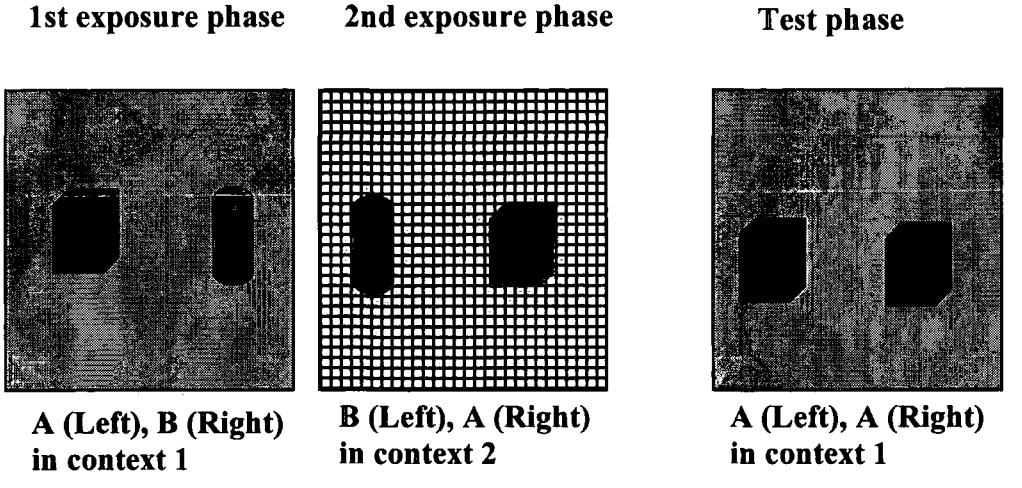
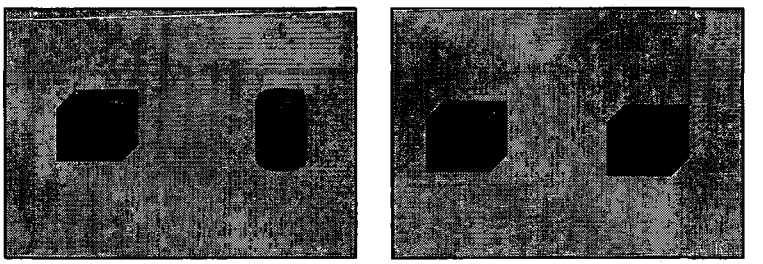


Figure 5.1: Exposure and test phases for experiment 5.1: episodic like memory.

Experiment 5.2

Experiment 5.2 was designed as a control for the effect of place alone on the familiarity of an object in experiment 5.1. The maze was configured as context 1 throughout this experiment. In the exploration phase the maze contained a copy of object (A) on the left and a copy of a different object (B) on the right. Animals were placed in the maze and allowed to explore until they had spent 15 seconds exploring each of the 2 objects. They remained in the maze for a minimum of 2 minutes. If they failed to explore the objects for 15 seconds each they were removed after 5 minutes and the time they had spent exploring was recorded. After a delay of 2 or 5 minutes the animal was returned to the maze for the test phase. For the test phase the maze contained 2 copies of object (A), one on the left and one on the right. The test phase lasted 3 minutes, and the time spent exploring each of the 2 copies of object A was recorded for each of three 1 minute time periods (Figure 5.2). The side of the maze on which each object appeared in the exposure phase was controlled for between animals, as was the object which was used in the test phase.



A(Left), B(Right)
Exposure phase

A(Left), A(Right)
Test phase

Figure 5.2: Exposure and test phases for experiment 5.2: object in place.

Data analysis

Following Ennaceur & Delacour (1988), the difference in exploration in seconds at a given time-point was calculated as the difference between the time spent exploring the object in the novel object-place-context combination and the time spent exploring the object in the familiar object-place-context combination (D1). Secondly, the difference between time spent exploring the objects in the novel and familiar object-place-context combinations was calculated as a proportion of the total time spent exploring both objects (D2). Repeated measures ANOVA's were carried out on each

of these measures, followed by post-hoc Tukey's test. The performance of each group was also analysed for difference from chance at each delay using one-sample T-tests.

5.3: Results

5.31: Histology

As the animals used in the experiments described here were those used in the experiments described in chapter 4, histological analysis can be found on pp126-132.

5.32: Behavioural

Experiment 5.1: "Episodic-like memory"

A 4 (lesion) x 2 (context) x 2 (delay) repeated measures ANOVA was performed. There was a large difference between the groups on both the D2 ($F = 17.581$, $df = 3,40$ $p < 0.001$) and the D1 ($F = 23.018$, $df = 3,40$, $p < 0.001$) measures. Post hoc analysis of these main effects of lesion showed that, as can be seen from figure 5.3, the fornix animals were severely impaired compared with the sham animals ($p < 0.001$) on both the D1 and the D2 measures. They were also severely impaired compared with the perirhinal and postrhinal animals ($p < 0.001$) on both measures, whilst these other lesion groups did not differ from the shams. There were no effects of delay ($F = 1.995$, $df = 1,40$ $p > 0.05$) or of context ($F < 1$, $df = 1,40$, $p > 0.05$) and no interactions between the variables, indicating that the fornix deficit was consistent across both delays and testing contexts.

Analysis of the absolute performance of the groups confirms this uniform and highly selective fornix deficit. One-sample T-tests showed that the fornix group was performing at chance in both contexts and at both the 2 minute and the 5 minute delays (see figure 5.3 and table 5.1), whilst all other groups performed well above chance levels in all conditions. Since there were no differences between any of the groups' performances in the two contexts, only data for the contexts combined are shown.

D2						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ	T	P	Differ
Sham	5.573	<0.001	Yes	6.450	<0.001	Yes
Perirhinal	5.468	<0.001	Yes	7.098	<0.001	Yes
Postrhinal	5.470	<0.001	Yes	7.692	<0.001	Yes
Fornix	0.0438	0.966	No	-1.112	0.292	No
D1						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ	T	P	Differ
Sham	5.226	<0.001	Yes	4.807	0.001	Yes
Perirhinal	4.864	<0.001	Yes	6.286	<0.001	Yes
Postrhinal	4.878	<0.001	Yes	8.440	<0.001	Yes
Fornix	0.205	0.841	No	-0.922	0.378	No

Table 5.1: One-sample T-tests for all groups at both delays.

Exploration

There was no difference between the groups in the amount they explored the objects ($F = 1.079$, $df = 3,40$, $p > 0.05$). There was also no effect of delay ($F = 1.483$, $df = 1,40$, $p > 0.05$) and no interaction between delay and lesion ($F < 1$, $df = 3,40$, $p > 0.05$) .

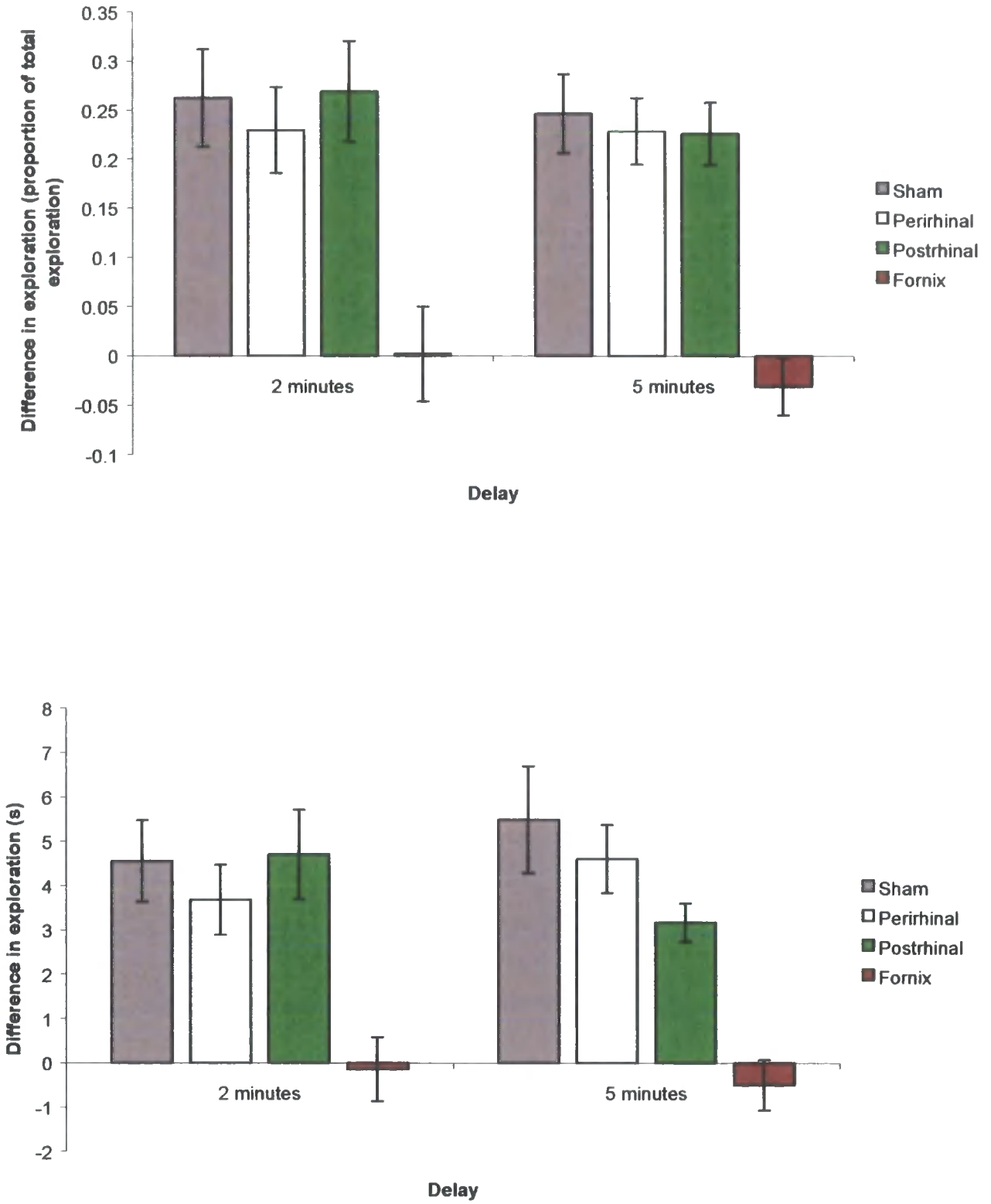


Figure 5.3: Discrimination of object in place in context, contexts combined. (a) D2 measure. (b) D1 measure.

Experiment 5.2: Place

This experiment was designed to control for the effect of place in experiment 1. A 4 (lesion) x 2 (delay) repeated measures analysis was performed. There were no differences between the groups on either the D2 ($F < 1$, $df = 3,40$, $p > 0.05$) or the D1 ($F < 1$, $df = 3,40$ $p > 0.05$) measure of performance. There was no effect of delay on either the D2 ($F < 1$, $df = 1,40$, $p > 0.05$) or the D1 ($F = 1.738$, $df = 1,40$, $p > 0.05$) measure of performance. As can be seen from figures 5.4a and 5.4b, this lack of difference between the groups reflects the fact that all groups perform well on this task with no lesion impairments.

Analysis of absolute performance using one sample T-tests largely supported this interpretation, although they did show that the perirhinal animals performed at chance at the 2 minute delay on both the D1 and the D2 measures of performance (see table 5.2). As all groups performed above chance at the 5 minute delay on both measures, this would seem to be a statistical anomaly, resulting from a high level of variance in this data point, when taken in conjunction with the lack of effects of lesion or of delay.

D2 measure						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ	T	P	Differ
Sham	4.541	0.001	Yes	7.511	<0.001	Yes
Perirhinal	2.027	0.070	**No**	3.287	0.008	Yes
Postrhinal	3.269	0.007	Yes	3.289	0.007	Yes
Fornix	3.570	0.005	Yes	3.954	0.003	Yes
D1 Measure						
Delay	2 minutes			5 minutes		
Lesion	T	P	Differ	T	P	Differ
Sham	4.197	0.002	Yes	6.393	<0.001	Yes
Perirhinal	1.937	0.082	**No**	3.442	0.006	Yes
Postrhinal	4.153	0.002	Yes	3.285	0.007	Yes
Fornix	2.401	0.037	Yes	3.946	0.003	Yes

Table 5.2: One-sample T-tests for all groups at both delays.

Exploration

There was no main effect of lesion ($F = 2.640$, $df = 3,40$, $p = 0.063$), but there was a main effect of delay ($F = 8.908$, $df = 1,40$, $p < 0.01$) and an interaction between delay and lesion ($F = 3.396$, $df = 3,40$, $p < 0.05$). This resulted from all groups except the

fornix animals showing less exploration at the 5 minute delay than at the 2 minute delay, with the fornix animals displaying the opposite pattern of behaviour.

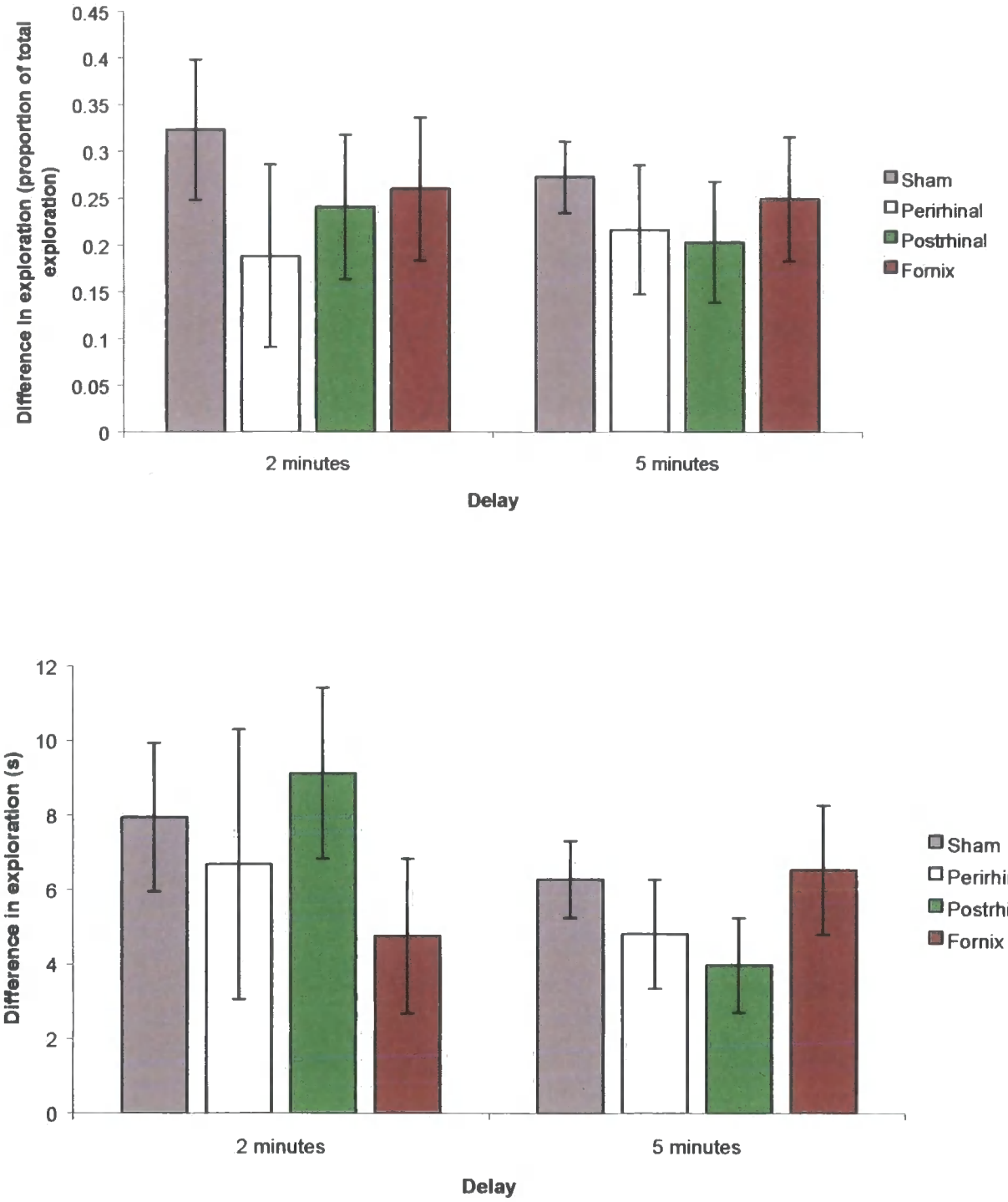


Figure 5.4: Discrimination of object in place. (a) D2 measure. (b) D1 measure

5.4: Discussion

The results of the experiments in this chapter are clear-cut. Whether the paradigm used in experiment 5.1 is in fact a test of episodic memory, it is clear that the animals with fornix lesions were severely impaired at both delays and whichever context the test session occurs in. Conversely, and contrary to our predictions the animals with perirhinal or postrhinal lesions showed no impairment compared with the controls.

The possibility that the fornix deficit was purely a result of the introduction of an "object-in-place" element into the task seemed a strong one, as fornix animals have repeatedly been found to be impaired on such a task (Ennaceur & Meliani, 1992b; Ennaceur, Neave & Aggleton, 1997). Indeed it was partly on the basis of such studies that the prediction of a fornix impairment was predicated. However, the delays used in these experiments were extremely short compared with the standard 15 minute delay used by many studies, so that the failure to find a deficit in any of the groups is less surprising than it might at first seem. An impairment at longer delays than were used here might reasonably be predicted.

The results of this attempt to model episodic-like memory in rats, although straightforward, are surprising. It had been predicted that the perirhinal animals would display only a mild impairment or no impairment at all, and it was also predicted that the fornix animals would be impaired. However, the impairment of the fornix animals was predicted to result primarily, at least at the 2 minute delay, from the effects of object-place combination. That this cannot be the case is demonstrated by the results of experiment 5.2, in which the fornix animals were not impaired at all. As the impairment shown by the fornix animals in the experiments in chapter 4 was neither as uniform nor as severe as that found in experiment 5.1 here, it must therefore be concluded that the fornix impairment is produced in large part by the combination of object, place and context, rather than singly by any one of these elements alone. At the very least it would appear that the combination of place and context – the "where" and the "context" of the configuration is not remembered in animals without an intact fornix.

However, the apparently paradoxical results of the animals with postrhinal lesions require further consideration. It is important to remember that the object recognition

and similar tasks contain no motivational element save for the intrinsic motivational properties of novelty (Ennaceur & Delacour, 1988). As has been discussed in chapter 4, lesions to the hippocampus may impair context recognition that is incidental to a motivated task, while sparing context recognition which is integral to task completion. It seems possible that the postrhinal animals retain the ability to discriminate context but do not actually do so when there is no motivation to so discriminate. This explanation is, however, rather problematic given the lack of an explicit motivational element in the current discrimination.

The problem of the fornix deficit on experiment 5.1, in conjunction with apparently intact performance on experiment 5.2 and only limited deficits on the experiments in chapter 4 remains. A possible explanation is that it is the simple additive effect of processing multiple aspects of a discrimination (what, where, context) which cumulatively produces a major impairment. However, this explanation is incompatible with the combination of intact postrhinal performance observed in experiment 5.1 and the severe postrhinal impairment observed on the object-in-context experiment (experiment 4.1) in chapter 4. In this case we find that the introduction of an additional element (place) to the discrimination effectively eliminates a postrhinal impairment. This would seem to render an explanation of the fornix deficit emerging purely due to an increased processing load in a more complex paradigm inherently unlikely.

A possible solution to this dichotomous performance would be that the situation in experiment 5.1 is in fact processed by a different memory system to that involved in processing the object-in-context (experiment 4.1) or the standard spontaneous object recognition (e.g. experiment 4.6) tasks. Such a memory system would appear to depend upon an intact hippocampus, either in and of itself, or as part of an extended memory system concerned with episodic and episodic-like memory. Under such an account the more restricted fornix impairments found in our studies of object-in-context paradigms and by other authors using hippocampal lesions in such tasks (Mumby et al., 2002) would reflect the lesser involvement of such a system in a "object in context" discrimination. The pattern of postrhinal impairments would reflect the fact that an intact hippocampus is both necessary and sufficient for discrimination in the test of episodic-like memory, but that, where such a system is

minimally engaged, postrhinal function becomes paramount in processing of the information involved in the object-in-context. This explanation is not particularly parsimonious in its account of the data, but it is both coherent and comprehensive. In the absence of an account which is both equally strong and also able to adhere more closely to the doctrine of Occam's razor it would appear deserving of serious consideration.

Some support comes from the work of Morris and colleagues (Day & Morris, 2002), who have developed a model of "what and where" which contains a motivational element and involves a "what, where" discrimination from a multiplicity of possible "wheres". Their work would also suggest that an intact hippocampus is not essential for the simple "where" element of the discrimination, but only for the combination of "place" with "object". It could be argued that this is a combination of two elements rather that the "what, where and context" used here, but it seems reasonable to suppose that a task in which the number of possible "where's" was very much greater (49) than the two used here would be more likely to engage an episodic memory system even without the additional demands of a contextual element.

The account proposed here is not necessarily that it is the presence of "what, where and when" alone, and only these three elements which would determine whether a hippocampally based system for episodic-like memory were engaged. The argument is rather that when a critical "situation remembering requirement" is reached – whether through the paradigm proposed here, that used by Clayton et al. (2001) or that being developed by Morris and colleagues, then the episodic-like memory system will become primarily engaged and an intact hippocampus correspondingly necessary for successful task completion. At levels of information where the spatial component is relatively low (e.g. context but not place information) processing may rely primarily on structures such as the postrhinal cortex, although a lesser role is played by the hippocampal formation, as evidenced by the milder fornix impairment found here and the hippocampal impairment reported by Mumby et al. (2002).

However, the argument is not that there is a difficulty criterion for hippocampal involvement. In fact it is arguable that, for intact animals, as for humans, episodic memory is more natural and effortless than other forms of memory. This view would

seem to be supported by the results of experiment 5.1, in which not only the control animals but also the perirhinal and postrhinal animals performed well above chance. This contrasts with the impairment (albeit sometimes mild) showed by all lesion groups on the object-in-context discrimination (experiment 4.1). The very fact that there is such a clear fornix impairment in the absence of any other lesion effects, and without an impairment of object-in-place at these delays, is perhaps supportive of the view that the "object, place, context" paradigm is in fact a model for episodic-like memory. In that it does not require either motivation, or, apparently, effort, the object, place, context task resembles not merely episodic-like memory but episodic memory itself. A further discussion of the relationship between object memory, memory for object-in-context and episodic-like memory can be found in chapter 7. Chapter 6, which follows, is somewhat tangential to the thesis so far in that it attempts to examine the role of calcium channels and the cholinergic system in object recognition.

Chapter 6: The role of calcium and the cholinergic system in object recognition: a neurochemical approach to the perirhinal cortex.

The majority of work in this chapter has appeared as:

Norman G, Brooks SP, Hennebry GM, Eacott MJ & Little HJ (2002). Nimodipine prevents scopolamine-induced impairments in object recognition. Journal of Psychopharmacology 16: 153-161.

6.1: Introduction

The experiments in this chapter aimed to examine the role of the cholinergic system in object recognition. It is clear from the discussion in preceding chapters that the perirhinal cortex is involved in object recognition, and that perirhinal lesions prevent discrimination between novel and familiar objects at delays of 15 minutes or longer (Ennaceur et al., 1996). The work of Ennaceur and colleagues (Ennaceur, Cavoy & Delacour 1989; Ennaceur & Meliani, 1992) has shown that the task can be disrupted by the systemic administration of nootropic or anticholinergic agents and, in particular, by scopolamine. The experiments reported in this chapter have therefore further investigated the mechanism by which the cholinergic system supports object recognition.

Support for the circumstantial evidence which had suggested the cholinergic innervation of the perirhinal cortex as a possible candidate for supporting object recognition was provided by studies which used intracerebral injections. Stimulus recognition in monkeys is severely impaired by local administration of the muscarinic acetylcholine (ACh) receptor blocker, scopolamine, when it is microinjected into the perirhinal cortex but not when it is injected into area TE or into the dentate gyrus (Tang, Mishkin & Aigner, 1997). This suggests that stimulus memory formation is critically dependent on cholinergic-muscarinic activation of the perirhinal area. This finding has been replicated in rats (Abe & Iwasaki, 2001). Brown, Warburton & Duguid (2000) found that scopolamine administered before initial exposure disrupted both spontaneous object recognition and differential fos expression in the perirhinal cortex of rats, following exposure to novel and familiar objects. Further support for the critical role of the cholinergic system in aspects of memory is provided by the finding that loss of cholinergic innervation of the hippocampus or neocortex (as a result of immunotoxic lesions of the basal nucleus of Meynert and/or the vertical limb of the diagonal band of Broca) causes damage which is functionally equivalent to

structural lesions of the areas which are targets of the cholinergic innervation (Ridley, Pugh, Maclean & Baker, 1999).

Dihydropyridine calcium channel antagonists have been found to prevent or reverse memory deficits that result from a variety of causes (Izquierdo, 1990; Schuurman, 1993). Nimodipine has been shown to ameliorate age-related memory impairments as measured by a trace conditioning response and by habituation to an open field (Deyo, Straube & Disterhoft, 1989). Performance following hippocampal lesions was improved by nimodipine on a variety of tasks including the radial arm maze (Nelson, Bawa & Finger., 1992), the holeboard test (Weichman, McMurray, Knuttinen & Mudd et al, 1994) and a delayed response latency task (Finger, Green, Tarnoff & Mortman et al., 1990). The amnesic effects of electric shock are alleviated by nimodipine as measured by a passive avoidance paradigm (Hoffmeister, Benz, Heise & Krause et al., 1982). Nimodipine also reduced the impairment in habituation to a novel environment that resulted from chronic administration of corticosterone (Dachir Robinzon, Grauer & Levy, 1995). Nimodipine has been found to reverse the impairment of object recognition produced by acute systemic injection of ethanol (Brooks, Hennebry, McAlpin & Norman et al., 2002). There is some evidence that nimodipine may also alleviate the symptoms of Alzheimer's disease in humans (Tollefson, 1990) and it is licensed as a treatment for Alzheimer's in a number of countries.

Despite the high density of dihydropyridine binding sites in temporal cortex and their alleviation of memory deficits; they have little effect on behaviour or neural activity in the normal brain, although there are reports that chronic administration may improve memory performance in normal animals. McMonaglestrucko and Fanelli (1993) studying spatial learning in the Morris maze; Deyo et al. (1989) studying associative learning; Vetulani, Bettaglia & Sansone (1997) studying acquisition of an avoidance response and Kane and Robinson (1999) using the Barnes circular platform task, all showed beneficial effects of nimodipine on learning in normal animals.

There is evidence that neuronal calcium channels may be critically involved in learning which is primarily centred on the perirhinal cortex. A possible neuronal basis for learning about objects, and object recognition in particular, is long-term

depression (LTD). By reducing future synaptic activation this inhibitory mechanism may form the basis for differential response of perirhinal neurons to novel and familiar stimuli (Zhu & Brown, 1995; Zhu et al., 1995). Long or short lasting LTD in the perirhinal cortex can be induced by electrical stimuli in the adjacent layers of the entorhinal or temporal cortex. The duration of synaptic depressions appears to be dependent on the type of metabotropic glutamate (MGlu) receptor involved, with group I or group III MGlu receptor agonists inducing long lasting depression, while group II MGlu receptors induce more transitory synaptic depression (McCaffery, Cho, Bortolotto & Aggleton et al., 1999). One form of LTD in the perirhinal cortex is dependent on the interaction of two groups of MGlu receptors, with group II MGlu receptors facilitating increases in intracellular calcium levels that are mediated by group III MGlu receptors (Cho, Kemp, Noel & Aggleton et al., 2000). However, if NMDA receptor function is enhanced by depolarisation this removes the requirement for the activation of group II MGlu receptors, and thus facilitates LTD. There is strong evidence (Cho, Aggleton, Brown & Bashir, 2001) that the magnitude of LTD in the perirhinal cortex is dependent on the magnitude of the increase in intracellular calcium concentration.

Abe & Iwasaki (2001) also found that retention of object discrimination is impaired when the NMDA antagonist D,L-2-amino-5-phosphopentanoic acid (AP5) is microinjected into the perirhinal cortex, which is suggestive of LTP within the structure being involved in object memory. Certainly long-term depression induced by low frequency stimulation following LTP in the perirhinal cortex is NMDA receptor-dependent and metabotropic glutamate receptor-independent (Ziakopoulos, Tillett, Brown & Bashir 1999). Conversely, the activation of muscarinic ACh receptors in the perirhinal cortex has been found to induce long-term depression (LTD) of synaptic transmission that is dependent on activation of muscarinic M1 receptors (Massey, Bhabra, Cho & Brown et al. 2001). This form of LTD requires neither NMDA receptor activation nor synaptic stimulation, nor can it be blocked by protein kinase C or protein phosphotase inhibitors. However, its magnitude is reduced by the administration of agents which either deplete intracellular calcium stores (cyclopiazonic acid) or inhibit protein synthesis (anisomycin), again suggesting a critical role for calcium channel activation in the support of such learning. In the light of these findings, this study attempted to examine the effects of administering a

dihydropyridine calcium channel antagonist in conjunction with the muscarinic antagonist scopolamine.

Dihydopyridine calcium channel antagonists are selective for the "L"-subtype of high voltage-activated calcium channel (Docherty and Brown, 1984; Nowycky, Fox & Tsien, 1985). Peripherally administered dihydropyridines easily enter the brain and have high affinity binding sites in the CNS. In human brain the highest density of [H3]nimodipine binding sites is in temporal cortex. The terminal half-life of nimpdipine is 8-9 hours, but the decline in plasma concentration is more rapid at 1-2 hours.

A task that relies on differential exploration of objects to provide a measure of amnesia, such as the spontaneous object recognition test used here, is potentially vulnerable to being confounded by other effects of an agent, such as alterations in locomotor activity. As scopolamine is known to decrease locomotor activity in rats in a dose-dependent manner (Sipos, Burchnell & Galbicka, 1999), the lowest dose of scopolamine (0.125 mg/kg) that has been reported to be effective on any mnemonic task (Sessions, Pilcher & Elsmore, 1998) was used. Sessions et al. (1988) reported no alterations in locomotor activity at this dose of scopolamine in rats. Scopolamine has a half-life of approximately 2.5 hours. Neither of the two doses of nimodipine used in this study have been found to cause changes in locomotor activity in mice when administered alone (Landauer, Castro, Benson & Hogan et al., 2001), although nimodipine at 10 mg/kg can cause decrements in activity when administered in conjunction with other agents such as phosphorothioates (Landauer et al., 2001). In order to assess any such effect when administered with scopolamine 0.125 mg/kg, locomotor activity tests were carried out at all the doses and post-injection delays used in the object recognition task. In assessing the object-recognition data itself, two indices of discrimination were used in order to minimise potential effects of any alterations in locomotor activity by providing both a direct and a proportional measure of the difference in exploration times of the novel and familiar object.

Scopolamine is an antagonist at muscarinic receptors and is known to disrupt memory on a variety of tasks including the object recognition task and the radial maze (Ennaceur and Meliani, 1992). Ennaceur and Meliani found that memory

impairments occurred only at the relatively high dose of scopolamine 0.50 mg/kg, compared with the 0.125 mg/kg used in this study, and that this was particularly the case on the object-recognition task. They concluded from this that the object recognition task was less sensitive to cholinergic function than radial-maze performance. Interestingly, Ennaceur and Meliani found that the cholinesterase inhibitor physostigmine also disrupted object-recognition performance at short delays at a dose of 0.20 mg/kg. This effect was the reverse of physostigmine's effect on other memory tests (Ennaceur, 1998). This may be indicative of extreme sensitivity of the task to cholinergic function, with imbalances in either direction producing deficits. The integrity of cholinergic transmission has been clearly implicated in the object recognition task by studies using nootropic drugs such as aniracetam. These restored cholinergic function simultaneously with object recognition, in rats which were hypocholinergic due to ageing, administration of scopolamine, or lesions of the nucleus basalis (Bartolini, Casamenti & Pepeu, 1996).

This study aimed to examine the possible effect of calcium channel blockade in reversing the impact on memory processes of muscarinic receptor antagonism induced by scopolamine. The general nature of nimodipine's efficacy is indicative of an action which impacts on several neural areas, including cerebral cortex as well as hippocampus. Endogenous acetylcholine has a role in regulating L-type calcium flow, causing reductions in the amplitude of L-type calcium current (Pemberton & Jones, 1997). This, along with other studies demonstrating the ameliorative effects of nimodipine on memory deficits, suggests that deficits induced by a cholinergic antagonist may be ameliorated by a dihydropyridine. It has been suggested (Bartolini et al., 1996) that decreases in cholinergic transmission in aged rats, which occur as part of extensive changes in brain neurotransmitter function (Pepeu & Giovannelli, 1994), may prevent acquisition rather than retention of object memories. In view of this, this study investigated the impact of administration of scopolamine both before and after the exploration period in which memories are formed. Two doses of nimodipine, 1 mg/kg and 10 mg/kg were used in order to investigate possible dose-dependency of its action

6.2: Methods and Materials

Subjects

Subjects were 6-8 month old male Lister hooded rats bred in house. Animals were housed under reversed phase lighting conditions (lights on 19.00 – 07.00; lights off 07.00 – 19.00) and all testing was carried out between 07.00 and 19.00. Animals had *ad libitum* access to both food and water throughout the experiment.

Drugs

All injections were made by the intraperitoneal route with a volume of 1 ml/kg. Scopolamine was dissolved in distilled water and nimodipine was suspended in Tween 80 (0.5% in distilled water) and sonicated. The suspension was protected from light throughout the studies.

In experiment 6.1 (retrieval) the drug treatment groups were:- scopolamine 0.125 mg/kg + tween vehicle; water plus nimodipine 10 mg/kg; scopolamine 0.125 mg/kg + nimodipine 10 mg/kg; water plus tween vehicle. In experiment 6.2 the groups were the same but with 1 mg/kg nimodipine substituted for the 10 mg/kg dose. All drugs were administered immediately after initial exploration was completed. The same doses were used for experiments 6.3 and 6.4 (acquisition) respectively. In these experiments all drugs were administered 15 minutes prior to initial exploration. In experiments 6.5 and 6.6 (scopolamine at long delay) two drug treatment groups were used. These were scopolamine 0.125 mg/kg and vehicle. In experiment 6.7 (locomotor activity) there were 6 drug treatment groups which were all those used in experiments 1 - 4. In all cases control animals received equal volumes of the corresponding vehicle. N values were 8 – 12 per treatment group for the object recognition experiments and 5 – 6 per treatment group for the locomotor activity experiment

Apparatus

Object recognition

As in the experiments in chapters 3, 4 and 5, the tests were carried out in an open field made of wood of base dimensions $1m^2$ and height 48cm. The base was painted matt black and the walls matt white. The objects were placed into the open field

equidistant from the sides of the maze. Objects used included bottles, jars, tubs and bowls.

Testing

Habituation

Prior to the test day animals received three habituation sessions. These took place in cage-mate pairs and lasted for 10 minutes. For each habituation session a different novel object was placed in the centre of the maze.

Experiment 6.1: Acquisition paradigm; nimodipine at 10 mg/kg

Animals were placed individually in the open field which contained 2 identical copies of a novel object, placed equidistant from the sides. They were allowed to explore freely until they had spent a total of 30 seconds exploring the objects. If they failed to explore for 30 seconds they were removed after 5 minutes and the actual time spent exploring was noted. Immediately after they were removed from the open field the rats were injected with one of four drug combinations.

Following injection they were returned to their home cage. 15 minutes after injection they were returned to the open field which now contained a new copy of the object they had previously explored and a novel object, placed equidistant from the sides of the maze. They were allowed to explore for 3 minutes and the time spent exploring each object was recorded for each of three 1 minute periods. There were 3 subsequent tests. These took place at 1 hour 15 minutes, 5 hours 15 minutes and 29 hours 15 minutes post-exploration. For each of these a new copy of the familiar object and a different novel object were used. These absolute delays represented relative delays since the last exploration of the familiar object of 15 minutes, 1 hour, 4 hours and 24 hours.

Intrinsic interest of objects was controlled for by varying the designated familiar and novel objects at each delay point between rats. Effects of place were controlled for by alternating the position of the novel and the familiar objects across both animals and delay points.

Experiment 6.2: Acquisition paradigm; nimodipine at 1mg/kg

For experiment 6.2 the methodology was identical with that employed for experiment 6.1, except that a dose of nimodipine 1 mg/kg was substituted for that of nimodipine 10 mg/kg.

Experiment 6.3: Retrieval paradigm; nimodipine at 10 mg/kg

For experiment 6.3 the methodology was identical with that employed for experiment 6.1, except that all injections were given 15 minutes before the initial exploration phase.

Experiment 6.4: Retrieval paradigm; nimodipine at 1 mg/kg

For experiment 6.4 the methodology was identical with that employed for experiment 6.3, except that a dose of nimodipine 1 mg/kg was substituted for that of nimodipine 10 mg/kg.

Experiment 6.5: Acquisition paradigm; testing at 29 hours 15 minutes only

For experiment 6.5 the methodology for the initial exploration phase was identical to that for experiment 6.1. Following initial exploration, and immediately on removal from the maze animals, were injected with one of two drugs. Following injection they were returned to their home cage. After a delay of 29 hours 15 minutes they were returned to the maze which now contained a new copy of the object they had previously explored and a novel object. They were allowed to explore for three minutes and the time spent exploring each object was recorded as described for experiment 6.1.

Experiment 6.6: Retrieval paradigm; testing at 29 hours 15 minutes only

For experiment 6.6 the methodology for the initial exploration phase was identical to that for experiment 6.3. Fifteen minutes before exploration animals were injected with one of two drugs. They were then returned to their home cage for fifteen minutes before being place in the maze for the initial exploration. Following initial exploration the animals were returned to their home cage. After a delay of 29 hours 15 minutes they were returned to the maze for the test phase as in experiment 6.5.

Data analysis

Following Ennaceur & Delacour (1988), the difference in exploration in seconds at a given time-point was calculated as the difference between the time spent exploring the novel object and the time spent exploring the familiar object (D1). Secondly, the difference between time spent exploring novel and familiar objects was calculated as a proportion of the total time spent exploring both objects (D2). Repeated measures ANOVA's were carried out on each of these measures, followed by post-hoc Tukey's test. One-sample T-tests were also carried out on each group at each delay to determine whether performance was better than chance.

Experiment 6.7 (Locomotor activity)

This experiment was designed to detect any change in locomotor activity associated with the drugs administered for the object recognition tests, which may have had an impact on exploration. Apparatus was Perspex boxes with removable lids, of base dimensions 42 x 46cm^2 and height 25cm equipped with infra-red beams which detected movement, and when broken consecutively, detected ambulatory activity which was the measure used to assess activity. Fifteen minutes prior to test animals were injected with one of 6 drug combinations. These were:

Nimodipine 10 mg/kg + scopolamine 0.125 mg/kg
Nimodipine 1 mg/kg + scopolamine 0.125 mg/kg
Nimodipine 10 mg/kg + vehicle
Nimodipine 1 mg/kg + vehicle
Scopolamine 0.125 mg/kg + tween
Vehicle + tween

Fifteen minutes after the injections animals were placed in one of the boxes for a period of 10 minutes during which their activity was recorded. Subsequent tests took place at 1 hour 15 minutes, 5 hours 15 minutes and 29 hours 15 minutes post-injection.

Data analysis

A repeated measures analysis followed by post-hoc Tukey's test and simple main effects was carried out on the ambulatory data.

6.3: Results

Experiment 6.1: Acquisition Nimodipine 10 mg/kg

There were significant differences between the drug groups on both the D2 ($F = 9.134$, $df = 3,36$, $p < 0.001$) and the D1 ($F = 4.470$, $df = 3,36$, $p < 0.01$) measures of performance. As the results on the two measures differ somewhat, they are presented separately.

D2

Post hoc analysis of the main effect of drug ($F = 9.134$, $df = 3,36$, $p < 0.001$) revealed that the scopolamine + tween group was impaired compared to all the other groups (compared with vehicle + tween group: $p < 0.001$; compared with the nimodipine 10 mg/kg + scopolamine group: $p < 0.01$; compared with the nimodipine 10 mg/kg + vehicle group: $p = 0.01$). There was also a main effect of delay ($F = 4.006$, $df = 3,108$, $p < 0.05$). There was no interaction between drug and delay ($F = 1.67$, $df = 9,108$, $p > 0.05$). However, as can be seen from figure 6.1a, the scopolamine + tween group did not appear to be impaired compared to the other groups at the 29 hour 15 minute delay. It seems probable that the failure to find an interaction was due to the variance in the control groups.

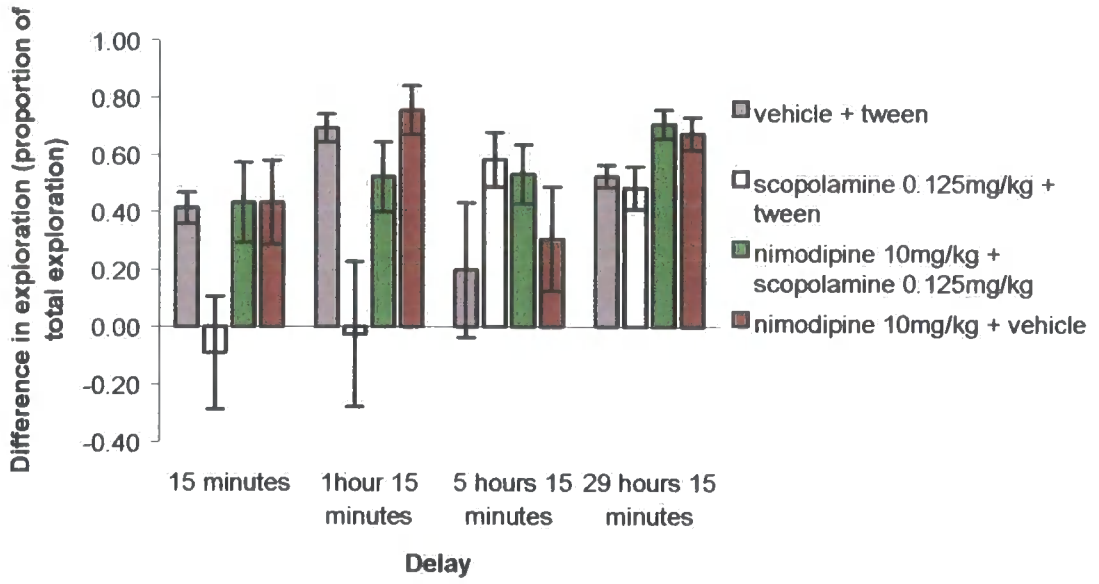
This interpretation was confirmed by the analysis of absolute performance of the groups. One sample T-tests showed that the scopolamine + tween group performed at chance at the 15 minute, the one hour 15 minute and the five hours 15 minutes delays, but was above chance at the 29 hour 15 minute delay. The nimodipine 10 mg/kg + vehicle also performed at chance at the five hours 15 minutes delay, although this would appear to be an anomaly in an otherwise strong performance (see figure 6.1a). All other groups performed above chance at all delays (see table 6.1).

D1

Post hoc analysis of the main effect of drug ($F = 4.470$, $df = 3,36$, $p < 0.01$) revealed that the scopolamine + tween group was impaired compared to the vehicle + tween group ($p < 0.01$) and compared with the nimodipine 10 mg/kg + scopolamine group ($p < 0.05$). There was also a main effect of delay ($F = 15.318$, $df = 3,108$ $p < 0.001$) although there was no interaction between drug and delay ($F = 1.15$, $df = 9,108$, $p > 0.05$). As with the D2 measure, the scopolamine + tween group did not appear to be

impaired compared with the other groups at the 29 hour 15 minute delay (see figure 6.1a). It seems probable that the failure to find an interaction was due to the variance in the control groups.

This interpretation was supported by analysis of the absolute performance of the groups, which showed that the scopolamine group performed at chance at all delays except the longest 29 hour 15 minute delay, whilst all other groups were above chance at all delays (see table 6.1).



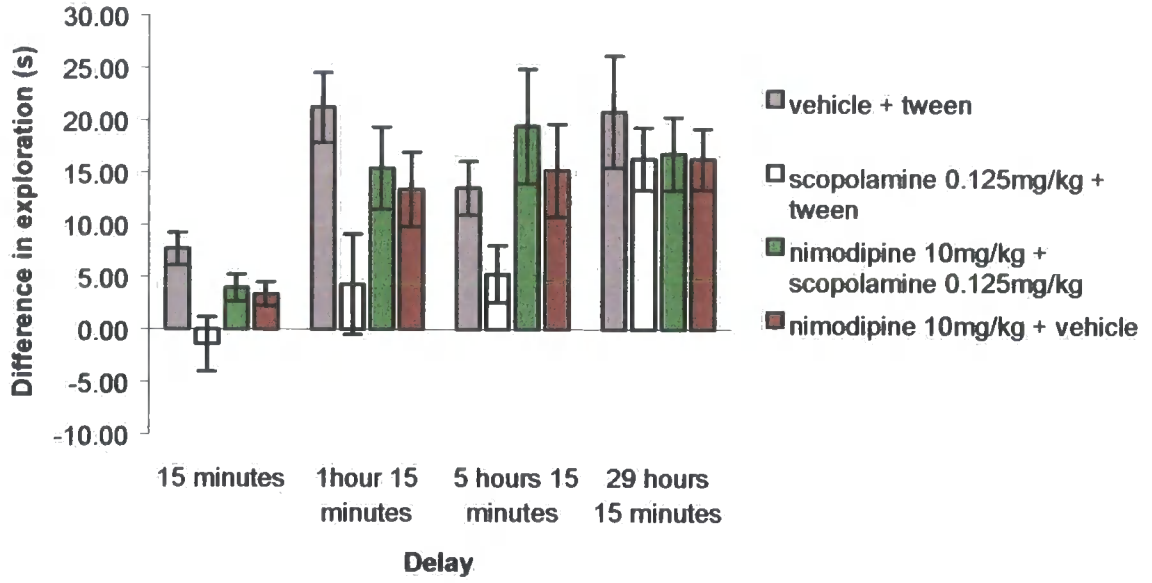


Figure 6.1: Discrimination when drugs were administered 15 minutes before initial exploration. Nimodipine at 10 mg/kg. (a) D2 measure. (b) D1 measure.

15 minutes						
Measure	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle + tween	8.122	<0.001	Yes	5.190	0.001	Yes
Scopolamine 0.125mg/kg + tween	-0.489	0.637	**No**	-0.567	0.584	**No**
Nimodipine 10mg/kg + scopolamine 0.125mg/kg	3.261	0.010	Yes	3.303	0.009	Yes
Nimodipine 10mg/kg + vehicle	3.126	0.012	Yes	3.219	0.011	Yes
1 hour 15 minutes						
Measure	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle + tween	15.027	<0.001	Yes	6.713	<0.001	Yes
Scopolamine 0.125mg/kg + tween	-0.109	0.916	**No**	0.948	0.368	**No**
Nimodipine 10mg/kg + scopolamine 0.125mg/kg	4.512	0.001	Yes	4.128	0.003	Yes
Nimodipine 10mg/kg + vehicle	9.404	<0.001	Yes	3.976	0.003	Yes
5 hours 15 minutes						
Measure	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle + tween	5.892	<0.001	Yes	5.560	<0.001	Yes
Scopolamine 0.125mg/kg + tween	1.150	0.280	**No**	2.066	0.069	**No**
Nimodipine 10mg/kg + scopolamine 0.125mg/kg	5.974	<0.001	Yes	3.742	0.005	Yes
Nimodipine 10mg/kg + vehicle	1.372	0.203	**No**	3.612	0.006	Yes
29 hours 15 minutes						
Measure	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle + tween	9.985	<0.001	Yes	4.102	0.003	Yes
Scopolamine 0.125mg/kg + tween	9.793	<0.001	Yes	5.731	<0.001	Yes
Nimodipine 10mg/kg + scopolamine 0.125mg/kg	10.072	<0.001	Yes	5.077	0.001	Yes
Nimodipine 10mg/kg + vehicle	18.471	<0.001	Yes	5.869	<0.001	Yes

Table 6.1: One-sample T-tests for each drug group at each delay with nimodipine at 10 mg/kg in an acquisition paradigm. "Differ" indicates whether performance differed from chance.

Total Exploration

The drug groups did not differ ($F = 1.663$, $df = 3,36$, $p > 0.05$). There was a main effect of delay ($F = 12.658$, $df = 3,108$, $p < 0.001$) but no interaction between delay and lesion ($F < 1$, $df = 9,108$, $p > 0.05$) as all groups explored the objects more with increasing delay.

Summary

The results of this experiment demonstrate that whilst the nimodipine 10 mg/kg + scopolamine group was unimpaired at all delays, the scopolamine + tween group was impaired relative to the control groups. At the first three delays the scopolamine + tween group did not differ from chance.

Experiment 6.2: Acquisition Nimodipine 1 mg/kg

The drug groups differed on both the D2 ($F = 10.69$, $df = 3,42$, $p < 0.001$) and the D1 ($F= 9.52$, $df = 3,42$, $p < 0.001$) measures. As the results on the two measures differ somewhat, they are presented separately.

D2

Post hoc analysis of the main effect of drug ($F = 10.69$, $df = 3,42$, $p < 0.001$) showed that the scopolamine + tween group was impaired compared with the vehicle + tween and the nimodipine 1 mg/kg + scopolamine groups ($p < 0.001$). The nimodipine 1 mg/kg + vehicle group was also impaired compared with the vehicle + tween and the nimodipine 1 mg/kg + scopolamine groups ($p < 0.05$). Although there was no main effect of delay ($F = 1.25$, $df = 3,126$, $p > 0.05$), and no interaction between drug and delay ($F = 1.05$, $df = 9,126$, $p > 0.05$), it can be seen from figure 6.2a that the scopolamine deficit was not uniform across all delays with no apparent impairment at the longest delay.

This interpretation was confirmed by analysis of the absolute rather than the relative performance of the groups. One sample T-tests showed that the scopolamine + tween group performed at chance at the 15 minutes, the 1 hour 15 minutes and the 5 hours 15 minutes delays (see table 6.2). The nimodipine 1 mg/kg + vehicle group also performed at chance at the 15 minutes, the 1 hour 15 minutes, and the 29 hour 15 minutes delays. This poor performance by the nimodipine 1 mg/kg + vehicle group as well as the chance performance of the scopolamine + tween group was surprising,

particularly in view of the strong performance of the nimodipine 1 mg/kg + scopolamine group. The fact that the nimodipine 1 mg/kg + scopolamine group performed above chance at all delays would seem to indicate that nimodipine at 1 mg/kg prevented a scopolamine-induced performance decrement.

D1

Post hoc analysis of the main effect of drug ($F= 9.52$, $df = 3,42$, $p < 0.001$) showed that the scopolamine 0.125 mg/kg + tween group was impaired compared with the vehicle + tween ($p < 0.001$), with the nimodipine 1 mg/kg + scopolamine 0.125 mg/kg group ($p < 0.001$) and with the nimodipine 1 mg/kg + vehicle group ($p < 0.01$). There was no main effect of delay ($F = 1.713$, $df = 2.49,129$ $p > 0.05$) and no interaction between drug and delay ($F < 1$, $df = 7.49,129$, $p > 0.05$).

Analysis of the absolute performance of the groups showed that, as can be seen from figure 6.2b, the scopolamine + tween group performed at chance at delays of 15 minutes, 1 hour 15 minutes and 5 hours 15 minutes delays, while the nimodipine 1 mg/kg + vehicle group performed at chance at the 15 minute delay. In contrast to the scopolamine + tween group, the nimodipine 1 mg/kg + scopolamine group performed above chance at all delays, indicating that nimodipine prevented the scopolamine-induced performance decrement whenever it occurred (see table 6.2).

Exploration

There was no effect of drug group ($F < 1$, $df = 3,43$, $p > 0.05$). There was also no effect of delay ($F = 2.217$, $df = 3,129$, $p > 0.05$) and only a trend towards an interaction between drug group and delay ($F = 1.924$, $df = 9,129$, $p = 0.54$) which results from the tendency for all groups except the vehicle + tween group to explore the objects more with increasing delay.

Summary

The results of this experiment demonstrate that whilst the nimodipine 1 mg/kg + scopolamine group was unimpaired at all delays, the scopolamine + tween group was impaired at the first three delays in absolute terms. Although it was not possible to isolate the delays at which the scopolamine + tween group was impaired relative to the control groups, figures 6.2a and 6.2b indicate that this may be limited to the shortest delay.

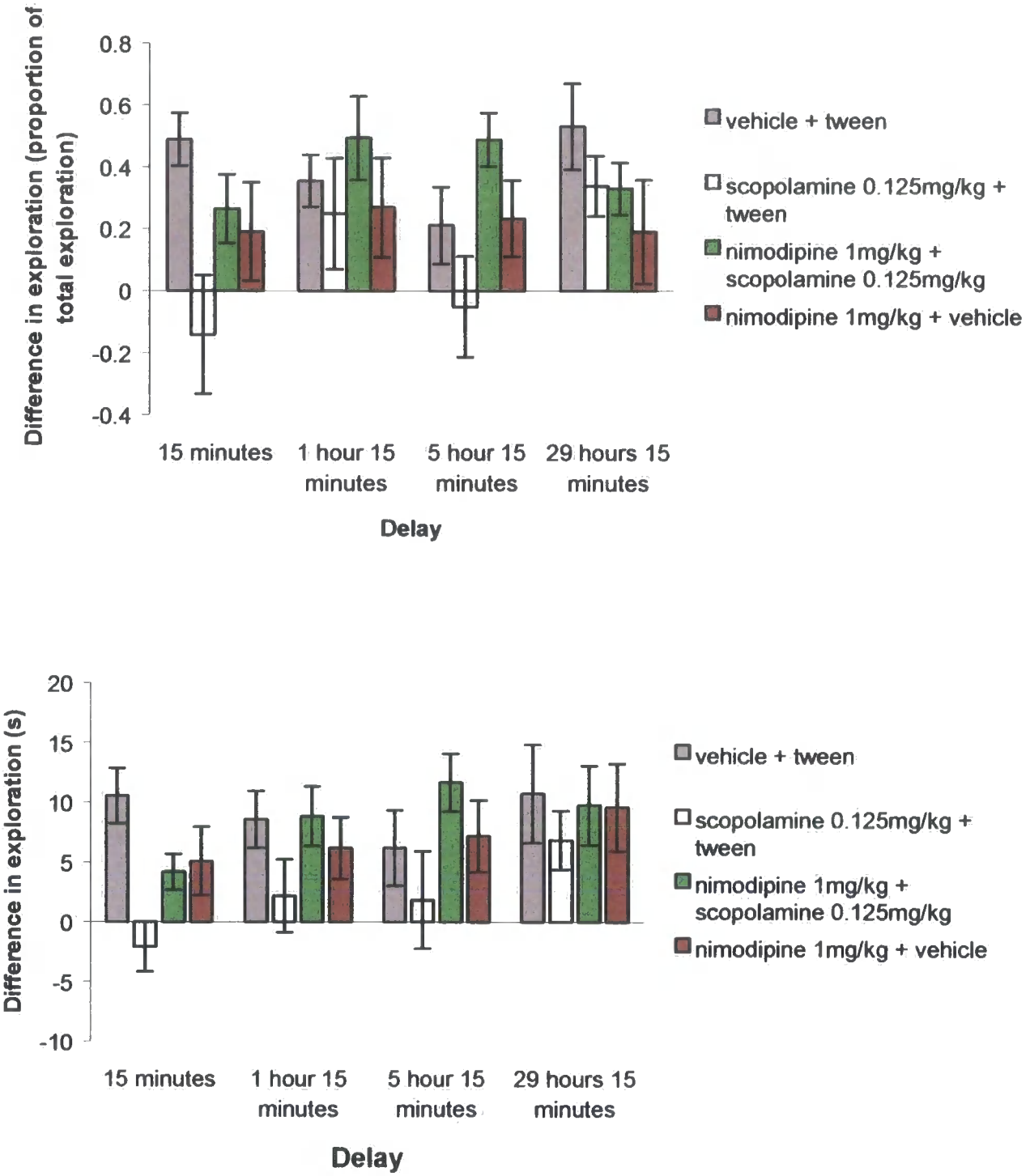


Figure 6.2: Discrimination when drugs were administered 15 minutes before initial exploration. Nimodipine at 1 mg/kg. (a) D2 measure (b) D1 measure.

15 minutes						
Measure	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle + tween	5.974	<0.001	Yes	4.801	0.001	Yes
Scopolamine 0.125mg/kg + tween	-0.773	0.456	**No**	-1.047	0.318	**No**
Nimodipine 1mg/kg + scopolamine 0.125mg/kg	2.500	0.030	Yes	2.863	0.015	Yes
Nimodipine 1mg/kg + vehicle	1.257	0.235	**No**	1.857	0.090	**No**
1 hour 15 minutes						
Measure	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle + tween	4.449	0.001	Yes	3.765	0.004	Yes
Scopolamine 0.125mg/kg + tween	1.445	0.176	**No**	0.739	0.475	**No**
Nimodipine 1mg/kg + scopolamine 0.125mg/kg	3.821	0.003	Yes	3.677	0.004	Yes
Nimodipine 1mg/kg + vehicle	1.759	0.106	**No**	2.502	0.029	Yes
5 hours 15 minutes						
Measure	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle + tween	1.788	0.104	**No**	2.055	0.067	**No**
Scopolamine 0.125mg/kg + tween	-0.335	0.744	**No**	0.471	0.647	**No**
Nimodipine 1mg/kg + scopolamine 0.125mg/kg	5.867	<0.001	Yes	5.935	<0.001	Yes
Nimodipine 1mg/kg + vehicle	1.978	0.074	**No**	2.488	0.030	Yes
29 hours 15 minutes						
Measure	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle + tween	4.008	0.002	Yes	2.745	0.021	Yes
Scopolamine 0.125mg/kg + tween	3.615	0.004	Yes	2.906	0.014	Yes
Nimodipine 1mg/kg + scopolamine 0.125mg/kg	4.160	0.002	Yes	3.060	0.011	Yes
Nimodipine 1mg/kg + vehicle	1.192	0.258	No	2.735	0.019	Yes

Table 6.2: One-sample T-tests for each drug group at each delay with nimodipine at 1 mg/kg in an acquisition paradigm. "Differ" indicates whether performance differed from chance.

Experiment 6.3: Nimodipine 10 mg/kg retrieval

The drug groups differed from each other on both the D2 ($F = 9.757$, $df = 3,28$, $p < 0.001$) and the D1 measures ($F = 5.135$, $df = 3,28$, $p < 0.01$). The results differ somewhat between the two measures so they are presented separately.

D2

Post hoc analysis of the main effect of lesion ($F = 9.757$, $df = 3,28$, $p < 0.001$) revealed that the scopolamine + tween group was impaired compared with the vehicle + tween group ($p < 0.01$) and that this group was also impaired compared with the nimodipine 10 mg/kg + vehicle ($p = 0.001$) and the nimodipine 10 mg/kg + scopolamine groups ($p < 0.001$). There were no other differences between the groups. There was only a trend towards an effect of delay ($F = 2.871$, $df = 2.31,84$, $p = 0.056$), and there was no interaction between delay and drug group ($F = 1.859$, $df = 6.94,84$, $p > 0.05$). As can be seen from figure 6.3a, the absence of an impairment at the 1 hour 15 minute delay would seem to be due to the poorer performance of the other groups, rather than to an improvement in the performance of the scopolamine + tween group.

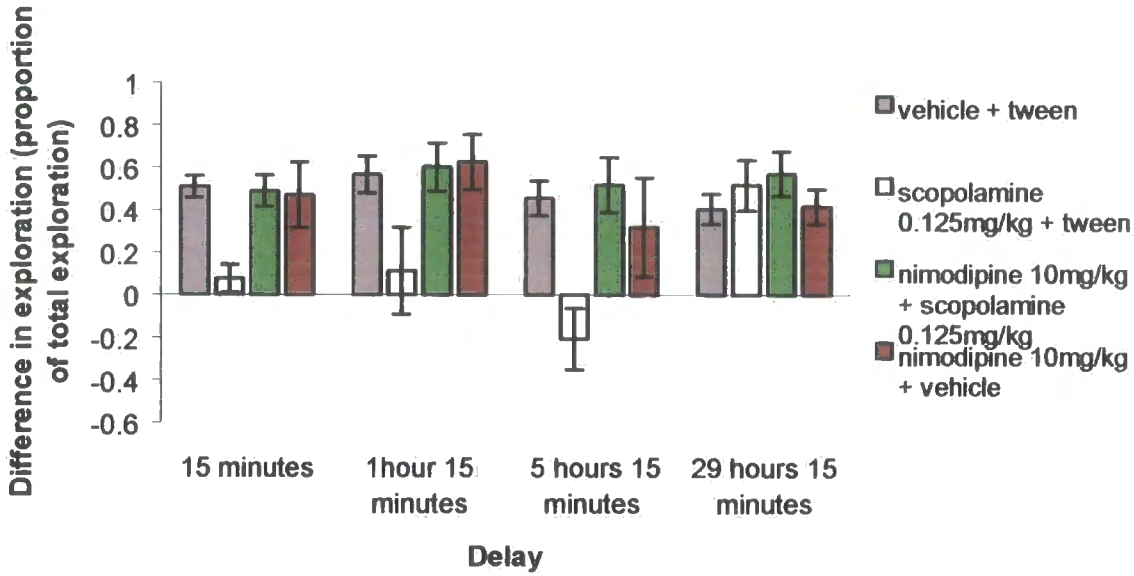
This view is supported by the analysis of the absolute performance of the groups (see table 6.3). One sample T-tests showed that the scopolamine + tween group did not differ from chance at delays of 15 minutes, 1 hour 15 minutes and 5 hours 15 minutes. They also showed that one of the control groups, the nimodipine 10 mg/kg + vehicle groups did not differ from chance at the 5 hour 15 minutes delay.

D1

Post hoc analysis of the main effect of lesion ($F = 5.14$, $df = 3,28$, $p < 0.01$) revealed that the scopolamine + tween group was impaired compared with the vehicle + tween group ($p < 0.05$) and that this group was also impaired compared with the nimodipine 10 mg/kg + vehicle and the nimodipine 10 mg/kg + scopolamine groups (in both cases $p < 0.05$). There were no other differences between the groups. There was no effect of delay ($F = 1.812$, $df = 2.32,84$, $p > 0.05$) and no interaction between drug and delay ($F = 1.59$, $df = 6.95,84$, $p > 0.05$). As can be seen from figure 6.3b, the scopolamine + tween group did not appear to be impaired at the longest delay, although there was an appearance of impairment at the shorter delays.

This view is supported by the analysis of the absolute performance of the groups (see table 6.3). One sample T-tests showed that the scopolamine + tween group did not

differ from chance at delays of 15 minutes, 1 hour 15 minutes and 5 hours 15 minutes, whilst all other groups differed from chance at all delays.



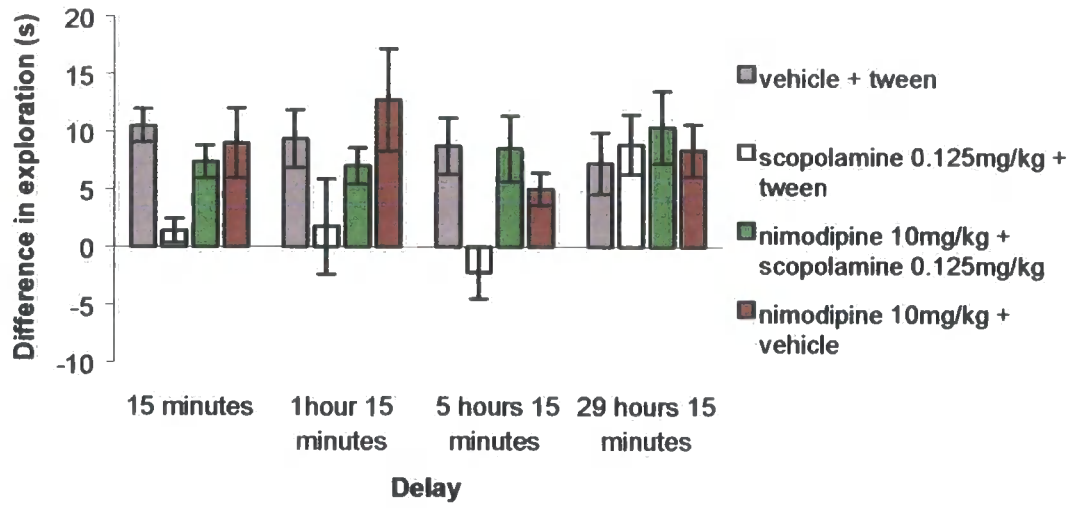
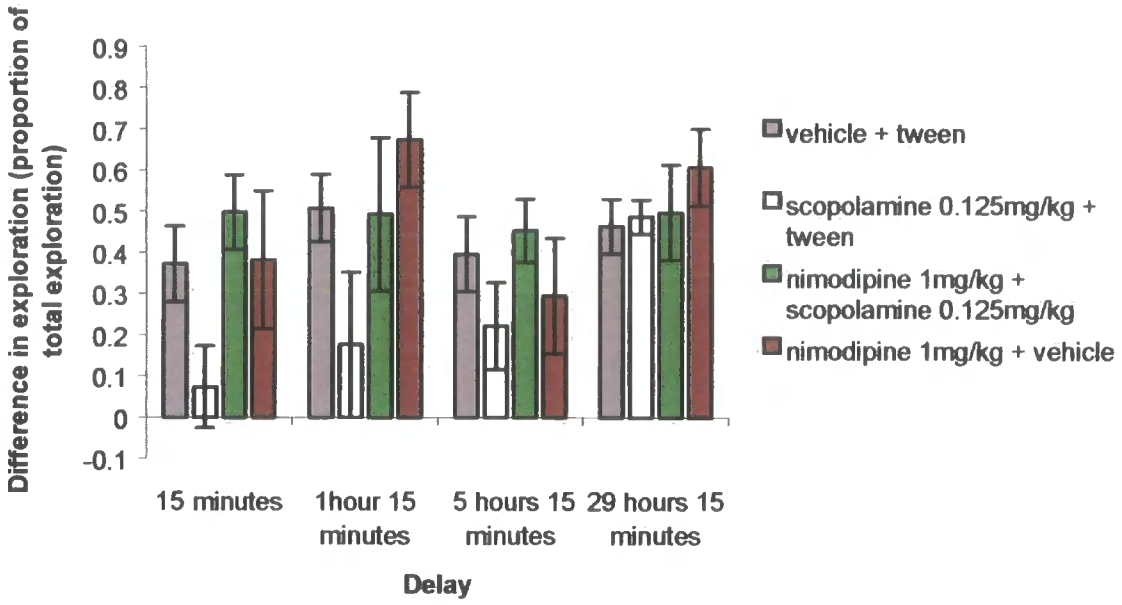


Figure 6.3: Discrimination when drugs were administered immediately after initial exploration. Nimodipine at 10 mg/kg. (a) D2 measure. (b) D1 measure.



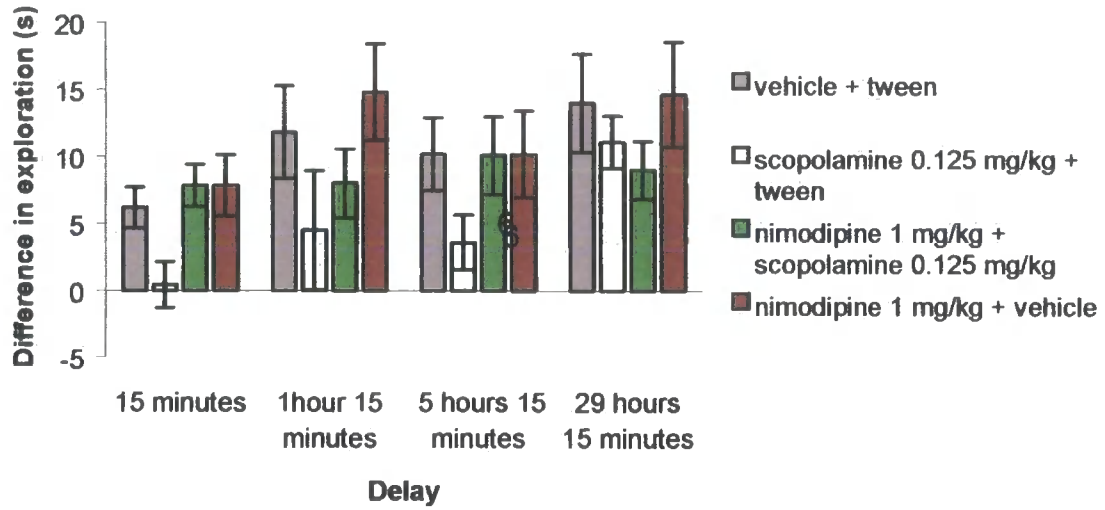
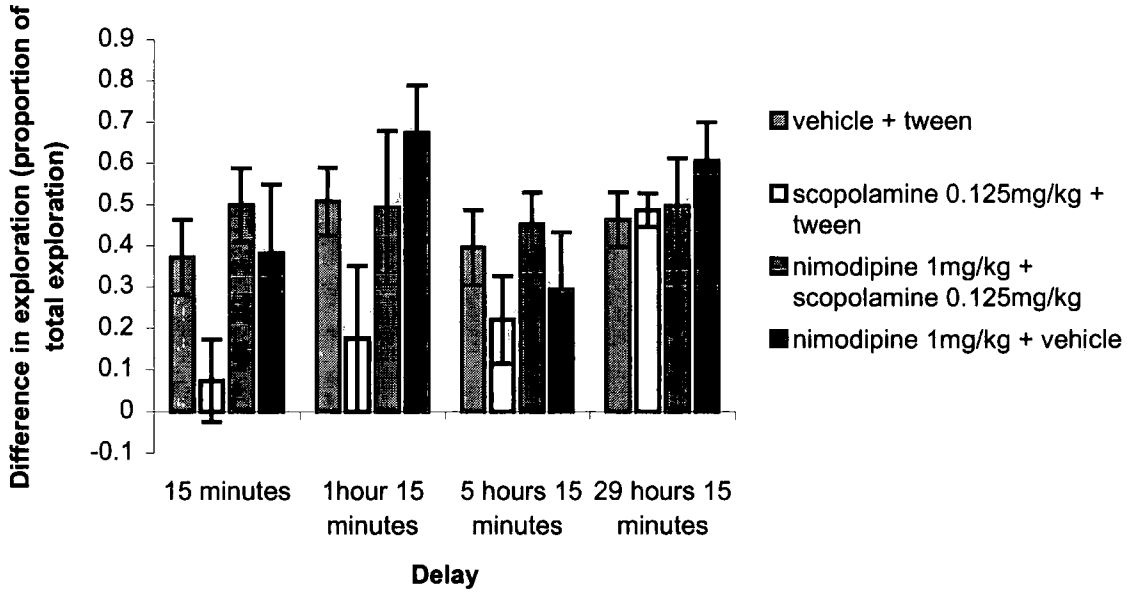


Figure 6.4: Discrimination when drugs were administered immediately after initial exploration. Nimodipine at 1 mg/kg. (a) D2 measure. (b) D1 measure.



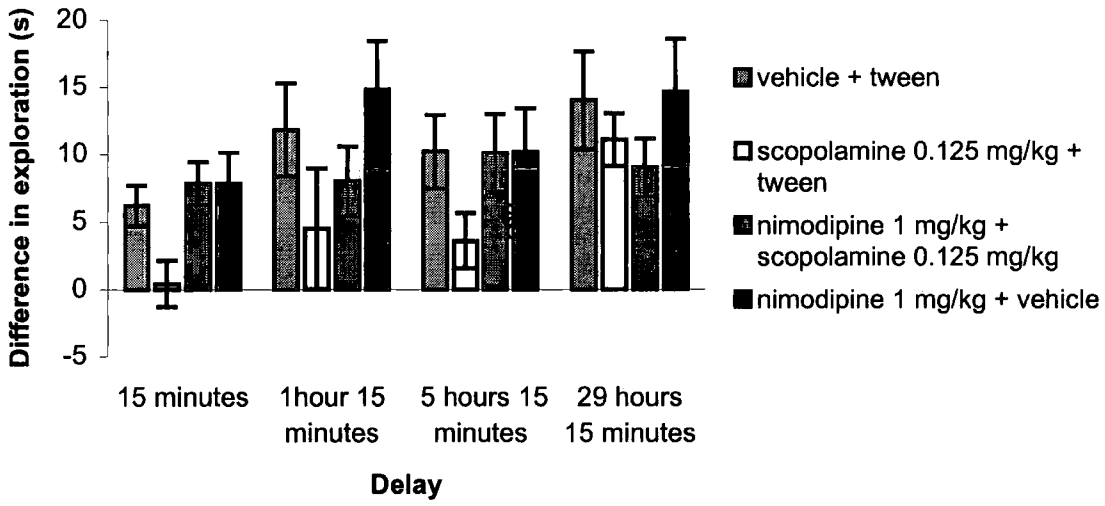


Figure 6.4: Discrimination when drugs were administered immediately after initial exploration. Nimodipine at 1 mg/kg. (a) D2 measure. (b) D1 measure.

15 minutes						
Measure	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle + tween	4.271	0.002	Yes	4.260	0.002	Yes
Scopolamine 0.125mg/kg + tween	0.771	0.457	**No**	0.255	0.804	**No**
Nimodipine 1mg/kg + scopolamine 0.125mg/kg	5.767	<0.001	Yes	5.148	<0.001	Yes
Nimodipine 1mg/kg + vehicle	2.387	0.036	Yes	3.558	0.004	Yes
1 hour 15 minutes						
Measure	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle + tween	6.508	<0.001	Yes	3.594	0.005	Yes
Scopolamine 0.125mg/kg + tween	1.035	0.323	**No**	1.052	0.315	**No**
Nimodipine 1mg/kg + scopolamine 0.125mg/kg	2.760	0.019	Yes	3.258	0.008	Yes
Nimodipine 1mg/kg + vehicle	6.158	<0.001	Yes	4.293	0.001	Yes
5 hours 15 minutes						
Measure	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle + tween	3.971	0.003	Yes	3.359	0.007	Yes
Scopolamine 0.125mg/kg + tween	2.219	0.048	Yes	2.061	0.064	**No**
Nimodipine 1mg/kg + scopolamine 0.125mg/kg	6.252	<0.001	Yes	3.626	0.004	Yes
Nimodipine 1mg/kg + vehicle	2.199	**0.050**	**No**	3.265	0.008	Yes
29 hours 15 minutes						
Measure	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle + tween	7.391	<0.001	Yes	4.017	0.002	Yes
Scopolamine 0.125mg/kg + tween	12.486	<0.001	Yes	5.944	<0.001	Yes
Nimodipine 1mg/kg + scopolamine 0.125mg/kg	4.459	<0.001	Yes	4.377	0.001	Yes
Nimodipine 1mg/kg + vehicle	6.728	<0.001	Yes	3.919	0.002	Yes

Table 6.4: One-sample T-tests for each drug group at each delay with nimodipine at 1 mg/kg in a retrieval paradigm. "Differ" indicates whether performance differed from chance.

Experiment 6.5: Acquisition: testing only at 29 hours 15 minutes delay.

This experiment was designed to establish whether the recovery of the scopolamine + tween group's performance seen at the longest delay in experiments 1 and 2 resulted from the disappearance of the scopolamine's effects or from learning which took place at the 5 hour 15 minutes test when those effects were diminishing. As can be seen in figures 6.5a and 6.5b, the groups differed due to an impairment of the scopolamine group compared to the vehicle group on the D2 measure ($T = 1.97$, $df = 30$, $p < 0.05$) and there was a trend for the groups to differ on the D1 measure ($T = 2.66$, $df = 30$, $p = 0.057$). The reported statistics are for a one-tailed T-test because there was a well-founded prediction, which was that the scopolamine group would be impaired compared with the vehicle group. As table 6.5 shows, this prediction was further confirmed by analysis of absolute performance which showed that the scopolamine group did not differ from chance on either measure, whilst the vehicle group was performing above chance.

	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle	2.236	0.041	Yes	2.536	0.023	Yes
Scopolamine 0.125mg/kg	-0.738	0.472	No	0.053	0.959	No

Table 6.5: One-sample T-tests for both groups on a 29 hour test in an acquisition paradigm. D1 and D2 measures. "Differ" indicates difference from chance.

Exploration

The groups did not differ in their exploration of the objects ($T = 0.97$, $df = 30$, $p > 0.05$).

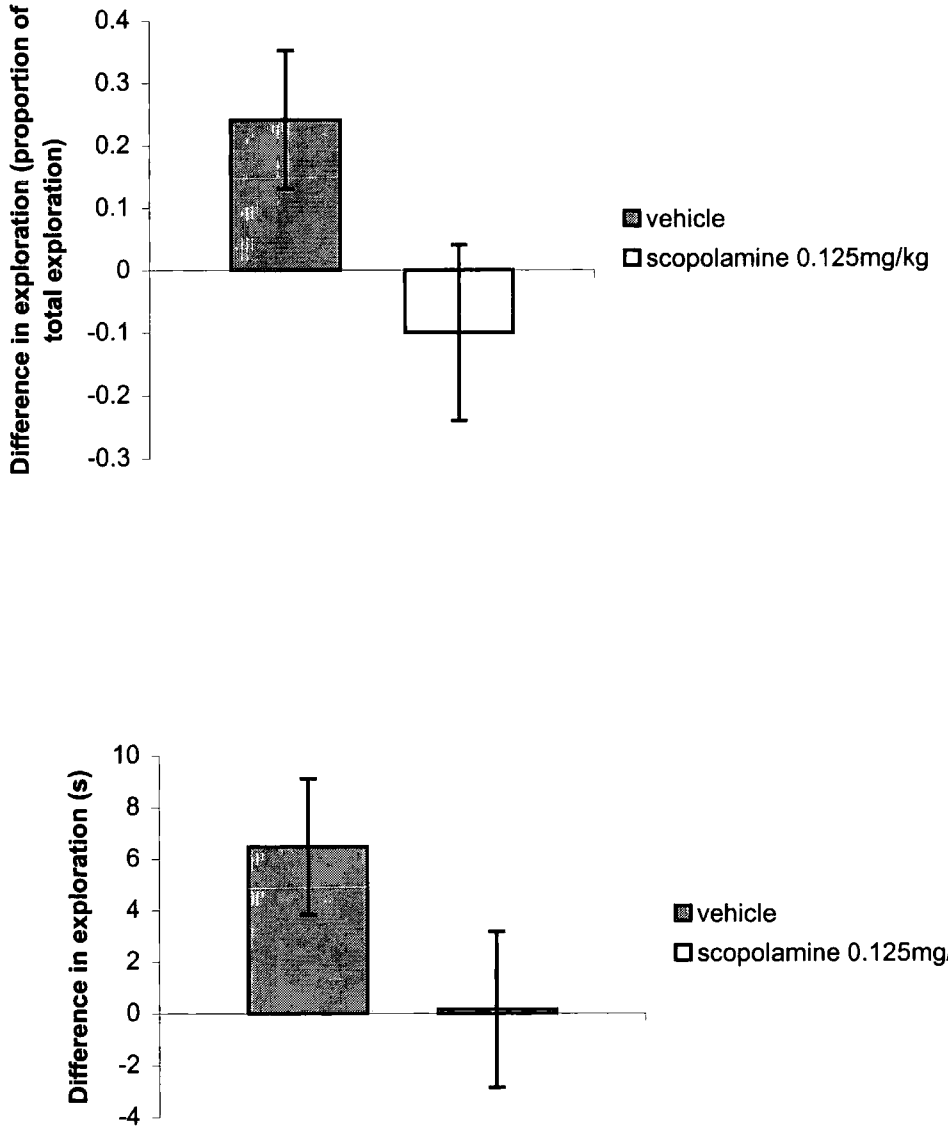


Figure 6.5: Discrimination when drugs were administered 15 minutes before initial exploration. Testing only at delay of 29 hours 15 minutes. (a) D2 measure. (b) D1 measure.

Experiment 6.6: Retrieval: testing only at 29 hours 15 minutes delay.

This experiment was designed to establish whether the recovery of the scopolamine + tween group's performance seen at the longest delay in experiments 3 and 4 resulted from the disappearance of the scopolamine's effects or from learning which took place at the 5 hour 15 minutes test when those effects were diminishing. As can be seen from figures 6a and 6b, there were no differences between the scopolamine and the vehicle groups, on either the D1 ($T = 0.76$, $df = 14$, $p > 0.05$) and D2 ($T = 1.21$, $df = 9.82$, $p > 0.05$) measures. One sample T-tests also showed that both groups were above chance on both measures (see table 6.6). This would indicate that the recovery seen in experiments 3 and 4 resulted from the disappearance of the scopolamine's effects.

	D2			D1		
Group	T	P	Differ	T	P	Differ
Vehicle	3.089	0.018	Yes	2.525	0.040	Yes
Scopolamine 0.125mg/kg	2.742	0.029	Yes	3.699	0.008	Yes

Table 6.6: One-sample T-tests for both groups on a 29 hour test in a retrieval paradigm. D1 and D2 measures. "Differ" indicates difference from chance.

Exploration

There was no difference between the groups in their exploration of the objects ($T = 0.40$, $df = 14$, $p > 0.05$).

Summary of experiments 6.5 and 6.6

When testing only took place at a delay of 29 hours 15 minutes the scopolamine group was impaired compared to the vehicle group in the acquisition paradigm (experiment 6.5) and performed at chance. However, in the retrieval paradigm (experiment 6.6) the groups did not differ and both performed above chance.

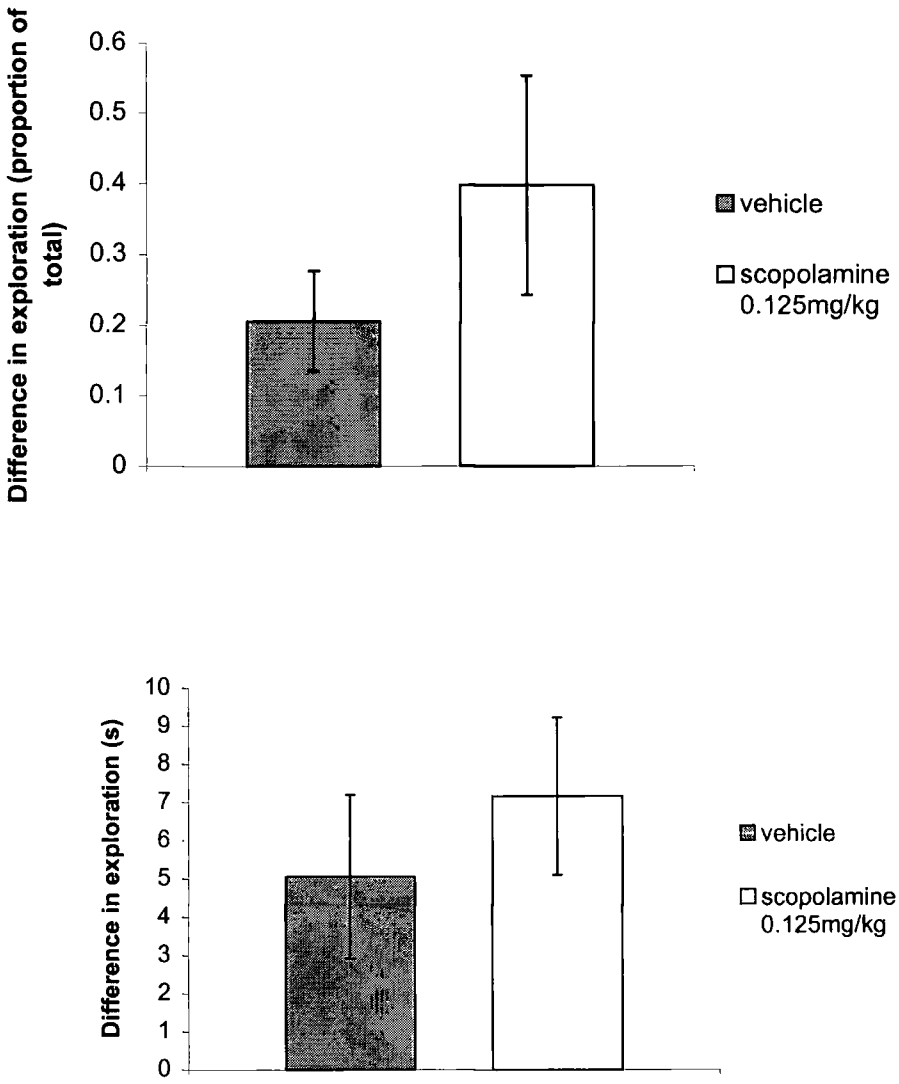


Figure 6.6: Discrimination when drugs were administered immediately after initial exploration. Testing only at delay of 29 hours 15 minutes. (a) D2 measure. (b) D1 measure.

Experiment 6.7: Locomotor Activity

The groups did not differ from each other overall ($F < 1$, $df = 1,5$, $p > 0.05$). There was, however, a strong effect of delay ($F = 20$, $df = 20,210$, $p < 0.001$) and an interaction between delay and drug group ($F = 2.453$, $df = 15,5$, $p < 0.01$). Analysis using simple effects revealed that the groups did not differ at any of the delays used. As can be seen from figure 6.7, any significance in the repeated measures ANOVA would appear to come from the lower activity shown by the nimodipine 10 mg/kg + scopolamine group at the 1 hour 15 minutes and the 5 hour 15 minutes delays.

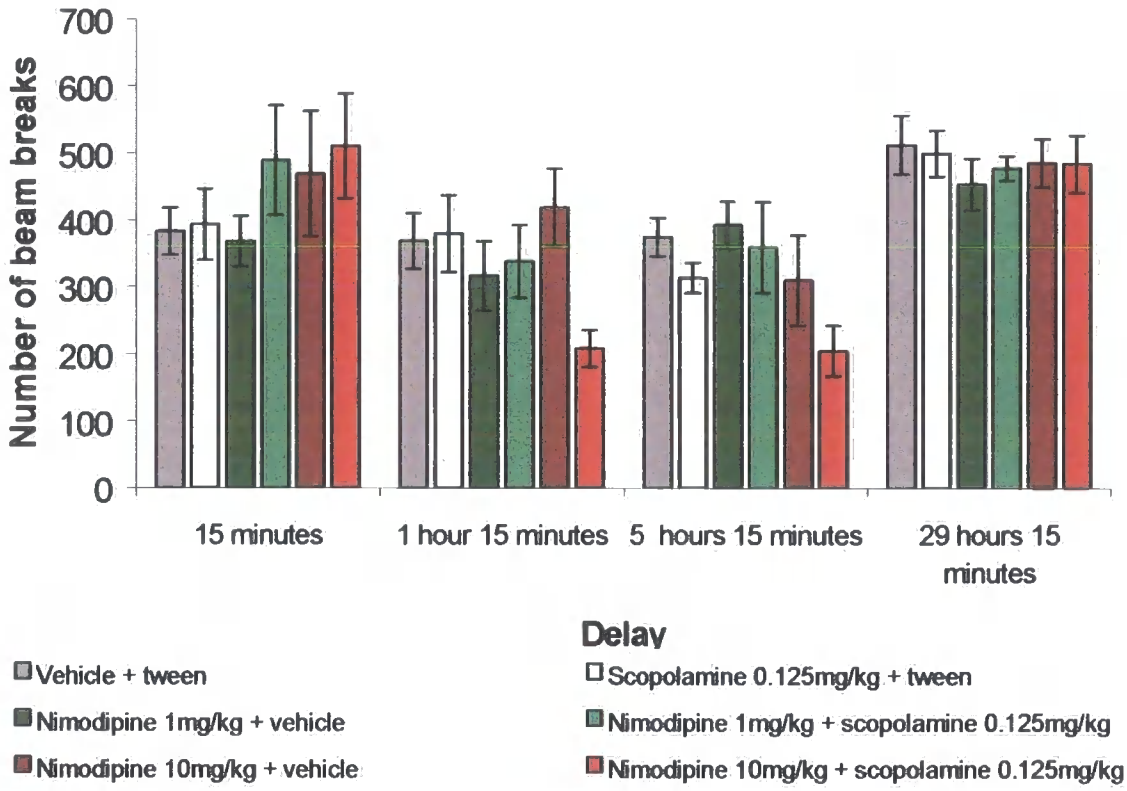


Figure 6.7: Number of consecutive beam breaks over a 10 minute period by animals in each of the 6 drug groups used in experiments 1-4.

6.4: Discussion

In the acquisition experiments (experiments 6.1 and 6.2), when both drugs were given 15 minutes before the initial exploration of the familiar object, nimodipine at both 10 mg/kg and at 1 mg/kg prevented the scopolamine-induced impairment. In the retrieval

experiments (experiments 6.3 and 6.4), when both drugs were given immediately after the initial exploration in the first exposure to the objects, nimodipine at both 10 mg/kg and at 1 mg/kg prevented scopolamine-induced impairment in retrieval of information about the familiar object. In each case, the higher dose of nimodipine had a longer and more consistent duration of effect. Nimodipine therefore completely prevented the effects of scopolamine in both in the retrieval and in the acquisition paradigms. The higher dose of nimodipine, 10 mg/kg, was in the same range as that reported in other studies (see Introduction) to have beneficial effects on learning and memory. The 1 mg/kg nimodipine dose clearly had some effect, but less than the higher dose.

The retrieval impairment produced by scopolamine corresponds well with that reported by Bartolini et al. (1996), in that it resulted from a relatively low dose compared with the 5 mg/kg found by Ennaceur and Meliani (1992) to be necessary. The duration of the impairment we report is also in keeping with that found in other studies which report impairments persisting at delays of 60 minutes but not of 24 hours (Ennaceur and Meliani, 1992; Bartolini et al., 1996).

On the basis of these experiments, it would be impossible to determine the effects of nimodipine on an acquisition deficit, as administration of scopolamine before initial exploration failed to produce a performance deficit at a delay of 29 hours 15 minutes. This might be taken to indicate that scopolamine administered 15 minutes before experience does not prevent memory formation, but merely renders the memories formed inaccessible for the time-course of scopolamine's action. However, another explanation is that acquisition of a representation of the familiar object took place at the 5 hour 15 minute test, when the action of scopolamine was diminishing, and it was as a result of this that the animals in the scopolamine 0.125 mg/kg group discriminated between the novel and familiar objects in the final 29 hour 15 minute test. This second interpretation is supported by the contrasting results of experiments 6.5 and 6.6 which used acquisition and retrieval paradigms respectively and tested only at a delay of 29 hours and 15 minutes. In experiment 6.5 the scopolamine 0.125 mg/kg group were impaired compared to the vehicle group and performed at chance, while in experiment 6.6 the two groups did not differ, and both discriminated between the novel and familiar objects. Therefore the results of this study support the view that scopolamine impairs both acquisition and retrieval of object memory, but must be

administered prior to the initial exploration of the objects in order to block acquisition.

It is unlikely that the effects of scopolamine were due to alterations of motor co-ordination or alertness, since the total exploration time in the object recognition test was not significantly altered. This contrasts with the Bartolini et al.'s (1996) and Ennaceur and Meliani's (1992) studies, which found decreases in exploration at the exposure and test sessions respectively. This is probably due to differences in intervals between injection and test and to differences in dose. Bartolini et al. used a comparable dose of scopolamine (0.20 mg/kg), but administered it 60 minutes prior to initial exposure, whilst Ennaceur & Meliani used a dose of 0.50 mg/kg and administered it 30 minutes prior to exposure.

When locomotor activity was measured at the post-injection delays used in the object recognition task, a difference was found only at 75 minutes post-injection when scopolamine was administered in conjunction with nimodipine at 10 mg/kg: this group differed from that given nimodipine 10 mg/kg and vehicle. This difference represented a reduction in activity, rather than the increase that would normally result from administration of scopolamine. Such an effect is clearly incapable of explaining the effects we report not merely at 75 minutes, but also at 15 minutes and 5 hours 15 minutes.

When given alone nimodipine neither altered behaviour in the object recognition test nor had any effect on locomotor activity. This was not due to a ceiling effect, as there was scope for measurement of improvements in memory, as well as deficits, in the test. The majority of studies have examined alleviation of memory and learning deficits by dihydropyridine calcium channel antagonists, but as discussed previously, Deyo et al. (1989) and McMonangle-Strucko and Fanelli (1993), Vetulani et al., (1997), and Kane and Robinson (1999) all showed beneficial effects of nimodipine on learning in the absence of any specific memory deficits.

It has not yet been fully established whether the anti-amnesic actions of nimodipine are due to neuronal actions or increased cerebral blood flow, but there is strong evidence indicating the former mechanism. The distribution of high affinity

dihydropyridine binding sites in the CNS is consistent with a neuronal rather than a vascular location. Parallel electrophysiological studies have demonstrated effects of nimodipine on hippocampal neurones that may be related to the improvements in learning and memory (Disterhoft, Moyer, Thompson & Kowalska, 1993). Administration of the dihydropyridine nifedipine directly into the hippocampus also had a beneficial effect on avoidance learning (Quevedo, Vianna, Daroit & Born et al., 1998). In addition, the dose relationship of the effects of nimodipine on avoidance learning in rabbits did not parallel that of changes in cerebral blood flow.

It is particularly interesting that we have found nimodipine preventing scopolamine-induced amnesic effects on an object memory task. The bulk of research on dihydropyridines has concentrated either on conditioning (e.g. eye-blink conditioning (Disterhoft, Golden, Read & Coulter et al., 1988)) or on forms of learning which are dependent on the septo-hippocampal system, and especially on spatial tasks such as the Morris maze (Bannon, McMonaglestrucko & Fanelli, 1993). There is a considerable, if sometimes contradictory, body of work, which implicates damage to the hippocampus, and its subcortical connections in spatial reference memory impairments, and nimodipine has been found to ameliorate or eradicate these impairments. However, as discussed extensively in previous chapters, the object recognition task is dependent on the integrity, not of the hippocampus, but of the rhinal cortices, and, specifically, of the perirhinal cortex.

While it is undoubtedly true that deficits in object recognition in animals can be obtained as a result of hippocampal lesions, relatively sophisticated tasks which require primates to perform tasks which engage episodic-like memory, such as the object-in-scene test (Gaffan & Harrison, 1989) are necessary. Spontaneous object recognition, however, is impaired by lesions to the perirhinal cortex (Ennaceur et al., 1996), with a more restricted contribution from the entorhinal cortex (Leonard et al., 1995; Meunier et al., 1993). In the light of this, any potential mechanism for the antagonism by nimodipine of the action of scopolamine is strengthened because this action is shown not to be limited to the hippocampus and its subcortical connections, but were also capable of including the perirhinal cortex.

Models of Alzheimer's disease have linked intra-cerebral infusions of β-amyloid to reductions in acetylcholine as well as dopamine (Nag, Yee & Tang, 1999), and there is clear evidence that spatial memory tasks which depend on the integrity of the septo-hippocampal system are severely disrupted by such β-amyloid infusions (Chen, Wright & Barnes, 1996; O'Hare, Weldon, Mantyh & Ghilardi et al., 1999; Sweeney, Luedtke, McDonald & Overmier, 1997). However, the object recognition task is not primarily dependent on hippocampal integrity (Ennaceur 1998; Aggleton and Brown, 1999), and performance on this has also been shown to be impaired by β-amyloid (Nag, Tang & Yee, 2001).

The impairments caused by β-amyloid cannot with certainty be ascribed to damage to cholinergic systems without further work. Nag et al's (2001) study found that such deficits were not reversible by administration of physostigmine, as might have been expected if the impairment were due to reduced synaptic levels of acetylcholine. However, as Ennaceur and Meliani (1992) demonstrated, the effect of physostigmine alone is not straightforward. The success of the cholinesterase inhibitor tacrine in reversing both reduced levels of extracellular acetylcholine and memory impairments on the object recognition and step-through tasks (Scali, Giovannini, Prosperi & Bartolini et al., 1997) supports the role of an intact cholinergic system in episodic memory in the rat. Nootropic drugs (piracetam and pramiracetam) have been shown to improve performance on the object recognition test (Ennaceur et al., 1989). Further evidence that the integrity of the cholinergic system is crucial to memory, not least non-spatial memory, is provided by the fact that such improvements co-occur with improvements in extracellular acetylcholine levels in previously hypocholinergic aged rats. This is the case when animals are treated, either with nootropic drugs such as aniracetam (Bartolini et al., 1996), or with intracerebroventricular perfusions of neural growth factor (Scali, Casamenti, Pazzagli & Bartolini et al., 1994). Aniracetam also reversed the deficit in object recognition performance, which resulted from scopolamine treatment.

Although there have been many studies demonstrating the anti-amnesic actions of nimodipine and other dihydropyridine calcium channel antagonists, the mechanism of this action is not yet fully understood. Some causes of cognitive deficits in which

dihydropyridines had beneficial effects, for example administration of glucocorticoids (Dachir, Kadar, Robinzon & Levy, 1997), and brain trauma (Westernbroek, Bausch, Lin & Franck et al., 1998), have been associated with increased calcium flux through voltage-activated channels. In old age, increased calcium-dependent hyperpolarisation has been suggested to be involved in the deficits in memory and learning (Campbell, Hao, Thibault & Balock et al., 1996), and this phenomenon is decreased by dihydropyridine calcium channel antagonists (Disterhoft et al., 1993; Norris, Halpain & Foster, 1998). If this hyperpolarisation were linked to the diffuse decrease in cholinergic transmission in aged brains (Pepeu & Giovannelli, 1994) then it would be reasonable to assume that it was nimodipine's direct action on the voltage-gated calcium channels of cholinergic neurons which was responsible.

That this is in fact the case is suggested by the fact that endogenous acetylcholine reduces the amplitude of L-type calcium current in cells transfected with muscarinic receptors. Different receptor subtypes appear to mediate this action by different mechanisms; with m2 receptors and m4 receptors inhibiting L-type calcium conductance through reduction of cAMP concentration, and m1, m3 and m5 receptors producing a reduction by their activation of PKC (Pemberton and Jones, 1997). As scopolamine is a muscarinic antagonist, and N-methyl-scopolamine binds to subtype m2 - m5 receptors (Tayebati, Codini, Gallai & Mannino et al., 1999) in both hippocampus and cortex, its application would be predicted to increase L-type calcium flux through both the proposed mechanisms. Nimodipine, as an L-type calcium channel antagonist, has an action which opposes that of scopolamine by preventing the excessive calcium current induced by scopolamine. As excessive calcium flow is known to be detrimental to learning and memory this is a plausible mechanism for the prevention of scopolamine-induced deficit by nimodipine. Such a mechanism would be predicted to occur in both hippocampal tissue and in cerebral cortex.

In summary, at a dose of 0.125 mg/kg, scopolamine caused clear effects in tests of both acquisition and retrieval. The fact that animals given scopolamine 0.125 mg/kg + tween in the acquisition task showed no deficit at the longest delay does not, as might at first be thought, suggest that we failed to block acquisition of information but rather that acquisition took place during a later test phase. When only one test was given at a

delay of 29 hours 15 minutes, scopolamine caused no deficits in a retrieval paradigm but a severe impairment in an acquisition paradigm. This would indicate that the lack of impairment of the scopolamine + tween group at 29 hours 15 minutes in experiments 1 and 2 resulted from new learning at earlier tests rather than from a genuine recovery. Nimodipine at 10 mg/kg prevented the deficits caused by scopolamine, while a smaller preventative effect was found for a dose of 1 mg/kg.

Nimodipine appears to have a protective effect on spontaneous object recognition which is otherwise impaired by scopolamine. This is the case when both drugs are administered prior to the exploration phase of the object recognition task and when both drugs are administered immediately following this exploration phase. This study confirms previous findings (Brooks et al., 2002) that nimodipine is able to prevent impairments on the spontaneous object recognition task. This is indicative of the fact that its effects are not limited to tasks which are primarily dependent on subcortical structures as might be indicated by previous work (Nelson et al., 1992; Weichman et al., 1994; Finger et al., 1990; Hoffmeister et al., 1982). Dachir et al., 1995). It is argued that the preventative effect found in this study results from nimodipine preventing excessive calcium flux induced by scopolamine's antagonism at muscarinic receptors. The general nature of nimodipine's efficacy and, in particular, the question of whether its action in the intact brain differs from that exerted on the damaged brain remain issues which require further work.

Chapter 7: General Discussion

7.1 Introduction

This discussion aims to give an overview of the findings and conclusions presented in the thesis and to discuss possibilities for future work which are suggested by these findings. A summary of the main results reported is provided in section 7.2 followed by a discussion of the implications and possible future work suggested by each broad area of findings. Section 7.3 deals with the implications for our understanding of the function of perirhinal cortex in the rat of the experiments presented here. Section 7.4 considers the involvement of the postrhinal cortex and the hippocampus in memory for object-in-context. Section 7.5 examines the evidence presented for a memory system, involving the hippocampus and its subcortical connections, which is engaged by situations which are episodic-like. Section 7.6 briefly considers the implications of the findings of chapter 6, that nimodipine prevents scopolamine-induced deficits in object recognition. Finally section 7.7 provides a broad conclusion to the work presented here and outlines some directions for future work which are suggested by it.

7.2 Summary of results

The prediction that perirhinal lesions would cause an impairment in the learning of visual-visual stimulus associations was not supported (experiment 2). On a number of measures perirhinal lesions were shown not to have a deleterious effect. The only evidence that there might be such an impairment was that the first post-operative problems produced a chance performance on trial 6 – the last trial before any primary reinforcement was received. However, the significance of this was negated by the fact that performance was above chance on the block of trials 2-6, for which poorer performance would have been expected. This in fact represented an improvement on pre-operative performance. In the light of this, the unimpaired performance over the whole problem and the lack of an effect of operated condition in the comparative analysis it must be concluded that lesions of the perirhinal cortex do not impair visual-visual associative learning.

Predictions that perirhinal lesions would cause impairments in object discrimination where configural processing was required were supported (Experiments 3.1-3.4). A clear relationship between the level of feature ambiguity in a discrimination and the severity of the perirhinal impairment was found. The lack of such a disproportionate

impairment in experiment 3.3, where difficulty was introduced by increasing the size of the stimulus set, suggested that the impairments observed in experiments 3.1, 3.2 and 3.4 were not merely the result of increased difficulty.

The prediction that lesions of both the fornix and the postrhinal cortex, but not of the perirhinal cortex, would cause impairments in memory for object-in-context were supported (experiments 4.1-4.4). However, the postrhinal impairment was more severe and more uniform than the fornix impairment. When a second object was used as a context there was a perirhinal impairment but no impairment in the other lesion groups. This therefore represents a double dissociation.

The prediction that both fornix and postrhinal lesions, but not perirhinal lesions would cause an impairment in the model of episodic-like memory was partially supported (experiment 5.1). There was a clear impairment of the fornix group but no other group was impaired. The fornix impairment was shown not merely a result of an impairment of memory for object-in-place, as experiment 5.2 showed no impairment of the fornix group on such a discrimination.

The prediction that nimodipine would prevent the scopolamine-induced deficit on object-recognition tasks was confirmed. The fact that it prevented deficits at a low dose (1 mg/kg; experiments 6.2 and 6.4) as well as at a higher dose (10 mg/kg; experiments 6.1 and 6.3) was interesting. Also of interest was the fact that it prevented impairments both of acquisition (experiments 6.1 and 6.2, see also 6.5) and of retrieval (experiments 6.3, 6.4, see also 6.6).

7.3 The object-centred role of the perirhinal cortex

The results of experiment 2 (chapter 2) strongly suggested that perirhinal cortex is not critically involved in the formation of visual-visual stimulus associations. This was contrary to the prediction made on the basis of much of the literature, which suggested that lesions to the perirhinal cortex disrupted the formation of associations between stimuli whether or not those stimuli were of the same modality (Bunsey & Eichenbaum, 1993; Goulet & Murray, 2001; Murray et al., 1993; Parker & Gaffan, 1998). It was also surprising in view of studies which have suggested that neurons within perirhinal cortex code the association between two visual stimuli (Higuchi and

Miyashita, 1996; Sakai and Miyashita, 1991; 1994). It is clear that the results of experiment 2 stand in contrast to those of Murray et al., (1993) who found that monkeys with perirhinal lesions were impaired on such a visual-visual stimulus association task, albeit only mildly impaired. This is not unreasonable given that visual afferents make up a substantially higher proportion of inputs to the monkey perirhinal cortex than is the case in the rat, and that the suggestion of pair-coding in the perirhinal cortex was provided by studies involving monkeys. It should also be noted that only Bunsey & Eichenbaum (1993) had previously demonstrated an impairment of stimulus-stimulus association learning following perirhinal lesions in rats. This result will be further considered and contrasted with later findings below.

Unlike experiment 2, the results of the experiments in chapter 3 are in accordance with the majority of the literature. They support the contention that the perirhinal cortex is required for the resolution of feature ambiguity, and therefore for configural processing of objects or stimuli. In this they are in accordance with those of Bussey et al. (2002) and Buckley & Gaffan (1998c), using monkeys, and Eacott et al. (2001), using rats, who have all found that perirhinal lesions cause impairments on tasks which require configural processing but not on control tasks of equal difficulty. In contrast to the results of Ennaceur et al. (1996), the adaptation of the object recognition task employed here produced results which clearly suggest that the severity of impairment following perirhinal lesions increases in relation to the level of feature ambiguity in a given discrimination. Moreover, it is shown that, independent of absolute difficulty, the introduction of feature ambiguity into an object discrimination task has a disproportionate effect on the performance of the perirhinal animals, compared to that of the control animals. Therefore, although this thesis cannot support the view that perirhinal cortex is crucial to the formation of stimulus-stimulus associations in the rat, it provides strong support for the view that one of the functions of perirhinal cortex is the formation of *within* object associations. To place the emphasis slightly differently, an intact perirhinal cortex is critical to the formation of a gestalt which corresponds to an object. In this the present study supports the work of Bussey & Saksida who proposed the PMFC model of perirhinal function (Bussey & Saksida, 2002). This hypothesised that as feature ambiguity within a discrimination increased the severity of impairment following perirhinal cortex lesions would also increase.

It would seem that this dependence upon perirhinal cortex for the resolution of problems containing sufficient feature ambiguity to require a configural solution is restricted to the processing of information which may reasonably be considered as an object, at least in the rat. Experiment 2 used stimuli which did not have a concurrent onset and which did not form unique features when the secondary reinforcer was super-imposed on to the S+. These elements of the design were deliberately included in order to prevent the formation of a single configural stimulus. In contrast experiments 3.1, 3.2 and 3.4 used single stimuli within which the feature configuration could be manipulated. The marked difference between the effects of perirhinal lesions in these two different paradigms suggests that it is integration of features within a single representation which requires an intact perirhinal cortex.

It should, however, be noted that experiment 2 contained a strong appetitive motivation for an animal to successfully discriminate between the stimuli. This was not the case with the experiments in chapter 3 which relied on the intrinsic value of novelty as a motivational element. There is another major difference between the two paradigms. This is that the discrimination in experiment 2 was extremely difficult for intact animals to learn, while the discriminations in the chapter 3 experiments were performed by sham animals without difficulty. This leaves open the possibility that with a sufficient motivation the perirhinal animals could perform discriminations in paradigms in which intermediate or absolute feature ambiguity was present at delays which caused a failure of discrimination in the present study. However, a number of studies, including that of Bussey et al. (2002) in monkeys and Buffalo et al.(1999) in rats suggest that this would not be the case.

However, a motivational version of the tasks used would still be of interest. As was noted in chapter 3, this was originally attempted using the automated Y-maze but was abandoned due to the failure of intact animals to acquire a version of the task which involved an intermediate level of feature ambiguity. It therefore seems that a version of the task using 3-dimensional stimuli and a motivational element would therefore be a reasonable approach. Based on the PMFC model, the fact that perirhinal animals failed to discriminate between objects at any delay in a condition of complete feature ambiguity, and the ease with which intact animals discriminated, it would be

predicted that the introduction of a motivational element would not affect their performance. Bussey et al.'s (2002) task included a motivational element and monkeys with perirhinal lesions nonetheless failed to discriminate where feature ambiguity was a factor in the discrimination.

Although there have been some studies which have suggested that the perirhinal cortex may be involved in memory for context (Bucci et al., 2002; Sacchetti et al., 1999), this thesis cannot support the contention that it is critical to memory for object-in-context. The experiments in chapter 4, taken together with those in chapter 3, indicate that the critical contribution of the perirhinal cortex to processing and memory for feature ambiguity is limited to feature ambiguity of objects or combinations of features that are perceived as objects. Experiment 3.1 demonstrated that the perirhinal animals were not disproportionately affected by a condition of complete feature ambiguity when that feature ambiguity was produced by the interaction of an object with a context, in the classical sense of "context" as a background feature. However, the perirhinal animals were disproportionately affected by an identical level of feature ambiguity, when that condition resulted from the combination of two objects where those objects together could be construed as a single gestalt (experiment 3.5). This clearly suggests that the introduction of feature ambiguity through reconfiguration of *objects* results in severe impairment of perirhinal animals. This is the case whether the reconfiguration is conceived of as within object (chapter 3) or between objects (experiment 4.5). There was also some evidence of impairment in experiment 3.3 which simply used context as an adjunct to a discrimination between novel and familiar objects, with the context as a modifier of object familiarity.

7.4 The postrhinal cortex is critical to memory for object-in-context

The above finding forms part of a clear double dissociation demonstrated by the experiments in chapter 4. Whilst perirhinal animals are able to perform the classical context based discrimination (experiment 4.1) at delays of 2 minutes, the postrhinal animals are severely impaired, to a greater extent even than the fornix group which was included for comparison purposes. Conversely the severe impairment of the perirhinal animals on the object based task (experiment 4.5) is in contrast to the complete lack of an impairment displayed by both the postrhinal and the fornix

groups on this task, even at the longer of the two delays. This is a striking result which serves to emphasise the primacy of perirhinal function in the processing and remembering of objects as unique gestalts. Along with the results of the experiments in chapter 3, it stands in contrast to the results of experiment 2, which demonstrated that the perirhinal cortex is not critical to the association of two stimuli which do not present as a single entity.

The impairment of the fornix and postrhinal groups in experiment 4.1 is in accordance with the findings of Bucci et al. (2002) who found impaired fear conditioning to context in both these groups, although they also found a perirhinal impairment. It is also in agreement with C-fos studies which supported the view that the postrhinal cortex was involved in spatial tasks while the perirhinal cortex was not (Aggleton et al., 2000; Vann et al., 2000). Wan et al., (1999) found that novel arrangements of familiar items caused increased fos activity in the postrhinal cortex, suggesting that it was involved in object-place memory. The evidence of lesion studies (e.g. Bussey et al., 2000) that postrhinal lesions do not cause impairment on a spatial paradigm such as delayed non-matching to place on a T-maze would again point towards the postrhinal cortex being involved with the combination of objects and locations.

The impairments seen in the fornix animals are in accordance with the bulk of the literature which suggests that fornix lesions cause impairment of memory for objects-in-context and scene memory, as well as for object-in-place (Gaffan & Harrison, 1989, Gaffan, 1994). The fornix impairments found are also in accordance with the findings of Mumby et al. (2002), who found a hippocampal impairment on a paradigm similar to experiment 4.1, although the animals in their study were still able to discriminate between the objects. This more severe impairment following fornix lesions in the present study may indicate that it is the hippocampus and its subcortical connections rather than the hippocampus alone which is involved in memory for object-in-context. This view would be supported by the results of Parker & Gaffan (1997a, 1997b) who found that lesions of the mammillary bodies or the anterior thalamic nuclei caused an impairment of object-in-context memory in monkeys equivalent to that produced by fornix lesions. The results of the present study are also in accordance with those of Simpson et al. (1998) who found impairment of what was

termed object-in-place encoding (which was effectively a test of object-in-context) following fornix transection in rats.

Given that, as with the experiments in chapter 3, there was no appetitive motivation involved in experiment 4.5 (object as context), it is possible that the perirhinal animals would show no impairment if such a motivational component were used. As has been discussed with reference to the experiments in chapter 3, this is certainly something that would bear investigation using 3-dimensional objects and a system of food rewards. However, the prediction would be that such an experiment would not find intact performance by the perirhinal animals although it might detect some savings, which were not apparent in the current paradigm. This prediction is based on two premises. First: that the discrimination is performed easily by intact animals and animals with postrhinal lesions; second: that there is no indication that the perirhinal impairment is delay-dependent. Both of these suggest that, while perirhinal cortex may not be essential to the discrimination, it is certainly the means of choice for the processing and memory involved. Again, Buckley & Gaffan (1998c)'s results indicated that monkeys with perirhinal lesions failed to learn a similarly configural task where there was a substantial motivational component, while Parker & Gaffan's (1997a, 1997b) work also indicated a failing in object-in-context memory following lesions to subcortical structures.

This double dissociation between the perirhinal impairment in chapter 3 experiments and in experiment 4.5 and the postrhinal impairment in experiment 4.1 (AB in incongruent context) is of considerable interest and provides a possible explanation for some of the rather contradictory evidence which exists as to the mnemonic role of the perirhinal cortex. It seems clear that the role of the perirhinal cortex is defined by the concept of the object as a single entity or gestalt. This capacity can be tested by the introduction of feature ambiguity into an object discrimination task. The fact that there was a perirhinal impairment in experiment 3.3 in which the element of context incongruity was secondary to a discrimination between a novel and a familiar object again suggests that it is object-based processing which is primarily processed by the perirhinal cortex.

Therefore what this thesis suggests is that there is a double dissociation between the effects of perirhinal and postrhinal lesions, with perirhinal lesions causing impairments on tasks which require the configuration of features within an object (or perceived object) while postrhinal lesions and, to a lesser extent, fornix lesions, cause impairments on tasks which require the configuration of objects and contexts. Both of these types of tasks rely on the intrinsic interest of novelty and, as previously discussed, it is possible that the animals could learn to perform discriminations of these types if there were a motivational component to the task. Nonetheless, it is clear that when relatively naturalistic behaviour is observed using these adaptations of the object recognition task (Ennaceur & Delacour, 1989) a clear double dissociation is observed. A clear possibility for future work is the exploration of whether postrhinal lesions cause impairments on the within-object configurations, which were employed in the experiments in chapter 3, or the between-object associations, which were used in experiment 2. However, on the basis of the work in this thesis, it would be predicted that such an impairment would not be found.

The postrhinal impairment in experiment 4.1 was found even at the shortest delay which could be tested using the experimental design employed. This means that it cannot be ascertained whether the deficit was primarily an impairment of memory or of processing. While this is of less interest than the issue of whether there is any saved performance to be tapped by a motivated task, it is a possible avenue for future work. It would be difficult to test memory for object-in-context in a "no-delay" condition but it would be possible to design an experiment which would permit testing at delays of 1 minute. If the impairment were as severe as these results indicate, it would be predicted that the postrhinal animals would not discriminate in such a condition.

7.5 Fornix lesions impair episodic-like memory

The postrhinal impairment on the object-in-context discrimination of experiment 3.1 forms part of a double dissociation between the effects of postrhinal lesions and the effects of fornix (and by extrapolation hippocampal?) lesions. Whilst postrhinal lesions caused a serious impairment on a task which was discrimination of an object in context, fornix lesions caused only a milder impairment. In contrast to this finding, experiment 4.1 found that postrhinal animals, like perirhinal animals, were

unimpaired on a task which used the combination of object, context and place to test episodic-like memory. Fornix animals, however, were more severely and more uniformly impaired on this task than they were on the object-in-context task. This presents something of a paradox. Since object-in-context recognition is clearly an essential part of the episodic-like memory task it seems counter-intuitive that a group which failed to discriminate this alone should perform normally when discriminating object-in-place-in-context. The explanation proposed by this thesis for this pattern of results postulates a separate system which processes episodic-like memory.

This explanation relies on the fact that there is no motivational element in the paradigm used in experiment 4.1. It is proposed that the postrhinal impairment observed in the object-in-context paradigm is dependent upon the fact that there is no benefit to the animal in discriminating between the objects. There is a clear method of testing this hypothesis, which would be to design a version of the task in which an appetitive reward was available on the choice of the novel object-context combination. If it is the case that postrhinal impairments on object-in-context tasks occur only when discrimination is not essential for a hedonic gain (c.f. Good et al., 1998 on hippocampal involvement in context processing) then it is possible that these functions can also be served by the hippocampus, and are preferentially processed by the hippocampus in an episodic-like scenario. Under this view the hippocampus is critical to episodic-like memory, though it is not critical to the individual aspects of it, at least at short delays. The evidence from the literature is in fact overwhelming in suggesting that the hippocampus is indeed not merely sufficient but necessary to episodic-like memory. Preliminary results from Clayton & colleagues (N. Clayton, personal communication) and Morris & colleagues (R. Morris, personal communication) both indicate that hippocampal lesions in rats impair place-learning and the flavour-place learning respectively. Certainly scene memory is vulnerable to lesions of the hippocampus and its diencephalic projections in both monkeys (Gaffan, 1994; Gaffan & Parker, 1996; Parker & Gaffan, 1997a, 1997b) and in rats (Gaffan & Eacott, 1997; Gaffan et al., 2000; Simpson & Gaffan, 1999; Simpson et al., 1998). Although there are some contradictory results which indicate that hippocampal damage may facilitate scene-learning ability (Gaffan et al., 2001; Simpson et al., 1998), these would seem to fall into the same category as Bussey et al's (1998) finding of facilitated configural learning following fornix transection.

7.6 Intracellular calcium is implicated in memory for objects

Whilst the experiments in chapter 6 are somewhat tangential to the bulk of this thesis, they are illuminating in their implications for the nature of the contribution of the perirhinal cortex to object recognition. It is clear that muscarinic antagonists injected into the perirhinal cortex (but not area TE or the dentate gyrus) impaired object recognition in both monkeys (Tang et al., 1997) and rats (Abe & Iwasaki, 2001). This confirmed the central role of the perirhinal cortex in object recognition, as did the finding that scopolamine simultaneously disrupted Fos expression in the perirhinal cortex of rats and object recognition (Brown et al., 2000). The fact that nimodipine prevented the scopolamine-induced deficit in object discrimination suggests that intracellular calcium is critical to the neural plasticity underlying stimulus recognition. This, together with the evidence of the cortically unique neuronal composition of the perirhinal cortex is in accordance with the possibility that long-term depression may form part of the basis of stimulus recognition. The relationship of these results to the general nature of nimodipine's efficacy in reversing or preventing memory deficits is unclear. However, the explanation advanced in chapter 6 would certainly be compatible with its efficacy in reversing the effects of septal lesions (McMonagle-Strucko & Fanelli, 1993) and its apparent usefulness in the treatment of Alzheimer's' disease. The suggestion that nimodipine's action on the cholinergic innervation of the medial temporal is critical is certainly worthy of further investigation.

7.7 Conclusion

The experiments reported in this thesis serve to expand an understanding of the types of memory in which the perirhinal cortex is critically involved. Of equal importance is the contribution they make in clarifying those aspects of object memory in which the perirhinal cortex appears to play at most a peripheral role. A number of doubly dissociated impairments are identified, contributing to understanding of the way in which information about objects and their surroundings is processed and remembered. Broadly speaking the experiments described in this thesis contribute to the conclusion that the perirhinal cortex is primarily involved in the perceptual and mnemonic processes which contribute to representation of an object. This includes aspects of processing which are internal to the object, and which concern relations between

features or elements of the object, but does not include those which are exclusively or primarily concerned with relations between objects or between objects and the scene or context and place in which they appear. Such relational processing would appear, from the comparative studies in this thesis to be the province of the postrhinal cortex and/or the hippocampal formation. There is a clear double dissociation between the effects of lesions to the perirhinal cortex, which cause object-related impairments, and those of lesions to the postrhinal cortex, which cause impairments of memory for objects-in-classical context.

This thesis also proposes that there may be a memory system which is engaged when a discrimination requires episodic-like memory, and which is, broadly speaking, hippocampally dependent. There is a double dissociation between the effects of fornix lesions and the effects of postrhinal lesions. While fornix lesions cause only mild impairments of memory for object-in-context but severely impair episodic-like memory, postrhinal lesions cause severe impairments of memory for object-in-context but do not impair episodic-like memory. Finally, the ability of a dihydropyridine l-type calcium channel antagonist to prevent both acquisition and retrieval scopolamine-induced impairments of object memory is demonstrated. The crucial contribution of the cholinergic system in object memory is confirmed, and its dependence upon intracellular calcium concentrations established.

This thesis opens up a number of interesting possibilities for future work. The double dissociations we have found are supportive of a distributed model of memory. However, the experiments of Parker & Gaffan (1995) suggest that a temporal lobe memory system, while distributed may nonetheless be integrated. The use of crossed unilateral lesions of the perirhinal cortex or postrhinal cortex with the fornix might provide an avenue for future research on the processing of, and memory for, objects-in-context. This would seem to be a particularly interesting direction to explore given that this thesis suggests (chapter 4) that context may form a critical aspect of episodic memory. In the light of work which has suggested that the hippocampus functions as part of a distributed system involving multiple subcortical structures (Aggleton & Brown, 1999) it would also be desirable to explore the possible function of structures such as the mammillary bodies and the anterior thalamic nuclei in the episodic-like discriminations described here. Finally, given that perirhinal cortex does not appear

to be critical to the learning of visual-visual stimulus associations (experiment 2), some studies of the role played by other structures thought to be involved in aspects of object memory would seem to be called for.

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