The functions of the retrosplenial cortex

Emma L. Hindley

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This work has not previously been accepted in any substance for any degree and is not being concurrently submitted in candidature for any degree.

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Summary

The functions of the retrosplenial cortex are not clearly understood, and the two current theories of retrosplenial cortex function, the translation and integration theories, are frequently very difficult to distinguish from each other, particularly in the spatial domain. The principal goal of this thesis was to differentiate between the integration and translation theories, and to explore the functions of the retrosplenial cortex in tasks that minimise or remove spatial demands, particularly navigation.

The work presented in this thesis demonstrates that the retrosplenial cortex is involved in tasks extending beyond the spatial domain into executive functions and cross-modal processing. The retrosplenial cortex has been shown to be required for visually determining location in an environment, in the absence of self-generated navigational cues. Further evidence has been presented for the differing roles of the retrosplenial sub-regions, which appear to work in conjunction with each other to combine information received from different sensory modalities. However, further work is required to fully understand the ways in which these areas work together and with other areas of the brain, and the implications that dysfunction in this area has for human cognition.

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Publications

This thesis contains the following publications:

Hindley EL, Nelson AJD, Aggleton JP and Vann SD (2014). The rat retrosplenial cortex is required when visual cues are used flexibly to determine location. Behavioural Brain Research, 263: 98 - 107. (Chapter 2)

Hindley EL, Nelson AJD, Aggleton JP and Vann SD (2014). Dysgranular retrosplenial cortex lesions in rats disrupt cross-modal object recognition. Learning and Memory, 21(3): 171 - 179. (Chapter 3)

Nelson AJD, Hindley EL, Haddon JE, Vann SD and Aggleton JP (2014). A novel role for the rat retrosplenial cortex in cognitive control. Learning and Memory, 21: 90-97. (forms part of Chapter 5)

Chapter 1

1. General introduction

1.1 Why investigate the retrosplenial cortex?

The purpose of the work contained in this thesis is to examine the functions of the retrosplenial cortex in the rat. The retrosplenial cortex is of intrinsic interest due to its importance in spatial navigation and episodic memory. It also has a clinical relevance, as it is involved in the most common neurological disorders that impair memory, notably Alzheimer's disease, mild cognitive impairment and vascular dementia. Research into the role played by this brain region will not only increase the understanding of intrinsic processes underlying a range of cognitive functions, but will increase the knowledge of an area of the brain that may become an important target for the development of new treatments for memory disorders.

1.2 Anatomy of the retrosplenial cortex

In primates, the retrosplenial cortex (areas 29 and 30) is found along the midline of the brain, forming part of the posterior cingulate region, which also includes areas 23 and 31 (Vann et al., 2009). In the rat, there are no clearly defined counterparts of areas 23 and 31, so the entire region is termed retrosplenial cortex. The rat retrosplenial cortex is also located along the midline but in contrast to the primate retrosplenial cortex, which is buried deep within the brain, the rat retrosplenial cortex is partly located on the dorsal aspect of the brain. The rat retrosplenial cortex

extends over the caudal half of the length of the brain making it one of the largest cortical areas in this species.

The rat retrosplenial cortex can be divided into two distinct sub-regions, the granular area 29 and dysgranular area 30 (Rdg). These sub-regions differ in their cellular morphology, connectivity, and lamination. The granular area can be further subdivided into granular a (Rga) and granular b (Rgb), based on work by Van Groen and Wyss (van Groen and Wyss, 2003, 1992, 1990a). Area Rdg can be distinguished from the adjacent area 18b in Nissl stained preparations by the lack of granule cells in layer IV. Area Rdg can be also be distinguished from Rgb by the wider layers II, III and IV (Wyss and van Groen, 1992), and by the loss of dense granule cells in layer II. At the border between Rgb and the more ventral Rga, layer II narrows and the cells become larger and less darkly staining. Additionally, layer III widens and the arrangement of cell bodies becomes more regimented (Wyss and van Groen, 1992; Figure 1.1).

Despite the differences in location and relative size of the rat and primate retrosplenial cortex, there are strong similarities in their connectivity. In both rat and primate, the retrosplenial cortex has dense interconnections with the hippocampal formation (Morris et al., 1999a; Wyss and van Groen, 1992) and outputs to frontal regions (Morris et al., 1999a; Shibata et al., 2004). Additionally, both have reciprocal connections with sensory areas, allowing them to receive visual and somatosensory information (Morris et al., 1999b; van Groen and Wyss, 1992).

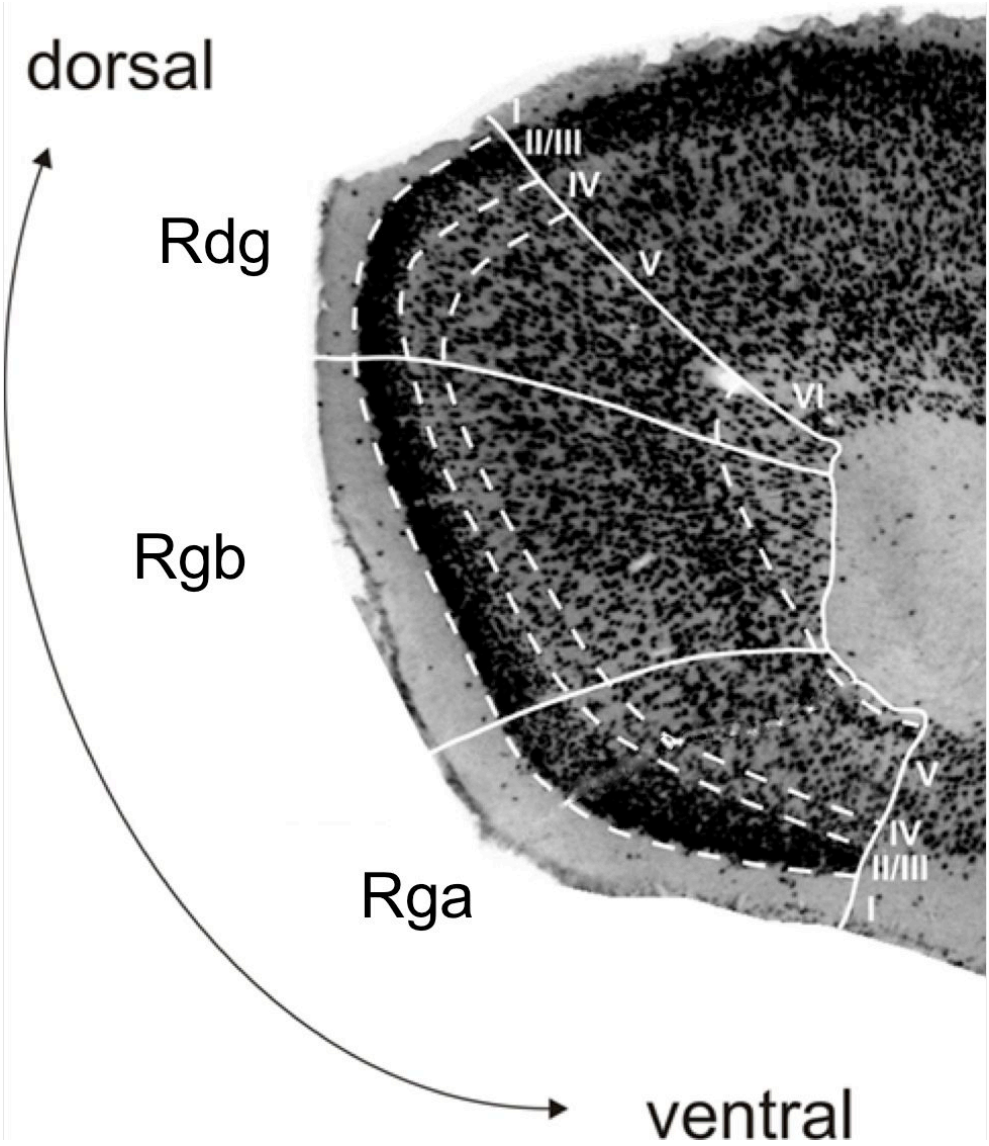

Figure 1.1

Photomicrograph of a coronal section of the retrosplenial cortex, stained for NeuN demonstrating the different cytoarchitectonic characteristics of the retrosplenial sub-regions. Rdg has wider layers II, III and IV, and lacks granule cells in layer II in comparison to Rga and Rgb. Rdg = dysgranular, Rgb = granular b, Rga = granular a. Figure modified from (Sugar et al., 2011), with permission.

Given the relative inaccessibility of the primate retrosplenial cortex for lesion studies, the rodent retrosplenial cortex provides a way to study the functions of this area of the brain in a controlled fashion. The rat retrosplenial cortex is located on the surface of the brain, allowing relatively easy access.

1.3 Connectivity of the rat retrosplenial cortex

There are many intrinsic connections between the different sub-areas of the retrosplenial cortex. Area Rdg projections from the entire rostrocaudal length of the area innervate the contralateral Rdg, as well as the caudal extent of Rga (Sugar et al., 2011). Neurones in Rdg also project to Rgb (Jones et al., 2005), and there are reciprocal projections from Rgb to Rdg, originating from and terminating in all layers. These are topographically organized, with rostral Rgb projecting to rostral Rdg, while caudal Rgb projects to the whole rostrocaudal length of Rdg (Sugar et al., 2011). Area Rgb also has intrinsic connections, arising from cellular layers II, III, V and VI along the entire length of the area and projecting to layers I – V, again along the entire length of the area. A similar pattern is seen with projections to Rga (van Groen and Wyss, 2003). Intrinsic connections within Rga follow a pattern of rostral-to-rostral and caudal-to-caudal projections (Shibata et al., 2009). Finally, area Rga projects to Rgb, along its entire rostrocaudal extent, and to Rdg, originating in layers III – V rostrally and in layer IV caudally (Sugar et al., 2011). To summarise, all subdivisions of the rat retrosplenial cortex have strong intrinsic connections, and are highly interconnected with each other.

Consistent with a role in memory and spatial navigation, the retrosplenial cortex also has extensive connections with the hippocampal formation, particularly with the subiculum. Neurones in layer V of Rga project to the subiculum (van Groen and Wyss, 1990a), with caudal Rga projecting to temporal subicular areas and rostral Rga to the septotemporal parts of the area (Sugar et al., 2011). Area Rgb also projects to the subiculum, again with a topographical organisation (van Groen and Wyss, 2003). Inputs arrive from the subiculum in all three sub-regions of the retrosplenial cortex. Those reaching Rga and Rgb are thought to be glutamatergic (Gonzalo-Ruiz and Bayona, 2001). Rgb and Rga, though not Rdg, also receive projections from the septal portion of CA1. There is evidence from anterograde labelling studies that at least some of these neurones are GABA-ergic, projecting to layer I (Miyashita and Rockland, 2007).

Further connections are found between the retrosplenial cortex and the parahippocampal regions. Area Rga projects to all the parahippocampal subdivisions. Layers II and V of Rga terminate in layers I, III, V and VI of the presubiculum, in an organised topographic manner so that while caudal Rga targets the whole extent of the presubiculum, neurones from the rostral end of Rga only project to the septal end of the area (Shibata, 1994, p. 199). Area Rga also projects to the parasubiculum, postrhinal and perirhinal cortices (Jones and Witter, 2007). Return projections to Rga from parahippocampal regions have so far only been detected from the presubiculum (Sugar et al., 2011). In contrast, Rgb has reciprocal projections with all of the parahippocampal areas, including the medial and lateral entorhinal cortices (Shibata, 1994; Sugar et al., 2011; Wyss and van Groen, 1992).

Similarly to Rga and Rgb, Rdg projects to the presubiculum, parasubiculum, perirhinal, postrhinal and entorhinal cortices (Jones and Witter, 2007). The rostral Rdg projects to the septal presubiculum and the caudal Rdg to the entire length of the presubiculum (Sugar et al., 2011), while projections to other areas do not appear to be topographically organized. Reciprocal projections have been observed from all of these areas (Sugar et al., 2011).

Again consistent with a role in memory and spatial navigation, reciprocal connections exist between layer VI pyramidal cells in the retrosplenial cortex, and the anterior thalamic nuclei (Shibata, 1998). Area Rga projects predominantly to the magnocellular sub-region of the ipsilateral anteroventral nucleus, and receives reciprocal connections back from the same sub-region (Shibata, 1998; van Groen and Wyss, 1995), though it does also have some sparse connections with the anterodorsal nucleus. The anterodorsal nucleus (AD) also receives information from Rgb, with the caudal AD targeted by neurones from the rostral Rgb and *vice versa*. In return, dense projections are found from AD to both Rga and Rgb (Shibata, 1993a). Projections are also found from Rgb to the anteroventral nucleus, to both the parvo- and magnocellular divisions of the nucleus (Shibata, 1998), although reciprocal fibres have so far only been found from the parvocellular sub-region. Area Rdg projects to both the anteromedial (van Groen and Wyss, 1992) and the anteroventral (Shibata, 1998) nuclei, as well as having weaker connections with AD. Although Rdg is the only area of retrosplenial cortex to project in any substantial way to the anteromedial thalamic nucleus, neurones from this same nucleus reach all sub-regions of the retrosplenial cortex (Shibata, 1993a). It has been estimated that

approximately 70% of the cells projecting to the retrosplenial cortex from the anterior thalamic nuclei, and about 90% of the reciprocal fibres, use glutamate or aspartate as a neurotransmitter (Gonzalo-Ruiz et al., 1997).

The retrosplenial cortex projects to frontal brain regions, known to be involved in executive functions such as task-switching, planning and working memory (Alvarez and Emory, 2006; Dalley et al., 2004). The retrosplenial cortex has predominantly ipsilateral projections to the anterior cingulate cortex, with Rga neurones projecting to the anterior cingulate areas 24a and 24b. These projections originate mostly in layer V and terminate in layers I and III (Shibata et al., 2004). Reciprocal projections from the anterior cingulate to Rga are arranged topographically, as caudal Rga received more inputs from the rostral anterior cingulate, and rostral Rga from the caudal anterior cingulate (van Groen and Wyss, 1990a). Area Rgb also projects to the anterior cingulate cortex, in a topographic manner, with caudal Rgb neurones targeting the anterior cingulate at the level of the anterior commissure, while rostral Rgb neurones tend to terminate more caudally (Shibata et al., 2004). Reciprocal connections from the anterior cingulate cortex back to Rgb mainly target the rostral Rgb, though some also reach more caudal Rgb (van Groen and Wyss, 2003). Area Rdg also targets areas 24a and 24b, with a similar topographic organisation as that seen for Rgb. Both Rdg and Rgb projections originate in layer V, but those from Rgb tend to terminate in layers I and III, while the neurones from Rdg target layers I and V (Shibata et al., 2004). Dense connections are also found running from the anterior cingulate cortex to Rdg (Fisk and Wyss, 1999). The retrosplenial cortex also has significant connections with the prefrontal cortex, thought to be involved in higher

cognitive and executive functions. Although there are both contralateral and ipsilateral projections, the ipsilateral projections are denser (Shibata et al., 2004). Area Rdg projects mainly to layers I – III of the ventral, medial and lateral orbital areas of the prefrontal cortex, as well as to the prelimbic area (Shibata et al., 2004). Area Rgb, on the other hand, projects to all areas of the medial frontal cortex, other than the medial precentral area (Condé et al., 1995). The retrosplenial cortex sends dense projections to the frontal medial agranular cortex, although the sub-regions that these projections arise from is not clear (Hoover and Vertes, 2007). Finally, the granular retrosplenial sub-regions project to the pre- and infralimbic cortices (Condé et al., 1995; Hoover and Vertes, 2007).

The retrosplenial cortex connects with several different major sensory areas, including visual, auditory and motor cortices. Area Rga has reciprocal connections with area 18b, part of the visual cortex (van Groen and Wyss, 1990a). Area Rdg receives inputs from visual areas 18b and 17, mostly terminating in layer I. These connections with areas 17 and 18b are reciprocated by projections from caudal, but not rostral Rdg (van Groen and Wyss, 1992, p. 199), targeting mainly the deeper cortical layers. Area Rdg also has some limited connections with area 18a (Vogt and Miller, 1983). However, few connections have been found between visual areas and the granular retrosplenial cortex (Vogt and Miller, 1983). Auditory inputs are also received by the retrosplenial cortex from layer IV of areas 41 and 36 (Vogt and Miller, 1983), while tactile information comes to the retrosplenial cortex in the form of reciprocal connections with the vibrissal area of the rat motor cortex (Miyashita et al., 1994). There are also projections from Rdg, Rga and Rgb to the superior

colliculus, an area that is involved in directing behaviour, particularly eye movements, towards specific points in egocentric space following a sensory stimulus (van Groen and Wyss, 2003, 1992, 1990a). Reciprocal connections are also found between the retrosplenial cortex and the posterior parietal cortex (Reep et al., 1994), thought to be involved in visual attention and spatial navigation (Corwin and Reep, 1998). Projections from the retrosplenial cortex to the posterior parietal cortex are both ipsilateral and contralateral, and involve all layers of the retrosplenial cortex (Reep et al., 1994).

In summary, the connectivity of the retrosplenial cortex appears to point to a role in spatial navigation or memory, due to the reciprocal connections found between areas important in these roles such as the hippocampal formation, anterior thalamic nuclei and the parahippocampal region (Figure 1.1). However, there are also notable connections between the retrosplenial cortex and areas of the prefrontal cortex, providing an indirect route via which the hippocampus can affect the prefrontal cortex and *vice versa* (Figure 1.2). This leaves the possibility that the retrosplenial cortex may have an additional role with functions aligned to those carried out by the prefrontal cortex. The granular and dysgranular sub-regions of the retrosplenial cortex can be distinguished by their different connectivity, suggesting that the sub-regions may have different functional roles. The granular retrosplenial cortex is more connected with those areas involved in navigation, while the dysgranular retrosplenial cortex has more connections with visual areas.

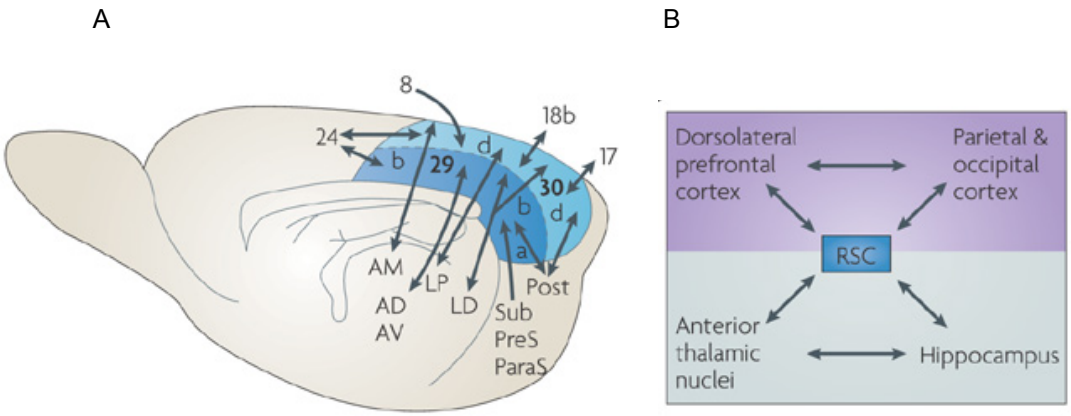

Figure 1.2

A: The location and principal connections of the rat retrosplenial cortex. The dysgranular sub-region (area 30) is marked 'd', while 'a' and 'b' show the granular a and granular b areas respectively. The arrows do not differentiate between the two granular sub-regions when indicating connections. AD, anterior dorsal thalamic nucleus; AM, anterior medial thalamic nucleus; AV, anterior ventral thalamic nucleus; LD, laterodorsal thalamic nucleus; LP, lateroposterior thalamic nucleus; ParaS, parasubiculum; Post, postsubiculum; PreS, presubiculum; Sub, subiculum. B: Major connections of the retrosplenial cortex. Figure reproduced with permission from (Vann et al., 2009).

1.4 Electrophysiology

Further evidence of retrosplenial cortex involvement in navigation comes from the presence of head-direction cells in both the granular and dysgranular sub-regions (Chen et al., 1994b). It has been estimated that approximately 8.5% of neurones in each of these sub-regions are responsive to head-direction information, and fire when an animal orients in a particular direction (Chen et al., 1994b). Additionally, many cells in the retrosplenial cortex are sensitive to spatial information such as angular velocity, place, direction of travel or running speed (Cho and Sharp, 2001). Some of the head-direction cells alter their directional preferences depending on the position of landmarks in the environment, showing that visual information is incorporated into the representation of space encoded by these cells (Chen et al., 1994a). However, all of the cells maintain directional sensitivity when the most obvious visual cues are removed, or even in the dark. Head-direction cells in the retrosplenial cortex are also responsive to ideothetic cues generated during movement, and lose directional sensitivity if the rat is passively rotated through the environment (Chen et al., 1994a), suggesting that a combination of visual and ideothetic cues are used to generate the head-direction signal.

Head-direction cells are also found in many of the areas with which the retrosplenial cortex is reciprocally connected, including the anterior thalamic nuclei (Taube, 1995), the dorsal presubiculum (Taube et al., 1990) and the entorhinal cortex (Sargolini et al., 2006). Lesions of the retrosplenial cortex have been shown to affect the head-direction cells in the anterodorsal thalamus, decreasing the stability of

their preferred firing direction (Clark et al., 2010). This decreased stability occurs even in the presence of prominent visual landmarks, suggesting that the retrosplenial cortex may be involved in integrating visual information into the head-direction cells in the anterodorsal thalamus. The effect of retrosplenial cortex lesions on head-direction cells in other areas has not yet been explored. However, retrosplenial cortex inactivation is known to alter the spatial coding of place cells in the hippocampus, reducing their location stability both across and within training days (Cooper and Mizumori, 2001).

Slow-wave theta activity has been recorded in cells in the granular retrosplenial cortex, similar to patterns seen in the hippocampus (Leung and Borst, 1987). Indeed, theta oscillations in the CA1 sub-region of the hippocampus are in coherence with those recorded in the retrosplenial cortex, although the amplitudes may differ (Young and McNaughton, 2009). Theta rhythms in both the hippocampus and retrosplenial cortex increase in frequency (7 – 9Hz) during more active movements such as rearing or walking, and are slower (6 – 8Hz) during head movements, or small postural changes (Leung and Borst, 1987). It is thought that the theta rhythm may be at least partially generated independently, in the retrosplenial cortex itself, as some septal lesions have been shown to abolish hippocampal theta rhythms while preserving, or even increasing, the theta rhythms in the retrosplenial cortex (Borst et al., 1987). This conclusion is supported by observations of theta activity in the retrosplenial cortex in the absence of hippocampal theta rhythms (Young and McNaughton, 2009). Synchronised theta activity has been postulated as an

important part of the way in which different cortical areas can interact with each other (Varela et al., 2001).

1.5 Clinical relevance of the retrosplenial cortex

In primates, the posterior cingulate and retrosplenial cortices are thought to correspond to the retrosplenial cortex in the rat (Brodmann's areas 29/30). This area has repeatedly been implicated in memory dysfunction in humans. During the very early stages of Alzheimer's disease, positron emission tomography (PET) scans have shown that glucose metabolism decreases in the area (Minoshima et al., 1997) in comparison to healthy control subjects. These metabolic changes are detectable in patients with Alzheimer's-like dementia even before certain cognitive symptoms develop (Haxby et al., 1986). Retrosplenial cortex atrophy is seen in the very earliest stages of Alzheimer's disease (Pengas et al., 2010), but this is not thought to be the reason for the hypometabolism observed in the area (Mosconi et al., 2006).

Metabolic changes have also been seen in the posterior cingulate cortex of patients with mild cognitive impairment (MCI), a prodrome of Alzheimer's disease with a conversion rate of between 6% to 25% per year (Petersen et al., 2001). Reduction in glucose metabolism is specific to the posterior cingulate region, with no changes seen in the amygdala, hippocampus or mammillary bodies at this stage in the disease, leading to the suggestion that posterior cingulate dysfunction may be the first clinical sign of emerging Alzheimer's disease (Nestor et al., 2003b). Further PET studies located the hypometabolism seen in MCI more specifically to Brodmann's

areas 29 and 30, the retrosplenial sub-region of the posterior cingulate cortex (Nestor et al., 2003a). This decreased metabolism in the retrosplenial cortex was the only abnormality common to all MCI subjects scanned.

Changes in the retrosplenial sub-region have in fact been found to predict the development of Alzheimer's disease from MCI. Decreased blood flow, detected by functional single photon emission computed tomography (SPECT) scanning, is seen in the posterior cingulate cortex in MCI patients who go on to develop full Alzheimer's (Huang et al., 2002; Johnson et al., 1998), when compared to MCI patients who do not convert to this disease. Evidence of retrosplenial cortex dysfunction is also present in other memory disorders, including vascular dementia (Martínez-Bisbal et al., 2004), epilepsy (Archer et al., 2003), Korsakoff's syndrome (Aupée et al., 2001; Reed et al., 2003) and schizophrenia (Laurens et al., 2005; Newell et al., 2005). The involvement of the retrosplenial cortex in Korsakoff's syndrome is paralleled by the pyrithiamine-induced thiamine deficiency model of the disorder in rats, in which significant reductions in acetylcholine efflux are seen in the retrosplenial cortex (Savage, 2012).

The involvement of the retrosplenial cortex in such a range of memory disorders highlights the potential clinical implications of a greater understanding of this region. Particularly, the fact that retrosplenial cortex dysfunction is reliably seen in the earliest stages of Alzheimer's disease may make the area an important target of future clinical research aimed at earlier detection and diagnosis of this condition.

1.6 Imaging studies of the retrosplenial cortex

The retrosplenial cortex has also been investigated in healthy subjects, with no cognitive impairments, using functional scanning techniques. The retrosplenial cortex is frequently activated during studies of memory (Gilboa et al., 2004), in particular episodic memory over semantic memory (Wiggs et al., 1998). The region was identified in a large meta-analysis as one of the brain areas most consistently activated during studies of autobiographical memory (Svoboda et al., 2006). However, this paper was forced to combine retrosplenial cortex and posterior cingulate cortex in the analysis, due to inconsistent reporting of the locations in imaging studies. Recent autobiographical memories in particular, rather than remote memories, appear to elicit activation in the retrosplenial cortex (Gilboa et al., 2004; Piefke et al., 2003; Steinvorth et al., 2006), although bilateral retrosplenial activation can be seen in both conditions.

The retrosplenial cortex is also often activated during mental navigation studies, including observing scenes, particularly those that are familiar (O'Craven and Kanwisher, 2000; Rosenbaum et al., 2004), and while learning new environments (Iaria et al., 2007). One recent study has shown that the retrosplenial cortex is preferentially activated by permanent landmarks, over those that could be expected to change (Auger et al., 2012). The same study demonstrated that people who navigate well, and make good judgements about landmark permanence, have increased retrosplenial cortex activity when viewing the landmarks compared to

poor navigators. The recognition of permanent landmarks may be how the retrosplenial cortex contributes to navigation.

Further evidence for involvement of the human retrosplenial cortex in navigation comes from fMRI studies showing that the area is highly sensitive to familiar scenes (Epstein, 2008). The retrosplenial cortex responds strongly when recognising a location as opposed to when organising images into their spatial categories (e.g. kitchens) or when recognising general situations such as parties (Epstein et al., 2007). This suggests that the retrosplenial cortex is specifically involved in recognising familiar scenes and localising them in the wider spatial context.

The retrosplenial cortex has been identified as a component the of the 'default network', the group of brain areas, including the lateral and medial parietal areas, and the medial frontal and medial temporal lobes, that are activated when the brain is not engaged in any particular task, i.e., during a "resting state". Although some brain areas in this network show decreased activity when a task commences (Buckner et al., 2008), the activity in the retrosplenial cortex does not appear to decrease, suggesting that it is involved in the tasks tested as well as when the brain is at rest (Maguire, 2001; Svoboda et al., 2006). During the resting state, subjects report a range of activities such as remembering past events, planning future events and other personal experiences (Andreasen et al., 1995), which may contribute to the activity of the retrosplenial cortex in the resting mode. The retrosplenial cortex, along with medial prefrontal and parietal regions, has been identified as one of a small group of brain areas that are activated during studies of autobiographical

memory, prospection, navigation and theory of mind (Spreng et al., 2009), highlighting the area's importance in a wide variety of tasks.

The deep location of the retrosplenial cortex in humans means that it is currently inaccessible to techniques such as magnetoencephalography and transcranial magnetic stimulation. At present, fMRI and PET scanning are not accurate enough to distinguish the different sub-regions of the retrosplenial cortex, and some studies even generalise activation in the retrosplenial cortex to the entire posterior cingulate cortex, making distinctions between the two regions difficult (Svoboda et al., 2006). Additionally, navigation in a scanner, using either imagination techniques or three-dimensional virtual reality programmes, is not the same as navigating in real space. For these reasons, lesion studies are extremely valuable in determining the role of the retrosplenial cortex.

1.7 Retrosplenial cortex lesions in humans

Neuroimaging studies alone will not be able to tell us the role of the retrosplenial cortex; that an area is activated during a particular task does not mean that it is necessary for the performance that task, or what its importance might be. Studies of patients with retrosplenial pathology allow us to learn about the functions of the brain in the absence of the retrosplenial cortex. Consistent with the findings from imaging studies, a common feature of retrosplenial cortex damage is memory impairment (Bowers et al., 1988; Katai et al., 1992; Maeshima et al., 2013; Masuo et al., 1999; Rudge and Warrington, 1991; Takayama et al., 1991), commonly, though

not exclusively (Maeshima et al., 2013; Masuo et al., 1999), in patients with bilateral lesions, or unilateral lesions to the left retrosplenial cortex (Maguire, 2001).

Patients with unilateral lesions of the right retrosplenial cortex more typically suffer from topographic disorientation and navigational difficulties (Iwasaki et al., 1993; Katayama et al., 1999; Sato et al., 1998; Takahashi et al., 1997; Yasuda et al., 1997), though these deficits often improve in the months following the lesion. Patients are usually able to landmarks, and have a sense of familiarity when entering locations that they have visited previously, but are unable to orient themselves using the landmarks in order to know in which direction they should travel (Maguire, 2001; Osawa et al., 2008; Takahashi et al., 1997). One particularly striking case study involved a man who had been working as a taxi driver in Kyoto for ten years when he suddenly suffered a haemorrhage in the left retrosplenial cortex, leaving him unable to calculate his route home. He reported being able to buildings and landmarks and so judge his current location, but that he was unable to take any directional information from these landmarks (Ino et al., 2007). A very similar report of the subjective effects of retrosplenial cortex damage has been made by a patient with an angioma affecting the area (Cammalleri et al., 1996). In this one case study, the patient was asked to point at a map to identify the position from which photographs of her house were taken. Although she could famous buildings, identify her house as her own, and identify viewpoints of single objects, the patient was completely unable to identify the viewpoint from which the photographs of her house were taken. This suggests that the topographical disorientation seen following many

retrosplenial cortex lesions may result from an inability to identify viewpoints of buildings and landmarks (Suzuki et al., 1998), leading to a navigation deficit.

While lesion studies in patients are extremely useful in indicating processes that the retrosplenial cortex might be essential for, cases of complete retrosplenial cortex damage are rare, as are cases of retrosplenial damage without involvement of adjacent cortical structures such as the posterior cingulate cortex or additional damage to subcortical structures such as the fornix or even the hippocampus. This makes it difficult to accurately determine whether the impairments seen result from the damage to the retrosplenial cortex itself, or to the other affected brain areas. For this reason, lesion studies in animals are used to further investigate what the retrosplenial cortex does.

1.8 Rodent lesion studies

1.8.1 Retrosplenial dysfunction in rats following distal lesions

In addition to showing changes in many disorders of memory, dysfunction in the rodent retrosplenial cortex can be induced by lesions in connected brain areas such as the hippocampus or anterior thalamic nuclei (Albasser et al., 2007; Jenkins et al., 2004; Poirier and Aggleton, 2009). Although there is no observable change in the number of retrosplenial cells or the organisation of the retrosplenial cortex following these lesions, expression of two immediate early genes (*c-fos* and *zif268*) in the retrosplenial cortex is dramatically reduced (Albasser et al., 2007; Jenkins et al.,

2004), sometimes by up to 90%. In the case of anterior thalamic nuclei lesions, the decrease is predominantly in the superficial layers of the granular retrosplenial cortex, and is combined with a decrease in long-term depression (Garden et al., 2009). These changes cannot simply be due to the effects of deafferentation, as similar results are seen following lesions in areas that do not have direct inputs to the retrosplenial cortex, such as the mammillothalamic tract and Gudden's ventral tegmental nucleus (Vann and Albasser, 2009; Vann, 2013).

Immediate early genes are activated in response to a wide range of stimuli, and are thought to have an important role in coordinating the neuronal response to changes in incoming information and, by extension, learning. One example of the evidence for this view is the abolishment of a long-term memory for food preference following an infusion of *c-fos* antisense oligodeoxynucleotides into the hippocampus (Countryman et al., 2005). The long-term maintenance of LTP - the long-lasting enhancement in the postsynaptic response following repeated stimulation - is disrupted in *zif268* knockout mice (Jones et al., 2001). This demonstrates the importance of this immediate early gene for synaptic plasticity. Behaviourally, the same *zif268* knock-out mouse strain shows deficits in long-term memory tasks, both spatial and non-spatial (Bozon et al., 2003; Jones et al., 2001). It is therefore likely that the reduction in retrosplenial immediate early gene expression following damage to other parts of the brain may have the effect of decreasing retrosplenial cortex function in the absence of any overt pathology in the region. In addition to changes in the expression of immediate early genes, deceases in cytochrome oxidase activity have been observed in the granular retrosplenial cortex following lesions of

the anterior thalamic nuclei (Mendez-Lopez et al., 2013), reflecting a decrease in metabolic capacity. Likewise, reductions in phosphorylated CREB indicated changes in granular retrosplenial plasticity after anterior thalamic damage (Dumont et al., 2012). This immediate early gene hypoactivity may have a similar root cause to the hypometabolism seen in the retrosplenial cortex in Alzheimer's disease and MCI. The retrosplenial cortex may have a particular sensitivity to damage within the extended network of brain areas dealing with memory, and it is possible that retrosplenial dysfunction contributes to the mnemonic impairments seen in these disorders.

1.8.2 Spatial memory

Given the connectivity between the retrosplenial cortex and hippocampus, rodent lesion studies on the retrosplenial cortex have understandably tended to focus on spatial memory and navigation, and on those tasks that are sensitive to lesions of either the hippocampus or anterior thalamic nuclei. There is now a general consensus that lesions of the retrosplenial cortex cause deficits in spatial memory. This is despite earlier uncertainty which was probably due to differences in surgical methodology (Vann et al., 2009). Lesions can either be made "physically" using techniques such as aspiration or electrolysis, which may damage adjacent white matter tracts (especially the cingulum bundle), thus disconnecting those brain regions that that have projections running through the lesioned area. More selective lesions can be made by injecting neurotoxins, which spare fibres of passage. There was initially some discrepancy between the behavioural impairments following these two different types of surgery as many early neurotoxic

lesions produced no deficits, or very mild deficits, on tasks that were more severely impaired following traditional lesions of the retrosplenial cortex (Aggleton et al., 1995; Neave et al., 1994; Warburton et al., 1998) giving the impression that the retrosplenial cortex was not important for spatial memory. However, early lesions tended to spare the most caudal part of the retrosplenial cortex, including most of Rga (Vann et al., 2003); given the apparent importance of the caudal retrosplenial cortex from both lesion (Vann et al., 2003) and immediate-early gene (Vann et al., 2000) studies, it is likely that this spared tissue was able to support spatial memory functions. While the caudal retrosplenial cortex was also spared in traditional lesion studies, the additional damage to the cingulum bundle (Meunier and Destrade, 1997; Whishaw et al., 2001), would disconnect the caudal retrosplenial cortex resulting in a "complete" retrosplenial cortex lesion.

Spatial memory deficits have been found following retrosplenial cortex lesions on standard reference memory (Harker and Whishaw, 2002; Sutherland and Hoesing, 1993; Vann et al., 2003) and working memory (Harker and Whishaw, 2004; Vann et al., 2003) versions of Morris water maze testing procedures. In the reference memory version, the escape platform remains in a constant position throughout the training sessions, while in the working memory version the platform remains stationary within a session but moves between sessions. Both of these tasks require the rat to use distal visual cues around the room to locate the escape platform.

Animals with complete retrosplenial cortex lesions take longer than surgical controls to learn the platform location during training, and spend less time in the correct area

during probe trials in which the platform is removed (Vann et al., 2003). Rats with lesions of only the caudal part of the retrosplenial cortex are impaired at acquisition, but not during probe trials, suggesting that these animals were able to successfully learn the location of the platform after extensive training (Vann et al., 2003). This is similar to the pattern seen in rats with lesions restricted to the more rostral parts of the retrosplenial cortex (Harker and Whishaw, 2002; Warburton et al., 1998), demonstrating that both regions must be damaged to affect probe performance.

The timing of surgery, with respect to water maze and spatial training, can affect the impairments seen following retrosplenial cortex lesions. Deficits in water maze tasks may reflect impairments in spatial navigation and memory, but may also be caused by problems with the non-spatial aspects of the task, such as knowing to use the platform as a refuge, or knowing to avoid swimming along the walls of the pool. If rats are trained in these aspects of the water maze before retrosplenial lesion surgery, impairments comparable to those seen in lesioned rats without pre-training are seen in the early stages of training (Cain et al., 2006), demonstrating that the retrosplenial cortex is involved in spatial navigation. Rats that have not had pre-training improve much more slowly during subsequent training than those that have been pre-trained on water maze strategies, suggesting that the retrosplenial cortex may also have roles in the learning of search strategies, or in allowing rats to move away from an unsuccessful strategy as quickly as control rats do (Cain et al., 2006). Non-spatial pre-training following retrosplenial lesion surgery also reduced the deficit in working memory water maze training, so although the retrosplenial cortex

may be involved about learning water maze strategies, loss of the area does not completely eliminate the ability to develop these skills (Lukoyanov et al., 2005).

Another task commonly used to study the effect of retrosplenial cortex lesions on spatial memory is the radial-arm maze task. This maze is typically made up of eight arms that radiate out from a central platform at equal intervals (Olton and Samuelson, 1976). Each arm is baited with a reward, which rats can collect by running to the end of the arm. Entries into arms that have already been visited are considered working memory errors. Retrosplenial cortex lesions have been shown to disrupt performance on this task (Cooper and Mizumori, 2001; Vann and Aggleton, 2004, 2002), but the deficit is not always present (Pothuizen et al., 2008; Vann and Aggleton, 2004).

Retrosplenial lesion deficits do, however, consistently appear on the radial-arm maze task if the maze is rotated after the first four rewards have been collected (Pothuizen et al., 2008; Vann and Aggleton, 2004, 2002; Vann et al., 2003). The arms are re-baited such that the remaining rewards remain in the same location relative to the distal room cues as they did prior to rotation, but their location has changed relative to intra-maze cues. Rats with retrosplenial cortex lesions make significantly more errors than sham animals following this rotation, even if they are unimpaired during the acquisition phase of the task (Pothuizen et al., 2008; Vann and Aggleton, 2005; Vann et al., 2003). Retrosplenial lesions affect performance on the task more if the maze is rotated than if the rat is rotated or a delay is imposed between the collection of the first four food pellets and the second four pellets (Vann and

Aggleton, 2004), suggesting that the setting of intra- and extra-maze cues in conflict is key in revealing the deficit.

A deficit in the selection of appropriate strategies when faced with conflicting information in the radial-arm maze is supported by several other studies that have also demonstrated strategy-switching problems in rats with retrosplenial cortex lesions. In one experiment, rats were trained to avoid one sector of a circular arena using electrical shocks as a deterrent (Wesierska et al., 2009). The sector in which the shock occurred could be defined by distal visual room cues, intra-maze cues or a combination of the two. In a version of the task when both distal and intra-maze cues were present but only distal visual cues defined the area to be avoided, retrosplenial cortex lesions caused a significant deficit, while performance was not affected if irrelevant stimuli were removed (Wesierska et al., 2009).

Although the first study of retrosplenial cortex lesioned animals involving alternation in the T-maze showed very severe deficits (Markowska et al., 1989), aspiration lesions were used that may have damaged fibres of passage. Surprisingly, given the effects of retrosplenial lesions on other spatial memory tasks, studies using cytotoxic lesions have struggled to find a consistent deficit on T-maze alternation (Aggleton et al., 1995; Neave et al., 1994). However, impairments emerge when the cue types that are available to solve the task are restricted, possibly indicating that retrosplenial cortex lesions only impair one or two of the multiple possible strategies available for solving the standard T-maze. Retrosplenial cortex lesion rats performed significantly worse than shams when allocentric cues were removed, but

performance did not differ when direction cues were removed as well, leaving only egocentric strategies for solving the task (Pothuizen et al., 2008). This suggests that retrosplenial lesions impair the ability to use directional information, possibly due to the loss of head-direction cells in the area.

Further evidence of retrosplenial cortex involvement in navigation comes from studies of path integration, the ability to derive information about the route taken from self-generated movement cues. This ability is known to be dependent on the hippocampus (Maaswinkel et al., 1999). The cues used may include information from receptors in the muscles, joints and tendons, as well as from vestibular cues and efference copy information from structures in the brain that generate movement. Retrosplenial cortex lesions made using suction ablation techniques reduced the ability of rats to navigate back to a starting position after foraging for food, both in the light and in the dark (Whishaw et al., 2001), though these results could be attributed to damage external to the retrosplenial cortex resulting from the lesion method. Inactivating the retrosplenial cortex with injections of tetracaine results in navigation impairments in darkness, when visual cues cannot be used to compensate for error, but does not affect performance in the light (Cooper et al., 2001). Similarly, a second inactivation experiment showed that the retrosplenial cortex is required to solve a familiar spatial task in the dark but not in the light (Cooper et al., 2001), again suggesting that the retrosplenial cortex may be required for navigation when the animal cannot rely on visual cues. In order to navigate successfully in the dark, an animal must be able to integrate information from

movement cues with knowledge about spatial location, a process that may rely on the retrosplenial cortex (Cooper et al., 2001).

1.8.3 Object recognition experiments

Experiments have also been carried out into the role of the retrosplenial cortex in object recognition. In the standard spontaneous object recognition task (Ennaceur and Delacour, 1988) rats are first presented with two identical objects and then, after a retention interval, allowed to explore the now familiar object and a novel, alternative object. Rats preferentially explore the novel object. Previous studies have shown that rats with retrosplenial cortex lesions are unimpaired on standard object recognition tasks (Ennaceur et al., 1997; Parron and Save, 2004; Vann and Aggleton, 2002), in which rats have access to visual, tactile and olfactory information about the objects involved.

Retrosplenial cortex deficits are, however, seen in tasks such as object-in-place, where the spatial location of an object becomes relevant. Importantly, the task has no navigational demands, allowing spatial processing to be separated from the ability to know how to get to a location. In the object-in-place task, four different objects are presented for exploration in the four corners of an arena. After exploration, and before testing, the positions of two of the objects are switched. Normal rats will spend more time exploring the objects that have moved, while rats with retrosplenial cortex lesions do not differentiate between the objects (Vann and Aggleton, 2002). This task requires rats to make a link between specific objects and

their location. In a similar task, where one object is moved and the others remain stationary, the rat only needs to realise that an object is in a novel location. Retrosplenial cortex lesions also cause a disruption in performance of this task, suggesting that the retrosplenial cortex may be involved in processing the spatial properties of an environment and linking that information to objects within it (Ennaceur et al., 1997; Parron and Save, 2004).

1.8.4 Fear conditioning

The retrosplenial cortex may have a role in fear conditioning, when the context in which the rat is placed determines whether a negative outcome will occur (Keene and Bucci, 2008a). Rats with retrosplenial cortex lesions are, however, able to learn fear conditioning paradigms that are not linked to contextual learning (Keene and Bucci, 2008a). The deficit may, therefore, be linked to the possible role of the retrosplenial cortex in processing spatial information as discussed above.

Studies of immediate early gene expression also support a role for the retrosplenial cortex in fear conditioning. Exposure to an environment in which a footshock had previously been received markedly increases Fos synthesis in the area (Beck and Fibiger, 1995; Robinson et al., 2012). A similar pattern is seen with *Arc* expression (Robinson et al., 2012). Control groups exposed to the context in the absence of shock or to shock in the home cages both showed increases in *c-fos* expression over a group that was exposed to neither the novel context nor to a shock. However, the context and shock group had significantly higher expression levels than the other

groups; the context-only group was also significantly higher than the shock-ony group (Robinson et al., 2012). This shows that the effects were due to the context, rather than to the emotional valence of the shock itself. A possible mechanism for the involvement of the retrosplenial cortex is suggested by a study in mice in which N-methyl-D-aspartate receptor blockade was carried out in the retrosplenial cortex during fear conditioning. This study showed that these receptors are required for the retrieval of both recent and remote contextual fear conditioning memories (Corcoran et al., 2011), supporting previous lesion work (Keene and Bucci, 2008a).

1.8.5 Sub-region studies

Retrosplenial cortex lesion studies do not typically differentiate between the different retrosplenial sub-regions. To date, very few studies have investigated the relative contributions of the granular and dysgranular sub-regions of the retrosplenial cortex, so the precise roles of the two areas are not yet clear. Lesions of the dysgranular retrosplenial cortex impair performance on the rotated radial-arm maze task, as described above, biasing the strategy chosen by the rats to solve the task from a visual one to a motor turning strategy (Pothuizen et al., 2010). This result is consistent with the fact that the dysgranular sub-region of the retrosplenial cortex receives the majority of inputs from visual areas. Selective granular sub-region lesions, however, also impair performance on the same task to a level comparable to complete retrosplenial cortex lesions (Pothuizen et al., 2010). This implies that the two sub-regions may work closely together during the rotation manipulation of the radial-arm maze task, and that both are required for it to be

successfully solved. That said, differences between the sub-regions are seen during the acquisition phase, when both rats with complete retrosplenial lesions and those with specific granular lesions showed an increase in the number of errors made (Pothuizen et al., 2010). Dysgranular lesions did not cause a deficit during acquisition (Vann and Aggleton, 2005), although the rats were more likely to select adjacent arms in sequence, suggesting that different strategies were being used.

Granular retrosplenial cortex lesions also caused deficits on a T-maze alternation task in which intra-maze cues were removed by the use of two adjacent, parallel mazes (Pothuizen et al., 2010). The deficit seen here was greater for granular lesions than for complete retrosplenial lesions when moving from one maze to two mazes. This move will have prevented the rats with granular lesions from using certain strategies such as intra-maze cues. Granular retrosplenial lesions caused a particular deficit in a condition that involved the animals running to the same absolute place in the room on both alternation trials, when the left hand arm of one T-maze was next to the right-hand arm of the other maze. This is consistent with an over-reliance on visual cues due to the sparing of the dysgranular cortex, leading to conflict between visual and proprioceptive cues (Pothuizen et al., 2010). This interpretation is supported by the fact that when the task is run in the dark, removing the visual cues and therefore the spatial conflict, performance by rats with granular retrosplenial cortex lesions improves. There is however still a mild deficit, indicating that proprioceptive information may also have been affected, possibly due to the loss of head-direction cells in the area (Pothuizen et al., 2010).

One study has investigated the effects of selective lesions to areas Rgb and Rga, the sub-divisions of the granular sub-region. This study found that removing Rgb impaired spatial memory performance, in both working and reference memory tasks in the Morris water maze (van Groen et al., 2004). Lesions of Rga, however, did not affect performance (van Groen et al., 2004). This disruption is consistent with the anatomical connections of each region, with Rgb receiving information from both visual area 18b and the parietal cortex, which are known to be involved in the processing of spatial information (DiMattia and Kesner, 1988). Rga does not have inputs from these areas (van Groen and Wyss, 2003, 1990a).

The roles of the retrosplenial sub-regions have also been investigated using immediate-early gene imaging of both *c-fos* and *zif268* following working memory tasks in the radial-arm maze run in both the light and the dark. *c-fos* and *zif268* activation was found to increase in the working memory groups, compared to controls, in the granular sub-region (both Rga and Rgb) whether the task was run in the light or the dark, suggesting that the granular sub-region has a role in spatial learning and navigation using both internal and external cues (Pothuizen et al., 2009). A very different pattern of results was seen in the dysgranular sub-region, where both *c-fos* and *zif268* activation increased in the light and decreased in the dark (Pothuizen et al., 2009). This result is consistent with a more selective role for the dysgranular retrosplenial cortex when performance is controlled by visual information. The connectivity of the region supports this view (van Groen and Wyss, 1992).

Immediate early genes have also been used to study changes in activity along the rostral-caudal axis during the working memory radial-arm maze task, again both in the light and in the dark (Pothuizen et al., 2009). *c-fos* and *zif268* increases were clearest in the caudal retrosplenial cortex, though this was more marked for *c-fos*. In the rostral retrosplenial cortex, changes in *zif268* activity were seen only in the dysgranular sub-region, and not in Rgb, while in the caudal area both regions showed changes in expression (Pothuizen et al., 2009). This highlights the importance of the caudal retrosplenial cortex in spatial memory, as previously shown by lesion studies (Vann and Aggleton, 2004; Vann et al., 2003). Understanding the roles of the retrosplenial cortex sub-regions is important, as while there may be overlap in the contributions that the different sub-regions make to spatial memory, each area may also have its own specific roles. Looking at the retrosplenial cortex only as a unitary structure may make it harder to interpret the function of this region.

1.9 Current theories of retrosplenial cortex function

The full impact of retrosplenial cortex lesions is most clearly seen in situations where retrosplenial cortex lesioned animals are forced to switch the types of cue that they use, or the strategy with which a task can be solved. Examples include solving a radial-arm maze task in the light and then the dark (Chen et al., 1994b), changing from allocentric to directional cues in a T-maze (Pothuizen et al., 2008), and switching from intra-maze to extra-maze cues when performing a radial-arm maze task in the light (Pothuizen et al., 2008; Vann and Aggleton, 2005, 2004). One

current theory of retrosplenial cortex function is that this area has a 'translational' role in transforming spatial codes, e.g., allocentric representations (North, South, East and West) into egocentric ones (left, right, etc.) and *vice versa* (Burgess et al., 2001; Byrne et al., 2007). This process would be required for navigation, either updating the current spatial position based on self-generated movement information, or based on visual information from the surroundings. Translation can also occur in the opposite direction, from an allocentric representation of location in space, probably based on a combination of activity in place cells, boundary vector cells and grid cells, to the egocentric representation during memory retrieval (Burgess, 2008). A related theory is that the retrosplenial cortex is involved in integrating information across stimulus modalities (Mizumori et al., 2000; Wolbers and Büchel, 2005).

1.10 What this thesis will cover

The functions of the retrosplenial cortex are still not clearly understood. The translation and integration theories of retrosplenial cortex function are frequently very difficult to distinguish from each other, particularly in the spatial domain. This is because a task that requires an animal to translate between different sources of spatial information will also require that animal to have integrated the information from the two representations, in order to make the switch easily. Demonstrating that rats with retrosplenial cortex lesions are impaired on tasks in which spatial cues are restricted (Chen et al., 1994b; Cooper and Mizumori, 1999; Pothuizen et al., 2008) could result from a deficit in either of these processes. Furthermore, the role

of the retrosplenial cortex in non-spatial tasks is not well understood at present. This thesis will attempt to differentiate between the integration and translation theories, and will also explore the functions of the retrosplenial cortex in tasks that minimise or remove spatial demands, particularly navigation.

The first chapter of this thesis examines the role of the retrosplenial cortex in spatial memory, using a novel appetitive paradigm that removes the navigational demands. This process might be expected to normally require the retrosplenial cortex for the effective processing of spatial information and its integration with self-movement cues. These task elements are less prominent in the procedure used in this thesis than in many of the standard spatial memory tasks that are impaired by retrosplenial cortex lesions (Cooper et al., 2001; Pothuizen et al., 2008; Vann and Aggleton, 2002).

Secondly, the role of the retrosplenial cortex in visual object recognition will be tested. The inputs to the dysgranular retrosplenial cortex from visual processing areas such as area 18 (van Groen and Wyss, 1992) suggest that the retrosplenial cortex has a role involving the use of visual information. Although previous studies have not found impairments on object recognition tasks resulting from retrosplenial cortex lesions, none of these studies controlled the cues available during the task (Parron and Save, 2004; Vann and Aggleton, 2002). It is possible that the animals were solving that task using tactile or olfactory cues, something that has been shown to depend on the posterior parietal cortex (Winters and Reid, 2010). Further, the role of the retrosplenial cortex in translating or integrating cues in a non-spatial context will be tested, using a cross-modal object recognition paradigm (Winters and

Reid, 2010) where rats can be forced to switch from using visual to tactile cues or *vice versa* to explore the objects.

From the object recognition tests, it is not possible to distinguish between the integration of cue types into a single representation, or the translation from a representation of a visual (or tactile) image based on exploration into an imagined representation of the corresponding tactile (or visual) properties that can be d during the test phase. As a result, the fourth chapter in this thesis will specifically examine the process of integrating cues from different types of stimulus, in situations where translation between stimulus types is not required.

Finally, the connections between the retrosplenial cortex and the prefrontal cortex will be investigated, using tasks that typically show deficits following prefrontal cortex lesions, specifically the Stroop task and intra- and extra-dimensional set-shifting. The potential role of the retrosplenial cortex in higher executive functions has received very little attention in previous experiments, despite its strong links with the areas carrying out these functions (Shibata et al., 2004).

Chapter 2

2. The rat retrosplenial cortex is required when visual cues are used flexibly to determine location

2.1 Introduction

There are multiple areas in the rodent brain that appear to support navigation and spatial learning (Mizumori et al., 1999). The assumption is that these sites contribute in different, but complementary, ways. The retrosplenial cortex is one such area involved in navigation. The connectivity of the retrosplenial cortex strongly suggests that its role in spatial learning and navigation is closely linked with those of the hippocampus and the anterior thalamic nuclei (Aggleton et al., 2012; Morris et al., 1999b; van Groen and Wyss, 2003, 1992, 1990b). Consistent with this notion is the finding of head-direction cells in the rat retrosplenial cortex (Chen et al., 1994b; Cho and Sharp, 2001). These neurones signal the direction an animal is heading, independent of location. Findings from patients with pathologies involving the retrosplenial cortex, particularly in the right retrosplenial cortex, also suggest a role for this area in spatial navigation. Many of these patients report an inability to use familiar landmarks to navigate despite retaining knowledge of the landmarks themselves, and some appear unable to orient themselves either in a novel or familiar environment (Maguire, 2001; Takahashi et al., 1997). These findings raise the question of what the spatial functions of the retrosplenial cortex are, beyond those of the hippocampus and anterior thalamus. However, a barrier to further

understanding the particular contributions of the retrosplenial cortex is the lack of patients with pathologies confined to this region (Vann et al., 2009).

To address this question, the impact of selective retrosplenial cortex lesions has been examined in rats. Previous studies have found spatial deficits in a variety of behavioural protocols, including water maze, T-maze and radial-arm maze tasks (Haijima and Ichitani, 2008; Pothuizen et al., 2008; Vann and Aggleton, 2004, 2002; Whishaw et al., 2001). These same lesion studies often reveal a reluctance to use allocentric visual cues when other spatial strategies are available. In addition, spatial tests in the dark have implicated the retrosplenial cortex in some forms of idiothetic learning (Cooper and Mizumori, 1999; Whishaw et al., 2001; Zheng et al., 2003), leading to the notion that this region may assist in the integration of visual with idiothetic spatial information (Mizumori et al., 2000). A related, broader notion is that the retrosplenial cortex has a 'translational' function in the integration and transformation between multiple spatial codes, including allocentric representations into egocentric ones and *vice versa* (Burgess et al., 2001; Byrne et al., 2007; Vann et al., 2009). Support comes from fMRI studies showing that the retrosplenial cortex is activated during a task requiring people to imagine looking at a scene from different viewpoints (Epstein et al., 2007; Lambrey et al., 2012).

In order to understand retrosplenial cortex function it is important to appreciate that this area is divided into two sub-regions, granular (area 29) and dysgranular (area 30). In the rat, the dysgranular area appears to be a leading candidate for the integration of visual information given its many connections with both cortical and

subcortical areas strongly linked with visual processing, e.g., area 17 and the lateral dorsal thalamic nucleus (Mizumori et al., 2000; van Groen and Wyss, 1992; Vogt and Miller, 1983). This view is supported by the finding that immediate-early gene expression increases selectively in the dysgranular retrosplenial cortex following spatial memory tasks performed in the light, compared to the same tasks performed in the dark (Pothuizen et al., 2009).

The present study sought to examine the effects of selective retrosplenial cortex lesions on two related spatial tasks where the use of distal visual cues could be assessed. The first task concerned the ability to use distal visual cues to distinguish the features of a room from a set viewpoint ('Perspective' task, Experiment 1). The second task concerned the ability to discriminate between two locations within a room, irrespective of the direction faced ('Location' task, Experiment 3). These closely related tasks were selected as the demands of the second task build onto those of the first task, including the ability to unite different perspectives from the same place. Two cohorts of rats were examined, one with lesions in both areas 29 and 30, the other with lesions targeted at just area 30 (dysgranular retrosplenial cortex). The goal was to test whether the dysgranular cortex is a critical access point for visual processing within the retrosplenial region. Finally, as both Experiments 1 and 3 used go/no-go discriminations where rats were only reinforced for digging when in the correct viewpoint or location, Experiment 2 tested whether such go/no-go procedures, with their emphasis on withholding responses, are appropriate for rats with retrosplenial damage. In this control study, the rats were trained on a non-

spatial go/no-go task involving the discrimination of different cups containing distinct digging media, where only one cup contained food (Experiment 2).

2.2 General Methods

Two cohorts of rats were trained and tested separately. Modifications were made to the procedures of Experiment 1 for Cohort 1 in light of the results from Cohort D, the dysgranular lesion cohort. Modifications included the number and type of probe trials, the use of background white noise, and the behavioural task carried out immediately beforehand. Further detail is given below. The methods for Experiment 1 are described together, although they were analysed separately. Both cohorts of animals were trained in the same manner for Experiments 2 and 3.

2.2.1 Animals

Subjects were 52 male Lister Hooded rats (Harlan, Bicester, UK), weighing 278-387g at time of surgery (Cohort D (dysgranular): 294-314g, 24 animals; Cohort 1 (combined) 278-387g, 28 animals). The rats were housed in pairs in a temperature-controlled room. Lighting was kept on a 12-hour light/dark cycle, from 08:00 to 20:00. Water was available *ad libitum* throughout the experiments. For all behavioural experiments, the animals were placed on a food-restricted diet where they were able to gain weight. Their weights did not fall below 85% of their free-feeding weights. All experiments were carried out in accordance with UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, and were approved by

local ethical committees (Cardiff University). Rats were provided with cardboard tubes and wooden chew sticks in their home cages. Subjects for the dysgranular cortex study (Cohort D) received either a bilateral excitotoxic lesion within area 30 of the retrosplenial cortex (RSdysg n = 14) or a sham lesion (ShamD, n = 10). Animals in the combined lesion study (Cohort 1) received either a bilateral excitotoxic lesion of both areas 29 and 30 (RScomb1, n = 16) or a sham lesion (Sham1, n = 12).

2.2.2 Surgical Procedures

Rats were deeply anaesthetised with an intraperitoneal (i.p.) injection of sodium pentobarbital (60mg/kg pentobarbital sodium salt; Sigma-Aldrich, U.K.). All subjects were given a subcutaneous injection of 0.06ml Metacam (Boehringer Ingelheim, Alkmaar, NL, USA) to reduce post-operative pain, as well as 0.1ml Millophylline (i.p.; Arnolds Veterinary Products Ltd, Shrewsbury, UK) to regulate breathing. The scalp was shaved and the animal then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) with the nose bar set at +5.0. The skull was exposed and a bilateral craniotomy extending from bregma to lambda was made in the skull using a dental drill. The more posterior areas of the retrosplenial cortex were revealed by drilling away two short strips of skull from the opened area, leaving a strip of bone approximately 2mm wide over the central sinus as protection.

Lesions were made by injecting 0.09M *N*-methyl-D-aspartate (NMDA; Sigma, Poole, UK) dissolved in phosphate buffer (pH 7.2), into 14 injection sites at a rate of 0.05µl per minute using a 1µl Hamilton syringe (gauge 25s; Bonaduz, Switzerland). The

stereotaxic coordinates of the lesion placements are stated relative to bregma in the anterior-posterior (AP) axis, and relative to the central sinus in the lateral-medial (LM) axis. Dorsal-ventral (DV) coordinates are taken relative to the surface of the cortex, using the eye of the needle.

Coordinates for the dysgranular lesion group (RSdysg) were: AP -1.6, LM±0.4, DV-1.0; AP-2.8, LM±0.5, DV-1.1; AP-4.0, LM±0.5, DV-1.1; AP-5.3, LM±0.5, DV-2.4; AP-5.3, LM±0.9, DV-1.4; AP-6.6, LM±0.9, DV-1.8; AP-7.5, LM±1.0, DV-1.1. At each site 0.25µl of NMDA was injected, apart from for the most caudal pair of injections, where the injections were 0.1µl NMDA. The coordinates for the combined lesion cohort (RScomb1) were AP -1.6, LM±0.4, DV-1.3; AP-2.8, LM±0.5, DV-1.4; AP-4.0, LM±0.5, DV-1.4; AP-5.3, LM±0.5, DV-2.6; AP-5.3, LM±0.9, DV-1.6; AP-6.6, LM±1.0, DV-2.0; AP-7.5, LM±1.1, DV-1.3. Injections of 0.25µl NMDA were made in the three most rostral pairs of sites, with 0.26µl injected in the next three pairs of sites. In the most caudal site 0.1µl was injected.

After each infusion the needle was left in place for 5 minutes before being slowly withdrawn. On occasion, animals received an additional dose of 0.05ml sodium pentobarbital to maintain anaesthesia. If further anaesthesia was still required <2% inhaled isoflurane was given. Oxygen was provided throughout the surgery. Following surgery the scalp was sutured, and a subcutaneous injection of 5ml glucose-saline was given to replace lost fluids. Lidocaine (Xylocaine, AstraZeneca, UK) and antibiotic powder (Dalacin C, Pharmacia, UK) were applied topically to the wound and animals were left to recover in a warm, quiet area before being returned

to their home cage. Sham animals underwent the same procedure, except that the needle was not lowered and injections of neurotoxin were not made. Post-operative care was identical for all groups. All animals recovered well following surgery.

2.2.3 Histological Procedures

At the completion of the experiments, rats were deeply anaesthetised using sodium pentobarbital (60mg/kg, i.p.; Euthatal; Merial Animal Health, Harlow, UK), then transcardially perfused with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M PBS (PFA). The brains were removed and placed in PFA for 4h before being transferred to 25% sucrose and left overnight at room temperature, with gentle agitation. Four adjacent series of coronal sections (40µm) were cut on a freezing sliding microtome.

One series was mounted directly onto gelatine-coated slides after slicing and stained using cresyl violet, a Nissl stain. A second series was stained for NeuN, which is a selective marker for neurones (Mullen et al., 1992) that helps visualise the extent of any lesion. This second series was collected in PBS. To visualise NeuN, the free-floating sections were rinsed in 0.1M PBST (PBS with 0.2% Triton X-100) and treated with 0.3% H_20_2 (hydrogen peroxide) in 0.1M PBST for 3min to suppress endogenous peroxidase activity. Sections were rinsed four times in 0.1M PBST for 10min each time, and then incubated for 48h at 4^{o}C in the monoclonal anti-NeuN serum (1:5000; Chemican, Temecula, CA, USA) diluted in PBST. After rinsing four times in 0.1M PBST for a further 10min each time, sections were incubated for 2h in the secondary

antibody, avidin-biotin-horseradish peroxidase complex (1:200; ABC-Elite, Vector Laboratories, Orton Southgate, Peterborough, UK) in PBST. After four rinses in 0.1M PBST and two rinses in 0.05M Tris buffer, sections were left for 1-2min in a chromagen solution consisting of 0.05% diaminobenzidine (Sigma; Poole, UK), buffer solution and 0.01% H_20_2 (DAB substrate kit; Vector Laboratories). The reaction was monitored visually and stopped by rinsing in cold 0.1M PBS. The sections were mounted and dried on gelatine-coated slides. All slides (Nissl and NeuN) were then dehydrated through an alcohol series, cleared with xylene and cover-slipped using the mounting medium DPX.

2.2.4 Statistical Methods

Statistical tests were carried out using SPSS 16.0 (SPSS Inc., Chicago). Where the assumption of sphericity was not met for parametric analysis, Greenhouse-Geisser corrections have been applied. In all statistical tests the critical alpha level is taken as $p \leq 0.05$.

2.3 Experiment 1 - Viewpoint discrimination ('Perspective' task)

2.3.1 Pre-Training

Pre-training started nine months after surgery for the dysgranular lesion rats and their controls. Cohort D had completed other spatial tasks, including a radial-arm maze task and a platform location task in the Morris water maze (see Annex A). For

the combined retrosplenial lesion rats (Cohort 1), pre-training started two months after surgery and followed testing on an object-in-place task. All rooms used in the study were novel to the rats.

During pre-training, two round digging cups (6.5cm tall and 7cm in diameter) made of black plastic were used to train rats to dig for food rewards. Each cup was filled with shredded paper. A false bottom, made of metal grille, was inserted into the base to allow Cheerios (Nestle, UK) to be hidden beneath where they could be smelled by the rats but not accessed. The cups could be fixed with Velcro to the floor of the pre-training arena, a rectangular white plastic box measuring 25cm x 42cm with walls 12cm high. The cups were placed 2cm away from the short end wall of the arena. Habituation took place in a room measuring 195 cm x 330 cm with walls 255 cm high. This room was different to that used for subsequent testing.

The RSdysg and ShamD rats (Cohort D) were habituated to the digging cups and arena over four days, receiving 10 minutes in the apparatus each day. The cups were filled with shredded paper mixed with crumbled Cheerios to mask the smell of a hidden Cheerio reward. One cup was placed at either end of the habituation arena. On the first day of habituation, half a Cheerio was placed on top of the paper in either cup. To encourage exploration of both cups, each cup was only re-baited after the other Cheerio had been eaten. Over the four sessions, the Cheerio was gradually buried deeper into the paper so that by the end of pre-training the rat would reliably dig to recover the food at the bottom of the digging cup. The training procedure for the RScomb1 and Sham1 rats was identical to that for Cohort D except

that white noise at approximately 75db was present throughout habituation. The source of the white noise was directly underneath the digging arena.

2.3.2 Apparatus

The same digging cup was used for the pre-training and test phases. The test arena consisted of a clear plastic box measuring 35cm by 55cm with walls 17cm high. The digging cup was placed 2cm from the middle of the short wall (Figure 2.1). The arena was placed on a table, 70cm high, in the centre of a room that measured 330cm x 250cm with walls 255cm high. There were salient visual cues on the walls, such as posters and shapes made from coloured paper. The holding boxes were kept on two tables (76cm high) that were located against the two walls of the room that the rats did not face during testing. For test trials in the light, the light level in the centre of the arena was 400 lux; for test trials in the dark the illumination was less than 1 lux.

2.3.3 Behavioural Protocol

During testing the rats were rewarded when the cup was in one direction relative to the animal's starting position e.g., East, but not if the rat had to travel in the opposite direction to reach the cup (e.g., West; see Figure 2.1A). The rewarded direction was counterbalanced across groups, as was the direction from which the rat was placed in the testing arena. Rats were carried to the test room in individual aluminium holding boxes, each with a hinged lid that prevented the rats from seeing the room when being brought in and when waiting between trials. Rats were tested

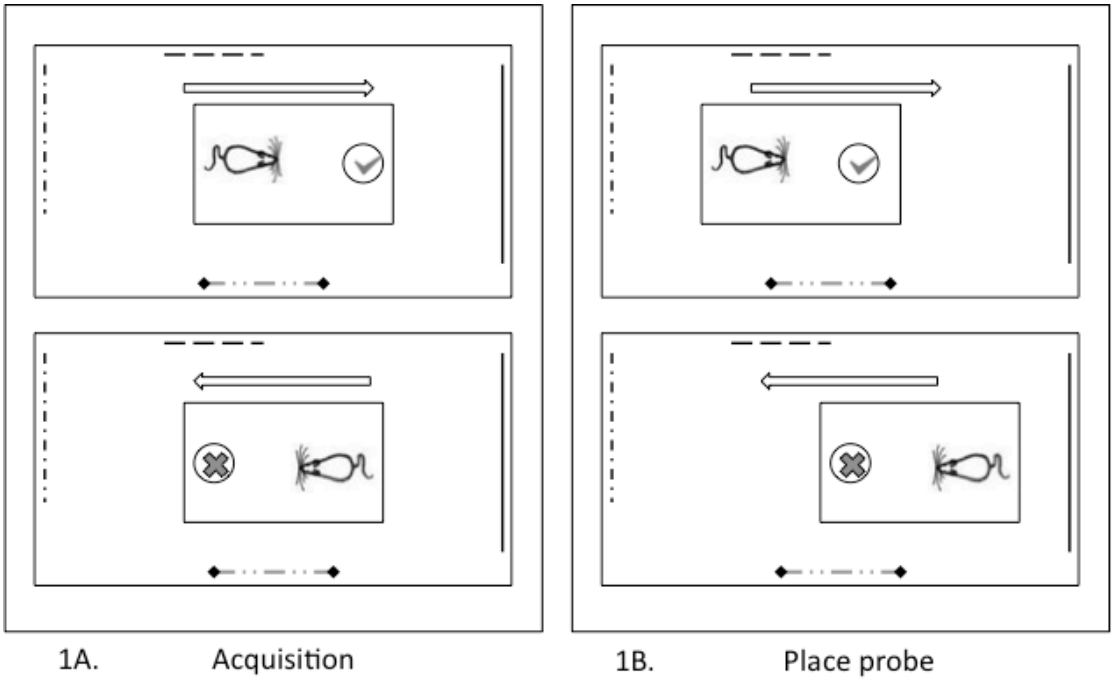

Figure 2.1

Perspective task (Experiment 1). A: A schematic of the acquisition trials in the Perspective task, which was used to test viewpoint discrimination. Rats were rewarded (tick) when the cup was in one direction relative to the animal's starting position (upper panel), but not rewarded (cross) if the rat had to travel in the opposite direction to reach the cup (lower panel). Visual cues (various dashed lines) were attached to the walls to help them to be distinguished. B: 'Place' probe: The location of the cup remained fixed regardless of the direction that the animal was facing. (The apparatus, room and test arena are not to scale).

in groups of four, each receiving one trial in turn. As a consequence, the inter-trial interval was approximately 90s. Between trials the holding box was rotated to deter the rats from building up internal direction cues that could allow them to solve the task in a non-visual manner.

On the first two days of training, each rat was placed in the test arena for 30s before the first trial. This additional period was to habituate the rats to the new arena. Every rat completed 16 trials per day, each trial lasting a maximum of 20s. Digging was defined as breaking the surface of the paper with the nose or paws. If the rat failed to dig on a correct (go) trial, the experimenter showed the rat the Cheerio at the end of the 20s period, and the animal was allowed to eat the reward. If the rat successfully found the Cheerio it was given 5s to eat the reward before being returned to the holding box. On any incorrect trial the rat was removed from the box after 20s or after finishing digging, whichever came first. The latency to dig was recorded for each trial and a difference score calculated, i.e., dig latency on non-rewarded (no-go) trials minus dig latency on rewarded (go) trials. A positive score was, therefore, evidence of discrimination between the rewarded and non-rewarded pots while a score of zero would indicate no discrimination. Between each trial, the entire test arena was rotated 180^{o} and the cup relocated to avoid directional odour cues developing. In addition, the arena and cup were cleaned with alcohol wipes between each group of four rats. The scores for each trial were summed to give a session score (maximum 320s).

When both groups of animals had reached asymptotic levels of performance, several probe sessions were introduced. Each probe involved two sessions. The first probe tested whether animals were using the absolute position of the cup in the room to determine whether or not to dig ('Place' probe). The rats were, therefore, run using the same procedure as before, with the exception that the digging arena moved so that although the rats could still approach the cup from different directions, the absolute location of the cup did not move (see Figure 2.1B). Only Cohort D was tested on this 'place' probe. Following the Place probe, rats were given two days of reminder training on the original acquisition ('Original').

During the subsequent probe sessions, external cues were occluded or removed. First, a plain white curtain was closed around the testing arena, to block distal visual cues on the far walls ('Curtain' probe). The room was rectangular in shape and the curtain followed the walls so that the general shape of the room and the positions of the two tables were still visible. Next, the sessions were run with both the curtain drawn closed and the room lights switched off in order to remove all visual cues ('Dark' probe). Between each probe session the RScomb1 and Sham1 rats were retrained for one session on the original task, i.e., with all cue types available. The intention was to ensure that performance on the probe session was not unduly affected by extinction. For the RSdysg and ShamD cohort each probe trial followed directly on from the previous probe, with no retraining in between other than after the Place probe. For the RSdysg and ShamD cohort, the final probe trial again took place in the dark with the curtains drawn, but now with a white noise generator playing directly beneath the testing arena at 75db to remove any potential

directional sound cues ('Dark plus Noise' probe). As the RScomb1 and Sham1 rats were tested throughout with white noise during the training and probe trials, no final probe (Dark plus Noise) was required.

2.3.4 Results

Histological evaluation of the lesions

Six rats in the dysgranular retrosplenial lesion group (RSdysg) were excluded as the lesions were either largely unilateral or because there was a high level of bilateral sparing of the dysgranular retrosplenial cortex. The final number of subjects in the RSdysg group was eight, with ten in the corresponding sham group (ShamD). None of the remaining animals had damage to the hippocampus or the subiculum. Five of the eight RSdysg animals had a very limited amount of unilateral damage to the granular retrosplenial cortex (see Figure 2.2). The lesions did not extend into any adjacent cortical areas (see Figure 2.3).

In the combined retrosplenial lesion cohort (RScomb1) seven rats were excluded due to sparing of the retrosplenial cortex or due to bilateral damage to the hippocampus, leaving nine rats in the RScomb1 group and twelve corresponding controls (Sham1) (see Figure 2.2). In these nine RScomb1 rats, extensive cell loss and gliosis was present throughout the retrosplenial cortex (see Figure 2.3) in both the granular and dysgranular sub-regions. Three of the nine animals had a very restricted area of cell loss and gliosis in the most dorsal medial tip of the CA1 subfield of the septal hippocampus. In two of these cases the CA1 cell loss was only unilateral. In one of

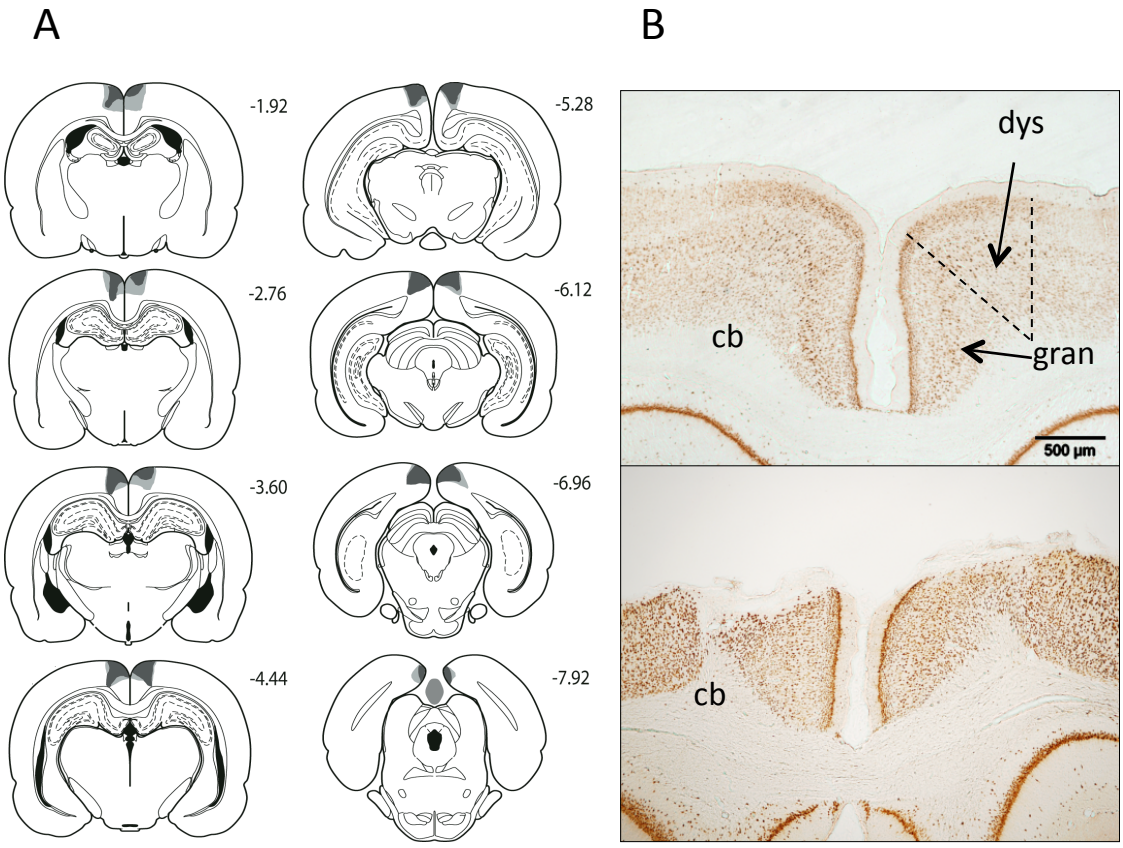

Figure 2.2

A: A series of coronal sections showing the cases with the largest and smallest lesions included in the dysgranular retrosplenial lesion group (RSdysg). Light grey represents the largest lesion, and dark grey the smallest. The numbers correspond the distance behind bregma in mm (Paxinos and Watson, 2006). B: Coronal NeuN sections showing the retrosplenial cortex (both hemispheres) in a sham surgery control rat (top), and a representative rat from the dysgranular (RSdysg) lesion group. The dashed lines show the limits of the retrosplenial cortex and of the granular and dysgranular sub-regions. The scale bar is 500µm long. Abbreviations: cb, cingulum bundle; dys, dysgranular retrosplenial cortex; gran, granular retrosplenial cortex.

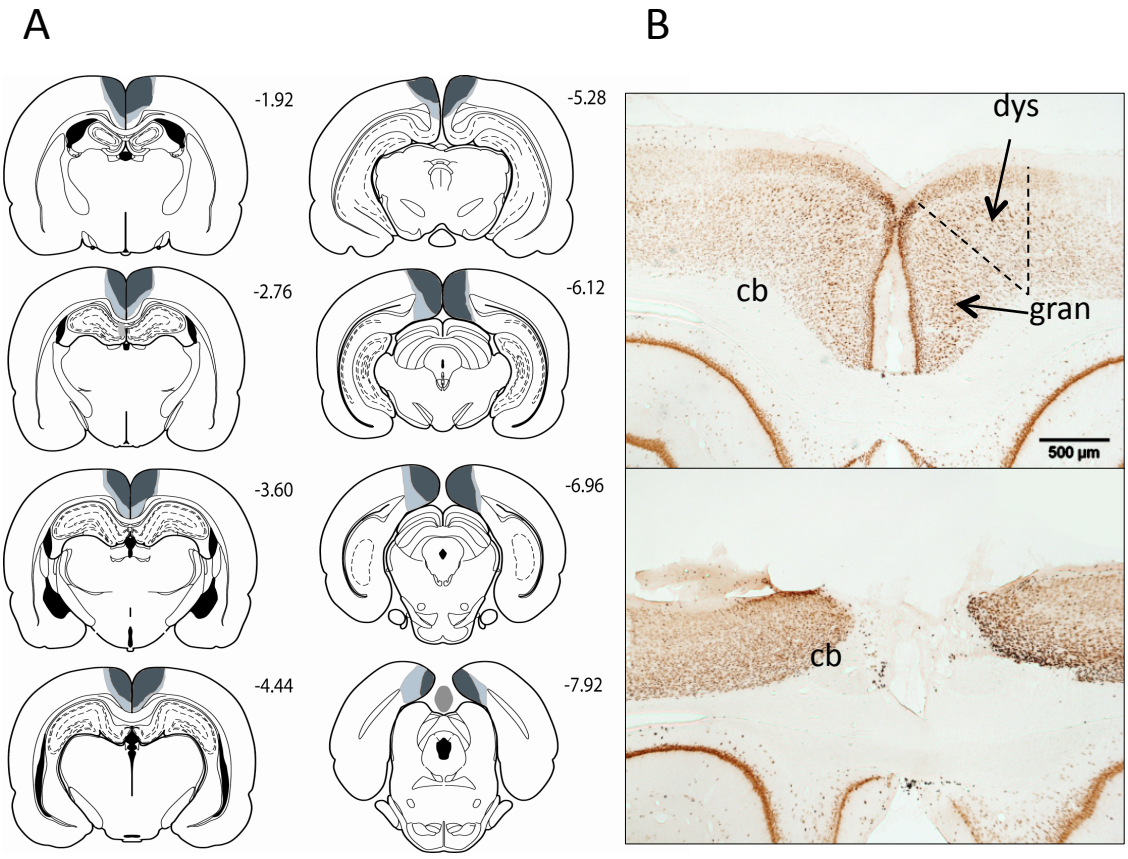

Figure 2.3

A: A series of coronal sections showing the cases with the largest and smallest lesions included in the combined dysgranular and granular retrosplenial lesion group (RScomb1). Light grey represents the largest lesion, and dark grey the smallest. The numbers correspond the distance behind bregma in mm (Paxinos and Watson, 2006). B: Coronal NeuN sections showing the retrosplenial cortex (both hemispheres) in a sham surgery control rat (top), and a representative rat from the combined dysgranular and granular (RScomb1) lesion group. The dashed lines show the limits of the retrosplenial cortex and of the granular and dysgranular sub-regions. The scale bar is 500µm long. Abbreviations: cb, cingulum bundle; dys, dysgranular retrosplenial cortex; gran, granular retrosplenial cortex.

these cases there was also some unilateral damage in the most medial part of the septal CA3. In all cases, the damage was restricted to fewer than 600µm in the AP direction. Four animals had partial sparing of the retrosplenial granular a region, particularly towards the caudal extent of the retrosplenial cortex. Three rats also had some limited sparing of retrosplenial granular b, typically only near its caudal limits. Only one animal showed a clear extension of the lesion into adjacent cortical areas, with some slight bilateral damage to the most caudal extent of the anterior cingulate cortex. A restricted area of gliosis was observed at the junction of the anterior medial and anterior ventral nuclei, as is consistently observed after extensive retrosplenial lesions (Gonzalez et al., 2003; Neave et al., 1994; Vann et al., 2003). No gliosis was seen in this area in the RSdysg animals.

Behavioural results

Analysis of the digging latencies for the separate go and no-go trials over the first block of four sessions, before any evident learning, showed no difference in baseline dig latency for either cohort (both $F < 1$). Only the first block could be analysed in this way, as in later blocks digging latency could be differentially affected by learning. As the baseline latencies of the two groups were found to be comparable, all further analysis has focused on the difference in the latencies of the go and no-go trials. With task acquisition, this latency difference should increase.

Retrosplenial dysgranular lesions

Based on the latency difference scores for go and no-go trials, both the lesion and sham groups acquired the discrimination at equivalent rates as indicated by the significant main effect of training block ($F(7, 112) = 16.7$, $p < 0.001$) with no main effect of lesion ($F(1, 16) = 1.86$, $p > 0.05$) or training block by lesion interaction ($F< 1$) (see Figure 2.4A).

Performance on the Place probe, when the absolute location of the cup remained the same for both rewarded and non-rewarded trials (Figure 2.1B) was assessed by comparing performance with that on the previous day of testing, when the location of the test arena remained stationary but the placements of the food cups differed. There was no effect of trial type ($F < 1$), no overall lesion effect ($F <1$), and no significant trial type by lesion group interaction ($F(1, 16) = 1.90$, $p > 0.05$). These findings show that neither the RSdysg nor ShamD animals relied on the absolute position of the digging cup to determine whether the trial would be rewarded or not. Following this probe rats were given two reminder days of the original acquisition training (Original, Figure 2.4B).

When the curtain was drawn around the test arena (Curtain probe) to hide salient visual cues attached to the walls, the go/no-go difference scores of both groups dropped significantly compared to the final day of acquisition training (Original) ($F(1, 16) = 10.7$, $p < 0.01$) (see Figure 2.4B), indicating that rats were less able to

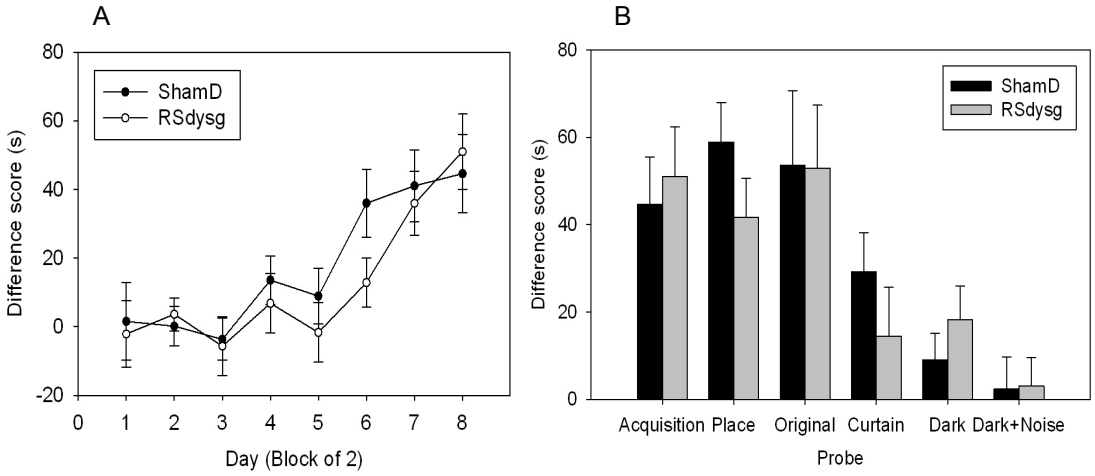

Figure 2.4

Experiment 1 - Perspective task (Cohort D). A: Graph showing acquisition of the Perspective task by ShamD and retrosplenial dysgranular lesion rats (RSdysg). Performance is shown by the latency difference between go and no-go trials. Rats discriminated between two spatial views, each with their own associated direction of travel. Error bars show the standard error of the mean. B: Probe trials are shown in the right hand graph. 'Acquisition' represents performance on the last day of training prior to the Place probe. Both ShamD and RSdysg lesioned animals' performance decreased significantly compared to the final day of training (Original) when visual cues were occluded (Curtain probe), with further falls for the ShamD group when tested in the dark with the curtain closed (Dark probe). Neither group was able to perform the task in the dark with background white noise (Dark+Noise). Error bars show the standard error of the mean.

distinguish the viewpoint that they were facing without these visual cues. There was no differential effect of lesion ($F < 1$) or trial type (Original vs. Curtain) by lesion interaction ($F < 1$). Both groups still performed above chance level (one sample t-test; RSdysg $t(7) = 3.37$, $p < 0.05$, ShamD $t(9) = 3.75$, $p < 0.01$). When further visual cues were removed by running the experiment in the dark, there was a significant trial type (Curtain vs. Dark) by lesion interaction ($F(1, 16) = 7.63$, $p < 0.05$) as the RSdysg group's performance did not change significantly ($F < 1$) while that of the ShamD animals decreased ($F(1, 9) = 12.3$, $p < 0.01$). During this Dark probe test, the RSdysg group ($t(7) = 5.01$, $p < 0.01$) still performed above chance while the ShamD group ($t(9) = 1.44$, $p > 0.05$) did not. The implication is that the ShamD animals could still use residual or peripheral visual cues during the Curtain probe and so were more disrupted when subsequently tested in the dark. For the final Noise probe, white noise was played (in the dark) for the first time, and although there was an apparent drop in performance when compared with the Dark probe this narrowly failed to reach significance ($F(1, 16) = 4.41$, $p = 0.052$). There was no effect of lesion on this final Dark plus Noise probe ($F(1, 16) = 1.73$), no lesion by trial type interaction ($F < 1$) and neither group was above chance (both groups, one sample test, $t < 1$).

Combined retrosplenial lesions

During the acquisition phase the animals' latency difference scores improved, as shown by a main effect of block ($F(6, 114) = 53.4$, $p < 0.001$). Although there was no overall difference between the lesion and control groups ($F(1, 19) = 2.00$, $p > 0.05$), there was a significant block by lesion interaction ($F(6, 114) = 2.58$, $p < 0.05$) (see

Figure 2.5A). Analysis of the simple main effects showed that although the two groups did not differ in the first 4 blocks (all $F < 1$), the Sham1 group performed significantly better than the RScomb1 rats in blocks 6 and 7 (minimum $F(1, 6.6) = 7.40$, $p < 0.05$).

Both the Sham1 and RScomb1 groups appeared to use the distal room cues to perform the task as the latency difference scores dropped significantly compared to the final day of acquisition when the curtain was drawn around the testing arena ($F(1, 19) = 54.6$, $p < 0.001$). While the lack of significant trial type by lesion interaction ($F1, 19) = 2.58$, $p > 0.05$) might indicate that both groups were similarly affected by the Curtain probe, this result is difficult to interpret as the performance of the two groups started from different levels. During this probe the Sham1 animals were still able to discriminate the two directions (one sample test, $t(11) = 4.05$, $p < 0.01$), while the lesion animals' performance fell to chance ($t < 1$). This difference in performance was also reflected by a significant group difference on this probe ($F(1, 19) = 11.6$, $p < 0.01$; see Figure 2.5B).

When further visual cues were removed by running the experiment in the dark there was a significant decrease in performance compared to the Curtain probe ($F(1, 19) = 10.7$, $p < 0.01$), but no trial type by lesion interaction ($F(1, 19) = 1.04$, $p > 0.05$). Neither group performed above chance level in this Dark probe (both $t < 1$) (see Figure 2.5). Between each probe trial the task was run again in the same way as during the acquisition phase, to prevent extinction. No difference was found between the final day of acquisition training and the two inter-probe days ($F(2, 38) =$

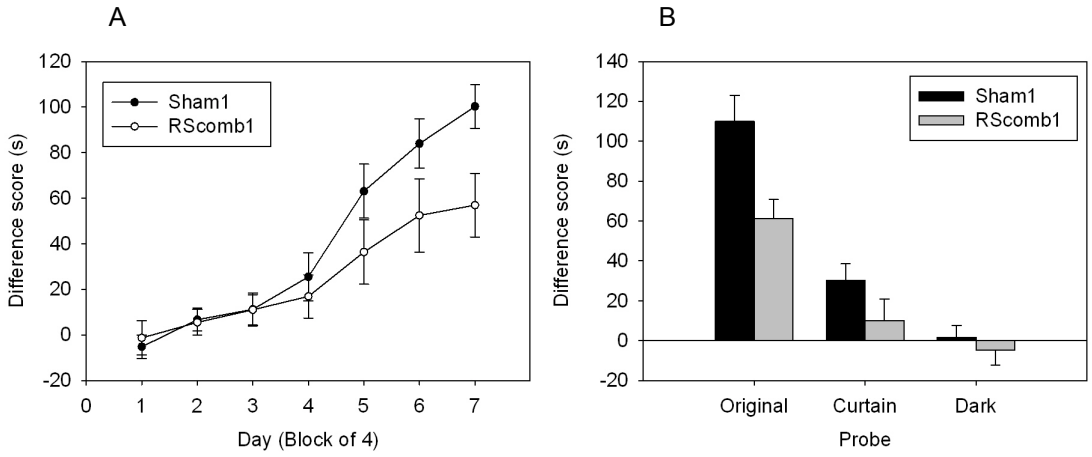

Figure 2.5

Experiment 1 - Perspective task (Cohort 1). A: Graph showing acquisition of the Perspective discrimination task by Sham1 and combined granular and dysgranular retrosplenial (RScomb) lesion animals. Rats discriminated between two spatial views, each with their own associated direction of travel. Performance is shown as the difference in dig latencies between correct ('go') and incorrect ('no-go') trials. Error bars show the standard error of the mean. B: A graph showing performance of the Sham1 group and RScomb1 groups on the probe trials administered following acquisition of the perspective discrimination task. 'Original' refers to performance on the final day of training. When visual cues were occluded ('Curtain') both groups' performance fell. Neither group carried out the task at above chance in the dark with the curtain drawn ('Dark'). White noise was played throughout training, so no noise probe was required. Error bars show the standard error of the mean.

2.09, p > 0.05), and no lesion by trial interaction (F < 1). However, there was still a main effect of lesion (F(1, 19) = 10.3, p = < 0.01), due to the poorer performance of the RScomb1 group compared to the sham group at the end of training.

2.4 Experiment 2 – Digging cup discrimination (non-spatial go/no-go)

2.4.1 Apparatus

Two different digging cups were used. One cup was the same as used in Experiment 1, and the other was a square cup made of white plastic, measuring 8cm x 8cm x 6cm. A metal grille was inserted into the base of both cups to allow Cheerios to be hidden inside without being accessible to the rats. The square cup was filled with small plastic beads (Hama beads, Malta Haaning Plastic A/S, Denmark) and the round cup with blue craft sand. The cup was fixed with Velcro onto the floor of the test arena, a rectangular white plastic box measuring 25cm x 42cm with walls 12cm high. The cup was placed 2cm away from one end of the arena, and was changed each time the digging medium changed. The arena was placed on a table at a height of 76cm, and against one wall of a room measuring 195cm x 330cm. The same room was used for both training and habituation, with white noise (75db) played throughout for both the RSdysg and RScomb1 cohorts.

2.4.2 Behavioural Protocol

Rats were presented with a digging cup filled with either craft sand or Hama beads, and were rewarded for digging in one medium but not the other. The rewarded medium was counterbalanced across groups. No habituation was given, as the experiment took place directly after Experiment 1. Rats were placed in the arena at the opposite end to the digging cup, and the time taken to start digging measured. During a rewarded (go) trial, rats that did not dig were shown the Cheerio. Rats were removed from the arena 5s after finding the reward. During no-go trials, the rat was removed from the arena after 20s if it had not dug or was removed after it had finished digging in the incorrect cup and found no reward. Digging was defined as breaking the surface of the digging medium with the nose or paws. Rats were given 16 spaced trials per day in groups of four animals, giving an inter-trial interval of approximately 90s. The arena and cups were cleaned with alcohol wipes after each session to help remove odour cues. A difference score was calculated in the same way as for Experiment 1.

2.4.3 Results

Retrosplenial dysgranular lesions

Analyses based on the latency difference scores showed that by the second test day both groups performed above chance (one sample test, RSdysg; $t(7) = 9.38$, $p < 0.001$; ShamD: $t(9) = 4.52$, $p < 0.001$). There was no significant difference in these scores between the two groups ($F(1, 16) = 2.52$, $p > 0.05$) and no day by lesion

interaction ($F < 1$). The main effect of day ($F(1, 16) = 20.6$, $p < 0.001$) demonstrated that the rats improved with training (see Figure 2.6A).

Combined retrosplenial lesions

Again, by the second day of testing both the combined retrosplenial lesioned animals and their sham group performed this task at above chance level (one sample t tests, RScomb1; $t(8) = 4.61$, $p < 0.01$; Sham1; $t(11) = 5.52$, $p < 0.001$). Again, there was no lesion effect ($F < 1$) when considering the latency difference scores, although there was a main effect of day ($F(1, 19) = 16.2$, $p < 0.001$) demonstrating an improvement in performance with training. There was no day by lesion interaction ($F< 1$) (see Figure 2.6B).

2.5 Experiment 3 – Place discrimination ('Location' task)

2.5.1 Apparatus

The same digging cups were used as in Experiment 1, while the test arena was the same as in Experiment 2. The testing room measured 300cm by 280cm, with a ceiling height of 240cm. Visual cues such as posters and shapes made of coloured paper were fixed to the walls of the testing room to differentiate between the different areas. Two identical tables, 70cm high, were placed in diagonally opposite corners of the room. The test arena was placed on one of these tables for each trial. The digging cup was filled with shredded paper. Light levels were at 260 lux in both

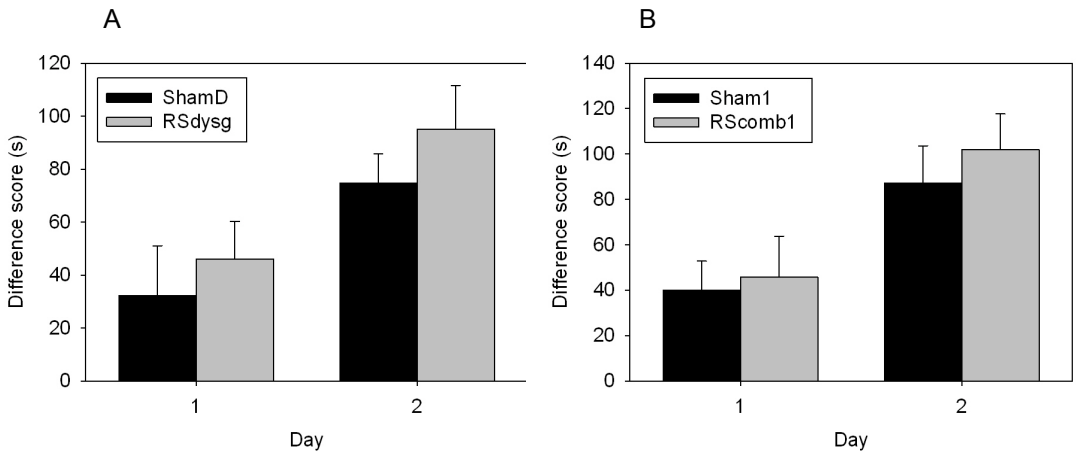

Figure 2.6

Experiment 2 - Digging cup discrimination (A shows Cohort D and B Cohort 1). The graph shows the mean latency difference scores between correct ('go') and incorrect ('no-go') trials for the two sessions of testing ± standard error of the mean. Neither dysgranular retrosplenial (RSdysg) nor combined granular and dysgranular retrosplenial (RScomb1) lesions affected performance on this non-spatial go/no-go discrimination.

test arena locations. Rats were brought to the testing room in individual metal holding boxes with lids. During testing, the holding box was located on a table 76cm high, in the centre of the room.

2.5.2 Behavioural Protocol

This experiment required rats to discriminate between two distinct locations (Figure 2.7). As this experiment directly followed Experiment 2, no habituation took place. There was only one test arena, which was moved between two identical tables in diagonally opposite corners of the room. Using the same arena throughout reduced the likelihood of using any local odour cues to discriminate location. To reduce odour cues further, the arena was cleaned with alcohol wipes after each session (the completion of a day's testing for four rats whose trials were interleaved with each other). Rats were rewarded for digging when in one arena location but not in the other, with the correct location counterbalanced across groups. Rats were placed in the arena so that they had to approach the cup from one of two directions in each location (see Figure 2.7), and so could not use direction *per se* as a discriminative cue. Following training, the RScomb1 and Sham1 rats were given a probe test in the dark, with light levels below 1 lux, to determine whether visual cues were critical for solving the discrimination. (Cohort D was not given this final probe.) Background white noise was played throughout training and testing for the combined lesioned cohort (RScomb1) and their controls (Sham1). All other running procedures were as previously described for Experiments 1 and 2.

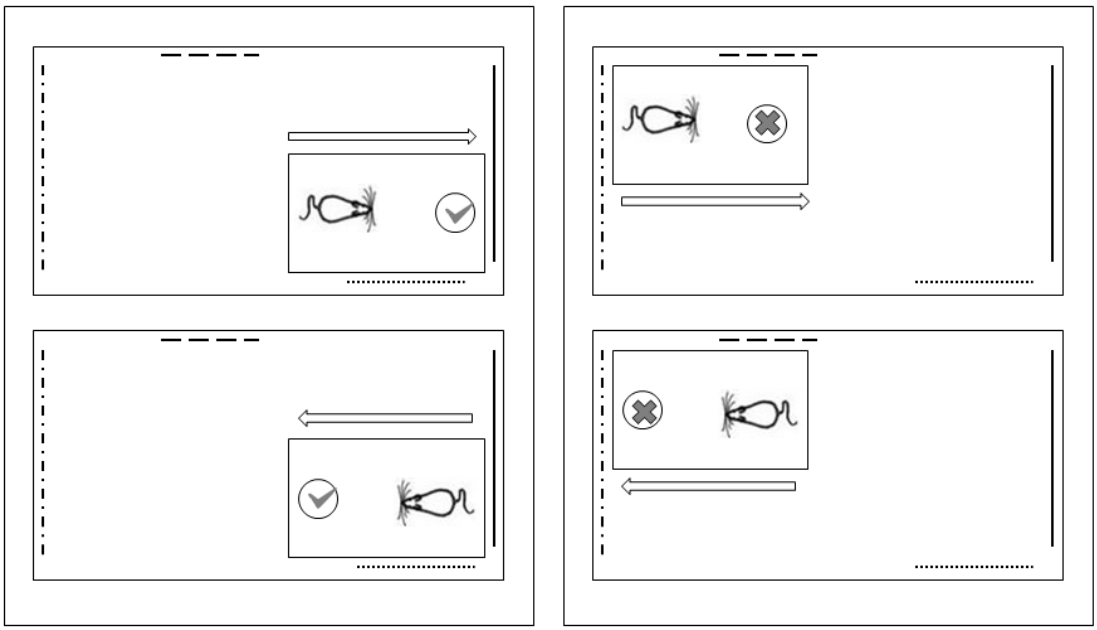

Figure 2.7

Location task (Experiment 3). In the Location task animals were rewarded for digging when the arena was in one corner of the room (left), but not if the arena was in the opposite corner (right). The animal could not use direction *per se* to solve the task, as both directions were equally rewarded and unrewarded. Various visual cues (dashed lines) were attached to the walls to allow them to be distinguished. (The apparatus, room and test arena are not to scale).

2.5.3 Results

Retrosplenial dysgranular lesions

The RSdysg lesion group was significantly poorer than their control group at discriminating between the two locations, as demonstrated by a main effect of group ($F(1, 16) = 6.28$, $p < 0.05$). There was a significant main effect of day ($F(5, 80) = 6.26$, $p < 0.001$), showing that overall performance, measured using difference scores, improved with training (Figure 2.8). However, there was no day by lesion interaction ($F(5, 80) = 2.13$, $p = 0.071$).

Combined retrosplenial lesions

Based on the latency difference scores, the Sham1 group performed significantly better than the lesioned animals, as shown by a main effect of lesion ($F(1, 19) = 11.3$, $p < 0.01$) as well as a significant day x lesion interaction ($F(7, 133) = 2.42$, $p < 0.05$) (see Figure 2.9). While there was also a main effect of day ($F(7, 133) = 21.2$, $p < 0.001$), indicating that the animals' performance on the place discrimination task improved with training, the lesion effect and interaction show that this improvement predominantly reflected the scores of the Sham1 rats.

When the animals were tested in the dark, performance decreased, giving a significant trial type by lesion interaction ($F(1, 26) = 25.7$, $p < 0.001$) as the two groups were significantly different on the last day of acquisition ($F(1, 19) = 13.4$, $p < 0.01$) but not on the Dark probe ($F < 1$). This interaction was, however affected by

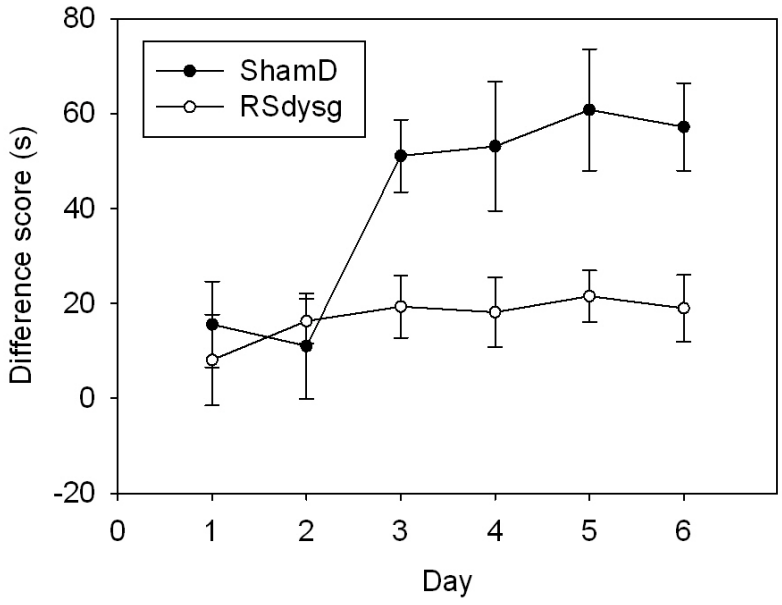

Figure 2.8

Experiment 3 – Location task (Cohort D). Graph showing acquisition of Location task, where rats were rewarded for digging in one corner of the room but not in the opposite corner. Performance is measured as the digging latency difference between correct ('go') and incorrect ('no-go') trials. A higher difference score indicates superior performance. Although both groups performed significantly above chance level by the end of training, dysgranular retrosplenial lesioned animals (RSdysg) acquired this location discrimination task more slowly than the ShamD animals. Error bars show the standard error of the mean.

both scaling effects and floor effects (Figure 2.9). Neither group performed above chance during this Dark probe (both $t < 1$) (Figure 2.9), indicating that rats had relied on visual cues to solve the task.

2.6 Discussion

The present study examined the effects of combined retrosplenial cortex lesions involving both areas 29 and 30, as well as the effects of more selective lesions largely confined to the dysgranular cortex (area 30). These two lesion groups, along with their respective controls, were trained on two sets of visuospatial tasks. The first task (Experiment 1, 'Perspective') taxed the ability to discriminate a particular room view, along with its associated direction of travel, from the opposite room view and its associated direction of travel (Figure 2.1). Only the rats with combined area 29 and 30 lesions (RScomb1) were significantly impaired at learning this Perspective task. Rats with dysgranular lesions (RSdysg) showed changes in their reliance on visual cues as they were less affected by being tested in the dark when compared to their prior performance in the light with a curtain around the walls of the room. In the second spatial task (Experiment 3, 'Location') both sets of lesions (i.e., RSdysg and RScomb1) impaired a place discrimination task that required the rats to determine their location in a room using distal visual cues. Unlike the first experiment, no particular direction of travel was associated with reward in the location task. As both of the spatial tasks in Experiments 1 and 3 involved go/no-go discriminations, Experiment 2 tested the ability of the rats to learn a non-spatial go/no-go discrimination. Neither group was impaired on this rapidly acquired

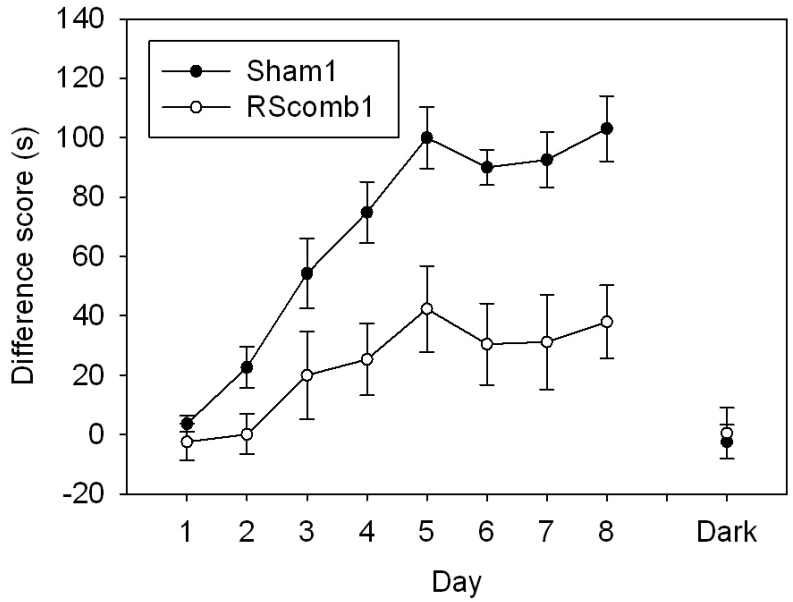

Figure 2.9

Experiment 3 – Location task (Cohort 1). Graph showing acquisition of the Location task, where animals were rewarded for digging if the arena was in one corner of the room, but not in the opposite corner. Performance is measured as the difference between digging latencies on correct ('go') and incorrect ('no-go') trials. Although both groups performed significantly above chance level by the end of training, animals with combined lesions of the granular and dysgranular retrosplenial cortex (RScomb1) acquired this perspective discrimination task far more slowly than the Sham1 animals. Neither group was able to perform the discrimination successfully in the dark. Error bars show the standard error of the mean.

control task, suggesting that the observed deficits in the other experiments were spatial in nature and not related to other task demands.

Two cohorts of rats were tested in all three experiments, making it possible to consider the roles of different retrosplenial sub-regions. The findings reinforced the importance of the dysgranular retrosplenial cortex (area 30) for the processing of visual information, as damage within this area consistently affected the use of distal spatial information. This result builds on anatomical studies showing that visual information first reaching the retrosplenial cortex appears to preferentially target area 30 (Mizumori et al., 2000; van Groen and Wyss, 1992; Vogt and Miller, 1983). Although minor procedural changes were introduced for the second cohort, in light of the findings from the first cohort, the profile of results for the two control groups appeared similar. An additional difference was that Cohort D (dysgranular lesions) was assessed longer after surgery than Cohort 1 (combined retrosplenial lesions) and had completed additional spatial tasks. These factors highlight the value of considering these two cohorts of lesioned rats with their own individual control rats.

The behavioural tasks were designed, in part, to help assess the potential importance of the dysgranular region for visuospatial tasks as well as considering the notion that the retrosplenial cortex has a 'translator' function, i.e., it aids the ability to change spatial frames of reference or coordinates (Burgess et al., 2001; Byrne et al., 2007; Vann et al., 2009). An example of this would be the interplay between allocentric and egocentric representations of the same location. To actively use the former representation, the viewer should be able to convert from an allocentric map

to an egocentric representation that can be matched to any heading direction, presumably using information from head direction cells in Papez' circuit, including the anterior thalamic nuclei (Bird and Burgess, 2008; Burgess et al., 2001; Byrne et al., 2007).

The 'Perspective' task (Experiment 1) should make few demands on coordinate translation as each of the two views to be discriminated had unique, salient wall stimuli, along with opposing directions of travel. There was no evidence, based on Cohort D, that the rats had learnt the potentially more complex solution of distinguishing the absolute locations of the rewarded and non-rewarded cups with respect to the room. Given that the demands of the Perspective task on stimulus integration and translation appear slight, it is all the more striking that the RScomb1 rats were impaired. One interpretation is that the retrosplenial cortex has additional spatial functions. Another is that optimal performance on the task involved the integration of both direction of travel and viewpoint information such that the control rats were superior to the RScomb1 rats. The unsatisfactory *post hoc* nature of this explanation indicates the need to develop a better specified translator model. While the present study does not allow us to distinguish the different possible explanations for the deficit seen in Experiment 1, this task supports other findings implicating the retrosplenial cortex in integrating visual and spatial information (Cooper and Mizumori, 2001; Cooper et al., 2001).

Clear evidence that all groups of rats were using distal visual stimuli to solve the Perspective problem (Experiment 1) came from the fall in performance when a

curtain was drawn around the test arena. Evidence that the RSdysg lesions had altered reliance on cue types came from the Dark probe, where they were less affected than the ShamD animals and still above chance levels. In contrast, the RScomb1 group did not perform above chance levels during either the Curtain or Dark probes, while the Sham1 animals remained above chance with the curtain closed, but not in the dark (Figure 2.5). The ability of the sham rats to perform above chance when the curtains were closed in the light, found in both cohorts of rats, suggests that the sham rats were able to use other visual room cues that the rats with retrosplenial damage could not exploit. Examples of potential visual cues include changes in light level at different distances from the overhead lighting, the positioning of equipment on the ceiling, or the overall geometry of the room, as the curtains did not obscure the room's dimensions. Not only is it known that rats can use geometric cues to identify locations, but this ability is disrupted by lesions in the hippocampus (Jones et al., 2007) and anterior thalamic nuclei (Aggleton et al., 2009), regions closely interconnected with the retrosplenial cortices.

One issue is the extent to which Experiment 1 performance was solved by using the different directions of travel. This issue was not tested directly as it would have required restraining the rats, and so qualitatively altering the task and its demands. It is, however, known that head direction cells, which provide compass-like information, can be maintained by both exteroceptive and interoceptive information (Taube, 2007). If task performance involved head direction information then it might be expected that when exteroceptive visual cues were removed by closing a curtain and switching off lights, there would be a loss of performance in normal rats.

It is also the case that retrosplenial lesions disrupt the stability of anterior thalamic head direction cells in the light (Clark et al., 2010), having less effect on self movement cues in the dark. Such findings, combined with the fact that head direction cells are present across both granular and dysgranular retrosplenial cortex (Chen et al., 1994a; Cho and Sharp, 2001), could help explain the results from Experiment 1. That is, the rats with dysgranular lesions might show a relative sparing due to preserved head-direction information in the granular retrosplenial cortex that is more reliant on interoceptive information. As a consequence these same rats might be less disrupted initially when performing in the dark. Their failure when finally tested in the dark with noise could then be explained by the disruption caused by the novel auditory stimulus, the more plausible as previous performance levels were low. However, there is also the possibility that RSdysg animals could have been impaired if white noise had been played throughout acquisition, as they were the only lesioned group able to discriminate the directions in the dark. Prior evidence that extensive retrosplenial lesions can impair a direction alternation task (Pothuizen et al., 2008) would again suggest that direction learning could underlie the pattern of lesion results of Experiment 1. It was partly for this reason that in Experiment 3 heading direction information was largely removed as a discriminatory cue, yet both retrosplenial lesion groups were still impaired.

The demands of Experiment 3 were more complex than those of Experiment 1. In this second spatial task, direction of travel within the test room is of little help on its own (Figure 2.7). Furthermore, a reliance on individual, distal room cues is more problematic as both the rewarded and non-rewarded locations will be associated

with overlapping common cues, albeit from difference distances. Thus, it is proximity to particular cue combinations that may best specify whether the rat is in the rewarded or non-rewarded location and so solving the task should, therefore, include the ability to unite different perspectives from the same place. That extensive retrosplenial lesions (RScomb1) can disrupt the ability to discriminate a location may seem unsurprising given the effects of this surgery on tasks such as platform finding in the Morris water maze (Cain et al., 2006; Vann and Aggleton, 2002; Whishaw et al., 2001) and object-in-place learning (Vann and Aggleton, 2002). Perhaps more striking is the severity of the apparent deficit (Figures 2.8, 2.9).

Other experiments using more standard radial-arm maze protocols for working memory (Olton and Samuelson, 1976), have often found that retrosplenial lesions have little impact on initial task acquisition (Pothuizen et al., 2009, 2008; Vann and Aggleton, 2005, 2004). At first sight this sparing may seem perplexing as spatial working memory tasks should be more demanding than the corresponding spatial reference memory task. It has, however, been found with radial-arm maze probe tests that rats with retrosplenial cortex lesions are unusually reliant on intra-maze cues and have difficulties when forced to use distal visual cues (Vann and Aggleton, 2005). The implication is again that the processing of distal visual inputs is a key component of normal retrosplenial functioning. Such an interpretation again focuses particular attention on the dysgranular cortex given its more direct interactions with visual areas (van Groen and Wyss, 1992; Vann et al., 2009). This interpretation was supported by the behaviour of the RSdysg group, who were markedly impaired on the spatial task in Experiment 3.

As mentioned earlier, the present experiments can be interpreted in light of the theory that the retrosplenial cortex has a key role in translating between allocentric and egocentric representations of space (Bird and Burgess, 2008; Byrne et al., 2007). According to this theory, the presentation of a single landmark or cue can lead to the retrieval of a complete representation of a place, including the viewpoint of the observer, through pattern completion. This involves the development of an allocentric representation from an egocentric stimulus. In order to then use this representation, the viewer must be able to convert it back from an allocentric map to an egocentric representation that can be matched to any heading direction, presumably using information from head-direction cells in Papez' circuit (Burgess et al., 2001). In view of the demands of the Location task it is of particular relevance that place-by-direction cells have been recorded in the presubicular and parasubiculuar cortices of the hippocampal formation (Cacucci et al., 2004). These subicular regions innervate the retrosplenial cortex, in particular the granular sub-region (van Groen and Wyss, 2003, 1992, 1990b), while both the granular and dysgranular retrosplenial cortices project to the subicular cortices (van Groen and Wyss, 2003). Thus, these electrophysiological and anatomical findings add further weight to the notion that the retrosplenial cortex is involved in information translation, and so it would be expected that the Location task (Experiment 3) should be particularly sensitive to retrosplenial cortex damage. A limitation with this interpretation is that it may sometimes prove difficult to specify when information integration and translation is or is not required.

In summary, the current experiments clearly reveal the importance of the rat retrosplenial cortex for the usage of distal visuospatial information. The present results also reveal how the two major retrosplenial sub-regions appear to work in conjunction for some spatial functions, as the rats could find a way of solving the Perspective problem (Experiment 1) after losing just the dysgranular cortex, but this area was required for the Location task (Experiment 3). One explanation that would fit the pattern of results is that the two regions work together to combine and integrate exteroceptive (e.g., visual stimuli, area 30) and interoceptive (e.g., proprioceptive stimuli, area 29) cues to solve spatial tasks that involve the resolution of multiple viewpoints from the same location.

Chapter 3

3. Dysgranular retrosplenial cortex lesions in rats disrupt cross-modal object recognition

3.1 Introduction

Research into the functions of the retrosplenial cortex has often considered its potential roles in spatial memory (Vann et al., 2009), reflecting its dense interconnections with the hippocampus and anterior thalamic nuclei (Van Groen and Wyss, 2003, 1992, 1990). However, other connections of the retrosplenial cortex in both rodents and primates suggest a broader role in multimodal processing. For example, the retrosplenial cortex receives visual information directly from the geniculostriate and tecto-cortical visual systems (Van Groen and Wyss, 1992; Wyss and Van Groen, 1992). The retrosplenial cortex also has reciprocal connections with parietal and parahippocampal cortices that may, respectively, provide somatosensory and olfactory information (Aggleton, 2010; Insausti et al., 1997). The former inputs are of additional interest given recent evidence of the importance of the parietal cortex for tactile to visual cross-modal transfer in the rat (Winters and Reid, 2010). Taking these results together, the retrosplenial cortex becomes a plausible candidate for the integration of different classes of sensory information to help form multisensory representations.

The retrosplenial cortex is divided into two major sub-regions, granular (area 29) and dysgranular (area 30), which differ in their connectivity and cellular morphology. In the rat, the dysgranular area may be of particular importance for the integration of visual information given its many connections with both cortical and subcortical areas strongly linked to visual processing, e.g., area 17 and the lateral dorsal thalamic nucleus (Van Groen and Wyss, 1992; Vogt and Miller, 1983). This view is supported by the finding that immediate early gene expression increases selectively in the dysgranular retrosplenial cortex, rather than the granular retrosplenial cortex, following spatial memory tasks performed in the light, compared to the same tasks performed in the dark (Pothuizen et al., 2009).

Many studies have demonstrated the involvement of the retrosplenial cortex in spatial memory, including when the tasks involve cue switching. Examples of the latter involve solving a radial-arm maze task in the light and then the dark (Chen et al., 1994b), changing from allocentric to directional cues in a T-maze (Pothuizen et al., 2008), and switching from intra-maze to extra-maze cues when performing a radial-arm maze task in the light (Pothuizen et al., 2008; Vann and Aggleton, 2005, 2004). It has been argued that the retrosplenial cortex has a 'translational' function in transforming spatial codes, e.g., allocentric representations into egocentric ones and *vice versa* (Burgess et al., 2001; Byrne et al., 2007; Vann et al., 2009). This translation function has, however, largely been confined to spatial processing. The present study used tests of object recognition to explore stimulus translation across a broader domain.

In the standard spontaneous object recognition task (Ennaceur and Delacour, 1988) rats are first presented with two identical objects and then, after a retention interval, allowed to explore the now familiar object and a novel, alternative object. Rats preferentially explore the novel object. Previous studies have shown that rats with retrosplenial cortex lesions are unimpaired on standard object recognition tasks (Ennaceur et al., 1997; Parron and Save, 2004; Vann and Aggleton, 2002). This same task has, however, been modified to examine cross-modal object recognition (Winters and Reid, 2010). In this task variant, the types of sensory cue available to the animal at the sample and test phases are switched, e.g., forcing the rats to rely on tactile cues in the sample phase but on visual cues in the test phase. To ensure tactile processing, rats were exposed to objects in the dark, while the subsequent visual discriminations were enforced by placing a clear Perspex barrier between the rat and the test objects in the light (Winters and Reid, 2010). Using these same methods (Figure 3.1), the present experiment determined whether retrosplenial lesions impair performance when rats are forced to use cross-modal strategies to solve recognition problems. Closely related experiments also examined object recognition memory when rats could only use visual cues or tactile cues throughout the object recognition test.

Finally, an object-in-place experiment was run with the combined lesion group and their corresponding sham animals that required rats to integrate information about the identity of an object with its spatial location in the testing arena. Rats with retrosplenial cortex lesions have previously been shown to exhibit deficits on this task (Parron and Save, 2004; Vann and Aggleton, 2004), which uses spatial

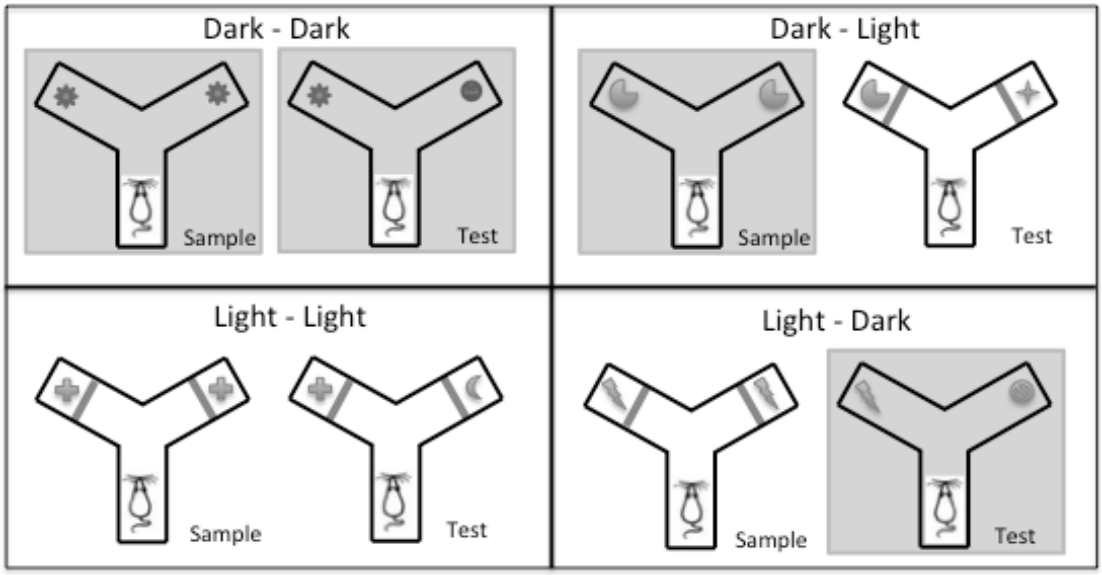

Figure 3.1

Schematic diagram showing the four different trial types in the cross-modal object recognition task. Left hand images in each pair show the sample phase, while right hand images show the test phase. The different symbols represent different objects. In any light phase condition, lights were turned on and barriers were placed between the rat and the object, to prevent tactile exploration; during dark phases these barriers were removed but the lights were turned off. Light-light and dark-dark trials do not require a cross-modal switch, while dark-light and light-dark trials do.

information without the complicating factor of navigation, as all the objects can be viewed by the rats from any point in the testing arena.

3.2 General Methods

The study comprised two cohorts of rats. The first cohort consisted of rats with dysgranular retrosplenial lesions and their sham surgical controls, the second cohort consisted of rats with combined granular and dysgranular retrosplenial lesions (RScomb2) and their surgical controls. The methods for Cohorts D and 2 were almost identical, with a few minor alterations made for Cohort 2 based on the results from Cohort D.

3.2.1 Animals

The two cohorts (dysgranular retrosplenial and granular plus dysgranular) comprised 52 experimentally naive male Lister Hooded rats (Harlan, Bicester, UK). Housing and feeding conditions for both cohorts were as described in Chapter 2. All experiments were carried out in accordance with UK Animals (Scientific Procedures) Act, 1986 and associated guidelines. The rats in the combined retrosplenial lesion cohort weighed from 278 -387g at the time of surgery. These animals received either a bilateral excitotoxic lesion of both areas 29 and 30 (RScomb2, n = 16) or a sham lesion (Sham2, n = 12). The rats in the dysgranular lesion cohort (Cohort D) have been described in Chapter 2.

3.2.2 Surgical Procedures

Surgical and post-surgical procedures were as described in Chapter 2.

The coordinates for the RScomb2 combined lesion cohort were AP -1.6, LM±0.4, DV-1.3; AP-2.8, LM±0.5, DV-1.3; AP-4.0, LM±0.5, DV-1.3; AP-5.3, LM±0.5, DV-2.6; AP-5.3, LM±0.9, DV-1.6; AP-6.6, LM±1.0, DV-2.0; AP-7.5, LM±1.1, DV-1.3. In each of the three most rostral injection sites, 0.25µl NMDA was injected. In the next three pairs of sites, 0.26µl NMDA was injected. In the most caudal site only 0.1µl NMDA was injected.

3.2.3 Histological Procedures

Histological procedures were as described in Chapter 2.

3.2.4 Statistical Methods

Statistical tests were carried out using SPSS 16.0 (SPSS Inc., Chicago). Where the assumption of sphericity was not met for parametric analysis, Greenhouse-Geisser corrections have been applied. The criterion alpha level was $p \leq 0.05$.

3.3 Experiment 1 - Cross-Modal Object Recognition

3.3.1 Apparatus

The Y-maze was constructed of wood and painted white. Each arm was 27cm long and 10cm high; the walls were 40cm high. In the start arm there was a wooden sliding door set 18cm from the end of the arm which, when closed, created an area within which an animals could be held until the beginning of a trial. There were also two clear Perspex sliding doors, positioned 9cm from the end of each sample arm that could be lowered during trials in the light to prevent the animal from using tactile cues to explore the objects (see Figure 3.1). These same barriers were removed during trials in the dark. During light trials, room illumination was provided by overhead fluorescent lights. During dark trials, room illumination was provided by infrared spotlights, with the experimenter using night-vision goggles (Productive Firm Dipol Ltd.) in order to see. Mean light intensity in the centre of the maze during light trials was 590 lx, and during dark trials was less than 1 lx. A video camera sensitive to infrared light was mounted on the ceiling to record each trial. This camera, which was connected to a monitor/DVD recorder, was used to record the rats' behaviour.

For the recognition tests, three identical copies of objects made from plastic, glass, aluminium or ceramic were used. These objects varied in height from 10 – 40cm, and differed in their tactile and visual properties. Before any object was placed in the maze it was wiped down with 50% ethanol to limit odour cues.

3.3.2 Pre-training

The cross-modal object recognition task started four months after surgery for the RSdysg and ShamD cohort. Prior to this experiment, the rats had been tested on spatial tasks in a radial-arm maze and water maze, on a biconditional task in an operant box and on an object recognition problem in the bowtie maze (Albasser et al., 2010). None of the objects used for that final task was similar to an object used in the experiments reported here. For the RScomb2 and Sham2 cohort, cross-modal object recognition testing began six months after surgery. These animals had previously been tested on a set-shifting task, negative patterning, conditioned inhibition and an object in place task (see Annex A). The object in place task has taken place three months prior to the cross modal recognition experiment, and did not use any of the same objects.

Habituation to the Y-maze took place over two consecutive days. No objects were present in the maze during habituation. On each day the rats were brought to the testing room from the holding room in an individual carrying box made of metal, with a lid that prevented the animal from seeing the room. Each rat spent two sessions in the maze consisting of five minutes in the dark with the Perspex barriers removed and five minutes in the light with the barriers in place. The rats were removed from the maze between each session and the order in which the sessions took place was counterbalanced across days and by lesion group.

3.3.3 Recognition testing

The experiment consisted of either four different trial types (Cohort D) or three different trial types (Cohort 2), each of which was repeated twice. Each test trial consisted of two phases, a sample phase and a choice phase. The sample phase was 3 minutes long and the choice phase lasted 1 minute. The greatest preference for the novel object is usually seen during the first minute, diminishing after that time (Dix and Aggleton, 1999; Moscardo et al., 2012), so this duration was chosen to give the clearest preference. The sample and choice phases were separated by a retention interval of 15 minutes for the RSdysg and ShamD groups, and by 5 minutes for the RScomb2 and Sham2 groups. The rats in Cohort 2 were given a shorter retention interval to help counter potential floor effects. There were four different classes of recognition test, reflecting the fact that both the sample phase and test phase could be in the light or the dark. During light trials a barrier was in place to prevent tactile exploration and to reduce olfactory cues; during dark trials this was not present. The four trial types were designated: light-light, dark-light, light-dark, dark-dark, where the first word refers to the sample condition and the second word refers to the test condition. While Cohort D received all four trial types it was found that the rats could not objects in the light-dark condition. This trial type was not given, therefore, to Cohort 2.

At the beginning of the sample phase the rat was placed in the start area of the Y-maze and the sliding door was raised. The trial was considered to have started when the whole of the rat's body, excluding the tail, left the start area. The sliding door

was then closed and the rat was allowed to explore the objects. During the sample phases, identical objects were placed at the end of each arm. After 3 minutes the rat was removed from the maze and returned to the carrying box for the duration of the retention interval.

During the retention interval one of the objects in the Y-maze was replaced with an identical copy, and the other replaced with a novel object that the rat had not yet explored. The arm in which the novel object was placed was counterbalanced across animals and across trials. To begin the choice phase the rat was placed back in the start area of the Y-maze and the sliding door opened. Once the rat had left the start area it was given one minute to explore the two different objects. Rats were tested twice on each of the various trial types (light-light, dark-dark, dark-light and light-dark), with a minimum of 48 hours between tests (Figure 3.1). Different objects were used for each of the rat's two trials of each test. All objects and the testing arena were wiped down with alcohol after each rat had been removed, to minimise the effect of olfactory cues not inherent to the objects themselves.

Videos of the rats' behaviour during the sample and choice phases were used to score how long rats spent exploring each object. In the dark, exploration was defined as time spent with the nose pointing towards the object at a distance of less than 1cm; in the light exploration was defined as time spent with the nose pointing to the area of the Perspex barrier directly in front of the object. Recognition performance was assessed using the D2 index (Ennaceur and Delacour, 1988), which was calculated as a measure of novel object preference, using the formula (time

exploring novel object – time exploring familiar object) / (total exploration). This ratio index was preferred to the absolute difference between time spent exploring the novel and familiar objects as the different lighting conditions were associated with differences in overall exploration time. Behavioural scoring was carried out with the experimenter blind to the lesion status of the animal.

3.3.4 Results

Histological evaluation of the lesions

The RSdysg lesions are described in Chapter 2 (see Figure 2.2 for details).

In the combined retrosplenial lesion cohort (RScomb2), three rats were excluded due to sparing of the retrosplenial cortex or due to bilateral damage to the hippocampus, leaving thirteen rats in the RScomb2 lesion group and twelve corresponding shams (Sham2). In the RScomb2 group, extensive cell loss and gliosis was seen throughout the retrosplenial cortex, in both the granular and dysgranular sub-regions. Three animals had restricted damage or gliosis in the most dorsal medial tip of the CA1 subfield of the hippocampus (two unilateral). In the remaining case the bilateral damage was very restricted. Over all cases, the maximum extent of anterior posterior hippocampal damage was 600μm. Seven animals, including the three with CA1 damage, had slight unilateral thinning of the medial blade of the dentate gyrus just caudal to the splenium. Nine animals had partial sparing of Rga, particularly at its caudal limit. Four rats also had some limited sparing of Rgb (see Figure 3.2). One rat had slight damage to the anterior cingulate cortex at the

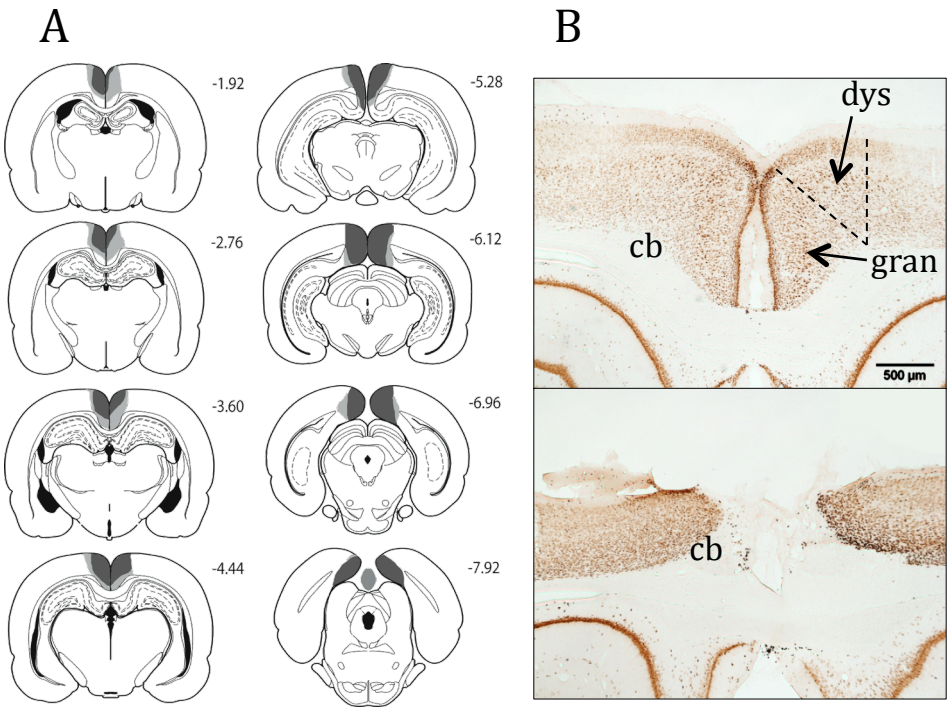

Figure 3.2

A: Series of coronal sections showing the cases of the largest (light grey) and smallest (dark grey) lesions included in the combined dysgranular and granular retrosplenial (RScomb2) lesion cohort. The numbers correspond to the distance behind bregma in mm (Paxinos and Watson, 2006). B: Coronal NeuN sections showing the retrosplenial cortex (both hemispheres) from a sham surgery control rat (top), and a representative rat from the RScomb2 lesion group (bottom). The scale bar represents 500um. Abbreviations: cb, cingulum bundle; dys, dysgranular retrosplenial cortex; gran, granular retrosplenial cortex.

junction with retrosplenial cortex, and two showed limited unilateral damage to the secondary motor cortex, lateral to the retrosplenial cortex. A restricted area of gliosis was observed at the junction of the anterior medial and anterior ventral nuclei, as is consistently observed after extensive retrosplenial lesions (Gonzalez et al., 2003; Neave et al., 1994; Vann et al., 2003). No gliosis was seen in this area in the RSdysg cohort.

Experiment 1 – Cross-modal object recognition

The cross-modal object recognition task required restricting the cue modalities available in both the sample and the test phases. This was achieved either by running the task in the dark, to remove visual cues, or by inserting a clear Perspex barrier between the animal and the object, to prevent tactile and olfactory exploration. Recognition performance was assessed using the D2 index (Ennaceur and Delacour, 1988), a measure of novel object preference. The D2 score is the difference in time spent exploring the novel object from the familiar object, divided by the total time spent exploring these objects. This ratio index was preferred to the absolute difference in the time spent exploring the novel object and the familiar object, as the contrasting light conditions used in the various tests were associated with changes in overall exploration times.

Cohort 1 - Dysgranular retrosplenial cortex lesions

Across the sample phases there was no difference in the amount of time that the RSdysg and ShamD animals spent exploring the objects (main effect of lesion on sample exploration time, $F < 1$). However, total exploration times during the sample phase did vary significantly depending on whether visual cues (light) or tactile cues (dark) were available ($F(1, 16) = 94.4$, $p < 0.001$, with greater exploration times in the dark (Table 3.1). There was no lesion by lighting type interaction ($F < 1$). The different durations of exploration in the light versus the dark reinforced the decision to focus on the D2 index of recognition for subsequent analyses. Furthermore, as no differences were found in the D2 scores when the same trial type was repeated (all $F < 1$) the two sessions of each trial type were combined prior to comparisons with other trial types.

In the test phase, differing levels of overall exploration were again seen in the dark and light tests ($F(1, 16) = 230.8$, $p < 0.001$), with more exploration in the dark (Table 3.1). The surgical groups did not, however, differ in their total amounts of exploration time ($F < 1$), nor was there was a group by lighting condition interaction on this measure ($F < 1$).

An ANOVA based on the D2 scores with factors of modality (two levels, intra- or cross-modal) and test (two levels, dark or light sample phase) revealed a main effect of modality as the rats discriminated the intra-modal recognition trials more readily than the cross-modal trials ($F(1,16) = 32.9$, $p < 0.001$; Figure 3.3). There was also an interaction between modality and test ($F(1,16) = 6.5$, $p < 0.05$), as performance was

	Sample exploration time (s)		Test exploration time (s)	
	Light	**Dark**	**Light**	**Dark**
ShamD	38.6 ± 14.0	71.6 ± 22.6	12.8 ± 4.05	27.9 ± 8.83
RSdysg	37.8 ± 11.9	71.2 ± 25.2	14.9 ± 5.26	28.7 ± 10.2
Sham2	24.1 ± 1.33	50.8 ± 1.61	9.47 ± 1.30	22.5 ± 2.01
RScomb2	26.0 ± 1.61	54.9 ± 2.27	7.81 ± 1.33	21.8 ± 1.58

Table 3.1

Experiment 1. Mean exploration times in the light and in the dark, for both the sample and the test phases of the cross-modal object recognition task. Times are shown in seconds ± SEM. Exploration times in the light were consistently lower than in the dark.

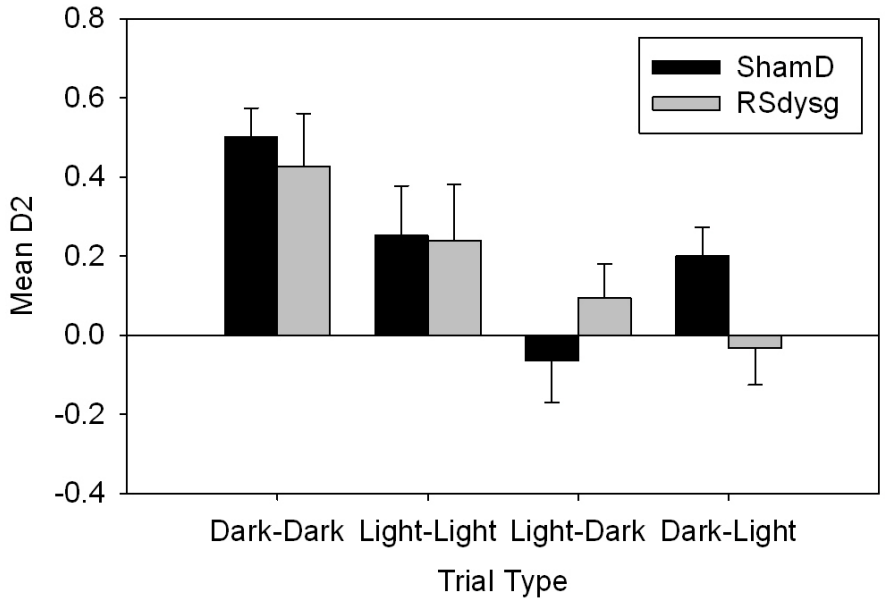

Figure 3.3

Cohort 1: Mean recognition (D2) scores seen at test for the four trial types. Two trial types (dark-dark and light-light) did not require a switch between cue modalities as the same cue types were available at both sample and test. The other two trial types were designed to force the rats to switch between different cue modalities between the sample and the test phases (light-dark and dark-light). Both the ShamD and RSdysg groups performed above chance levels on the light-light and dark-dark trials, but only the ShamD animals were above chance for the dark-light switch. Error bars show SEM.

better on dark-dark than on light-light trials ($F(1,16) = 9.8$, $p < 0.05$). Neither of these trends was affected by lesion group (max $F(1,16) = 1.6$, $p = 0.2$). However, there was a three-way interaction between modality, test, and lesion ($F(1,16) = 4.6$, $p < 0.05$). This interaction arose because the RSdysg group performed worse on the dark-light trials relative to shams ($F(1,16) = 12.8$, $p < 0.01$) but there were no statistically reliable differences between the groups on any of the other trial types (all $Fs<1$). There was also no overall effect of lesion ($F(1,16) = 1.4$, $p = 0.26$).

On the dark-light (cross-modal) trials ($F(1, 16) = 12.8$, $p < 0.01$), tactile and olfactory cues were available during the sample phase while only visual cues were available during the test phase. This group difference was due to the ShamD group significantly discriminating the novel object during the recognition phase ($t(9) = 4.18$, $p < 0.01$), while the RSdysg group's performance was at chance ($t <1$). It was also found that neither group discriminated the novel object in light-dark trials, where the sample object was explored in the light behind a barrier (visual only), and the test session took place in the dark with tactile and olfactory cues available (ShamD $t < 1$, RSdysg $t(7) = 1.34$, $p > 0.05$).

In dark-dark trials, where rats had access to tactile and potential odour cues, but not visual cues, both groups of animals showed a significant preference for the novel object (one-sample t-test vs. zero; ShamD $t(9) = 12.6$, $p < 0.001$; RSdysg $t(7) = 5.48$, $p < 0.001$) and there was no effect of lesion (D2, $F < 1$). Similarly, no group difference for D2 was found in light-light trials, when only visual cues could be used ($F < 1$). Again, both groups spent more time exploring the novel object (ShamD $t(9) = 3.14$, $p < 0.01$; RSdysg $t(7) = 2.52$, $p < 0.05$).

Cohort 2 – Combined granular and dysgranular retrosplenial cortex lesions

As only three trial types were used for the combined granular and dysgranular retrosplenial cohort, the statistical analyses were modified from those used for Cohort 1. Across both sample phases there was no difference in the amount of time that the RScomb2 and Sham2 animals spent exploring the objects (main effect of lesion on sample exploration time, $F(1, 23) = 1.56$, $p > 0.05$). However, total exploration times during the sample phase varied significantly depending on whether the trial was in the light or the dark ($F(1, 23) = 236.0$, $p < 0.001$), as more exploration occurred in the dark (Table 3.1). There was no lesion by lighting type interaction ($F < 1$). Once again, the different durations of exploration in the light versus the dark reinforced the decision to focus on the D2 index of recognition for subsequent analyses.

Exploration times during the test phase were found to differ significantly between the light and dark tests ($F(1, 23) = 194.4$, $p < 0.001$), with exploration levels in the dark higher than in the light (Table 3.1). There were, however, no differences in exploration times between the two groups ($F < 1$) nor was there an interaction with lighting condition ($F < 1$).

As for the RSdysg cohort, no differences were found in the D2 scores when the same trial type was repeated (all $F < 1$), so the two sessions of each trial type were combined and analysed together. An ANOVA based on the D2 scores revealed an effect of trial type ($F(1,23) = 10.9$, $p < 0.001$) as both groups performed best on the dark-dark trials (Figure 3.4). There was, however, no interaction between lesion

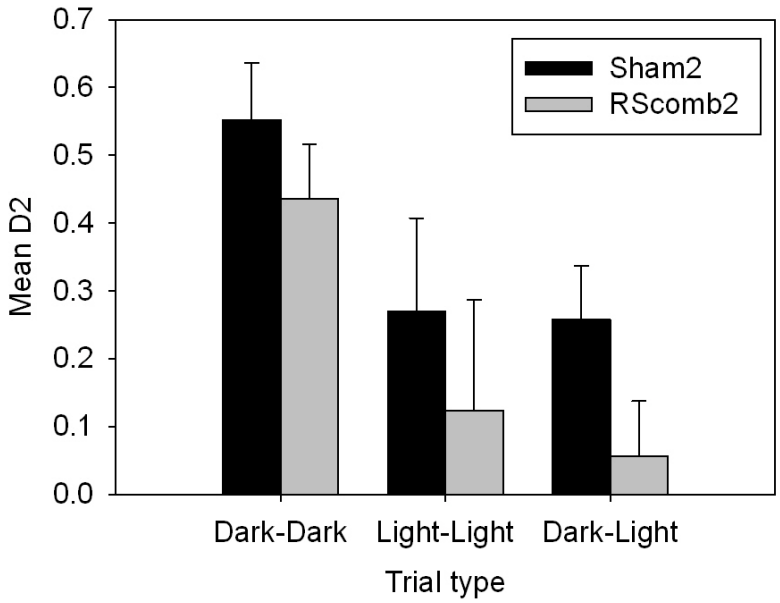

Figure 3.4

Cohort 2: Mean recognition (D2) scores achieved on dark-dark, light-light and dark-light trials. While both Sham2 and RScomb rats spent significantly more time with the novel object in dark-dark trials, only the Sham2 rats performed above chance levels in the light-light and dark-light trials on this task. Error bars show SEM.

group and trial ($F < 1$), though there was an effect of lesion group ($F1,23) = 10.5$, $p < 0.01$). This group difference reflected the lower overall D2 scores of the RScomb2 rats.

On the dark-light (cross-modal) trials, where rats were forced to switch between different cue types, the Sham2 rats had significantly higher D2 scores than RScomb2 rats ($F(1, 23) = 6.37$, $p < 0.05$). While the RScomb2 animals failed to show a preference for the novel object ($t(12) = 1.12$, $p > 0.05$) the Sham2 animals scored significantly above chance ($t(11) = 4.43$, $p < 0.01$), indicating that they were able to recognise the familiar object across modalities (see Figure 3.4). When all three trial types are compared (Figure 3.4) in an overall ANOVA there was not a significant group by condition interaction ($F < 1$).

3.4 Experiment 2 – Visual object recognition

One concern with the cross-modal object recognition task is the reduced levels of exploration when an object is placed behind a barrier in the 'visual' trials. The aim of Experiment 2 was to identify and test three-dimensional stimuli that would provide just visual cues for a recognition memory test, even though the rats could interact directly with the objects (there was no barrier). Through an initial screening stage, objects were selected that the rats could not distinguish in the dark, i.e., could not be distinguished by their olfactory or tactile cues. By allowing more direct examination in the light, object exploration times should increase over those seen in Experiment 1 for the light conditions, so enabling visual based recognition.

3.4.1 Object screening (dark–dark testing)

The choice of objects for this experiment was critical as the goal was to use pairs of different objects that could only be distinguished by their visual appearance. For this reason, all pairs of objects were intended to be indistinguishable in terms of shape, material and smell. Examples included a Coke can versus a Diet Coke can, or two glass containers of different flavours of the fruit drink, Schloer (Merrydown, Belfast; see Figure 3.5 for examples). These were all sealed to reduce odour cues, with different labels and different coloured contents. These objects should, therefore, be identical aside from their visual features. A second, related criterion was that these objects should not be discriminable in the dark and any such examples were excluded (see below). To determine which objects were suitable for this task, the first stage of the experiment consisted of a series of screening trials run in the dark, using the same dark – dark protocol described for Experiment 1. The D2 scores for each pair of objects were calculated following testing, and any objects that the rats could in the dark were removed from the experiment as they could be distinguished non-visually. Object pairs that could not be distinguished in the dark were subsequently used for testing in the light.

Experiment 2 took place immediately after Experiment 1 using the same test room and Y-maze apparatus, so no additional habituation was required. On each test day the rats were brought to the testing room from the holding room in an individual carrying box as previously described. During the sample phase, identical objects

Figure 3.5

Examples of objects used during the visual object recognition task. Objects were indistinguishable with regards to shape, material, texture and smell, as determined by the failure of the rats to recognise these objects in the dark. However, these same objects differed in colour and pattern, and so could potentially be visually distinguished when subsequently tested in the light. The scale bar represents 5cm.

were placed at the end of each arm of the Y-maze. After 3 minutes the rat was removed from the maze and returned to the holding box for 5 minutes. During this time, one of the objects was replaced with an identical copy, and the other with a novel object to which the rat had not yet been exposed. The arm in which the novel object was placed was counterbalanced across animals and across trials. At the end of 5 minutes the rat was then returned to the maze, which now contained two objects (one familiar) that were visually different but very alike in regard to their other sensory properties. The rat was allowed to explore these objects and was removed from the maze after 1 minute. The mean light intensity in the centre of the maze during dark trials was less than 1 lx.

Rats were tested with five different sets of suitable objects, with an interval of at least 48 hours between sessions. Of these five, two sets of objects could be distinguished in the dark and so were not tested in the light.

3.4.2 Non-visual recognition (light-light testing)

The three sets of objects that rats had not previously been able to distinguish in the dark during the screening trials were used in this task. At least 48 hours were left between testing on each set of objects. The procedure was identical to that followed during pre-screening, except that the room was illuminated. The light intensity in the apparatus during these trials was 590 lx.

3.4.3 Results

Cohort 1 - Dysgranular retrosplenial cortex lesions

Any pairs of objects that animals were able to discriminate in the dark-dark trials were removed from the experiment on the grounds that they had distinctive textures or odours that allowed them to be distinguished non-visually. Three sets of objects that could not be discriminated in the dark were subsequently used for testing in the light. The criterion for selection was that the D2 scores of both groups failed to be above chance (one-sample t test, $p \geq 0.05$, one tailed). For purposes of comparison with the light-light trials, the data from the dark – dark screening trials for those same three pairs of objects are presented and analysed.

During the sample phase no differences in exploration time were found between the two lesion groups ($F < 1$), or between the dark and light sample phases ($F(1, 16) = 2.48$, $p > 0.05$). There was no sample lighting by lesion interaction ($F < 1$). Consequently, sample exploration times were similar across both lesion groups and both lighting types (Table 3.2).

No differences were found between the three test sessions in either trial type ($F < 1$), so these three sets of results were combined for analysis. No group differences were found in exploration time during the choice sessions ($F < 1$), or between the light and the dark (screening phase) trial types ($F(1, 16) = 3.18$, $p > 0.05$). As intended, the mean time spent exploring the objects in the light-light test phase (20.11s ± 2.76s) proved to be considerably higher than the light-light exploration times in Experiment 1 (13.8s ± 2.47s).

	Sample exploration time (s)		Test exploration time (s)	
	Light	**Dark**	**Light**	**Dark**
ShamD	36.3 ± 1.87	38.1 ± 3.95	19.7 ± 3.18	17.7 ± 4.20
RSdysg	36.1 ± 1.13	40.9 ± 4.94	17.8 ± 2.69	20.6 ± 3.32
Sham2	29.8 ± 1.27	32.13 ± 1.13	23.3 ± 3.77	25.3 ± 3.42
RScomb	33.9 ± 1.61	35.2 ± 1.12	28.2 ± 3.48	25.9 ± 3.35

Table 3.2

Experiment 2. Mean exploration times in the light and in the dark, for both the sample and the test phases of the visual only object recognition task. Times are shown in seconds ± SEM. Exploration times did not differ between light and dark sessions in either the test or the sample phases.

There was no overall difference in recognition (D2) performance between the two groups ($F < 1$, see Figure 3.6), though there was a main effect of trial type ($F(1, 15) = 11.5$, $p < 0.01$), with performance on the light-light trials superior to the dark-dark trials. This is to be expected, as all of the objects that were distinguished in the dark were removed from the analysis. As a consequence, neither group performed above chance on the combined dark-dark trials (ShamD $t < 1$; RSdysg $t(7) = 1.73$, $p > 0.05$). In contrast, both groups of animals showed a significant preference for the novel object in light-light trials (ShamD $t(9) = 3.27$, $p < 0.01$; RSdysg $t(7) = 3.38$, $p < 0.05$) indicating that they were able to discriminate the objects on the basis of visual cues alone.

Cohort 2 – Combined granular and dysgranular (area 29/30) retrosplenial cortex lesions

During the sample phase the lesioned animals tended to explore the objects for slightly longer than the shams ($F(1, 23) = 11.26$, $p < 0.05$; Table 3.2). However, there was no significant difference between the dark and light sample phases, and no sample lighting by lesion interaction (both $F < 1$).

During the test phases, the dark-dark and light-light trials did not differ in the amount of time that the rats spent exploring objects in these two conditions ($F < 1$) and no differences were seen between the exploration times of the two groups ($F < 1$; see Table 3.2). As expected, given that any objects that could be d in the dark were removed from the analysis, neither group performed above chance level

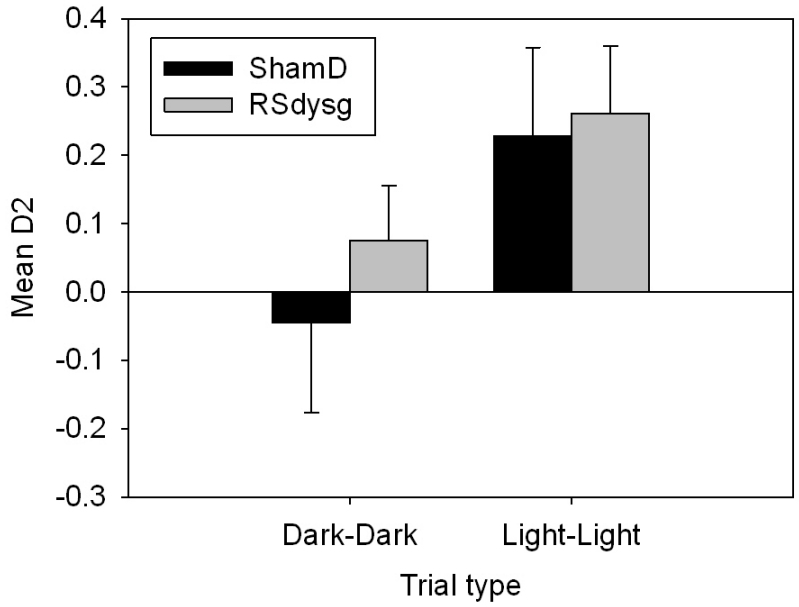

Figure 3.6

Cohort 1: Mean recognition (D2) scores on the dark-dark and light-light sessions of the object recognition task without barriers. Any objects that could be recognised in the dark were removed from the analysis. Consequently, neither the ShamD nor the RSdysg group was above chance discrimination on the dark-dark trials. On the light-light trials, where the same objects are used as in the dark-dark trials, both groups spent significantly more time with the novel object. Error bars show SEM.

on the dark-dark trials (Sham2 $t < 1$, RScomb2 $t < 1$). However, both groups had D2 scores significantly above chance on the light-light trials, showing they were able to distinguish the novel from familiar object when reliant on visual cues (Sham2 $t(11) = 3.53$, $p < 0.01$, RScomb2 $t(12) = 3.37$, $p < 0.01$) (see Figure 3.7). There was no difference between the D2 scores achieved by the Sham2 and RScomb2 groups on the light-light trials ($F < 1$).

3.5 Experiment 3 – Object-in-place

3.5.1 Procedure

Only the RScomb2 and Sham2 animals were tested on this experiment. Rats were tested in a 'bowtie' maze, which had a wooden floor and steel walls, 50cm high. The arena was placed on a table at a height of 76cm, and against one wall of a room measuring 195cm x 330cm. The maze was 120cm long, with two triangular ends 50cm wide at their widest point. A corridor, 12cm wide, joined the apices of these two triangles. The wide end of each triangle had two food wells recessed into the floor, 3.5cm in diameter and 2cm deep, which were covered by the objects being explored. Each of the objects used in this task was heavy enough that they could not be displaced by the rat, and were tall enough that the animals could not easily jump on top of them. On one side of the maze, the food wells were separated from each other by a steel dividing wall 48cm high, that extended 15cm from the middle of the back wall of the maze. On the other side of the maze the dividing wall was not present. In addition, a sheet of white bench-guard paper was attached to one end maze wall, to allow the animal to differentiate between different ends of the maze.

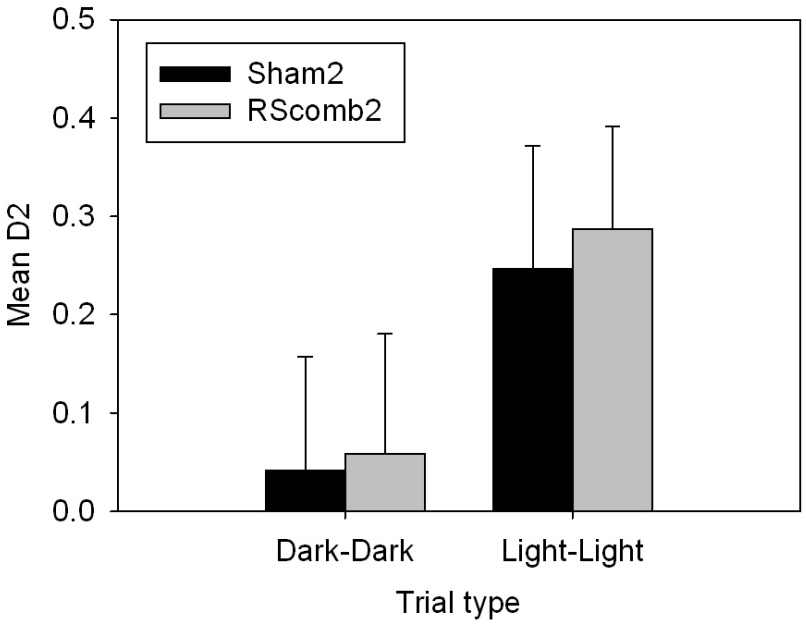

Figure 3.7

Cohort 2: Mean recognition (D2) scores of the Sham2 and RScomb2 groups on the dark-dark and light-light sessions of the object recognition task without barriers. Any objects that had been recognised in the dark were removed from the analysis. Consequently, neither group was above chance on the dark-dark trials. On the light-light trials, where the same objects are used as in the dark-dark trials, both groups spent significantly more time with the novel object. Error bars show SEM.

Rats were brought to the testing room in individual metal carrying boxes, with a lid to prevent the animal from seeing outside the box. Prior to the test day, all animals were given two habituation sessions, during which the rat was placed in the arena for 10 minutes. No objects were present in the maze during habituation.

During the sample phase, four different objects were placed in the maze, one in each corner. Rats were placed in the centre of the maze to start the sample phase, and allowed to explore the objects for 5 minutes. Exploration was defined as time spent with the nose pointing towards the object at a distance of less than 1cm. At the end of the sample phase the rat was removed from the maze and returned to the carrying box. During the 15 minute inter-trial interval the maze was wiped down with 50% ethanol to reduce odour cues as far as possible, and the objects were removed and replaced with identical copies. The positions of two of the objects, diagonally opposite each other, were swapped, while the other two objects remained in the same location as they had been during the sample phase (see Figure 3.8). Following the inter-trial interval the rat was returned to the maze for a 3-minute test phase, during which the time spent exploring each object was recorded. Each rat had two test sessions that were 48 hours apart, and involved different sets of objects. The positions of the objects that were moved for the test phase were counterbalanced across sessions.

3.5.2 Results

During the sample phases no difference was found between the two groups in the amount of time spent exploring the objects ($F < 1$). Mean exploration levels (±

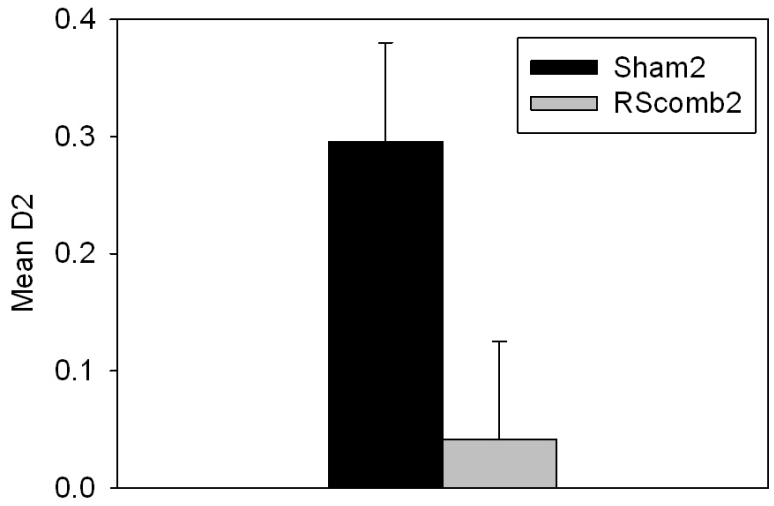

Figure 3.8

Mean recognition (D2) scores achieved on the object-in-place task. While Sham2 rats spent more time with the displaced object, RScomb2 rats did not discriminate between the objects that had changed location and those that remained stationary. Error bars show SEM.

standard error of the mean) were 59.2s ± 2.11s for the RScomb2 group, and 58.8s ± 2.52s for the Sham2 group. During the test phases there was also no lesion group difference in exploration levels ($F(1, 23) = 1.08$, $p > 0.05$). The mean exploration times were 44.1s ± 2.16s for the RScomb2 group and 39.8s ± 3.61s for the Sham2 animals.

For analysis, D2 scores were calculated using the formula (time exploring novel object – time exploring familiar object) / (total exploration). No differences were found in the D2 scores between the two repetitions of the test ($F(1, 23) = 2.42$, $p > 0.05$) and there was no lesion by repetition interaction ($F < 1$). The two test phases were therefore analysed together. Overall, across the test phases there was a significant difference in D2 scores between the two lesion groups ($F(1, 23) = 16, 9$, $p < 0.001$). While Sham2 animals spent more time exploring the objects in a novel position ($t(11) = 10.51$, $p < 0.001$), lesioned animals showed no preference for the objects that had been displaced over those that were not displaced ($t < 1$; Figure 3.8).

3.6 Discussion

Studies into the functions of the rodent retrosplenial cortex have often focused on its potential role in navigation and spatial memory. Part of the rationale has arisen from the prominent, reciprocal connections between the retrosplenial cortex, hippocampus and anterior thalamic nuclei (Van Groen and Wyss, 2003, 1992, 1990). While mild deficits are sometimes seen after retrosplenial lesions on standard spatial

memory tasks (Aggleton, 2010; Pothuizen et al., 2008; Vann and Aggleton, 2005), these deficits often become more apparent when rats are forced to switch between different types of spatial information. Examples include moving from using visual to non-visual cues when navigating in the light and in the dark (Cooper and Mizumori, 1999), or when switching from local to distal cues (Pothuizen et al., 2008; Vann and Aggleton, 2004). From these tasks, however, it is difficult to determine whether the lesion deficit stems from the need to integrate different classes of spatial information or from the related need to switch effectively between these cue types. It is also unclear whether the putative involvement of the retrosplenial cortex in these translational abilities is restricted to spatial information. For these reasons, the present task examined the importance of the retrosplenial cortex for a recognition task that involves integrating and switching between non-spatial cues from different sensory modalities.

The first issue is whether recognition memory within a constant modality was affected by retrosplenial cortex lesions, as any such deficit could confound interpretation of the cross-modal task. Previous studies have shown that rats with retrosplenial cortex lesions can perform at normal levels on standard object recognition memory tasks in the light (Ennaceur et al., 1997; Parron and Save, 2004; Vann and Aggleton, 2002). In these tasks, rats are typically given three-dimensional objects so that the animal could potentially use visual cues, tactile cues, olfactory cues, or a combination to help recognise the same object when it is presented again. There is, however, a lack of information about the importance of the retrosplenial cortex when performance is limited to just one of these sensory modalities. This

focus on just one modality may prove important as studies of object recognition in the dark have not only confirmed the ability of rats to use non-visual cues (Albasser et al., 2013b; Winters and Reid, 2010) but have also shown that, unlike object recognition in the light, this form of recognition need not be dependent on the integrity of the perirhinal cortex (Albasser et al., 2013b; Winters and Reid, 2010). Rather, it has been found that tactile-based recognition memory is dependent on the posterior parietal cortex (Winters and Reid, 2010). Such findings raise the question of whether the retrosplenial cortex might also be involved in non-visual object recognition, despite the lack of any lesion effects when tested in the light.

The present study first examined object recognition memory when the animals were restricted to using either visual cues (by use of a clear Perspex barrier) or tactile cues (by testing in the dark). It is, of course, important to be confident that the rats could not see in the dark conditions, which were run using infrared illumination. It is known that rats are unable to see in the infrared spectrum (Burn, 2008; Deegan and Jacobs, 1993), and the results from both experiments help to confirm that the rats could not use visual cues in the dark. Thus, in Experiment 1 the rats failed to solve the light-dark cross-modal task, which could have been solved had they been able to use visual cues in the 'dark'. Likewise, neither group performed above chance on the selected dark-dark trials in Experiment 2, where visual cues would have aided performance, as demonstrated by their performance with the same objects in the light.

It was found that both rats with dysgranular retrosplenial cortex lesions (RSdysg) and rats with combined lesions of the granular and dysgranular sub-regions (RScomb2)

performed as well as their respective sham groups when restricted to the use of tactile or olfactory cues to perform an object recognition task (Experiment 1). That neither lesion impaired recognition in the dark provides a contrast with posterior parietal cortex lesions (Winters and Reid, 2010). Despite not differing from their control group, the RScomb2 group did fail to perform significantly above chance in the light-light trials in Experiment 1, suggesting a potential, selective problem with visual recognition. It was, however, observed that the object exploration times in Experiment 1 were low whenever the object was placed behind a Perspex barrier. This reduction in activity, which is presumably a consequence of the rat not being able to physically explore the object, would explain the lower D2 scores by all groups on such trials, and may partly explain the seemingly poor RScomb2 performance. For this reason, a separate visual object recognition task was carried out in Experiment 2, where animals were able to interact directly with the objects. Objects were first screened to select those that could only be distinguished visually, i.e., animals were unable to discriminate the same objects in the dark. When then tested in the light, the rats showed increased exploration times and both the RScomb2 and RSdysg groups, as well as their respective control groups, were able to discriminate the novel objects (Experiment 2). This result indicates that neither the RScomb2 nor RSdysg lesions affected visual object recognition, consistent with previous findings from standard object recognition testing in the light (Ennaceur et al., 1997; Parron and Save, 2004; Vann and Aggleton, 2002).

The main goal was to determine the rats' ability to discriminate novel objects when required to switch between cue modalities. The retrosplenial cortex was examined

as it is interconnected with sites providing sensory information from multiple modalities, including areas implicated in rodent cross-modal recognition (Reid et al., 2013, 2012; Winters and Reid, 2010). A shorter retention interval was used than in these previous cross-modal studies in order to avoid floor effects and facilitate comparisons with other studies of object recognition that have also used intervals of 15 mins or less (Ennaceur et al., 1997; Parron and Save, 2004; Vann and Aggleton, 2002). These intervals are sufficient to reveal impairments in the object-in-place task described here.

Sham animals were found to perform at above chance levels in the dark-light trials, consistent with previous studies (Winters and Reid, 2010). The light-dark trials proved, however, to be particularly difficult for all rats, but this is to be expected as the cross-modal switch followed a sample phase in the light that typically involved significantly less sample exploration of the objects than found in the dark. In contrast, the sham rats from both cohorts showed cross-modal recognition when tested on the dark-light trials. However, both the RSdysg and RScomb2 animals were impaired on this same trial type, i.e. on the condition that forced a switch in cue modalities from tactile or olfactory cues to visual between sampling the objects and being tested. For the RSdysg group this deficit is unlikely to reflect particular problems with either visual or tactile and olfactory object recognition, as the retrosplenial lesion groups did not differ from their respective sham controls on such tasks. It is notable that posterior parietal cortex lesions also disrupt performance on dark-light cross-modal recognition (Winters and Reid, 2010), given the connections between the two regions. Parietal lesions also disrupt dark-dark object recognition

(Winters and Reid, 2010), and so the present findings for the dysgranular retrosplenial cortex provide a more selective deficit of cross-modal transfer. The finding for the rats with combined granular and dysgranular retrosplenial lesions are harder to interpret, as in Experiment 1 the cross-modal deficit was not selective. In this respect, the results were more like those for parietal cortex lesions (Winters and Reid, 2010).

The results for the RScomb2 and RSdysg lesioned animals were strikingly similar on the cross-modal recognition task. This similarity suggests not only that the different experimental histories of the two lesion groups did not affect their performance on this task, but more importantly shows that the dysgranular sub-region of the retrosplenial cortex is critical for the cross-modal aspect of this task. This involvement may reflect the fact that the dysgranular retrosplenial cortex is especially closely connected with brain regions receiving visual information (van Groen and Wyss, 1992) and the way that the present study deliberately isolated demands on vision. It is not possible to determine fully the involvement of the granular retrosplenial cortex in this task. That both groups showed a deficit on the cross-modal task does, however, demonstrate that the small amount of hippocampal damage seen in several of the RScomb2 rats cannot be responsible for their changes in performance on this task, as no hippocampal damage was present in any of the animals in the RSdysg group. This conclusion is further supported by experiments showing that excitotoxic hippocampal lesions do not impair cross-modal object recognition (Murray and Mishkin, 1986; Reid et al., 2012), though this specific sparing for cross-modal recognition does not preclude a role for the hippocampus in

wider aspects of intra-modal or cross-modal binding (Hannula et al., 2006; Mayes et al., 2007, 2004; Pertzov et al., 2013).

Other relevant information comes from studies of cross-modal matching by monkeys with lesions in the medial temporal lobe (Goulet and Murray, 2001) and from fMRI and PET studies of humans performing cross-modal recognition tasks (Banati et al., 2000; Holdstock et al., 2009; Vargha-Khadem, 1997). In addition to the perirhinal cortex, which has been implicated in selective aspects of cross-modal recognition in animal studies (Albasser et al., 2011; Goulet and Murray, 2001; Winters and Reid, 2010), human imaging studies have implicated the anterior cingulate cortex and dorsolateral prefrontal cortex in tactile to visual cross-modal recognition (Banati et al., 2000) along with the insula (Holdstock et al., 2009). It is notable that many of these areas are closely connected with the retrosplenial cortex (Deacon et al., 1983; Kobayashi and Amaral, 2007, 2003). These results suggest a network of cingulate and frontal areas that work together to perform cross-modal transfer (Reid et al., 2013; Winters and Reid, 2010).

Experiment 3, the object-in-place task, tested the ability of rats with combined granular and dysgranular retrosplenial lesions to associate the object's identity with its location within the testing arena. While Sham2 animals spent significantly more time exploring objects that had been displaced during the test session, the RScomb2 group showed no preference. Both Experiment 1 and Experiment 2 demonstrated that retrosplenial cortex lesions have no effect on object recognition when a cross-modal switch is not required, indicating that the deficit shown on this task cannot be explained by an inability to the objects themselves. Instead, the deficit must be due

to the added challenge of integrating information about spatial location into the representation of each object, or in recognising that the spatial arrangement has changed.

The current study indicates that the rodent retrosplenial cortex has a selective role in integrating or switching between stimuli across modalities, which includes but is not limited to the spatial domain (Byrne et al., 2007). It remains, however, difficult to determine whether this cross-modal object recognition deficit stems from an inability to associate cue types from different modalities into a single representation, or to then switch between cue types. While a strict division between these two processes may prove over-simplistic, some preliminary evidence comes from the combined retrosplenial deficit on the object-in-place task, where integration of information is required but a translation or switch between cue types is not. However, that fact that spatial information is required means that it is still difficult to separate a spatial deficit from a deficit in cue integration *per se*. Other evidence comes from the finding that rats with retrosplenial damage are impaired on acquiring a serial feature negative discrimination task, where rats formed an association between a visual stimulus and a tone (Robinson et al., 2011). The implication is that the retrosplenial cortex might be involved in the initial stimulus-stimulus association process for cross-modal learning. At the same time, retrosplenial cortex lesions do not bring about a general inability to form associations (Keene and Bucci, 2008a; Pothuizen et al., 2008; Vann and Aggleton, 2002). Such findings highlight the value of examining further the roles of the retrosplenial cortex in stimulus integration and translation, while trying to narrow

down the specific nature of its contribution. The cross-modal task with its intra-modal controls also made it possible to reveal a selective impairment following retrosplenial damage, where loss of the dysgranular cortex was sufficient. The deficit for cross-modal recognition, which contrasted with apparently spared intra-modal recognition, may reflect the fact that in the former task the stimulus for comparison is self-generated. This emphasis on stimulus control has strong echoes in the notion that effective human navigation, which involves the retrosplenial cortex, also involves multimodal representations of space in which there is a role for visual imagery when switching from the light to the dark or *vice versa* (Byrne et al., 2007). For this reason, the present results point to a potential precursor role within the rodent retrosplenial cortex for navigation.

Chapter 4

4. Retrosplenial cortex lesions affect cue integration

4.1 Introduction

The role of the retrosplenial cortex in spatial memory has been demonstrated in many studies, both in humans and in rats. However, its involvement appears particularly clear when the tasks involve switching between different groups of cues. Examples of the latter involve solving a radial-arm maze task in the light and then the dark (Chen et al., 1994b), changing from allocentric to directional cues in a T-maze (Pothuizen et al., 2008), and switching from intra-maze to extra-maze cues when performing a radial-arm maze task in the light (Pothuizen et al., 2008; Vann and Aggleton, 2005, 2004). Based on these results, it has been suggested that the retrosplenial cortex has a function in 'translating' between different spatial codes, e.g., allocentric representations into egocentric ones and *vice versa* (Burgess et al., 2001; Byrne et al., 2007; Vann et al., 2009). There is a related hypothesis that states that the retrosplenial cortex may be required for integrating different types of cues before they can be used in combination (Cooper et al., 2001; Vann et al., 2009). Tests of these functions have, however, largely been confined to those related to spatial processing. As a result, it is uncertain whether the retrosplenial cortex has a more general role in integrating and switching between information streams or whether it is selective to spatial cues.

The connectivity of the retrosplenial cortex leaves it well placed for a role in the integration of multiple cue types. For example, the retrosplenial cortex receives visual information directly from the geniculostriate and tecto-cortical visual systems (Van Groen and Wyss, 1992; Wyss and Van Groen, 1992). Additionally, the retrosplenial cortex has reciprocal connections with the parahippocampal and parietal cortices that may provide olfactory and somatosensory information (Aggleton, 2010; Insausti et al., 1997). The inputs to the parietal region are of particular interest for cue integration given recent evidence of the importance of the parietal cortex for tactile to visual cross-modal transfer in the rat (Winters and Reid, 2010). A role for the retrosplenial cortex in processing information from multiple stimuli is suggested by previous studies that have demonstrated a deficit in animals with retrosplenial cortex lesions on acquisition of both a compound feature negative discrimination task, requiring animals to learn about multiple cues simultaneously (Keene and Bucci, 2008b), and a serial version of the same task where one stimulus occurred just prior to the other (Robinson et al., 2011). However, both of these studies used electrolytic rather than excitotoxic lesions, leaving open the possibility that the deficits result from damage to fibres of passage running through and adjacent to the retrosplenial cortex, rather than from damage to the retrosplenial cortex itself. The most notable of these fibres of passage is the cingulum bundle, which connects the anterior thalamus with the cingulate cortices and hippocampus, among other areas (Shibata, 1993a, 1993b).

The current chapter involves two experiments that test the processing of multiple cues in a non-spatial setting to determine whether the role of the retrosplenial

cortex is limited to spatial cues, or whether it has a more generic role in cue integration. Additionally, a latent structural learning experiment has been included that tests the integration of spatial cues in the absence of navigational demands. All experiments were carried out with rats that have undergone excitotoxic lesions, to prevent any damage to fibres of passage that might inadvertently disconnect communications between other brain regions.

With the experiments already presented in this thesis, it is difficult to separate the 'integration' and 'translation' theories of retrosplenial cortex function. In both of the digging cups experiments where retrosplenial lesion deficits were found, rats needed to extract spatial information from visual cues. Deficits could arise because lesions of the retrosplenial cortex affect the ability to integrate visual and spatial information with each other or because the ability to translate from an egocentric viewpoint to an allocentric representation is impaired. Equally, the deficits seen in the cross-modal object recognition task could be due to an inability to integrate information from visual and tactile sensory modalities, or to a problem in translating from one sensory modality to a representation of the other modality that can then be d during the test phase. The experiments presented in this chapter deliberately separate integration from translation, to test integration alone.

Rats were tested on the formation of simple associations using a conditioned inhibition experiment, where the presence of an 'inhibitor' stimulus signalled a lack of reward when presented in combination with a previously rewarded cue. Involvement of the retrosplenial cortex in this task has previously been indicated in rats by the observation of changes in glucose metabolism in the area, measured by

fluorodeoxyglucose autoradiography, in response to an inhibitory tone (Jones and Gonzalez-Lima, 2001). This task is, in many ways, similar to the feature negative discrimination task on which excitotoxic retrosplenial cortex lesions impair performance (Keene and Bucci, 2008b). If animals with lesions to the retrosplenial cortex are unable to combine information from different cue types, they would be expected to demonstrate a deficit on this task. This experiment was run in operant boxes, to remove spatial cues as far as possible.

The role of the retrosplenial cortex in combining information from multiple stimuli was further tested using a negative patterning task. This requires the rat to discriminate two rewarded elemental cues (A+ and B+) from an unrewarded compound cue made up of each elemental cue presented simultaneously (AB-). This task is more complex and demanding than the conditioned inhibition task, as for this discrimination to be carried out successfully the AB- compound stimulus must be represented as a single unique stimulus that can then acquire the reward associations separately from the elemental stimuli (Harris et al., 2009; Myers et al., 2001). This is not the case in the conditioned inhibition task, where configural processing is not required. The ability to combine information from multiple stimuli in this way is thought to be key for spatial navigation, where the combinations of cues available from any particular location will differ, but are likely to overlap with the cues visible in other nearby locations. Pattern separation, the process of distinguishing between overlapping representations, is thought to be carried out by the hippocampus (Bakker et al., 2008; Gilbert et al., 1998; Leutgeb et al., 2007), with

which the retrosplenial cortex has dense reciprocal connections (Wyss and Van Groen, 1992).

Additionally, a latent structural learning task was carried out in which rats were required to learn the location of a submerged platform in a water maze without actively navigating around the area during training. Boards were inserted into the water maze to create a square arena with the platform at one corner, distinguished by a striped wall. To correctly locate the platform, the rat should learn the conjunction of the striped wall with the plain wall, and their arrangement (whether the striped wall is on the left or the right of the plain wall). This is an example of structural learning, a type of configural learning that is particularly closely linked to the hippocampus (Aggleton et al., 2009, 2007; Sanderson et al., 2006). The latent training reduces the likelihood that rats will use a stimulus-response strategy to learn (e.g. go to the striped wall and turn left), as they have no experience of navigating to the platform prior to the test day. Spatial learning that occurs must take place latently (Horne et al., 2012), as there is no opportunity to explore the arena before the test session, and on the test session all four corners of the arena are fully visible, decreasing the navigational component of the task as far as possible. Traditional spatial learning experiments are unable to differentiate between deficits caused by an inability to learn about spatial elements of the environment and those caused by an inability to use spatial information to navigate to the correct place. Removing the navigational elements of a task removes this ambiguity.

These three experiments test, in increasing complexity, the ability of retrosplenial cortex lesioned animals to use information from multiple stimuli, and to combine that information in appropriate ways to direct behaviour. The negative patterning experiment was carried out before the conditioned inhibition experiment as the conditioned inhibition could build on the training given during the negative patterning.

4.2 General Methods

4.2.1 Animals

A description of Cohort 2 (RSComb2 and Sham2) can be found in the Methods section of Chapter 3. After exclusions were made due to sparing or damage outside the retrosplenial cortex there were 13 rats in the RSComb2 group and 12 in the Sham2 group.

4.2.2 Surgical Procedures

Surgical procedures are provided in Chapter 2, and the coordinates used for the RScomb2 cohort can be found in Chapter 3. Testing took place two months after surgery. Before this, animals had been tested on the object in place experiment detailed in Chapter 2 (see Annex A).

4.2.3 Histological Procedures

Histological procedures are the same as those presented in Chapter 1.

4.2.4 Statistical Methods

Statistical tests were carried out using SPSS 16.0 (SPSS Inc., Chicago). Where the assumption of sphericity was not met for parametric analysis, Greenhouse-Geisser corrections have been applied. Criterion alpha level has been taken as $p \leq 0.05$. All significant interactions found following ANOVA testing were explored with simple effects, and where appropriate with pairwise comparisons. Bonferroni corrections were applied to control for multiple comparisons (Howell, 2011).

4.3 Negative patterning

4.3.1 Apparatus

Eight operant boxes measuring 30cm wide x 24cm deep x 21cm high (Med Associates, George, VT) were used. Each chamber had three aluminium walls with the fourth wall composed of a Perspex door. The floor of the chamber was made up of 19 stainless steel rods, 1.6 cm apart. Each rod was 3.8mm in diameter. Beneath the rods was a removable metal tray. Illumination in each chamber was provided by a 3W house light located at the top centre of the left-hand wall. In the centre of the right-hand wall, a recessed magazine (5cm tall by 5cm wide) was located, via which Noyes food pellets (45mg; Noyes, Lancaster, NH) or 15% (w/v) sucrose solution could

be delivered to the rat. To either side of the magazine was a flat-panel retractable lever, each with a 2cm diameter circular panel light located above it. Auditory stimuli were delivered via speakers in the ceiling, and consisted of a 2kHZ tone or a series of clicks at 10Hz. Visual stimuli were either the two panel lights flashing (0.1s on, 0.1s off), or steady simultaneous illumination of both panel lights and magazine light.

4.3.2 Behavioural procedures

Rats were trained to lever press, so that after four training sessions each rat would lever press for a single food pellet on a random interval schedule (RI15) such that on average once in every 15s a reward became available following a lever press.
Rats were then trained to lever press in response to a stimulus. Stimuli were either a tone (A+) or flashing panel lights (B+). Each of these stimuli was presented twelve times, with a varying inter-stimulus interval (mean 60s). During the inter-stimulus interval both levers were retracted, and were extended again at the start of the subsequent trial. Each stimulus presentation lasted for 60s. During the first 10s of stimulus presentation, no reinforcement was available to the rat, so that lever pressing performance was unaffected by the presence of a food reward. In the remaining 50s food was given on an RI15 schedule where, on average, a reward would be given once in every 15s following a correct lever press. Training on A+ B+ continued for four days, by which time the number of lever presses in response to the stimuli had stabilised.

Once responding to A+ and B+ had been established, an unrewarded compound AB- was introduced. In each training session AB- was presented twelve times, while A+ and B+ were presented six times each. The order of these trial presentations was pseudorandom, with the constraint that no more than three trials of the same type could occur consecutively. Rats were trained on this negative patterning task for 24 sessions.

Following acquisition of the negative patterning (A+ B+ AB-), two transfer tests were carried out. First, animals were given three sessions of training with a novel rewarded stimulus, illumination of the magazine light (C+). The stimulus was presented twenty four times in each session, and the same inter-stimulus intervals and stimulus duration were used as during the negative patterning training. Once lever pressing to C+ had stabilised, C+ was introduced into the original negative patterning problem, giving A+ B+ AB- C+. The compound AB- was presented twelve times in each session, while the three simple rewarded stimuli were presented four times each. Rats were given four sessions of training on this discrimination. Training on A+ B+ AB- C+ was followed by a transfer test, in which C+ was presented in combination with the tone or the flashing panel lights, the original A+ and B+ stimuli. This tested whether animals had learnt the negative patterning discrimination based on the number of stimuli presented simultaneously, instead of creating a configural representation of the AB- compound. If this were the case, responding to AC- and BC- would be expected to drop. If, however, a configural method of solving the task were used, responding to AC- and BC- would be expected

to be higher than to AB-. The session consisted of four trials of each of the compound and individual stimuli.

Finally, to demonstrate that rats are able to suppress responding to compounds containing the magazine light, a final transfer test was carried out in which C+ was combined with AB- to produce an unrewarded compound, ABC-. During this session, the three original simple stimuli were each presented four times, with the two compounds AB- and ABC- presented six times each. As ABC- contains the previously trained, unrewarded stimulus AB-, responding to the ABC- compound is expected to be low, showing that rats can suppress lever pressing in the presence of the magazine light.

4.3.3 Results

For all analyses only lever presses during CS1 were measured, as during this time no rewards were given and so responses are uncontaminated by reward.

Acquisition of A+ B+

Both groups learnt to lever press to the A+ and B+ stimuli, shown by a significant main effect of day ($F(3, 66) = 24.5$, $p < 0.001$). There was no main effect of lesion or lesion by day interaction (both $F < 1$). No differences were found between the number of lever presses made to each stimulus ($F(1, 22) = 4.02$, $p = 0.058$), and no

lesion by stimulus interaction ($F < 1$). Rats learnt to respond to both stimuli at the same rate ($F(3, 66) = 1.22$, $p > 0.05$).

Acquisition of negative patterning

A discrimination ratio was calculated using the formula (lever presses during CS1 of reinforced stimuli) / (total lever presses during CS1). A ratio of 0.5 would reflect a lack of discrimination between reinforced and non-reinforced stimuli; the better the discrimination, the higher the ratio score.

Discrimination scores increased with training, demonstrating that rats were acquiring the negative patterning discrimination ($F(7, 154) = 54.0$, $p < 0.001$). There was no significant difference in discrimination ratio between the two groups ($F(1, 22) = 3.58$, $p = 0.05$; see Figure 4.1), although there was a significant day by lesion interaction $F(7, 154) = 2.068$, $p = 0.050$).

Acquisition of C+

Both groups acquired lever pressing to the C+ stimulus within a session (mean lever presses per minute (± standard error of the mean) during CS1 was 34.54 ± 2.86). There was no difference in the number of lever presses made by each group ($F(1, 22) = 1.03$, $p > 0.05$). There was no main effect of day on lever presses per minute, presumably due to ceiling effects, and there was no day by lesion interaction (both $F < 1$).

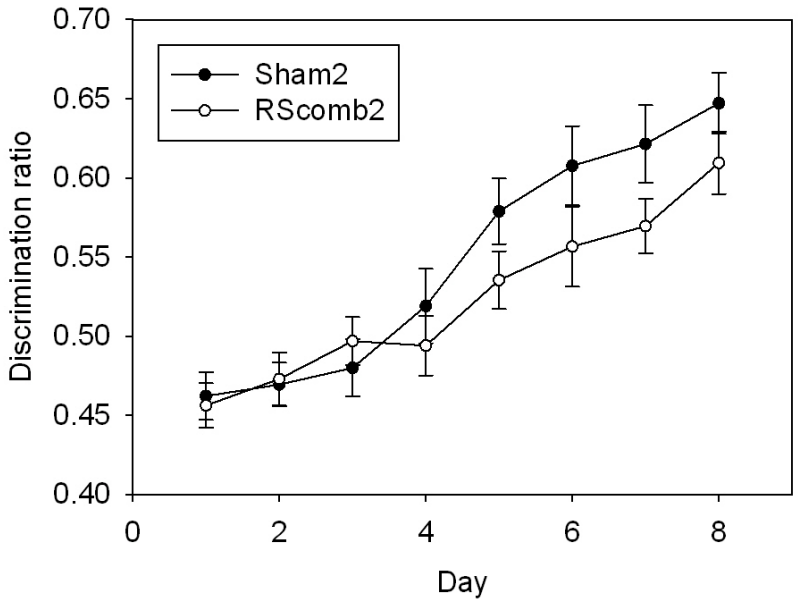

Figure 4.1

Acquisition performance on the negative patterning task. Results are expressed as a discrimination ratio (lever presses during CS1 of reinforced stimuli) / (total lever presses during CS1), with a higher score showing increased discrimination performance. RScomb2 rats acquired this task at the same rate as Sham2 animals. Error bars show SEM.

Negative patterning with C+

When the C+ stimulus was added into the negative patterning task, animals were still able to discriminate the elemental and compound stimuli (see Figure 4.2). Discrimination improved with training, as shown by a main effect of day ($F(3, 66) = 10.7$, $p < 0.001$). There was, however, no main effect of lesion ($F(1, 22) = 1.76$, $p > 0.05$) or lesion by day interaction ($F < 1$).

Transfer test (A+ B+ AB- C+ AC- BC-)

To determine whether the rats might have solved the negative patterning discrimination on the basis of the number of stimuli presented, a transfer test was carried out using novel compounds involving the C stimulus. For analysis, a mean of lever press activity during CS1 was taken across the original elemental stimuli (A+/B+) and the compounds containing the magazine light (AC-/BC-). Overall, there was no significant main effect of lesion or trial type by lesion interaction (both $F < 1$; see Figure 4.3). However, there was a significant main effect of trial type ($F(3, 66) = 17.4$, $p < 0.001$). Pairwise comparisons showed that responding to AB- was lower than to any of the other stimuli (all comparisons $p < 0.001$). There were no differences between any of the other trial types (all $p > 0.05$), demonstrating that animals had not learnt to withhold responding based simply on the number of elements present in the stimulus.

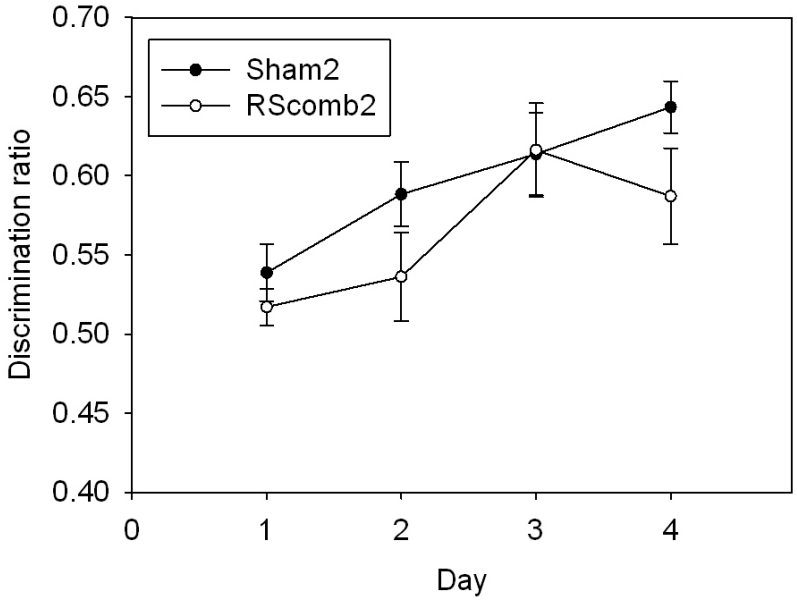

Figure 4.2

Acquisition of the negative patterning task after the C+ stimulus was added. Both groups of rats were still able to distinguish the elemental from the compound stimuli.

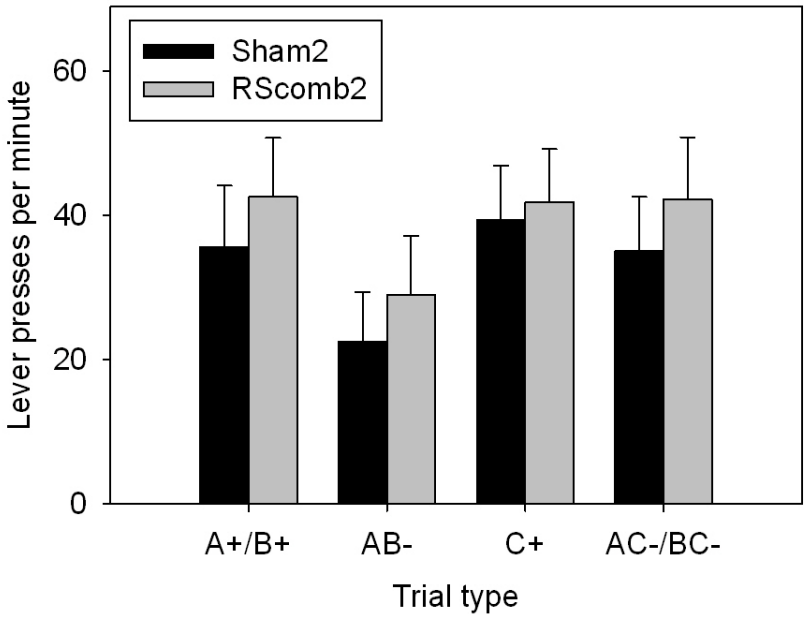

Figure 4.3

Transfer test (A+ B+ AB- C+ AC- BC-). To determine whether the rats might have solved the negative patterning discrimination on the basis of the number of stimuli presented, a transfer test was carried out using novel compounds involving the C stimulus. For both groups, responding to AB- was lower than to any of the other stimuli. There were no differences between any of the other trial types, showing that animals had not learnt to withhold responding based simply on the number of elements present in the stimulus.

Transfer test (A+ B+ AB- C+ ABC-)

To determine whether rats were able to supress responding to compound stimuli containing the C stimulus, a final transfer test involving the compound ABC- was completed. Rats were expected to reduce responding to this compound, as it also contains the unrewarded compound AB-. Rats responded significantly more to C+ than to ABC- ($F(1, 21) = 66.7$, $p < 0.001$; see Figure 4.4), with no main effect of lesion or stimulus by lesion interaction (both $F < 1$). This demonstrates that rats were able to supress responding to C+ compounds.

4.4 Conditioned Inhibition

4.4.1 Behavioural Methods

Conditioned inhibition testing immediately followed the negative patterning experiment, using the same A+ and B+ stimuli that rats had been trained on previously. All timings and conditioning schedules were the same as for the negative patterning experiment. In the first session a novel unrewarded stimulus, X-, was introduced. This was the extinguishing of the house light, which was on at all other times, leaving the operant box in darkness. Stimulus X- acted as the conditioned inhibitor, and if it was presented in conjunction with either A+ or B+, lever pressing was not rewarded. The fourteen training sessions consisted of six trials of each of the four trial types, A+, B+, AX- and BX-. Following acquisition of this discrimination a neutral stimulus was added to the procedure for a single session. This novel

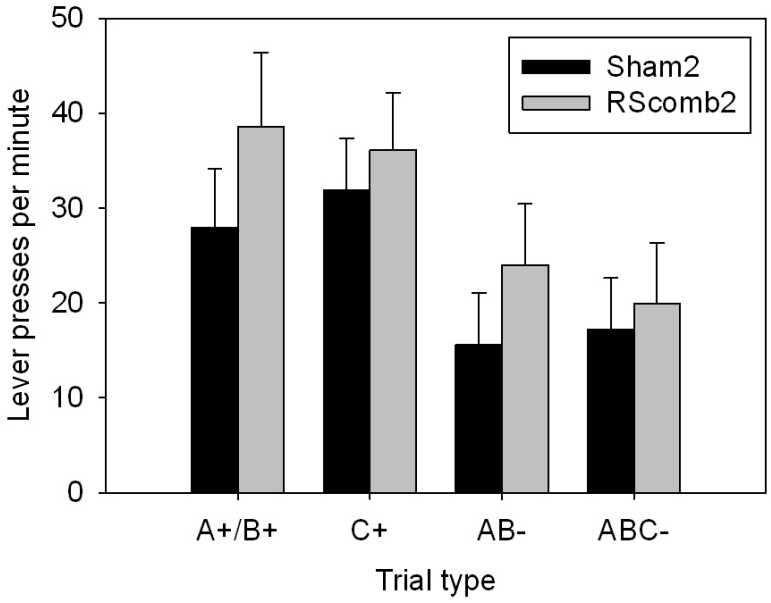

Figure 4.4

Transfer test (A+ B+ AB- C+ ABC-). This test was carried out to determine whether rats were able to supress responding to compound stimuli containing the C stimulus. Both groups of rats responded significantly more to C+ than to ABC-, demonstrating that rats are able to supress responding to C+ compounds.

stimulus, Y, was a train of clicks at 10Hz. All five stimuli were presented equally. During presentation of Y the levers were not extended, so rats were unable to lever press in response to the stimulus.

On the two subsequent sessions a retardation test was carried out, where only X and Y were presented, in the absence of A or B. Both stimuli were rewarded. This compares the ability of X to become an excitor with that of a neutral stimulus. The number of lever presses that rats made to each stimulus was recorded, with rats expected to make fewer lever presses to stimulus X, which has previously signalled a lack of reward, than to stimulus Y, which has neutral connotations. This result would demonstrate that X has been learnt as an inhibitor.

4.4.2 Results

During acquisition of the A+, B+, AX-, BX- discrimination, animals' ability to suppress responding to the conditioned inhibitor improved with training ($F(6, 150) = 12.56$, $p < 0.001$; see Figure 4.5). There was no main effect of lesion ($F(1, 25) = 1.59$, $p > 0.05$) or block by lesion interaction ($F < 1$). By the final day of testing, both groups were above chance at both the auditory (RScomb2 $t(14) = 25.41$, $p < 0.001$; Sham2 $t(11) = 32.36$, $p < 0.001$) and the visual discrimination (RScomb2 $t(14) = 28.17$, $p < 0.001$; Sham2 ($t(11) = 26.33$, $p < 0.001$), although performance on the auditory discrimination was superior ($F(1, 25) = 127.3$, $p < 0.001$). There was no type by lesion interaction ($F < 1$) or block by type interaction ($F(6, 150 = 2.15$, $p = 0.051$), although the latter came very close to significance.

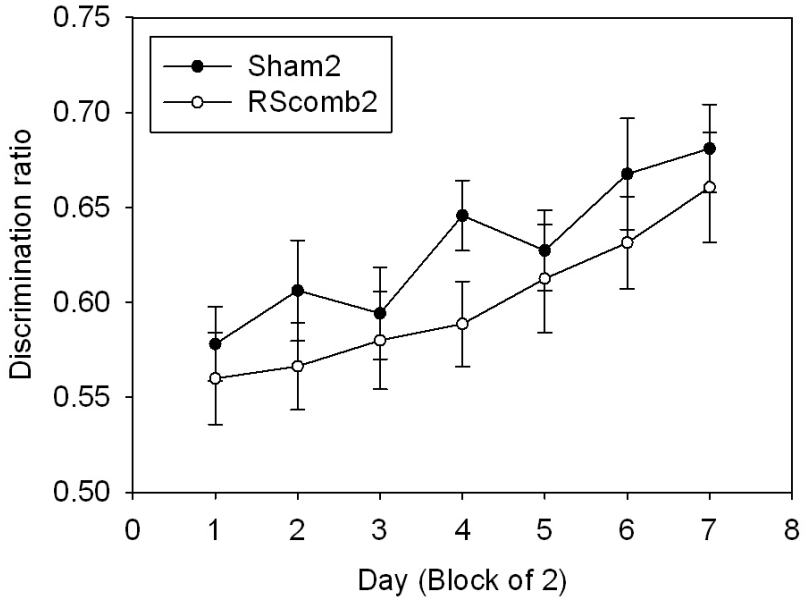

Figure 4.5

Acquisition of the conditioned inhibition task. Both groups' performance improved with training, and both groups were able to suppress responding to the compounds containing the conditioned inhibitor compared to the elemental stimuli. Error bars show SEM.

When the neutral stimulus, Y, was included, the performance of both groups of rats continued to improve, as shown by a significant main effect of day ($F(1, 25) = 19.72$, $p < 0.001$) and a lack of lesion effect or day by lesion interaction (both $F < 1$). During the retardation test sessions, when X and Y were both presented in combination with rewards, lever pressing was higher in response to Y, the previously neutral stimulus, than to X, the stimulus previously used as an inhibitor ($F(1, 24) = 24.73$, $p < 0.001$; see Figure 4.6). This was the case for both groups (RScomb2 ($t(14) = 2.50$, $p < 0.05$); Sham2 ($t(11) = 4.65$, $p < 0.001$), showing that learning about X as an excitor was slowed by its previous role as an inhibitor. There was no main effect of lesion ($F < 1$), and no lesion by day interaction ($F(1, 24) = 1.96$, $p > 0.05$).

4.5 Latent structural learning

4.5.1 Apparatus

Pre-training

Rats underwent pre-training in a white, circular pool measuring 2m in diameter and 0.6m in depth. The pool was raised 0.6m from the floor in the centre of the testing room (300cm wide x 360cm long x 240cm high). The water in the pool was maintained at a temperature of $25^{o}C \pm 2^{o}C$, and at a depth of 27cm and was changed daily. A platform measuring 10cm in diameter was used during all training trials. The platform was mounted on a column 25cm long, such that the platform was always 2cm below the surface of the water. In order to prevent the rats from

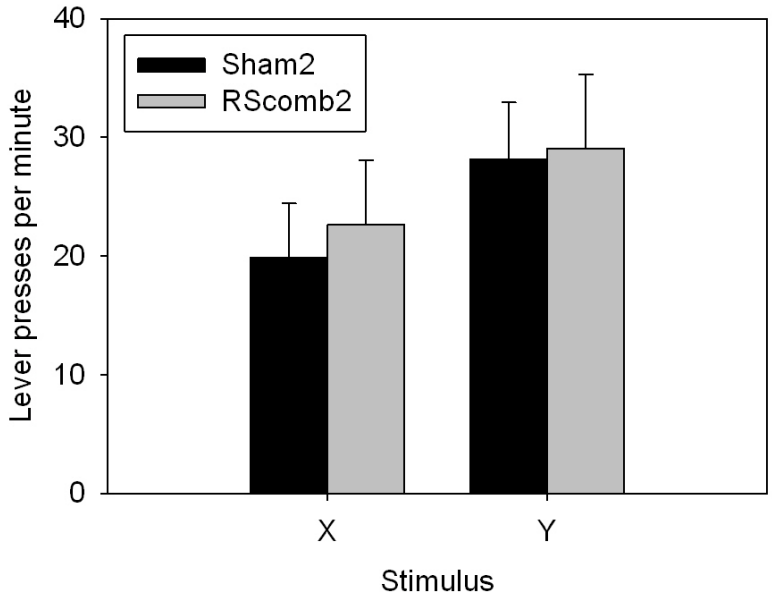

Figure 4.6

Mean responding to the conditioned inhibitor (X) and the neutral stimulus (Y). In this retardation test both stimuli were rewarded. Responding to X was lower than to Y, showing that both groups of animals were slower to learn about a stimulus that had previously been a conditioned inhibitor than about the neutral stimulus.

seeing the platform location through the water, 0.5l of white E308 opacifier (Roehm and Haas, UK Ltd., Dewsbury) was added to the pool. For two of the pre-training trials, a beacon was attached to the platform to guide the rats. This beacon was a black and white striped stick 1cm in diameter that extended 15cm above the platform, topped by a white circular wooden marker 4cm in diameter. The stripes on the stick were 2cm wide.

A white circular board was suspended 1.75m above the base of the pool. In the centre of this board a 30cm diameter hole was cut, through which a wide-angle lens video camera could film the pool. The video camera was connected to a video monitor and a computer in an adjacent room. The board also held eight 45W lights in a 1.6m diameter circle around the video camera, which provided illumination for the pool. Additional lighting came from two 1.53m strip lights on each of the East and West walls. A door (1.75m x 2m) was located in the centre of the South wall. During all pre-training, a circular white curtain was drawn around the pool. This curtain was attached to the edge of the white board, and fell to 25cm below the edge of the pool, completely blocking any visual cues in the testing room. During training rats' movements were analysed using Watermaze software (Morris & Spooner, 1990).

Latent structural learning

The latent structural learning training was carried out in a novel test room that measured 425cm wide x 400cm long x 245cm high. The dimensions of the pool,

curtain and platform were the same as those used for pre-training. Four Perspex boards (1.5m long, 0.59m high, 2mm thick) were inserted into the pool to form a square arena. The boards were suspended vertically in the pool by horizontal bars that extended over the sides of the pool. Three of these boards were white while the fourth board had black and white vertical stripes. The stripes were made with matt black Fablon (DCFix, UK) and each black stripe was 10cm wide, with a 10cm gap between each. At either end of the board was a white stripe 5cm wide, i.e., all the corners of the square were white. During testing rats' movements were analysed using Watermaze software (Morris & Spooner, 1990).

4.5.2 Behavioural procedures

Pre-training

In order to train rats to swim and climb onto the platform, four pre-training days were carried out in a circular pool (without the square insert). The rats were carried to and from the maze room in groups of four, in individual lidded travelling boxes made of metal. For the pre-training, there were eight possible platform locations (at two different distances from the edge of the pool) and eight different start locations. Rats completed four trials a day and a different platform and start location were used for each. The rats completed four trials each day. For the first two days of pre-training a beacon was attached to the platform to guide the rat. For days 1-3, rats were given 120s per trial to find the platform and on day 4 this time was reduced to 90s. If the rats had not located the platform within this time they were guided to the platform by the experimenter. The animals then had to remain on the platform for

30s before being removed and returned to the travelling box. The four rats in each group were run in an interleaved fashion, leaving an inter-trial interval of approximately five minutes. Throughout pre-training the curtain was drawn closed around the maze to occlude visual cues around the edges of the room.

Latent structural learning

During latent structural learning training rats were carried to the testing room in the same travelling boxes as for the pre-training, and were kept in an ante-room adjacent to the testing room when not in the pool. The curtain was drawn around the pool during testing, and the platform was placed in the water, 25cm diagonally from the corner of the square insert. For half of the rats, the corner in which the platform was located was made up of a striped wall on the left and a white wall on the right, while for the other half of the group the platform was found in the corner made up of a striped wall on the right and a white wall on the left (Figure 4.7).

Rats were given eight days of training with four trials a day. For each trial they were placed on the platform where they had to remain for 30s before being returned to the travelling box. If the rat jumped off during the 30s, it was returned immediately to the platform. Any rats that managed to swim away after jumping off were excluded from the data analysis, as they had actively explored the arena. Between each trial the square was rotated clockwise through 90^{o}, 180^{o} or 270^{o}, in a pseudorandom fashion. Each orientation of the square was used only once in each

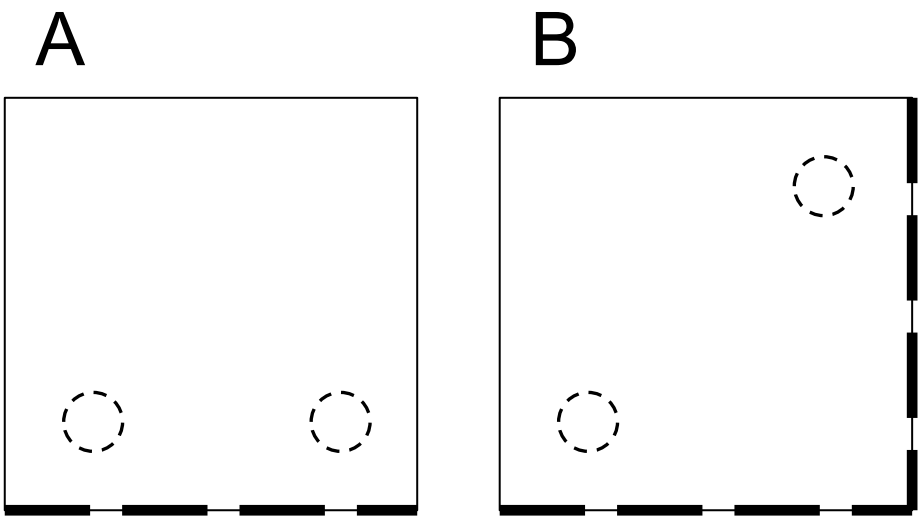

Figure 4.7

The arena used for the latent structural learning task and first probe trial (A) and for the second probe trial (B). The dashed circles show the two possible positions of the platform. These were counterbalanced across groups.

session. Again, rats were tested in groups of four and the trials were interleaved, giving an inter-trial interval of approximately five minutes.

On the eighth day of training, the first three trials were conducted as described above. On the fourth trial the platform was removed from the pool. Rats were allowed to swim in the pool for 60 seconds before being removed. The time spent in each corner of the pool was recorded as an indicator of learning about the platform location.

Following this probe trial, one day of reminder training was given with the platform in place, using the same procedure as the original training days. On the following day the first three trials were run as normal. For the fourth trial, a second probe was run with the platform removed, but this time one of the white walls was replaced with a second striped wall. This striped wall was positioned at right angles to the original striped wall (Figure 4.7). This was to test whether rats were solving the task by learning about the combination of walls making up the correct corner, or by using a stimulus-response strategy, e.g. go to the striped wall and turn left. Again, rats were allowed to swim for 60 seconds before being removed from the pool.

Active training

The day following completion of the latent structural learning task, rats were given four days of active training on the same task. For this, the original maze with a single striped wall was used and the platform was in position for all trials. Rats were

released in the centre of the maze, with the direction they were facing varying across the four trials each day. Rats were given 60s to locate the platform and if they had not found the platform within this time, the experimenter guided them there. The rats then had to remain on the platform for 30s before being dried and retuned to the travelling box. All other procedures were identical to during the latent structural learning task.

On the fourth day of active training, the first three trials were carried out as normal but the fourth trial was replaced with a probe test where the platform was removed from the pool, for the 60s trial. The time spent in each corner of the square insert was recorded as a measure of learning about the location of the platform.

4.5.3 Results

Pre-training

No difference was found in swim speeds between the two lesion groups ($F < 1$). Swim speeds did not change with training ($F(3, 69) = 1.02$, $p > 0.05$), and there was no day by lesion interaction ($F(3, 69) = 1.15$, $p > 0.05$). Over the course of pre-training, the rats became faster at locating the platform as revealed by a main effect of day ($F(3, 69) = 10.47$, $p < 0.001$; see Figure 4.8). There was no main effect of lesion or day by lesion interaction (both $F < 1$) reflecting the equivalent improvement in both lesion and sham animals.

Latent structural learning

To determine what the rats had learnt about the location of the platform during training, rats underwent a probe trial with no platform present; the amount of time spent in each corner was measured (Figure 4.9). One animal was removed from the analysis after repeatedly jumping off the platform during training. Repeated measures analysis found a main effect of corner ($F(3, 66) = 45.9$, $p < .01$) as well as a corner by lesion interaction ($F(3, 66) = 5.71$, $p < 0.01$). For the main effect of corner in the lesioned animals ($F(3, 66) = 18.5$, $p < 0.001$), pairwise comparisons showed that there was no difference between the correct and incorrect corners. The time spent in both the correct and incorrect corners differed from the two white corners ($p < 0.01$). In the sham animals, the main effect of corner ($F(3, 66) = 31.5$, $p < 0.001$) was due to a significant difference between the correct and incorrect corners ($p < 0.05$). Time spent in the correct was also significantly higher than that spent in either of the white corners ($p < 0.001$). Time spent in the incorrect corner did not differ from time spent in the white corners (minimum $p = 0.08$; Figure 4.9).

Further simple effects confirmed that the shams spent more time in the correct corner than the lesioned animals ($F(1, 22) = 6.12$, $p < 0.05$), but time spent in the other corners did not differ by lesion (minimum $F(1, 22) = 3.84$, $p = 0.063$). Overall, there was no main effect of lesion ($F(1, 22) = 2.15$, $p > 0.05$), showing that both groups spent the same amount of time exploring the corners rather than the centre of the maze or its walls.

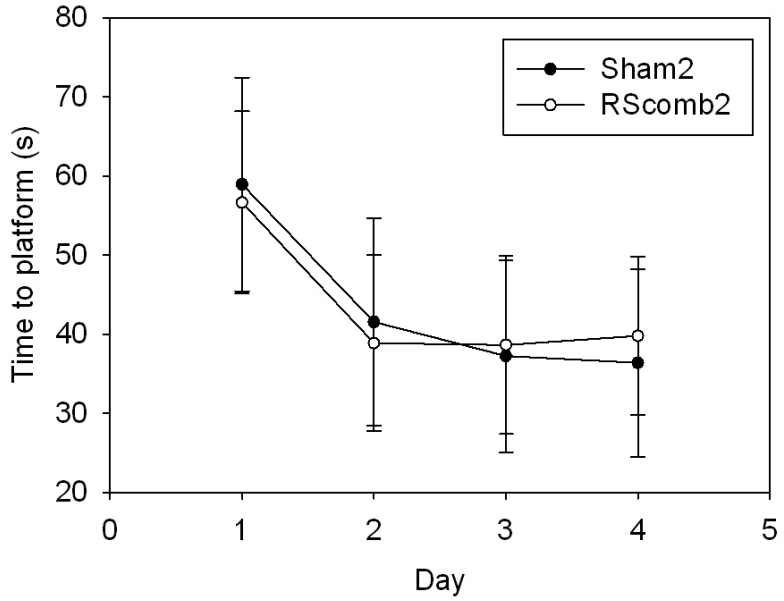

Figure 4.8

The mean time taken for RScomb2 and Sham2 rats to find the platform on each of the four days of pre-training. The RScomb2 group showed no deficits in their ability to swim or to climb onto the platform once it had been located.

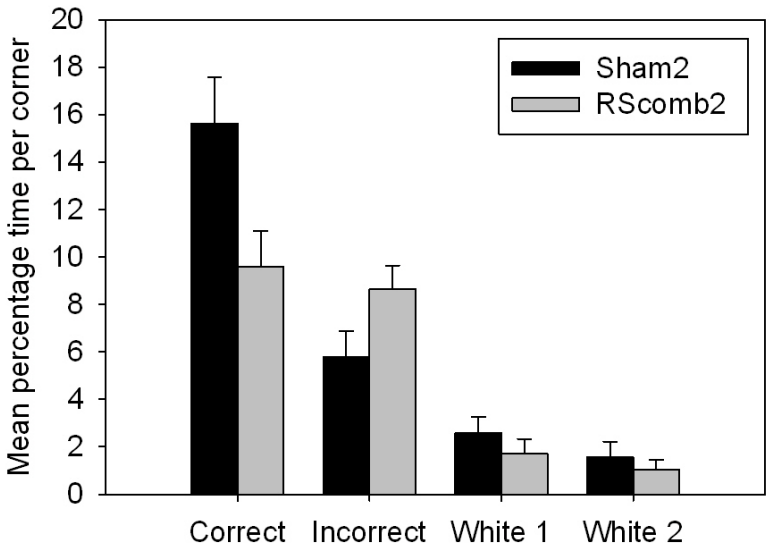

Figure 4.9

The mean percentage time that RScomb2 and Sham2 rats spent in each corner of the arena on the probe trial, when the platform was removed. The arrangement of the walls was identical to the arrangement used during the latent structural learning training, with one striped wall and three white walls. While Sham2 rats spent more time searching for the platform in the correct corner, RScomb2 rats did not distinguish between the correct and incorrect corners. The incorrect corner was defined as the corner made up of a junction between a striped wall and a white wall in the opposite orientation to that in the correct corner.

During the second probe trial, when two striped walls were used, there was an overall main effect of corner ($F(3, 66) = 7.59$, $p < 0.001$; Figure 4.10), but no corner by lesion interaction or main effect of lesion (both $F < 1$). Pairwise comparisons showed that there were no differences in the amount of time spent in the correct and incorrect corners, or in the correct and striped corners. It is, therefore, not possible to say whether animals were navigating using a combination of walls, or a single wall. More time was spent in both the correct and striped corners than the white corner (maximum $p = 0.009$), but the time spent in the incorrect corner did not differ from time spent in the white corner ($p = 0.054$). No other comparisons were significant.

Active Training

When the rats were tested on an active version of the latent structural learning task with a single striped wall, there was a significant main effect of day when analysing escape latencies ($F(3, 66) = 27.26$, $p < 0.001$; Figure 4.11). As swim speed did not differ by group ($F < 1$), escape latency was used as a measure of the rats' accuracy at navigating to the platform. There was a main effect of lesion ($F(1, 22) = 5.12$, $p < 0.05$), but no day by lesion interaction ($F < 1$), meaning that the RScomb2 group took longer to locate the platform, but both groups improved across training days. On the final day of training there was no difference between the two lesion groups ($F < 1$).

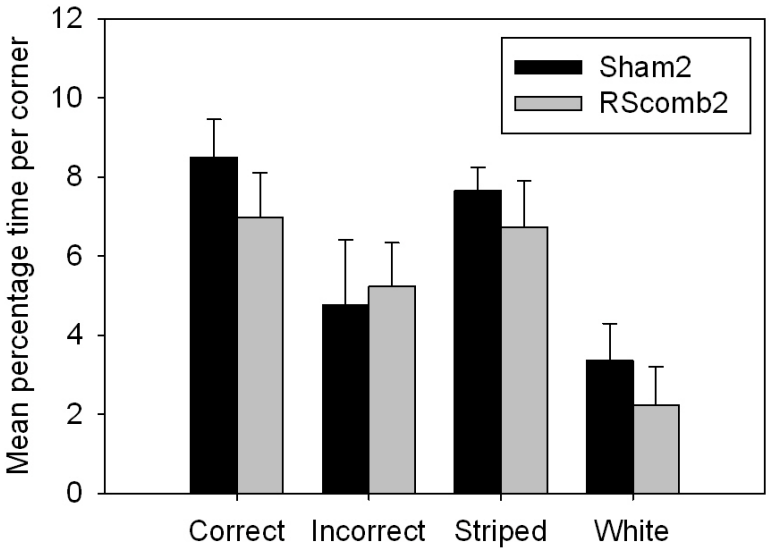

Figure 4.10

The mean percentage time that RScomb2 and Sham2 rats spent in each corner of the arena on the probe trial, when the platform was removed. The arrangement of the walls differed from that used during the latent structural learning training, with two striped walls and two white walls instead of one striped wall and three white walls. Neither group of rats spent more time searching for the platform in the correct corner than in the stripes-white corner.

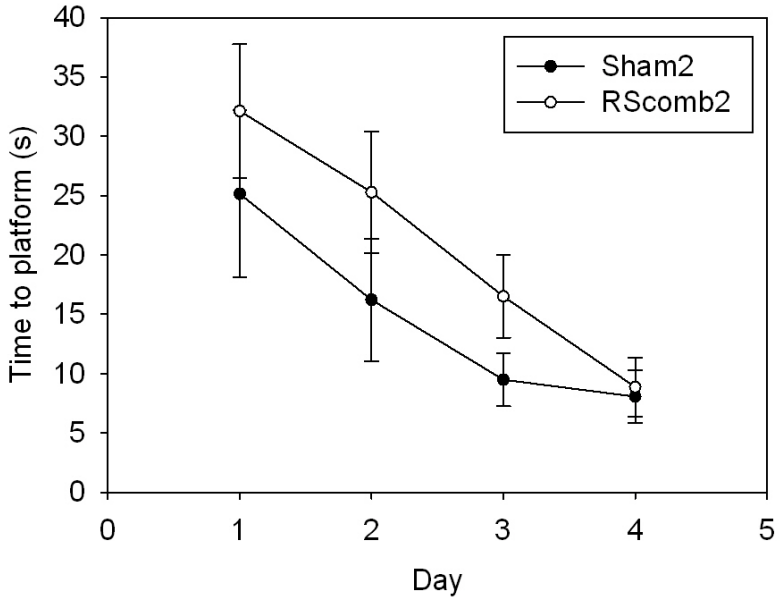

Figure 4.11

The mean time taken for RScomb2 and Sham2 rats to find the platform on each of the four days of active training in an arena with one striped wall and three white walls, identical to the arena used for latent structural learning training. By the end of training there was no difference between the RScomb2 and Sham2 groups.

On the probe trial following the active training there was no main effect of lesion or lesion by corner interaction (both $F < 1$; Figure 4.12) on the percentage time spent in each section of the arena. However, there was a significant main effect of corner ($F(3, 66) = 234.4$, $p < 0.001$). Both groups spent more time in the correct corner than in any other corner ($p < 0.001$). No other comparisons were significant, showing that both groups are able to learn the correct location of the platform when actively trained.

4.6 Discussion

The results of the present chapter show that retrosplenial cortex lesions had no effect on forming simple associations between stimuli and reward, or lack thereof. This was demonstrated by their normal acquisition of the conditioned inhibition experiment, and the lack of impairment when learning about a conditioned inhibitor. A retardation test carried out after acquisition of the task demonstrated that both the Sham2 and RScomb2 rats showed decreased responding to the inhibitor stimulus, X, when compared to a neutral stimulus, Y. This demonstrates that the rats are slower to learn about X as an excitor than about a neutral stimulus, indicating that they had learnt about X as an inhibitor.

Other studies have found a deficit when retrosplenial cortex lesion rats were tested on a compound feature negative discrimination (A+ / AX-), both when the cues were presented simultaneously (Keene and Bucci, 2008b) and when they were shown in

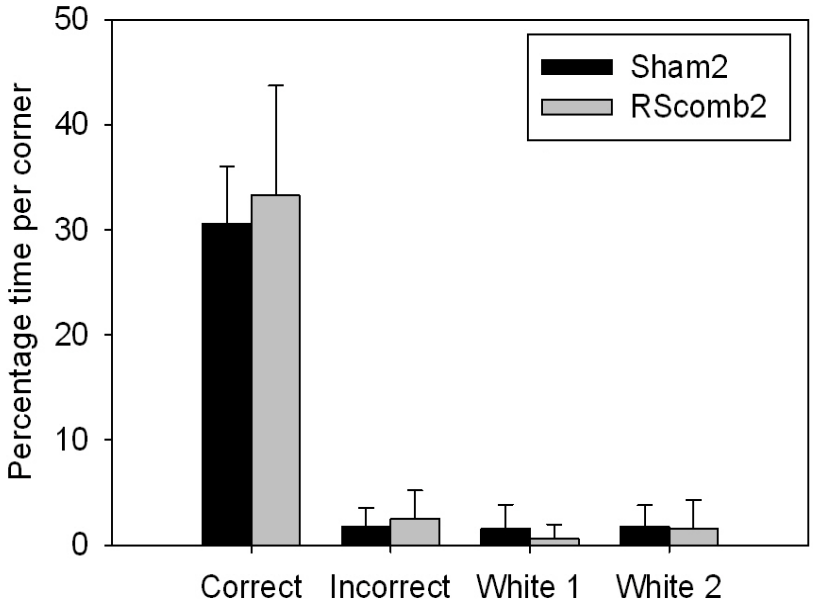

Figure 4.12

The mean percentage time that RScomb2 and Sham2 rats spent in each corner of the arena on the probe trial following active training, when the platform was removed. The arrangement of the walls was identical to the arrangement used during both the active training and the latent structural learning training, with one striped wall and three white walls. Both groups spent significantly more time in the correct corner (where the platform had previously been located) than in any of the other corners.

sequence (Robinson et al., 2011). However, this study did not carry out either a retardation or a summation test, and so it is impossible to confirm whether the rats had learnt about X as an inhibitor or whether they were using some other method such as the number of stimuli presented to solve the task. Another important difference is the way in which the lesions were made. The current study used excitotoxic lesions, while the previous experiments mentioned used electrolytic lesions, which are likely to damage fibres of passage running through and adjacent to the retrosplenial cortex. Damage to the cingulum bundle, which runs immediately below the retrosplenial cortex, can have behavioural effects distinct from those caused by excitotoxic lesions (Warburton et al., 1998). Direct comparisons of posterior cingulate lesions (analogous to rostral retrosplenial lesions) made in mice using radiofrequency and excitotoxic lesions show significantly greater deficits if fibres of passage are damaged (Meunier and Destrade, 1997). Overall, this evidence suggests that the deficits seen by Robinson *et al* (2011) and Keene and Bucci (2008b) may be explained by inadvertent disconnections between brain areas other than the retrosplenial cortex, due to the surgical methods used.

The negative patterning experiment tested a more complex way of combining information across cue types. Configural processing, where the features of a stimulus or object are drawn together into a single representation, is required for solving the negative patterning task (Rudy and Sutherland, 1989). Results from the negative patterning experiment show that rats with retrosplenial cortex lesions learnt the negative patterning discrimination, where they must learn not to press a lever in response to an unrewarded compound stimulus made up of two rewarded

elemental stimuli presented simultaneously, at the same rate as sham animals. Both sham and lesion groups improved at the task with training. This suggests that animals are able to carry out simple configural processing tasks in the absence of the retrosplenial cortex. Transfer tests carried out after acquisition of the negative patterning task confirmed that rats had not used the number of stimuli present at any one time to solve the task, and that they were able to suppress responding to stimuli containing the C+ element. These transfer tests confirm that the animals were using configural processing, in which multiple cues are combined to form a single representation, to solve the task.

Finally, rats with retrosplenial cortex lesions were tested on a structural learning problem, using a latent structural learning task in the water maze. In this task, the spatial configuration of two walls signalled the location of an escape platform. Rats had to learn the location of the escape platform latently, through being placed on the platform during training sessions, rather than through actively navigating to the platform from a start location. This removed the possibility that rats could learn the task through path integration, and instead ensured that knowledge of the platform location would be based on the surrounding visual cues. Retrosplenial cortex lesions caused a severe deficit on a probe trial carried out immediately after the latent structural learning training. The RScomb2 rats were unable to learn the correct location of the platform during latent structural learning training while Sham2 rats showed a significant preference for the correct corner. Although RScomb2 rats did not show a preference for the correct corner over the incorrect corner, which could only be distinguished by learning about the configuration of the white with the

striped wall, they were able to learn that the platform was located near the striped wall. This is demonstrated by the lack of time spent in either of the two corners that did not contact that striped wall.

However, neither the Sham2 nor the RScomb2 rats showed a preference for the correct corner in the second probe trial, when the arena was changed to one with two striped walls instead of just one. This may be because rats were not using a structural strategy to solve the task, or may have been because rats were attracted to the novelty of the corner made from the junction of two striped walls, which they had not seen before. Equally, sham animals may not have been able to transfer the information they had acquired in the original arena to the novel arena. This explanation, though, seems less likely as previous latent learning experiments in rats have shown the ability to transfer information gained through latent learning in a water maze between arenas of different shapes (Horne et al., 2012).

The results of these experiments suggest a role for the retrosplenial cortex in structural learning. That retrosplenial cortex lesions could affect configural processing in the spatial domain is consistent with the probable role of the hippocampus in certain subsets of configural learning, most notably structural learning (Aggleton et al., 2007; Albasser et al., 2013a; Sanderson et al., 2006), given the close connections between the retrosplenial cortex and hippocampus. However, further testing would be required to confirm this, given the lack of a deficit on the negative patterning task – albeit very close to significant - and the fact that Sham2 animals failed to demonstrate that they were using a structural strategy on the

transfer test following the latent structural learning task. Structural learning has been linked to spatial processing, and is thought to be essential for visual navigation, as the unique arrangements of overlapping cues and their spatial distribution are used to determine location. A deficit in some forms of configural processing could therefore contribute towards some of the deficits on spatial tasks seen after retrosplenial cortex lesions, particularly those with a visual component.

The experiments presented in this chapter were chosen to remove elements of translation as far as possible, and test solely integration, in an attempt to separate the two major theories of retrosplenial cortex function discussed earlier. The deficits seen on the latent structural learning tasks demonstrate that aspects of integration are impaired in animals with retrosplenial cortex lesions. However, although this shows that the retrosplenial cortex must have an involvement in integrating different spatial cues, the experiments presented in this thesis cannot rule out the existence of an additional or complementary role for the retrosplenial cortex in translating between different representations.

Chapter 5

5. A role for the retrosplenial cortex in cognitive control

5.1 Introduction

Previous studies on the retrosplenial cortex have tended to focus on its roles in spatial navigation and memory tasks (Vann et al., 2009), due to its dense interconnections with the hippocampal formation (Wyss and van Groen, 1992) and anterior thalamic nuclei (Shibata et al., 2004). However, the retrosplenial cortex also has connections with a number of other regions, including the prefrontal cortex (Kobayashi and Amaral, 2003) and the anterior cingulate cortex (Jones et al., 2005; Wyss and van Groen, 1992). Both of these areas have repeatedly been shown to be involved in detecting and resolving response conflict (de Wit et al., 2006; Haddon and Killcross, 2005, 2011, 2006a; MacDonald et al., 2000; Marquis et al., 2007; Pardo et al., 1990) and in selectively attending to behaviourally relevant stimuli (Birrell and Brown, 2000; MacDonald et al., 2000). Additionally, strong connections are found between the retrosplenial cortex and the parietal cortex, which has roles in attention (Kobayashi and Amaral, 2007; Mesulam, 1983) and, more specifically, in switching between cue types when one cue becomes behaviourally irrelevant (Fox et al., 2003). This connectivity suggests that the retrosplenial cortex may have a role in similar processes.

To date, very few studies have been carried out on the ways that retrosplenial cortex dysfunction impacts decision-making, response choice and other higher functions involved in cognitive control. A task that has typically been used in humans to test cognitive control and flexibility is the Wisconsin Card Sorting Test. Here, participants are given cards with different shapes on them e.g., a square, cross or circle. The cards differ in the number of shapes present and the colour of the shapes (e.g., three blue squares). The participants initially have to organise the cards according to one rule (e.g., "colour") and are subsequently required to switch rules and organise the cards according to a different features (e.g., "shape"). A patient with unilateral pathology to the retrosplenial cortex, as well as to parts of the splenium and cingulum bundle, was reported to have deficits in the Wisconsin Card Sorting Test (Valenstein et al., 1987), although another patient with a retrosplenial cortex lesion was unimpaired on the task (Yasuda et al., 1997). Patients with prefrontal cortex damage are frequently shown to be impaired at the Wisconsin Card Sorting Test when compared to healthy controls or to patients with non-frontal brain damage (Barceló and Knight, 2002; de Oliveira-Souza et al., 2001; Grafman et al., 1990). Involvement of the anterior cingulate cortex is also demonstrated through functional imaging studies (Lie et al., 2006; Monchi et al., 2001).

In the current study, the impact of rodent retrosplenial cortex lesions on an attentional set-shifting task analogous to the Wisconsin Card Sorting Test was investigated. This task requires animals to carry out two-choice discriminations between complex stimuli, which vary in more than one perceptual dimension. Both intra-dimensional and extra-dimensional attentional set-shifting were tested. An

intra-dimensional shift would be one where novel stimuli must be discriminated based on the same perceptual dimension as has been previously trained, while for an extra-dimensional shift the novel stimuli must be discriminated based on the perceptual dimension that has not previously been attended to. Performance on the rodent attentional set-shifting task is specifically impaired on extra-dimensional shifting by medial frontal lesions (Birrell and Brown, 2000).

Following the set-shifting trials, a reversal test was carried out, where the stimuli do not change but the previously rewarded stimulus becomes non-rewarded and *vice versa*. Rather than testing attentional set-shifting this last reversal trial investigated the ability of retrosplenial cortex lesioned animals to carry out affective shifts. This is the ability to alter the associations attached to various stimuli in order to change behaviour if reinforcement contingencies shift. An impairment on this aspect of the task could imply that retrosplenial lesioned animals are unable to flexibly alter their responses as task demands change, which could also explain the deficits seen when switching between cue types in spatial tasks. There is some previous evidence that retrosplenial cortex lesions impair reversal performance (Cain et al., 2006; Meunier and Destrade, 1997; Meunier et al., 1991), but as these experiments all used spatial tasks, it is difficult to distinguish the spatial from the reversal components of the lesion effect. While one previous study (Neave et al., 1994) had found no effect of retrosplenial cortex lesions on a non-spatial reversal task, the lesions in this study were not complete and spared the caudal-most part of the structure, thus leaving retrosplenial-frontal connection intact (Shibata et al., 2004). For these reasons, it is

important to test rats with reversal function on a non-spatial task, to clarify the role of the retrosplenial cortex in this process.

Further evidence that the retrosplenial cortex may contribute to cognitive control processes in humans comes from a diffusion MRI study that demonstrated significant correlations between performance on the Stroop task and the microstructure of the left cingulum bundle, particularly those parts of the cingulum bundle most connected to the retrosplenial cortex (Metzler-Baddeley et al., 2012). In the Stroop task, participants are presented with the names of different colours (e.g. the word "red") written in coloured ink and are required to name the colour of the ink. People take longer to name the colour of the ink when the word and colour are incongruent (e.g., the word "red" is written in yellow ink) than when the word and colour are congruent (e.g., the word "yellow" is written in yellow ink). The increase in time taken on the incongruent trials and the number of errors made are used as a measure of cognitive flexibility (Uttl and Graf, 1997). Patients with prefrontal cortex lesions have been repeatedly shown to perform significantly worse on this task than control subjects or patients with non-frontal brain damage (Demakis, 2004; Stuss et al., 2001; Vendrell et al., 1995). Anterior cingulate lesions also impair performance on this task (Swick and Jovanovic, 2002). The involvement of these areas in the Stroop task has also been identified in functional imaging studies (Bench et al., 1993; Leung et al., 2000; MacDonald et al., 2000; Pardo et al., 1990). Further investigation of the role of the retrosplenial cortex in tasks requiring cognitive control is difficult, as there are very few case studies of patients with retrosplenial damage, and those that do exist are not selective or complete.

To clarify the role of the retrosplenial cortex in cognitive control and the flexible selection of relevant cues, rats with excitotoxic lesions of the retrosplenial cortex were tested on a rodent analogue of the Stroop Test (Haddon and Killcross, 2005, 2006a; Haddon et al., 2008), which looks at choice behaviour when conflicting cues are present. Instead of using words and colours to create congruent and incongruent stimuli, two instrumental biconditional discriminations, one auditory and one visual, are trained simultaneously in different contexts. At test, audio-visual compounds of the stimuli are presented, which can either direct the rat to the same instrumental response, or to conflicting responses. In this latter situation, rats are expected to use contextual cues to disambiguate correct from incorrect lever press choices. The rat medial prefrontal cortex, an area analogous to the prefrontal areas implicated in human studies, is critical for successful completion of this task (Haddon and Killcross, 2005).

The prefrontal cortex in rodents is thought to contribute to executive function, including the cognitive control processes that are required for attentional selection, task switching, and behavioural inhibition (Brown and Bowman, 2002; Dalley et al., 2004). Several theories have suggested that the different roles of the prefrontal cortex can be arranged hierarchically, with different sub-regions contributing to different processes. In primates, two different theories suggest either a two-level division based on the ways in which working memory is accessed (Petrides and Baddeley, 1996) or a three-level division based on the complexity of the rules required for performance on various tasks (Wise et al., 1995). This latter theory has a parallel in the discussions of rodent prefrontal cortex function (Kesner and

Churchwell, 2011). Under this theory, a three-level hierarchy of function is established, with lower-order rules including those used during reversal learning, higher-order rules those involved with set-shifting and the highest order rules including paired-associate learning and other tasks that require resolution of cognitive interference (Kesner, 2000). This hierarchy may reflect the increasing demands of the tasks, as more independently varying elements are introduced that must be processed simultaneously to generate the correct rules and responses (Halford et al., 1998). The experiments presented here test the role of the retrosplenial cortex in all of these levels of processing. The set-shifting task includes both lower- and higher-order processing in the reversal and intra-dimensional shifting, both of which ensure that the animal is paying attention to the target stimulus features at the time of the shift, and a further test of higher-order processing in the extra-dimensional shifting, in which irrelevant features are targeted. The Stroop task tests the highest order functions by adding a level of ambiguity of response in the incongruent trials, which must be resolved using contextual cues.

5.2 General Methods

5.2.1 Animals

Subjects were 56 experimentally naive male Lister Hooded rats (Harlan, Bicester, UK), making up two cohorts of 28 animals each. The Stroop cohort (Cohort 3) weighed 272-284g at time of surgery, and the set-shifting cohort (Cohorts 1 + 2)

weighed 278-387g. Housing and feeding procedures were as described in Chapters 2 and 3. Animals in each cohort were randomly assigned to surgery groups, receiving either a bilateral excitotoxic lesion to the retrosplenial cortex (Cohort 3, n = 16) or a sham lesion (Cohort 3, n = 12). All animals were given at least ten days to recover after surgery before any behavioural testing began. Final animal numbers in Cohorts 1 and 2 after exclusions were RSComb1 = 9, Sham1 = 12, RSComb2 = 13, Sham2 = 12.

The rats in Cohort 3 were experimentally naïve when the Stroop task was carried out, two weeks after the completion of surgery (see Annex A). Rats in Cohort 2 were also experimentally naïve when undergoing the set shifting experiment, immediately after they had recovered from surgery. Cohort 1 had been tested on an object-in-place task and the perspective, viewpoint and medium discrimination tasks described in Chapter 1 before the set-shifting experiment was conducted, and were eight months post-surgery (see Annex A). Two cohorts of animals were used during the set-shifting experiment to confirm the results found with the first cohort in the light of previous findings (Ng et al., 2007) and the findings from the Stroop task.

5.2.2 Surgical Procedures

Rats in Cohort 3 were injected with atropine (0.03ml of a 600µg/ml solution delivered via the intraperitoneal (i.p.) route, five minutes before being deeply anaesthetised with an i.p. injection of sodium pentobarbital (60mg/kg pentobarbital sodium salt; Sigma-Aldrich, U.K.). Otherwise, surgical and post-surgical procedures were as described in Chapter 2.

The stereotaxic coordinates of the lesion placements are stated relative to bregma in the anterior-posterior (AP) axis, and relative to the central sinus in the lateral-medial (LM) axis. Dorsal-ventral (DV) coordinates are taken relative to the surface of the cortex, using the eye of the needle. The coordinates for Cohort 3 were AP -1.8, LM±0.4, DV-1.0; AP-2.8, LM±0.4, DV-1.1; AP-4.0, LM±0.4, DV-1.1; AP-5.3, LM±0.4, DV-2.4; AP-5.3, LM±0.9, DV-1.4; AP-6.6, LM±0.9, DV-1.8; AP-7.5, LM±1.0, DV-1.1. 0.27µl of neurotoxin was injected in the three most rostral pairs of sites, with 0.29µl injected in the next three pairs of sites. In the most caudal site 0.1µl was injected. The coordinates for Cohorts 1 and 2 can be found in Chapters 2 and 3 respectively.

5.2.3 Histological procedures

At the completion of all experiments, rats were deeply anaesthetised using sodium pentobarbital (60mg/kg, i.p.; Euthatal; Merial Animal Health, Harlow, UK), then transcardially perfused with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M PBS (PFA). The brains were removed and placed in PFA for 4h before being transferred to 25% sucrose and left overnight at room temperature, with gentle agitation. Four adjacent series of coronal sections (40µm) were cut on a freezing sliding microtome. One series was mounted directly onto gelatine-coated slides after slicing, and was stained using cresyl violet, a Nissl stain, for verification of the specific brain regions. A second series from each animal was collected in PBS then transferred into 10 mM citrate buffer (pH = 6) dissolved in deionised H_2O. The slices were incubated in a water bath at 70^{o}C for 30 minutes, then processed for NeuN immunohistochemistry. NeuN stains selectively for

neurones (Mullen, Buck and Smith 1992), and so allows the extent of the lesion to be more accurately determined. The further processing of the tissue was as described in Chapter 2.

5.2.4 Statistical Methods

Statistical tests were carried out using SPSS 16.0 (SPSS Inc., Chicago). Where the assumption of sphericity was not met for parametric analysis, Greenhouse-Geisser corrections have been applied. In all statistical tests the critical alpha level is taken as $p \leq 0.05$.

5.3 Experiment 1 - Attentional Set-shifting

5.3.1 Apparatus

Testing was carried out in a black Perspex box measuring 69.5cm long x 40.5cm wide x 18.6cm deep. Approximately one third of the length of the box was divided into two smaller compartments, with the remaining area of the box being a single open space. The two smaller sections could be separated from the larger area by removable black Perspex panels that could be used by the experimenter to control access. Each of the three sections had a separately hinged transparent Perspex lid. One glass pot (75mm in diameter, 45mm deep) that was used to contain digging media was placed in each of the two smaller areas. A third identical glass pot containing water was placed against the opposite wall of the larger area (see Figure

5.1). As test sessions could last several hours, this was to prevent the rat from getting thirsty, particularly as this could reduce motivation to dig for food rewards.

5.3.2 Behavioural Testing

During this task, rats were presented with a series of two-choice discriminations. The stimuli used were multi-dimensional, with only one stimulus dimension (odour or texture) signalling reward. This experiment was run twice with two different cohorts, to increase the number of animals in the task and allow detection of smaller effects.

Pre-training began three days prior to testing, when each rat was placed in the arena for 10 minutes to habituate to the apparatus. For the first pre-training day, both removable panels were taken out of the arena, so animals had access to all three chambers, but none of the glass pots was present. The following day, rats were returned to the arena with both smaller chambers closed off and all three of the glass pots in place. The two glass pots in the smaller chambers were filled with bedding sawdust and baited with half a Cheerio (Nestle, UK). The day before testing rats were again placed in the arena and pre-exposed to the stimuli that would be used in the set-shifting task. Each odour that would be used was presented mixed with bedding sawdust. Digging media were presented without odours added. Each rat was required to retrieve a buried Cheerio from each pot of digging medium, and from each pot of odour-laced sawdust. This was repeated in each of the smaller chambers, to avoid spatial bias.

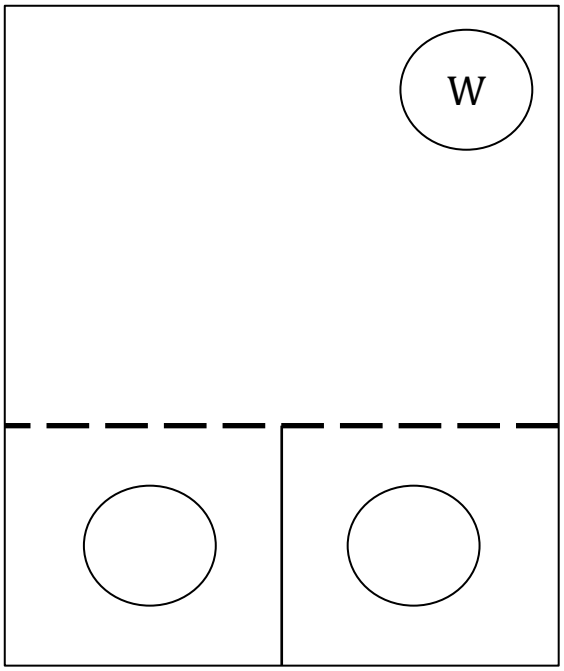

Figure 5.1

A diagram of the box used for the set-shifting experiments. Approximately one third of the length of the box was divided into two smaller compartments, with the remaining area of the box being a single open space. The two smaller sections could be separated from the larger area by removable black Perspex panels (represented by the dashed line) to control access. One glass pot (the circles) was placed in each of the two smaller areas, to contain digging medium. A third identical glass pot containing water was placed against the opposite wall of the larger area (the circle marked with a W).

On the test day, rats were trained on a series of discriminations (see Table 5.1 for examples). The rat did not move onto the next discrimination until it had acquired the present discrimination, defined as making a correct choice on six consecutive discriminations. All tests were completed in a single day for each rat. The first discrimination was either between two different odours or between two different digging medium (the choice of stimuli was counterbalanced between groups). For these discriminations the glass pots in each of the smaller areas were filled with either two different digging media or with sawdust infused with two different scents. Only one odour (or one medium) contained a buried Cheerio. The location of the rewarded bowl was pseudo-randomly allocated in each trial. Once the dividing doors were removed, the rat had 10 minutes to find the Cheerio. If the rat dug in the rewarded pot – defined as breaking the surface of the digging medium with the paws or nose - the trial was marked as correct. If the rat dug in the incorrect pot, the trial was marked as incorrect. For the first four trials of each discrimination the rat was allowed access to the correct pot to uncover the reward following an incorrect initial dig. On subsequent incorrect trials, the trial was terminated as soon as the rat returned to the large waiting area of the arena. The ITI was approximately 5 seconds long, during which time the cups were re-baited. The criterion performance to move on to the next discrimination was six correct choices in a row. An experimenter, blind to whether the rat was in the RScomb or Sham group, scored the number of trials to criterion. Throughout training, rats were carried to and from the testing room in individual carrying boxes with a lid, so that the animal was unable to see outside.

Trial type	Odours	Media	Relevant
Simple	Oregano (+), Cloves (-)	Sawdust	Odour
Complex	Oregano (+), Cloves (-)	Confetti, Paper	Odour
Intra 1	Cinnamon (+), Ginger (-)	Coarse tea, fine tea	Odour
Intra 2	Tarragon (+), Fenugreek (-)	Coarse cork, fine cork	Odour
Intra 3	Marjoram (+), Sage (-)	Shavings, wood chip	Odour
Intra 4	Cumin (+), Dill (-)	Short filters, long filters	Odour
Extra	Mint, Turmeric	Polystyrene (+), beanbag pellets (-)	Medium
Reversal	Mint, Turmeric	Beanbag pellets (+), polystyrene (-)	Medium

Table 5.1

An example of combinations of media and odours that were used in the set-shifting experiment, for a rat shifting attention from odour to digging media. The rewarded element is indicated by a (+), and the non-rewarded element by a (-). The same two odours were always paired with the same two media, but the order in which these were presented and the rewarded element was varied to avoid preference bias.

During the simple discrimination (SD) trial, the digging media was made up either of scented sawdust or of a digging medium with no odour added. In the subsequent complex discrimination (CD), the same odour or texture rewarded in the simple discrimination trials was still rewarded, but the irrelevant dimension was added. For all subsequent discriminations other than the reversal trial both the odour and the texture presented changed.

5.3.3 Results

Histological evaluation of the lesions

Descriptions of Cohorts 1 and 2 can be found in Chapters 2 and 3 respectively. No animals were removed from Cohort 3 after histological evaluation, leaving final numbers of 16 lesions (RScomb3) and 12 sham animals (Sham3). Marked cell loss and gliosis could be seen throughout almost the entire retrosplenial cortex (Figure 5.2). Rostrally, the lesions were largely complete, with the exception of two cases where some granular sparing was evident (one bilaterally). The anterior cingulate cortex was not affected. Caudally to the splenium Rga was partially spared in five cases; in three of these cases the sparing was bilateral. Additional cell loss was seen in the most medial dorsal tip of CA1 in the septal hippocampus in six animals. One case had bilateral damage to CA1. In six cases contraction of cells in the medial blade of the dentate gyrus was observed at the level of the splenium; in a further three cases this narrowing was bilateral. These cases also showed very limited cell loss in the dorsal subiculum at the same level. A restricted area of gliosis was

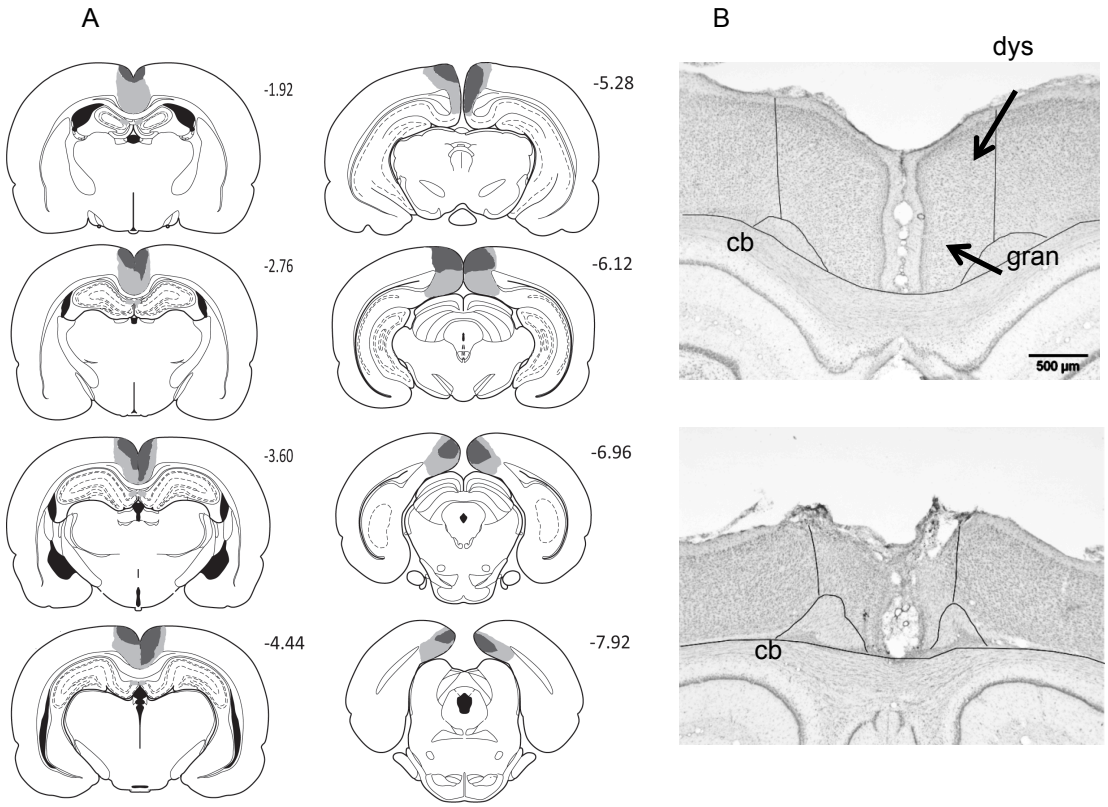

Figure 5.2

A series of coronal sections showing the cases with the largest and smallest retrosplenial lesions included in Cohort 3. Light grey represents the largest lesion, and dark grey the smallest. The numbers correspond the distance behind bregma in mm (Paxinos and Watson, 2006). B: Coronal Nissl sections showing the retrosplenial cortex (both hemispheres) in a sham surgery control rat (top), and a representative rat from the Cohort 3 retrosplenial lesion group. The dashed lines show the limits of the retrosplenial cortex and of the granular and dysgranular sub-regions. The scale bar is 500µm long. Abbreviations: cb, cingulum bundle; dys, dysgranular retrosplenial cortex; gran, granular retrosplenial cortex.

observed at the junction of the anterior medial and anterior ventral nuclei, as is consistently seen after retrosplenial lesions (Gonzalez et al., 2003; Neave et al., 1994; Vann et al., 2003).

Behavioural Results

The data from the two cohorts was analysed together, with a factor of repetition. A main effect of repetition was found, reflecting a baseline difference in performance, with rats in Cohort 2 taking more trials to reach criterion than those in Cohort 1. There was no lesion by repetition interaction ($F < 1$), or repetition by trial type interaction ($F(7, 280) = 1.82$, $p > 0.05$), so repetition was removed as a factor and both cohorts were analysed together. Both the number of trials taken to reach criterion and the number of errors made were analysed.

No difference was found in the number of trials taken by the lesion and sham groups to acquire the simple (single dimension) discrimination ($F < 1$; Figure 5.3, SD), nor in the number of incorrect choices made prior to reaching criterion ($F < 1$; Figure 5.4, SD). Similarly, there was no difference in the number of trials taken to subsequently acquire the complex (two dimension) discrimination ($F(1, 42) = 3.52$, $p = 0.068$; Figure 5.3, CD), when a further tactile or olfactory stimulus was added to the original stimuli used for the simple discrimination. There was also no difference in the number of incorrect choices made during this trial ($F < 1$; Figure 5.4, CD).

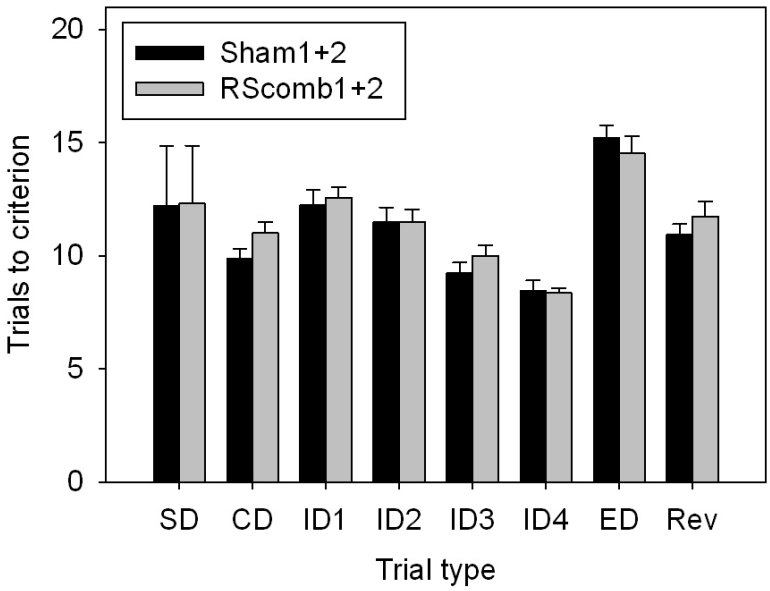

Figure 5.3

The number of trials taken to reach criterion (six correct choices in a row) on the set-shifting task. SD = simple discrimination, CD = complex discrimination, ID1-4 = intra-dimensional shifts 1-4, ED = extra-dimensional shift, Rev = reversal. No differences were found between the two groups.

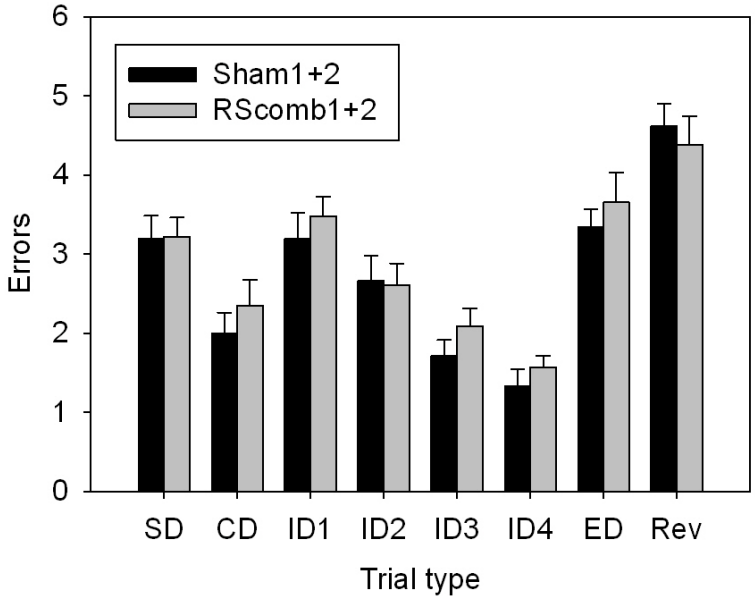

Figure 5.4

The number of errors made before reaching criterion (six correct choices in a row) on the set-shifting task. SD = simple discrimination, CD = complex discrimination, ID1-4 = intra-dimensional shifts 1-4, ED = extra-dimensional shift, Rev = reversal. No differences were found between the two groups.

The rats' performance improved across the four successive intra-dimensional discriminations (Figure 5.3, ID1-4) as they required fewer trials to reach criterion on the later discriminations ($F(3, 126) = 34.15$, $p < 0.001$), and made fewer errors ($F(1, 42) = 61.79$, $p < 0.001$). This pattern is consistent with the rats acquiring an attentional set. The RScomb animals did not differ from Shams in number of trials taken to acquire the discriminations ($F < 1$) or in the number of incorrect choices ($F < 1$; Figure 5.4) and there was no lesion by discrimination interaction on either measure (both $F > 1$).

On the extra-dimensional trials, when rats were shifted from one dimension of stimulus (e.g., odour) to the other (e.g., texture), an increase was seen in the number of trials taken to reach criterion compared to the final intra-dimensional shift ($F(1, 42) = 131.9$, $p < 0.001$). This increase helps to confirm that an attentional set had been formed across the intra-dimensional discriminations and demonstrates a cost in switching away from this set. There was no difference between the groups in the number of trials taken to reach criterion ($F < 1$; Figure 5.3, ED), and no lesion by shift type (ID vs. ED) interaction ($F < 1$). This same pattern was also seen when analysing the total number of incorrect choices made (Figure 5.4, ED).

During the final reversal test the number of trials taken to acquire the discrimination did not differ between the two lesion groups ($F < 1$; Figure 5.3, Rev), and nor did the number of incorrect choices made before reaching criterion ($F < 1$; Figure 5.4, Rev).

5.4 Experiment 2 - Stroop task

5.4.1 Apparatus

Eight operant boxes measuring 30cm wide x 24cm deep x 21cm high (Med Associates, George, VT) were used. Each chamber had three aluminium walls with the fourth wall being made up of a Perspex door. In the four 'white' chambers, the walls and ceiling of the boxes were lined with white laminated paper with a single black strip 5cm across, fixed behind transparent Perspex. In the other chambers, the walls were left bare. The floor of the chamber was made up of 19 stainless steel rods, 1.6 cm apart. Each rod was 3.8mm in diameter. Beneath the rods was a removable metal tray that was filled with sawdust. In the four 'white' chambers the sawdust was mixed with cumin powder, and in the remaining 'plain' chambers with paprika powder. This was to provide the rats with an additional non-visual way to distinguish between the two contexts.

A 3W house light located at the top centre of the left-hand wall provided illumination in each chamber. In the centre of the right-hand wall, a recessed magazine (5cm tall by 5cm wide) was located, via which Noyes food pellets (45mg; Noyes, Lancaster, NH) or 15% (w/v) sucrose solution could be delivered to the rat. To either side of the magazine was a flat-panel retractable lever, each with a 2cm diameter circular panel light located above it. Auditory stimuli were delivered via speakers in the ceiling, and consisted of a 2kHZ tone or a 10Hz train of clicks. Visual

stimuli were either the two panel lights flashing (0.1s on, 0.1s off), or steady illumination of the panel lights and magazine light simultaneously.

5.4.2 Behavioural Procedures

Lever press training

The rats received four training sessions, at the end of which each rat would lever press for a single food pellet or 0.1ml of the sucrose solution on a random interval schedule (RI15) such that on average once in every 15s a reward became available following a lever press.

Conditional discrimination training (Experiment 2a)

Rats were trained for 18 days on two concurrent conditional discriminations (see Fig 5.5), with two sessions of training per day, one in each of the contexts (white/cumin and plain/paprika). Correct responses were rewarded in one context with food pellets, and in the other with sucrose solution. The contexts, stimuli and rewards were counterbalanced across animals, as far as possible given the size of the cohort.

In one context, rats were presented with the two visual cues (flashing or steady lights). Each visual cue was paired with a rewarded lever, for example during flashing lights only responding on the left lever would be rewarded; during steady lights only responding on the right lever would result in a reward. In the other

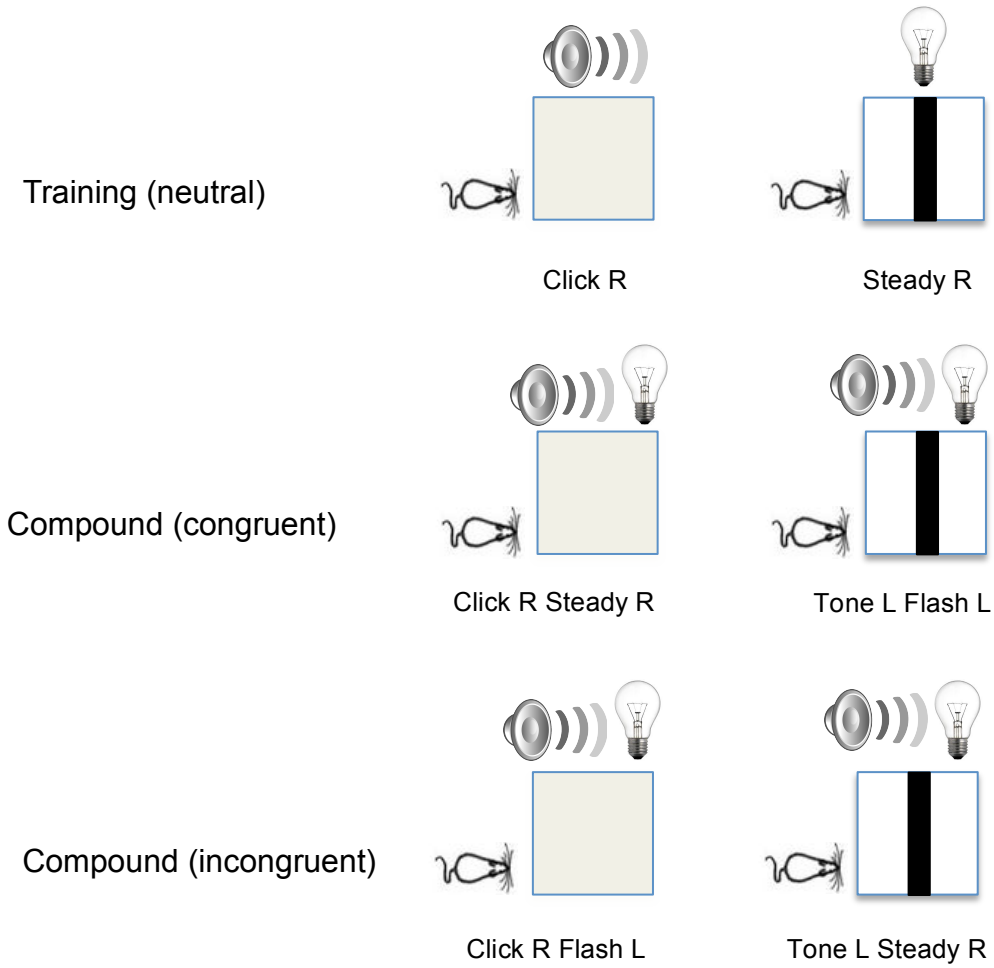

Figure 5.5

Experimental design. The letters R and L indicate the rewarded lever (left or right). This shows as example of what one rat could have experienced; in the experiment, the lever associated with each of the training stimuli were counterbalanced. Animals acquired two conditional discriminations concurrently, one auditory and one visual. These took place in different contexts (one metal and one white with a black strips), and with different rewards (one with a food pellet and the other with sucrose solution). During extinction tests animals received audio-visual compounds of the training stimuli, comprising either elements that had elicited the same response (congruent trials) or different responses (incongruent trials) during training.

context, the two auditory stimuli (click or tone) were presented, each associated with rewards for responding only on one lever.

Each training session consisted of 24 trials (12 of each stimulus type) with a mean inter-stimulus interval of 60s. During the inter-stimulus interval both levers were retracted, and were extended again at the start of the next trial. Each stimulus presentation lasted 60s. During the first 10s of each trial no reinforcement was given, so that discrimination performance was uncontaminated by the presence of a reward. In the remaining 50s reinforcement was given on an RI15 schedule, where on average a reward would be available once in every 15s following a correct lever press.

Extinction sessions

Following discrimination training, all rats underwent four extinction sessions: two in each of the two training contexts. After the first two sessions (one in each context) rats were given two days of reminder training on the original conditional discriminations, during which rewards were available, before continuing to the second two sessions. The order in which the contexts were tested was counterbalanced across animals. During the extinction test, rats were presented with either individual training stimuli or with audio-visual compounds of two training stimuli. Stimulus duration was 60s, with a mean inter-stimulus interval of 60s. If both of the stimuli making up a compound stimulus had been rewarded after lever

presses on the same lever, the pair of stimuli was 'congruent'; if the rewarded levers were different, the stimuli were 'incongruent'. During these presentations both levers were available but responses were not reinforced. As congruent stimuli were made up of individual stimuli that had both signalled a response on the same lever, when presented together the stimuli should elicit responding on this same lever, regardless of context. In contrast, incongruent stimuli consisted of auditory and visual components that had been trained to elicit responses on *different* levers. When incongruent stimuli were presented, the two stimuli of which they were made up would elicit responses on different levers. In this instance, rats should use the context in which the stimulus is presented to determine which lever to press.

Selective reward devaluation (Experiment 2b)

Rats were trained for two reminder days on the original conditional discriminations. Following this training the rats were given free access to one of the two rewards (15% sucrose solution or food pellets) for 30min, in a room adjacent to the testing room. For this period rats were kept in a cage identical to their home cage. Rats were then placed in the operant boxes for an extinction session, lasting 15min. During this session the house lights were on and both levers were available. Each rat was tested twice in the same context, counterbalanced across the groups. Testing order (devalued versus non-devalued) was also counterbalanced.

Selective reward devaluation – effect of contextual control of conflict behaviour (Experiment 2c)

All animals carried out two days of reminder training on the original conditional discriminations. Each animal was then given four extinction tests, two in each context. After the first two extinction tests a further two days of reminder training were given. Before each extinction test animals were pre-fed (see previous section) either the reward associated with that context, or the reward associated with the other context. Testing order was fully counterbalanced. Each session consisted only of congruent and incongruent trials, with no individual stimulus trials given; for this reason the test session was only 12 minutes long.

5.4.3 Results

Both Sham3 and RScomb3 groups acquired the visual and auditory discriminations, shown by their preference for the rewarded lever over the non-rewarded lever ($F(1, 25) = 121.6$, $p < 0.001$; Figure 5.6A). This preference emerged over training, resulting in a significant main effect of day ($F(8, 200) = 13.7$, $p < 0.001$). There was no main effect of lesion and no lesion group by day interaction (both $F < 1$).

Extinction test (Experiment 2a)

For the analysis of the three trial types (single element, congruent and incongruent), the mean response rates on the correct and incorrect levers were combined over the

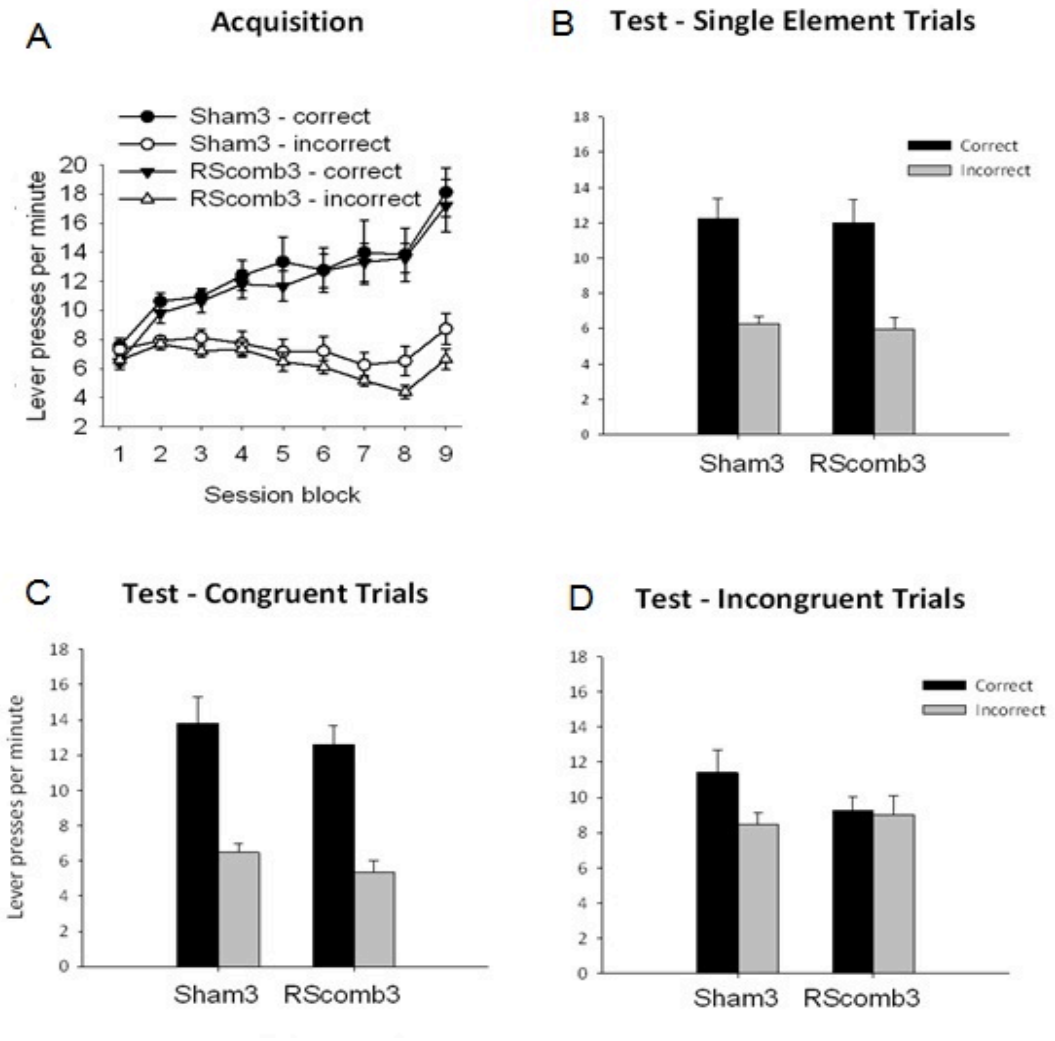

Figure 5.6

Correct and incorrect lever presses per minute:

A: during acquisition of the conditional discrimination.

B: during presentation of the single element training stimuli in extinction.

C: for congruent compound stimuli, using elements that were rewarded during training for a response on the same lever.

D: for incongruent compound stimuli, made up of elements that were rewarded for responses on different levers during training.

RScomb3 rats were unable to use contextual cues to disambiguate the correct lever during an incongruent stimulus presentation, unlike the Sham3 animals. Error bars show SEM.

four extinction test sessions. On the single element trials, both groups exhibited a greater level of responding on the correct than the incorrect lever ($F(1, 25) = 53.3$, $p < 0.001$; Figure 5.6B). There was no main effect of lesion ($F < 1$) or lesion by lever response interaction ($F < 1$).

When congruent compound stimuli (i.e., a compound in which both elements directed the rat to the same lever) was presented, both groups again produced more responses on the correct lever ($F(1, 25) = 103.9$, $p < 0.001$; Figure 5.6C). There was no effect of lesion ($F < 1$) or interaction ($F < 1$).

When incongruent compound stimuli were presented (i.e., those in which the individual stimulus elements would direct the animals towards different levers), Sham3 animals tended to respond according to the stimulus that had been trained in that context, indicating the use of contextual cues to disambiguate the conflicting information ($F(1, 25) = 9.2$, $p < 0.01$; Figure 5.6D). In the RScomb3 group, however, there was no difference in the rate of responding on the two levers ($F < 1$), suggesting contextual cues were not used to determine the correct lever. There was a lever by group interaction, reflecting this difference ($F(1, 25) = 4.4$, $p < 0.05$). However, there was no overall main effect of lesion ($F < 1$) or lever presses ($F(1, 25) = 5.8$, $p < 0.05$). Simple effects analysis confirmed that the Sham3 group responded appropriately for the context they were tested in ($F(1, 25) = 9.2$, $p < 0.01$), but the RSC1 rats did not ($F < 1$; Figure 5.6D).

Selective reward devaluation (Experiment 2b)

During the reminder sessions on the original discrimination both groups pressed the rewarded lever more frequently than the non-rewarded lever ($F(1, 25) = 187.9$, $p < 0.001$). There was no main effect of lesion or lesion by lever choice interaction (both $F < 1$). During the extinction sessions, both groups pressed the lever less frequently when they had been pre-fed the reward associated with the context that they were in than when they were pre-fed the other reward ($F(1, 25) = 12.9$, $p < 0.001$). This demonstrates an ability to form and use context-reward associations. There was no effect of lesion ($F < 1$).

Selective reward devaluation – effect of contextual control of conflict behaviour (Experiment 2c)

During the reminder training both groups of rats solved the conditional discriminations correctly ($F(1, 25) = 133.8$, $p < 0.001$; Figure 5.7). There was no main effect of lesion or interaction (maximum $F(1, 25) = 2.7$, $p > 0.05$). Additionally, there was no effect of test order or the context in which the test took place ($F < 1$), so the data were combined and analysed together.

When tested on the congruent compounds after pre-feeding, there were no effects of lesion or lesion interactions (all $F < 1$). Rates of responding were found to be lower after pre-feeding with the 'same' reward (that associated with the test context) than with the 'different' reward (that associated with the other context) ($F(1, 25) = 37.1$, $p < 0.001$, see Figure 5.7). Overall, rats were found to make a

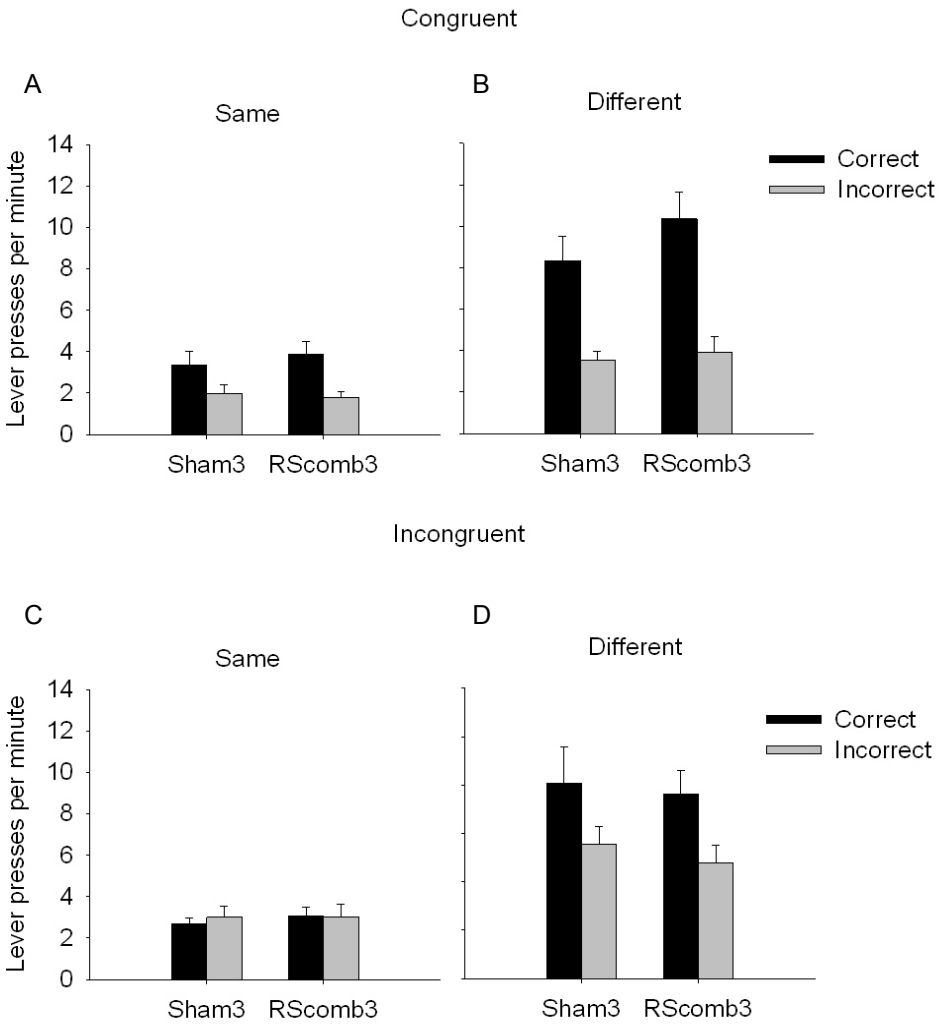

Figure 5.7

Selective reward devaluation on the contextual control of conflict behaviour. The graphs show correct and incorrect lever presses per minute for Sham and RScomb 3 lesioned animals when:

A: responding to congruent compounds when pre-fed with the reward that had previously been earned in the test context ('same'), and B: when pre-fed with the reward that had not previously been earned in the test context ('different').

C: responding to incongruent compounds in the 'same' context or D: the 'different' context.

Rates of responding decreased in the 'same' compared to the 'different' condition. Rats maintained accurate performance on congruent trials in both conditions. However, context-appropriate responding to incongruent stimuli was abolished in both groups when tested in the 'same' condition. Both groups responded appropriately to incongruent stimuli after pre-feeding in the 'different' condition, although RScomb3 lesioned animals did not do this without pre-feeding (Figure 5.4).

significantly greater number of correct than incorrect responses ($F(1, 25) = 80.3$, $p < 0.001$, see Figure 5.7). Although this effect was larger in the 'different' condition ($F(1, 25) = 16.3$, $p < 0.001$), animals responded more on the correct lever in both the 'same' ($F(1, 25) = 30.5$, $p < 0.001$) and the 'different' ($F(1, 25) = 47.5$, $p < 0.001$) conditions.

Responding to the incongruent stimuli was reduced following pre-feeding with the 'same' reward (that associated with the context in which the test was carried out) more than following pre-feeding with the 'different' reward, (associated with the other context) ($F(1, 25) = 27.9$, $p < 0.001$). During the 'different' condition, both groups of rats showed more responding on the correct than the incorrect lever ($F(1, 25) = 8.9$, $p < 0.01$, see Figure 5.7B and D). However, during the 'same' condition, in which rats were pre-fed with the reward associated with the test context, rats were less able to use context to disambiguate the correct response to the incongruent stimuli, as they no longer differentiated between the correct and the incorrect lever in their responding ($F < 1$; see Figure 5.7A and C).

5.4 Discussion

The experiments presented here tested the role of the retrosplenial cortex in a range of tasks known to be impaired by lesions of the prefrontal cortex, an area with which the retrosplenial cortex has strong connections (Kobayashi and Amaral, 2003; van Groen and Wyss, 2003). The first of these experiments was an attentional set-shifting task, which investigated reversal learning as well as intra- and extra-

dimensional set-shifting. No differences were found between the sham and lesion groups on any of these trial types.

Animals with lesions of the retrosplenial cortex were not impaired in their ability to acquire reversal learning during the attentional set-shifting task. This is contrary to previous evidence that retrosplenial cortex lesions impair reversal performance (Cain et al., 2006; Meunier and Destrade, 1997; Meunier et al., 1991), but as these experiments all used spatial tasks, it is possible that the deficit stems from an inability to accurately learn about or navigate between spatial locations, rather than from reversal learning *per se*. A previous test of reversal learning carried out in an operant box, and therefore minimising the demands of navigation, has shown no effect of retrosplenial cortex lesions (Neave et al., 1994). It seems likely from these results that the retrosplenial cortex is not involved in reversal learning itself, but is required for reversal learning within certain classes of spatial problem.

Retrosplenial cortex lesions had no effect on the number of trials taken to learn an intra-dimensional shift, nor on the number of mistakes made before reaching criterion. This is contrary to a previous experiment that tested rats with posterior cingulate lesions (analogous to the rostral retrosplenial cortex) on a version of the attentional set-shifting task, and found a selective deficit on intra-dimensional set-shifting (Ng et al., 2007). However, the precise location of the lesion in this study is unclear, as there appears to be a mismatch between the surgical coordinates used and the location of the posterior cingulate lesions given in the diagrams. Additionally, the paper did not look at the number of errors made to confirm the

deficit. Given the design of the experiment, with six correct approaches in a row required before reaching criterion, the placement of a single error early or late in a run of correct responses can have a large effect on the number of trials needed before criterion is reached. In order to confirm the null effect seen during the current study, two cohorts of rats were run on the task, with no differences seen between their performances.

Additionally, no effect of retrosplenial cortex lesions was seen on extra-dimensional set-shifting. This is unlike the specific deficit seen on the same task following medial prefrontal cortex (Birrell and Brown, 2000) or posterior parietal cortex lesions (Fox et al., 2003). Previous experiments in rats with retrosplenial cortex lesions have shown a consistent deficit in tasks that require rats to switch from one cue type to another, such as from navigating a radial-arm maze in the light to the dark (Chen et al., 1994b), changing from allocentric to directional cues in a T-maze (Pothuizen et al., 2008), or switching from intra-maze to extra-maze cues when performing a radial-arm maze task in the light (Pothuizen et al., 2008; Vann and Aggleton, 2005, 2004). It has been suggested that the retrosplenial cortex has a function in 'translating' between different spatial codes, e.g., allocentric representations into egocentric ones and *vice versa* (Burgess et al., 2001; Byrne et al., 2007; Vann et al., 2009). There is a related hypothesis that states that the retrosplenial cortex may be required for integrating different types of cues before they can be used in combination (Cooper et al., 2001; Vann et al., 2009). These deficits could be linked to a problem with attending to the correct, behaviourally appropriate, cues during these tasks. However, the lack of a deficit in extra-dimensional set-shifting implies

that animals without a retrosplenial cortex are still capable of covertly attending to seemingly irrelevant cues, and shifting their attention selectively to them when the task demands it. The previously observed deficits, therefore, may be specific to certain spatial situations, rather than due to an overall impairment in shifting attention between different cue types. Together, the findings from the set-shifting task would suggest that the retrosplenial cortex is not necessary for lower- or higher-order executive processes.

In order to test the involvement of the retrosplenial cortex in the highest-order executive processes, animals were tested on a version of the Stroop task, modified for suitability for rats (Haddon and Killcross, 2005, 2006a). Both groups of rats were able to learn the initial conditional discriminations in different contexts (Experiment 2a). When compound stimuli were introduced, sham animals used contextual cues to disambiguate the correct response during an 'incongruent' stimulus, each element of which directed the animal to give an opposing response. This is consistent with previous studies (Haddon and Killcross, 2005, 2006a). However, retrosplenial lesions impaired this ability, with the RScomb3 group showing no difference in responding between the contextually-appropriate and the contextually-inappropriate levers. This could reflect a deficit in selecting the correct response in the face of conflicting cues, a difficulty in forming associations with contextual cues, or a failure to learn about the contextual cues as they were irrelevant during the initial acquisition of the conditional discriminations. The latter explanation seems less likely given the lack of a deficit on the set-shifting task, showing that animals with retrosplenial lesions are able to attend to and learn about task-irrelevant

stimuli in the same way as their sham controls. Two further experiments were carried out to further explore the retrosplenial lesion deficit displayed on the Stroop task.

Experiment 2b (selective reward devaluation) demonstrated that both RScomb3 and Sham3 animals were able to reduce responding in a context-appropriate manner following pre-feeding with the reward associated with that context. As no stimuli were present during the extinction session, this reduction cannot be explained by the formation of a stimulus-reward association, and must be explained by a context-reward association. This demonstrates that, contrary to previous suggestions that the retrosplenial cortex is required for contextual discrimination (Bar and Aminoff, 2003; Robinson et al., 2012), animals with lesions of the retrosplenial cortex are able to use contextual information. If the deficit seen during incongruent Stroop trials is to do with contexts, it may be in how contextual information is used, rather than in the ability to use contextual information *per se*, or an inability to inhibit responding appropriately.

Experiment 2c (selective reward devaluation on contextual control of responding) further investigated the basis of the deficit on incongruent Stroop trials seen in Experiment 2a. Similarly to Experiment 2b, both groups again reduced their responding significantly in the context associated with the pre-fed reward. This again demonstrates that RScomb3 rats are able to form an association between contextual cues and their associated rewards. Additionally, when the context-inappropriate reward was pre-fed, RScomb3 animals were then able to make the

contextually-appropriate correct response on presentation of an 'incongruent' compound stimulus. That correct responding was restored when the influence of competing cues was reduced by pre-feeding suggests a role for the retrosplenial cortex in supressing alternative behavioural choices. When the reward associated with the test context was pre-fed neither group of animals were able to correctly disambiguate the cue conflict to press the correct lever.

The results of Experiment 2c can be interpreted in two ways. These results may show that the retrosplenial cortex is involved in the use of contextual cues to disambiguate competing cue types and their associated responses in a non-spatial environment. However, it is not clear that animals were in fact using contextual cues to make the contextually-appropriate response. It is possible that, instead, animals were responding to the expected reward outcome of the compound stimulus. One of the two elemental stimuli presented will elicit a response associated with an outcome that is devalued, while the other is associated with a response that has not been devalued by pre-feeding. Rats may have been choosing the response associated with the desired reward, rather than that associated with the context that they were being tested in. If this were the case, however, one might expect to see an increase in contextually-inappropriate responding following pre-feeding with the reward associated with the test context, a result that is not seen here for either the RScomb3 animals or their sham controls.

The results of these experiments suggest that the retrosplenial cortex is unlikely to have a necessary role in either the lower- or the higher-order processes governed by

the prefrontal cortex. However, the current data does suggest that the retrosplenial cortex may be involved in the highest-order levels of processing, associated with the anterior cingulate and dorsolateral prefrontal cortex (Kesner and Churchwell, 2011). This is consistent with the connectivity of the retrosplenial cortex, which has direct reciprocal links with the dorsolateral prefrontal cortex (Morris et al., 1999b) and a series of dense connections with the anterior cingulate cortex (Jones et al., 2005). Although the exact involvement of the retrosplenial cortex in executive function is not yet clear, the current results do indicate the possibility of a function in decision making in the presence of ambiguous cues, that merits further investigation.

Chapter 6

6. General discussion

The retrosplenial cortex is one of the largest cortical structures in the rat, yet there is still very little known about how it contributes to aspects of cognition. The present body of work sought to provide a greater understanding of the role of the retrosplenial cortex by testing two current theories of retrosplenial function: translation and integration (Burgess et al., 2001; Byrne et al., 2007; Vann et al., 2009). In addition, the retrosplenial cortex has been principally linked to spatial memory (Cain et al., 2006; Cooper et al., 2001; Vann et al., 2003); in this thesis, I have looked at how the retrosplenial cortex may also contribute to non-spatial aspects of cognition such as object recognition and executive functions such as disambiguating conflicting cues.

6.1 Summary of findings

A summary of the findings in this thesis can be seen at Table 6.1 While many experiments have attempted to define the role of the retrosplenial cortex in spatial navigation, these studies have typically been unable to distinguish between the translation and integration theories of retrosplenial function (Burgess et al., 2001; Byrne et al., 2007; Vann et al., 2009). This is because a task that requires an animal

6.1 – Summary of findings

Task	Chapter	RScomb	RSdysg	Notes
Go/no-go perspective task	2	Impaired	Unimpaired	RSdysg - altered use of distal visual cues during 'dark' probe.
Go/no-go location task	2	Impaired	Impaired	
Go/no-go medium task	2	Unimpaired	Unimpaired	
Cross-modal object recognition	3	Impaired (lower scores on all trials)	Impaired on dark-light trials	RScomb – not above chance on dark-light or light-light trials.
Visual-only object recognition	3	Unimpaired	Unimpaired	
Object in place	3	Impaired	--	
Conditioned inhibition	4	Unimpaired	--	
Negative patterning	4	Unimpaired	--	
Latent structural learning	4	Impaired	--	Sham2 - no preference for correct corner on second probe. May not use a structural strategy.
Set-shifting	5	Unimpaired	--	
Stroop - acquisition	5	Impaired on incongruent trials	--	
Stroop – selective reward devaluation	5	Unimpaired	--	
Stroop – selective reward devaluation on contextual control of responding	5	Unimpaired	--	

to translate between different sources of spatial information will also require that animal to have integrated the information from the two representations, in order to make the switch between them appropriately. The observation that rats with retrosplenial cortex lesions are impaired in comparison to rats with sham lesions on tasks in which spatial cues are restricted (Chen et al., 1994b; Cooper and Mizumori, 1999; Pothuizen et al., 2008) could result from a deficit in either of these processes. Traditional spatial navigation tasks are also unable to distinguish between a deficit resulting from impaired learning about the layout of the environment, and an impaired ability to use that knowledge to navigate. To separate these different elements of spatial learning, Chapter 2 of this thesis examined the role of the retrosplenial cortex in a novel spatial learning task that removed the demands of navigation. The task did not require rats to integrate spatial information with self-motion cues, as many navigational tasks do, and focussed on the ability of the rat to learn about its location from visual information about its surroundings.

In the initial experiment, testing discrimination of two viewpoints of the same room, rats with combined lesions involving both areas 29 and 30 were significantly impaired. Those with lesions affecting only the dysgranular retrosplenial cortex (area 30) were not impaired (although procedural differences mean that they may have been able to use non-visual cues to solve the task. This is discussed in more detail in chapter 2). However, changes were seen in the extent to which the rats relied upon visual cues, with testing in the dark having only a mild effect on performance. In the version of the task testing location discrimination, both dysgranular and combined retrosplenial lesioned animals showed poorer

performance than their respective sham groups. These go/no-go experiments demonstrate a role for the retrosplenial cortex in spatial learning in the absence of navigational demands. The results do not rule out a further role for the retrosplenial cortex in navigation, but do show that the retrosplenial cortex is required for the efficient extraction of spatial information from distal visual cues.

Chapter 3 of this thesis further tested the role of the retrosplenial cortex in processing visual information, this time in the non-spatial context of object recognition. The availability of visual and tactile cues was carefully controlled throughout, to allow recognition of an object based on visual information to be distinguished from recognition using tactile or olfactory cues. Additionally, the role of the retrosplenial cortex in translating or integrating cues in a non-spatial context was tested. Although this experiment was unable to distinguish between the two processes, a specific deficit was found in visual-tactile cross-modal recognition in the dysgranular lesion cohort. A deficit was not seen on either visual-only or tactile-only recognition, indicating that the impairment must be related to the process of switching between cue modalities, or integrating them into a single representation.

The results seen from the complete retrosplenial cortex lesioned rats were less clear than for the dysgranular lesioned rats. The “combined” retrosplenial cohort showed poorer recognition than their sham group, regardless of whether a switch in modality was required or not. However, when tested on visual-only recognition with barriers removed, so that exploration times were increased, all groups of rats performed at above chance, showing that the retrosplenial cortex is not required for

recognising objects visually. When taken in combination with the go/no-go experiment results, this demonstrates that the visual processing deficit is not global, and is either restricted to the extraction of spatial information from visual cues, or to processing distal visual cues as opposed to proximal cues.

While the role of the granular retrosplenial cortex in cross-modal processing is not clear from these experiments, the dysgranular retrosplenial cortex is clearly involved in the integration or translation of cues between modalities in a non-spatial domain. Further tests, preferably with a greater number of animals to reduce the variation inherent in tasks that rely on spontaneous behaviour, would be required to evaluate the exact nature of the contribution of each sub-region to the deficit in visual and cross-modal processing.

As the first two experimental chapters of this thesis were unable to distinguish between the roles of the retrosplenial cortex in translation and integration, Chapter 4 specifically tested integration in controlled environments where translation would not be required. Rats with retrosplenial cortex lesions were able to carry out simple stimulus-reward associations and to integrate different cues in more complex situations such as the negative patterning task. In a latent structural learning task in the water maze, thought to test skills essential for visual navigation, retrosplenial lesioned rats showed a severe deficit, consistent with the deficit seen in the location task in Chapter 2. The spatial configuration of two walls signalled the location of the escape platform during training, but probe testing was unable to confirm that the sham group were in fact using a structural method of solving the task. Although

strong conclusions cannot be drawn from the evidence presented in this chapter, due to the very close to significant result found in the negative patterning experiment and the inability to confirm that a structural strategy was used in the water maze, the results do indicate a possible role for the retrosplenial cortex in configural, specifically structural, learning. These deficits in solving tasks that rely on the integration of different cue types do not rule out an additional role for the retrosplenial cortex in translating between different representations.

Finally, Chapter 5 investigated the effect of retrosplenial cortex lesions on executive functions typically associated with the prefrontal and anterior cingulate cortices, due to the connections between these areas. Roles carried out by the prefrontal and anterior cingulate cortices include detecting and resolving response conflict (de Wit et al., 2006; Haddon and Killcross, 2005, 2011, 2006b; MacDonald et al., 2000; Marquis et al., 2007; Pardo et al., 1990) and selective attention to behaviourally relevant cues (Birrell and Brown, 2000; MacDonald et al., 2000). It is possible that the retrosplenial cortex may also contribute to these functions. To investigate this, rats with excitotoxic retrosplenial cortex lesions were tested on two tasks that taxed a range of processes known to be susceptible to prefrontal cortex damage, across the hierarchy of prefrontal function. An attentional set-shifting task tested reversal learning, intra- and extra-dimensional set-shifting, while a rodent version of the Stroop task investigated the role of the retrosplenial cortex in cognitive control in response to ambiguous cues. Results showed that retrosplenial cortex lesions caused no deficit on either reversal learning or set-shifting. This suggests that the deficit in reversal learning seen after retrosplenial cortex lesions in spatial tasks (Cain

et al., 2006; Meunier and Destrade, 1997; Meunier et al., 1991) may be the result of a deficit in learning about the spatial environment rather than reversal learning itself. This is consistent with both the results of the go/no-go location experiment, where retrosplenial lesions caused an inability to discriminate spatial locations based on overlapping cues, and with a task showing normal reversal performance in an operant box, where spatial demands are minimised (Neave et al., 1994). The retrosplenial cortex lesioned animals showed no deficits in either intra- or extra-dimensional set-shifting, showing that they are able to attend to cues that are seemingly behaviourally irrelevant, and to learn about them.

During the Stroop task, the retrosplenial cortex lesions did not impair learning about the conditional discriminations in each context. However, they did bring about an impairment on the 'incongruent' trials of the Stroop task, once the compound stimuli were introduced. Retrosplenial cortex lesions prevented the rats from responding in a contextually appropriate way to the compound stimuli. Further tests showed that following pre-feeding, animals were able to selectively reduce their responding in the context where the pre-fed reward was given. This occurred in the period before rewards were provided, even in the absence of stimuli. It therefore cannot be due to stimulus-reward associations, or to a reaction to the reward given, and so must be due to context-stimulus associations. The retrosplenial cortex is, therefore, not required for learning about contextual cues. When the rats were tested with compound stimuli following pre-feeding, the retrosplenial lesioned animals were able to respond correctly to incongruent compound stimuli. The reduction in response conflict caused by the pre-feeding appears to allow the

retrosplenial cortex lesioned animals to use contextual cues to disambiguate the incongruent cues, possibly indicating a role for the retrosplenial cortex in decision making in the presence of ambiguous cues. These results suggest that, despite the dense connections with the prefrontal cortex and anterior thalamic nuclei, the retrosplenial cortex is involved only at the highest order levels of cognitive processing. The deficits seen in the Stroop task may be related to the deficits seen in the location discrimination go/no-go experiment, where determining location requires the rat to disambiguate a series of overlapping spatial cues.

6.2 Limitations and future experiments

Due to issues with task development and restrictions of time, there are some limitations to the studies presented within this body of work. One concern is that, in the location and viewpoint tasks carried out using digging cups, the experimental methodology was refined between testing the two cohorts. This led to differences that make it impossible to draw direct comparisons between the two lesion groups. In the viewpoint experiment, these changes meant that the RScomb and Sham2 cohort learnt the task in about half of the time taken by the RSdysg and Sham1 group. This slower acquisition may have given the RSdysg group time to develop alternative strategies to solve the task. White noise was played throughout acquisition of the viewpoint task for the RScomb and Sham2 group, but not for the RSdysg and Sham1 cohort. This may have allowed RSdysg and Sham1 rats to include directional auditory information in their initial acquisition of the task. From the results of the location task, it seems likely that the dysgranular retrosplenial cortex is

required for the normal processing of distal visual cues for spatial information. The presence of directional sound cues in the viewpoint experiment may have masked a potential deficit by providing rats with a non-visual method of solving the task.

Further differences were present during the probe trials. The RScomb and Sham2 cohort were given a day of reminder training on the original task set-up between each probe trial, whereas for the RSdysg and Sham1 cohort each probe followed immediately after the previous one. This may have resulted in RSdysg and Sham1 animals showing decreased performance on later probes - if the probes are consistently more difficult than the acquisition trials, and the rats are not reminded of the correct digging rule, they may give up due to extinction and stop discriminating between go and no-go trials. Rats that are given the reminder trials in between probes will be less likely to stop using the original rule, and the probe trials that they do will therefore be more reflective of their default method of solving the task.

Despite the experimental differences, however, the task can still advance our understanding of the involvement of the retrosplenial cortex in spatial processing, particularly when each cohort is compared to its sham group. The rat retrosplenial cortex appears to be involved in the processing of distal visuospatial information to solve tasks that involve the resolution of multiple viewpoints from the same location. The exact contributions of each sub-region to these tasks cannot be determined, although they do seem to work in conjunction to some extent, as the

deficits seen after a selective dysgranular lesion are different to those seen following a combined granular and dysgranular retrosplenial lesion.

In the cross-modal object recognition task, exploration levels are much lower in the light when barriers are present, making it very difficult for rats to objects in the light-light condition. That rats with both combined retrosplenial lesions and those with selective dysgranular lesions are able to carry out visual-only object recognition is evident from the control experiment, where no barriers were in place. However, this was not demonstrated for the combined lesions in the cross-modal task when barriers are present, leading to uncertainty as to whether the deficits seen with this group are due to low exploration levels when barriers are there, or to a decreased ability to objects visually.

In a number of experiments there are potential order effects. In an ideal situation, order of testing would be counterbalanced, however due to time limitations this is not possible, and it is arguably not ethical to increase numbers of animals for testing in this manner. In particular, in the two versions of the go/no-go task with the digging cups, the shams could have potentially learnt a schema in the first task. Order effects are also possible in the operant box experiments, conditioned inhibition and negative patterning. Rats may have learnt a schema in the conditioned inhibition experiment that assisted learning in the negative patterning task; ideally, the order of the two experiments would have been counterbalanced.

Several experiments presented here, such as the go/no-go location task and the latent structural learning experiment, suggest that the retrosplenial cortex may be involved in using visual cues to determine the animal's current location. However, it was not possible to determine definitively that retrosplenial cortex lesions disrupt structural learning, as the sham animals may not have been using a structural learning method of solving the task in the water maze. An alternative method of testing this problem could be carried out, using visual cues in an operant box. This would remove the requirement for navigating during the probe trial, and would not permit the development of alternative strategies for solving the task.

The lack of a granular retrosplenial cortex lesion group presents further difficulties in determining the functions of the retrosplenial cortex sub-regions. Although some inferences can be drawn from comparing the performances of the combined and the dysgranular lesion groups, testing rats with granular sub-region lesions on the same tasks and with the same experimental methodologies would give a clearer picture of the way the areas interact. This would be of particular interest in the cross-modal object recognition task. In spatial learning experiments the roles played by the dysgranular and granular sub-regions appear to vary depending on whether tasks are run in the light or the dark (Pothuizen et al., 2009), so the roles in the cross-modal object recognition task may change depending on light condition.

To further understand the roles of the retrosplenial cortex sub-regions in these tasks, the activity of immediate early genes such as *c-fos* could be investigated. *c-fos* is expressed in neurones that have recently been active (Dragunow and Faull, 1989;

Sagar et al., 1988), and so can be used to indicate which brain areas were involved in the previous task. Staining for *c-fos* could be used either on tasks where the retrosplenial cortex in known to be involved, in rats without lesions, or using rats with retrosplenial cortex or sub-region lesions to see how activity changes in connected areas of the brain.

The studies presented in this thesis suggest that the retrosplenial cortex is involved in executive function typically associated with the prefrontal cortex. Crossed lesion studies could be used to further examine the interaction between the prefrontal and retrosplenial cortices. Crossed lesions would remove the retrosplenial cortex on one hemisphere and the prefrontal cortex on the other. Any tasks that required only the retrosplenial cortex or the prefrontal cortex would be likely to be spared, as one hemisphere of each of these areas would remain intact. However, any tasks that depended on the interaction of the two areas would be affected.

One problem with using lesions to investigate the retrosplenial cortex is that, in the time between surgery and experiments, the rat may have developed compensatory methods of carrying out the tasks. This is particularly likely in experiments that take place several months after surgery. One way of reducing the opportunity for rats to develop alternative strategies is to use temporary inactivation to disable to retrosplenial cortex just for the duration of an experiment. This could be used during the set-shifting task, where no deficit was found in this thesis. This would allow verification that the lack of a deficit, which is surprising given the overall

pattern of results presented in this thesis, is the correct result and does not arise from compensation.

The results presented here are consistent with findings from humans with retrosplenial cortex lesions. Patients, particularly those with lesions of the right retrosplenial cortex, suffer from topographic disorientation and problems with navigation. This includes patients who report being unable to orient themselves using landmarks so as to know which direction they should travel in (Maguire, 2001; Osawa et al., 2008; Takahashi et al., 1997). This is consistent with the results seen in the go/no-go tasks. To further investigate the similarities between human and rat retrosplenial cortical functions, ideally one would want to test patients with retrosplenial cortex lesions on a similar task, involving discrimination of both perspective and location. However, there are very few patients with retrosplenial cortex lesions, and even fewer where the damage is selective. A possible alternative would be to use fMRI scanning to see when the retrosplenial cortex is activated in such a task in humans.

Experiments testing the involvement of the retrosplenial cortex in cross-modal object recognition and cross-modal processing more generally could also be carried out in patients with retrosplenial cortex lesions. This is not an area of retrosplenial function that has been tested in humans before. Previous experiments using fMRI have however demonstrated a role for the retrosplenial cortex in analysing the contextual associations of an object, but not for recognising the object itself (Fenske et al., 2006), unless that object is personally familiar (Sugiura et al., 2005).

6.3 Conclusions

In conclusion, the results shown in this thesis demonstrate that the involvement of the retrosplenial cortex extends beyond spatial tasks into executive functions and cross-modal processing. Although the precise contribution to spatial processing is not yet clear, the retrosplenial cortex is evidently required for visually determining location in an environment in the absence of self-generated navigational cues. This has been demonstrated both by the go/no-go experiments and by the latent structural learning task. Further evidence has been presented for the differing roles of the retrosplenial sub-regions, which appear to work in conjunction with each other to combine information received from different sensory modalities. However, further work is required to fully understand the ways in which these areas work together and with other areas of the brain, and the implications that dysfunction in this area has for human cognition.

Annex A – Experimental history

Cohort	Experiment	Chapter	Date
Cohort D (n = 8 lesion, 10 sham)	Surgery		Sep-10
	Radial arm maze	Not included in thesis	Oct-10
	Cross modal bow tie maze	Not included in thesis	Dec-10
	Working memory water maze	Not included in thesis	Jan-11
	Biconditional operant box	Not included in thesis	Mar-11
	Object recognition	Not included in thesis	Mar-11
	Cross modal object recognition	3	Apr-11
	Visual only object recognition	3	May-11
	Biconditional water maze	Not included in thesis	Jun-11
	Perspective discrimination	2	Aug-11
	Medium discrimination	2	Oct-11
	Viewpoint discrimination	2	Oct-11
Cohort 1 (n = 9 lesion, 12 sham)	Surgery		Nov-11
	Object in place	Not included in thesis	Dec-11
	Perspective discrimination	2	Jan-12
	Medium discrimination	2	Mar-12
	Viewpoint discrimination	2	Jul-12
	Set shifting	5	Aug-12
Cohort 2 (n = 13 lesion, 12 sham)	Surgery		Nov-12
	Set shifting	5	Nov-12
	Object in place	3	Mar-13
	Negative patterning	4	Mar-13
	Conditioned Inhibition	4	Apr-13
	Cross modal object recognition	3	May-13
	Latent structural learning	4	Jun-13
	Visual only object recognition	3	Jul-13
Cohort 3 (n = 16 lesion, 12 sham)	Surgery		Aug-12
	Stroop	5	Sept-12

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