Published in final edited form as:

J Comp Neurol. 2008 November 10; 511(2): 257–270. doi:10.1002/cne.21821.

Rhinal and Dorsolateral Prefrontal Cortex Lesions Produce Selective Impairments in Object and Spatial Learning and Memory in Canines

Lori-Ann Christie¹, Richard C. Saunders², Danuta, M. Kowalska³, William A. MacKay⁴, Elizabeth Head¹, Carl W. Cotman¹, and Norton W. Milgram⁵

¹Institute for Brain Aging & Dementia, University of California Irvine, Irvine, CA, USA ²Laboratory of Neuropsychology, NIMH/NIH, Bethesda, MD, USA ³Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland ⁴Department of Physiology, University of Toronto, Toronto, ON, Canada ⁵Division of Life Sciences, University of Toronto at Scarborough, Scarborough, ON, Canada

Abstract

To examine the effects of rhinal and dorsolateral prefrontal cortex lesions on object and spatial recognition memory in canines, we used a protocol in which both an object (delayed non-matching to sample, or DNMS) and a spatial (delayed non-matching to position or DNMP) recognition task were administered daily. The tasks used similar procedures such that only the type of stimulus information to be remembered differed. Rhinal cortex (RC) lesions produced a selective deficit on the DNMS task, both in retention of the task rules at short delays and in object recognition memory. By contrast, performance on the DNMP task remained intact at both short and long delay intervals in RC animals. Subjects who received dorsolateral prefrontal cortex (dlPFC) lesions were impaired on a spatial task at a short, 5-sec delay, suggesting disrupted retention of the general task rules, however, this impairment was transient; long-term spatial memory performance was unaffected in dlPFC subjects. The present results provide support for the involvement of the RC in object, but not visuospatial, processing and recognition memory, whereas the dlPFC appears to mediate retention of a non-matching rule. These findings support theories of functional specialization within the medial temporal lobe and frontal cortex and suggest that rhinal and dorsolateral prefrontal cortices in canines are functionally similar to analogous regions in other mammals.

Keywords

object recognition; spatial; medial temporal lobe; entorhinal cortex; perirhinal cortex; dorsolateral prefrontal cortex; frontal lobe; canine; delayed non-matching to sample; delayed non-matching to position

Introduction

The present study was conducted to determine the roles of the rhinal cortex (RC) and dorsolateral prefrontal cortex (dlPFC) in object recognition and visuospatial function in canines. Canines represent an important animal model of human aging and cognitive dysfunction as they emulate many features of mild cognitive impairment and early Alzheimer's disease (AD). Canines express the same 1 to 42 amino acid sequence beta-amyloid peptide as humans (Johnstone et al., 1991; Selkoe et al., 1987), and naturally exhibit diffuse beta-amyloid plaque pathology in analogous brain regions with age (Cummings et al., 1996c; Head et al., 2000). As in humans, the accumulation of beta-amyloid in canines is linked to age-related cognitive dysfunction (Cummings et al., 1996a; Cummings et al., 1996b; Head et al., 1998). In addition, dogs show age-related cortical atrophy, ventricular enlargement (Su et al., 1998; Tapp et al., 2004) and reduced neurogenesis (Siwak-Tapp et al., 2007), which are also associated with cognitive impairment. Thus, canines are appropriate models of AD with advantages over nonhuman primates, as monkeys predominantly express the shorter form of the beta-amyloid peptide (Gearing et al., 1996), and the plaques they develop do not reliably predict cognitive dysfunction (Sloane et al., 1997).

Previous studies show that age-related cognitive deficits in dogs can be ameliorated by pharmacological interventions (Milgram et al., 2000). Further, made possible by their relatively long life span, dogs have been used in longitudinal studies examining the effects of antioxidant dietary supplementation and environmental enrichment, which both improve cognition in aged animals (Milgram et al., 2004; Milgram et al., 2005) and reduce age-related pathology, such as cell loss (Siwak-Tapp et al., 2008). Thus, dogs can be used to study aging and AD, as well as the effects of possible therapeutics and interventions to slow or prevent cognitive decline.

Previous studies report that beta-amyloid plaques accumulate in both the rhinal (Head et al., 2000) and prefrontal cortices of aged beagles (Head et al., 2000; Satou et al., 1997), and that cortical atrophy occurs in these regions as well (Tapp et al., 2004). These brain changes are associated with deficits on several object- and spatial-based tasks (Cummings et al., 1996a; Head et al., 1998; Tapp et al., 2004), however these findings are largely correlative. In order to understand the basis of age-related cognitive decline in dogs and determine if parallels in structure and function exist with humans and nonhuman primates, we conducted the present lesion study to assess the involvement of the rhinal (RC) and dorsolateral prefrontal (dlPFC) cortices in object recognition and visuospatial memory.

In humans (Buffalo et al., 1998; Holdstock et al., 2000; Lee et al., 2005) and non-human primates (Baxter and Murray, 2001; Gaffan, 1994; Malkova et al., 2001; Murray and Mishkin, 1986), damage to the RC, consisting of the entorhinal and perirhinal cortices (Murray and Mishkin, 1986), disrupts memory for object or form information. Therefore, we hypothesized that damage to the RC would disrupt object recognition memory. By contrast, damage to the dlPFC is often associated with spatial working memory deficits in nonhuman primates (Kowalska et al., 1991; Meunier et al., 1997; Oscar-Berman, 1978). Further, human neuroimaging studies report that the dlPFC is engaged during maintenance of spatial

information in working memory (Courtney et al., 1996; Kohler et al., 1995; Owen et al., 1996). Thus, we hypothesized that damage to the PFC would disrupt visuospatial memory. More recent evidence from monkey (Rainer et al., 1998; Rao et al., 1997) and human (Nystrom et al., 2000; Postle et al., 2000; Sala and Courtney, 2007) studies suggests that the PFC, including the dlPFC is important for integrating spatial and object information during working memory maintenance, however the precise organization and contributions of the PFC's subregions are still debated.

In the present study, we used a protocol in which two tasks differing only in the type of stimulus information to be remembered were administered pre- and post-surgery. Our protocol consisted of two delayed non-matching tasks, a delayed non-matching to sample (DNMS) task to assess object recognition and a delayed non-matching to position (DNMP) task to assess spatial memory. Both tasks were conducted in the same apparatus, required the same behavioral responses from the animals, used the same food reward for accurate performance, and the same progressively increasing delay sequence. Further, both tasks were administered daily, one in the morning and one in the afternoon, which avoided any post-surgery recovery time effects on performance. Testing in this manner on these two tasks allowed us to attribute any dissociation in lesion effects to the type of information being processed and remembered.

We report a dissociation in the RC's involvement in processing object versus spatial information; dogs with RC lesions were impaired only on a DNMS task at both short and long delays. By contrast, we found that RC lesions did not disrupt retention of or performance at long delays on a spatial task, suggesting that the RC processes object, but not spatial, information. DIPFC lesions disrupted retention of a DNMP task, but with continued training, the animals eventually performed at levels comparable to their pre-surgery performance.

Materials and Methods

Subjects

The eleven beagles (*Canis familiaris*) used in the present study (nine males and two females) were between four and six years of age. They were housed individually with continuous access to water, maintained on a 12:12-hour light/dark cycle and exercised daily. They were fed Lab Canine Chow #5006 (Agribrands Purina Canada, Inc., Woodstock, ON, Canada) once daily upon completion of cognitive testing. The care and use of the animals complied with Canadian regulations and with guidelines prepared by the University of Toronto's Animal Care Committee.

Experimental Design

Prior to the start of the present study, all dogs underwent a standard training protocol to familiarize them with the testing apparatus and procedures, consisting of reward and object approach learning, object discrimination learning, and discrimination reversal learning (for details, see Milgram et al., 1994). The subjects were also successfully pre-trained on a DNMS task (Callahan et al., 2000), and a DNMP task (Chan et al., 2002).

These 11 subjects were then tested on the DNMP task in the morning and DNMS task in the afternoon, with sessions separated by a minimum of three hours, to obtain both relearning scores and indices of maximal working memory. Following completion of baseline testing, four dogs (RC) received bilateral aspiration lesions of the rhinal cortex, three dogs (dlPFC) received bilateral aspiration lesions of the dorsolateral prefrontal cortex, and the remaining four canines served as unoperated controls (CON). Following a 14-day post-surgical recovery period, RC, dlPFC and CON dogs were again tested on the DNMS and DNMP tasks.

Behavioral Procedures

Apparatus and materials—For both the DNMS and DNMP tasks, a canine adaptation of the Wisconsin General Testing Apparatus was used, as described previously (Milgram et al., 1994). Briefly, the testing chamber was equipped with a sliding Plexiglas stimulus tray with one center and two lateral food wells. A screen with a one-way mirror visually separated the experimenter from the dog. During inter-trial intervals (ITI's) and delay intervals, the stimulus tray was removed out of the animal's sight by sliding it through a hinged door at the bottom of the screen.

Approximately 1 g of wet dog food (Hill's® Prescription Diet® p/d®; Hill's Science and Pet Technology, Topeka, KS, USA) was used as the food reward. In order to mask the presence of the food reward in the food wells, the undersides of all stimuli were baited with large amounts of the same food such that, while able to smell it, the animals could not see or eat it.

Delayed non-matching-to-sample—DNMS testing was conducted to assess retention of the task rules and maximal object recognition memory performance. 101 small 3-D objects were used as stimuli for the DNMS task. Each trial started with the presentation of a single sample object covering a food reward over the center well. After the animal displaced the sample object and retrieved the food reward, the tray was withdrawn out of sight and a delay interval began. At the end of the delay, the sample object and a novel object were placed over the two lateral food wells and presented to the subject. Displacing the non-match, or novel object uncovered a food reward, displacing the sample object was not rewarded. The tray was then removed out of sight for an inter-trial interval (ITI) of 60 sec before the next sample was presented. Presentations of the non-match object over the left and right food wells occurred an equal number of times in a pseudorandom sequence. Subjects were presented with 24 objects per session over 12 trials such that no object appeared more than once per session. Thus, a given object could reappear during the following day's session, or as many as 5 sessions later.

The subjects were initially retrained with delay of 5 sec between the sample and test presentations for a maximum of 50 sessions (or 600 trials). If a subject made a minimum of 11 out of 12 correct responses over one session, or 20/24 over two sessions, or 29/36 over three sessions, followed by three 'stabilization' days in which performance remained above 70% correct, the delay was progressively increased to 10, and then to 20, 30, 50, 70, 110, 150, 180, 210, 240 and 270 sec over 50 test sessions. To pass from one delay to the next, the

subject had to meet the same performance criteria described above. The longest delay the animals successfully attained criterion performance within the 50 test sessions was taken as their maximal memory score.

Delayed non-matching-to-position—DNMP testing was conducted to assess retention of the task rules and maximal spatial recognition memory performance. The stimuli used for the DNMP task were two identical red blocks. Each DNMP trial, like the DNMS, consisted of both a sample and test phase. For each trial, one block was placed over the left, center, or right food well in a pseudorandom sequence. After the subjects displaced the sample block and retrieved the reward, the stimulus tray was withdrawn out of sight for the delay. Subjects were then presented with one block at the sample location along with the identical second block covering one of the other two food wells. To obtain the food reward, the subjects had to avoid approaching the location of the sample block and displace the block covering the non-matching spatial location. The tray was then removed out of sight for an inter-trial interval (ITI) of 60 sec before the next sample was presented. 12 trials per day were administered.

The subjects were tested for a maximum of 50 sessions on the DNMP task beginning with a delay of 5 sec. Maximal memory scores were determined using the same criteria and increasing delays as described above for the DNMS task.

Surgery

Following completion of pre-surgery cognitive testing, four subjects received lesions of the RC and three subjects received lesions of the dIPFC. All lesions were bilateral and made by aspiration of the cortical tissue.

On the day of surgery, subjects were administered an acepromazine (.03 to 0.25 mg/kg, i.m.) and atropine (.02 to .05 mg/kg, i.m.) mixture. Surgical anaesthesia was then maintained with 1 to 2% isoflurane, as required. All subjects were given isotonic fluids (i.v.) throughout the surgery and RC2, RC3 and RC4 received mannitol (.25g/kg i.v., over a 2 hr period) to reduce brain volume. Heart rate, blood pressure, blood oxygenation levels and body temperature were monitored during anaesthesia and recovery. Surgeries were conducted in aseptic conditions according to standard sterile surgical procedures.

Each subject was secured in a head holder for stability, the scalp was disinfected, a midline incision made, and the connective tissue and temporal muscles were gently retracted in anatomical layers. For both RC and dIPFC ablations, the tissue at the sites was removed by aspiration with the aid of a surgical microscope, with the major sulci and gyri serving as references to guide the lesion placements.

RC lesions were intended to include removal of both the entorhinal and perirhinal cortices (Murray and Mishkin, 1986), as shown in Figure 1. The entorhinal was defined as the posterior extent of the piriform cortex, with the anterior border at the intersection of the prepiriform cortex and cortical amygdaloid nucleus and the posterior border at the retrosplenial sulcus. Laterally, the intended entorhinal lesions extended from the medial

bank of the rhinal sulcus, and medially to the parasubicular region of the hippocampus (Kowalska et al., 2001; Kreiner, 1968).

The entorhinal cortex is characterized by a bilaminar, 6-layered cell arrangement, with lateral and medial areas that differ in their cell arrangement (Kowalska et al., 2001). The lateral entorhinal cortex consists of a prominent layer II with large, darkly stained stellate neurons and well-developed layers III, V and VI. Layer IV contains few cells. The medial entorhinal cortex is characterized by broad, well-defined layers. Layer II consists of pale, continuous sheets of neurons, layer III has medium-sized lightly-stained neurons, and layers V and VI contain more densely packed cells than the lateral entorhinal cortex (Kowalska et al., 2001; Woznicka et al., 2006). At its anterior extent, the entorhinal cortex borders the prepiriform cortex and the cortical amygdaloid nucleus. The main distinguishing feature at this border is a gradual transition from the 6-layered entorhinal cortex to the 3-layered prepiriform cortex (Kowalska et al., 2001; Woznicka et al., 2006). Medially, the entorhinal cortex meets the parasubicular region of the hippocampus, and a disappearance of layer II in the parasubiculum is the main cytoarchitectonic feature that differentiates these two regions (Kowalska et al., 2001; Woznicka et al., 2006). The posterior extent of the entorhinal cortex is bordered by the retrosplenial sulcus.

The perirhinal cortex was defined as the cortical tissue along the lateral bank of the posterior rhinal sulcus, with the anterior extent of the target lesion site just caudal to the Sylvian sulcus and the posterior border at the caudal limit of the posterior rhinal sulcus (Kowalska et al., 2001; Kreiner, 1968; Woznicka and Kosmal, 2003). The perirhinal cortex consists of Brodmann's areas 35 and 36 (Woznicka and Kosmal, 2003). Area 35 consists of a broad layer I with an increasing number of small, densely packed neurons in layer II. Layer III is narrow and consists of round, medium size, lightly stained cells that blend with the cells of layer V. Layer V's cells are larger than layer III and are darkly stained in Nissl sections. The number of these cells gradually increases in the lateral part of the fundus and bank of the posterior rhinal sulcus (Kowalska et al., 2001; Woznicka and Kosmal, 2003). Area 35 borders the lateral entorhinal cortex, and this transition is marked by a disappearance of fibers in the lamina dissecans (layer IV).

Area 36 is a continuation of area 35 in the lateral bank of the posterior rhinal cortex. It is poorly-developed in dogs and its border with area 35 is unclear in Nissl stained sections, however, it can be distinguished by a gradual increase in the total thickness of its cortex due to an increased number of neurons in layers III and V (Kowalska et al., 2001). Area 36's lateral border with the posterior composite gyrus of the temporal cortex is also difficult to distinguish based on cytoarchitectonic features. Again, the total thickness of the cortex and cell number in layers III and V gradually increases further into the temporal cortex, and the radial arrangement of cellular columns is more distinct (Kowalska et al., 2001).

Our intended dIPFC lesion site (Figure 2) included the entire preoreal gyrus, extending from the posterior end of the presylvian sulcus to the frontal pole (Lawicka et al., 1966; Rosenkilde, 1978; Stasiak and Lawicka, 1990). The preoreal gyrus contains a thin, relatively dense layer II, and layer III consists of moderately sized neurons (Tanaka, 1987). Of the six major cytoarchitectonic subregions of the canine prefrontal cortex, the preoreal gyrus is the

only area in which a distinct layer IV can be identified (Tanaka, 1987; Kreiner, 1961), which consists of small, densely-arranged granular cells with groups of medium-size neurons interspersed amongst them at regular intervals (Tanaka, 1987). Layer V merges gradually with layer VI, and consists of small and medium pyramidal cells, while layer VI is thin and contains small cells in short columns or palisades. In the preoreal gyrus, layer VI's border with the underlying white matter is easily distinguished (Tanaka, 1987).

Our anterior border was the frontal pole, or polar region of the prefrontal cortex. The polar region, by contrast with the preoreal gyrus, does not have a distinct layer IV and, overall, its cortex is relatively thin (Tanaka, 1987). The lateral border of the intended dlPFC site was the orbital gyrus. The cortex of the orbital gyrus is thicker than the preoreal gyrus, and contains a poorly defined layer IV with scattered clusters of small cells. In addition, the orbital gyrus differs from the preoreal gyrus in that all of its cells are more widely spaced with individual layers that are more difficult to distinguish (Tanaka, 1987). On the medial surface, the preoreal gyrus is bordered by the pregenual gyrus, which is distinguished by its dense accumulation of neurons in layer II and VI, widely spaced, medium sized neurons in layer III, and densely packed neurons and pyramidal cells of layer V. Layer IV is undetectable in the pregenual gyrus (Tanaka, 1987).

We also intended to damage the dorsoanterior aspect of the paraorbital gyrus, which is thought to be a transitional zone between the dlPFC and ventromedial prefrontal cortex based on its connectivity and cytoarchitecture (Kosmal, 1981; Markow-Rajkowska and Kosmal, 1987; Rajkowska and Kosmal, 1988). The paraorbital gyrus has a thin overall width, and no detectable layer IV. Layers II to IV of the paraorbital gyrus are difficult to distinguish and layer VI is composed of large numbers of fusiform cells (Tanaka, 1987).

Buprenorphine hydrochloride (.02 mg/kg, sub. cut. and i.m.) was administered as an analgesic and enrofloxacin (2.5 mg/kg i.m.) as an antibiotic following surgery. All subjects were given a 14-day recovery period before resuming behavioral testing and daily exercise.

Data analysis

All analyses were conducted using SPSS 11 for Mac OS X (v. 11.0.2, © SPSS Inc., Chicago, Illinois, USA). Levene's test and the Shapiro-Wilk statistic demonstrated that the assumptions of homogeneity of variance and normality required for ANOVA were not met. Therefore, to assess overall group effects, non-parametric statistical procedures were employed with two-tailed p-values less than or equal to 0.05 designated as statistically significant.

If an overall significant effect was found between the groups, two post hoc Mann-Whitney U tests were conducted to compare the RC group with the CON group and the dlPFC group with the CON group. In order to adjust for inflation of Type I error due to multiple comparisons, p-values less than or equal to a Bonferroni adjusted p-value of 0.025 were designated as statistically significant for these post hoc tests.

Pre-surgery analyses—In order to ensure that rate of relearning did not differ between CON, RC and dlPFC subjects prior to the lesion surgeries, errors to criterion at the 5-sec

delay were compared between the groups. Also, the mean percentage of correct trials over three stabilization days was calculated at the maximal delay attained by each subject in order to demonstrate equivalent accuracy performance between the subjects prior to removal of the rhinal or dorsolateral prefrontal cortices. Surgical groups were matched for performance as was possible pre-surgery, such that subjects assigned to the surgical groups were neither the best nor worst performers in terms of their maximal memory scores.

Analyses for lesion effects—For the DNMS and DNMP tasks, a retention score was calculated using the number of errors made until criterion performance was attained at the 5-sec delay according to the formula: (pre-surgery errors – post-surgery errors / pre-surgery errors) $\times$ 100. Kruskal-Wallis tests were applied in order to determine if retention of the task rules differed significantly between the groups on either of the tasks.

The maximum delay reached on each task was coded for statistical analyses (i.e. 5 sec was designated as 1, 10 sec as 2,...,50 sec as 5,...). Each animal had two maximal memory scores for each task, one pre- and one post-surgery. The difference in coded maximal memory score on the DNMS and DNMP tasks were calculated (i.e. post-surgery score – pre-surgery score) and Kruskal-Wallis tests were conducted in order to determine if maximal memory differed significantly between the groups.

Histology

After completion of behavioral testing, the operated animals were sedated, given a lethal dose of sodium pentobarbital (RC1 and RC2) or deeply anaesthetized using isoflurane (RC3, RC4 and dlPFC1–4), and perfused transcardially with 0.9% saline, followed by either 10% buffered formalin (RC1 and RC2) or 4% paraformaldehyde (RC3, RC4 and dlPFC1–4). The brains were removed, photographed, cryoprotected, frozen, and 50 μ m coronal sections were cut on a freezing microtome. Every fifth section was mounted, stained with thionin, and examined microscopically. Regions of cell loss and gliosis were plotted on a series of normal coronal sections. The limits of the lesion area were mapped onto a normal brain from an age-matched control animal and used to calculate the total volume of intended and unintended damage using a digital tablet system (Wacom Intuos, Wacom Co., Ltd., Saitama, Japan) linked to a computer running ImageJ software (version 1.33, © NIH, Bethesda, MD, USA). The extent of damage was therefore expressed as a percentage of the total volume for each region.

Representative photomicrographs from the actual lesion sites in RC4 and dlPFC3 were obtained using a Nikon Macrophotography setup (Nikon, Inc., Melville, NY, USA) with a Fuji digital camera (Fuji Photo Film U.S.A., Inc., NY, USA). The images were collected and stored using Adobe Photoshop CS2 (version 9.0, Adobe Systems, Inc., San Jose, CA, USA). Brightness and contrast were adjusted using Adobe Photoshop CS2; no further modifications were made.

Results

Lesion extent

Table 1 shows the extent of damage to the RC and adjacent posterior composite gyrus expressed as a percentage of normal volume for each subject by hemisphere.

Reconstructions of individual RC lesions are shown in Figure 3. The extent of damage to the RC ranged from 58.6% to 69.7% (mean, 63.0%). All subjects sustained more damage to the perirhinal cortex (mean, 69.4%; range, 59.5 – 92.0%) compared to the entorhinal cortex (mean, 59.6%; range, 40.2% – 72.7%). RC1 and RC2 also had considerable damage to the adjacent posterior composite gyrus (87.1% and 53.6%, respectively), while RC3 and RC4 sustained only minor damage to this region (<20%). In all cases, unintended damage to the white matter and subiculum was minimal. Representative photomicrographs of the actual lesion taken from RC4 are shown in Figure 4.

Table 2 shows the extent of damage to the dorsolateral prefrontal cortex and adjacent orbitofrontal cortex expressed as a percentage of normal volume.

Reconstructions of individual lesions are shown in Figure 5. All subjects sustained substantial bilateral damage to the dlPFC (mean, 61.3%; range, 46.8 – 74.1%), while damage to the orbital cortex was minimal (<3%). Unintended damage to the white matter and adjacent cortical areas, including the frontal pole, pregeniculate, cruciate and presylvian cortices, was also minimal in all cases. Representative photomicrographs of the actual lesion taken from dlPFC3 are shown in Figure 6.

Object DNMS

Pre-surgery comparisons—Pre-surgery DNMS performance is summarized in Table 3. Analysis of the number of errors to criterion at the 5-sec delay revealed no significant difference in rate of relearning the DNMS task between the CON, RC and dlPFC groups prior to surgery. Further, there was no significant difference in performance accuracy between the groups pre-surgery, as measured by the mean percentage of correct trials over three stabilization days at the maximal delay attained by each subject.

Retention at 5-second delay—Post-surgery DNMS performance is summarized in Table 3 for all subjects on the object DNMS task. All RC subjects showed an increase in the number of errors required to relearn the object DNMS task at the 5 sec delay compared with controls and their own pre-surgery relearning. One subject (RC1) was unable to relearn the task at the 5 sec delay over 50 sessions (or 600 trials) post-surgery.

Indeed, retention of the DNMS task at the 5-sec delay differed significantly between the groups. Post hoc tests revealed that the RC group differed from the CON group ($p = 0.021$), while dlPFC animals did not differ significantly from CON animals in their retention scores (Figure 7A). However, as can be seen in Figure 7A, one animal (dlPFC2) did show a substantial disruption in retention of the DNMS task.

Two subjects, RC1 and RC2, sustained substantial damage (> 50%) to the posterior composite gyrus. However, post-surgical error scores and retention scores for these subjects overlap with those of RC3 and RC4, who sustained only minor damage (<20%) to the region, suggesting that the disruption in object recognition abilities can be attributed primarily to removal of the intended RC structures.

Maximal memory performance—Prior to surgery RC subjects were able to perform the object DNMS task up to a delay interval of 30 sec (Table 3). Post-operatively, maximal memory scores decreased for all RC subjects, with the best of the animals attaining criterion with a delay of only 10 sec. By contrast, three of the four CON animals and all three dIPFC subjects showed an increase in their maximal memory scores suggesting improved performance with repeated testing.

Difference scores in maximal memory varied significantly between the groups (Figure 7B). Post hoc tests revealed that RC subjects differed significantly from CON animals ($p = 0.025$), while maximal memory difference scores did not differ significantly between the dIPFC and CON groups.

Spatial DNMP

Pre-surgery comparisons—Pre-surgery DNMP performance is summarized in Table 4. Analysis of the number of errors to criterion at the 5-sec delay revealed no differences in rate of relearning the DNMP task between the CON, RC and dIPFC groups prior to the lesion surgeries. There was also no significant difference in performance accuracy between the groups prior to surgery.

Retention at 5-second delay—Post-surgery DNMP performance is summarized in Table 4 for all subjects on the spatial DNMP task. All dIPFC subjects showed an increase in the number of errors required to relearn the spatial DNMP task at the 5 sec delay compared to the CON and RC groups and their own pre-surgery relearning.

Retention differed significantly between the groups, and post hoc tests revealed that dIPFC subjects differed from the CON ($p = 0.025$) while the RC and CON groups did not differ from each other in their retention of the spatial DNMP task.

Maximal memory performance—Post-operatively, all three dIPFC subjects attained identical maximal memory scores to those obtained pre-surgery (Table 4). Difference scores pre- versus post-surgery for the maximum delay interval reached on the spatial DNMP task did not differ between the three groups (Figure 8B).

Discussion

The present study used a novel protocol to examine the roles of the rhinal and dorsolateral prefrontal cortices in object and spatial learning and memory in dogs. Beagles were trained daily on two working memory tasks, an object recognition task (DNMS) and a spatial recognition task (DNMP). After RC lesions, subjects were not impaired on a DNMP task, but showed disrupted performance on a DNMS task compared to the CON group, which

suggests that removal of the RC produced selective deficits in object recognition memory. By contrast, removal of the dlPFC disrupted retention of a DNMP task. This deficit appeared specific to relearning; with prolonged training, dogs were able to relearn the DNMP task and ultimately attained post-surgery maximal memory scores comparable to those obtained pre-surgery.

As far as we know, we are the first to use a protocol in which daily test sessions on both an object and spatial task were administered, and in which both tasks required use of a non-matching rule. Previous studies with major differences in task procedures complicate direct comparisons because it cannot be assumed that the type of stimulus information to be processed, and not some other factor, is leading to the dissociation in behavioural deficits. Further, daily testing on both a DNMS and a DNMP task in the present study avoided possible variations in lesion effects over time. Thus, differences in performance relative to lesion area are less likely to be due to order of testing or differences in post-surgical recovery time. Based on the present results, we cannot rule out the possibility that administration of both the DNMP and DNMS each day could have led to interference effects in learning and remembering the tasks. However, we took steps to minimize interference. In particular, we ensured a minimum of three hours rest between morning DNMP testing and afternoon DNMS testing. And, given the dissociated disruptions in performance following RC versus dlPFC lesions that we report, it seems unlikely that interference was a major factor. Further, we feel that the benefits of concurrent testing discussed above were worth any trade-off in terms of direct comparability to previous aging studies using the DNMP and DNMS tasks in dogs.

The present results suggest a dissociation in the RC's involvement in object versus spatial learning and memory in beagles. In support of our findings, several non-human primate lesion studies that directly compare the effects of RC damage on object and spatial cognition report selective deficits on object recognition, and not spatial, tasks (Alvarado and Bachevalier, 2005; Gaffan, 1994; Maclean et al., 2001). This dissociation is further supported by human functional neuroimaging studies (Moscovitch et al., 1995; Pihlajamäki et al., 2004) that show selective activation of temporal cortical regions, including the perirhinal cortex, when subjects identify objects but not spatial locations of objects, and by a study in brain damaged humans showing that patients with perirhinal cortex damage are impaired in discriminating objects, scenes and faces, while those with hippocampal damage are selectively impaired in discriminating spatial scenes (Lee et al., 2005). However, there are some reports of deficits in spatial learning and memory following damage to the RC (Buckmaster et al., 2004; Charles et al., 2004; Murray et al., 1998).

Murray and colleagues (1998) found that RC lesions in rhesus monkeys produce impairments in spatial scene learning that are comparable to those seen following hippocampal damage. Their task required animals to learn and remember which of two spatial locations in a complex scene was associated with food reward. Based on their findings, the authors propose that the RC is necessary not for the processing of spatial information for individual stimuli, but rather for the binding together of features of a scene into a coherent representation (Murray et al., 1998). This interpretation is consistent with similar impairments observed in object-in-place scene memory (Charles et al., 2004), and on

a spatial delayed recognition span task following entorhinal cortex lesions in monkeys (Buckmaster et al., 2004). Buckmaster and colleagues (2004) report that entorhinal lesions severely impair acquisition of a spatial delayed recognition span test in which monkeys must encode several types of information available to solve the task, including local spatial cues provided by the test stimuli and themselves, and by the configuration of these items in relation to the broader setting in which they appear. By contrast, entorhinal lesions did not impair performance on the DNMS task used in their study, or on other procedures that could be solved on the basis of memory for individual stimuli (Buckmaster et al., 2004). These findings suggest that spatial tasks that engage more complex, relational processes than those engaged by the DNMP task employed in the present study might be impaired by RC lesions in dogs, however, additional studies are necessary to directly assess this hypothesis.

In the present study, the observed DNMS deficits were likely caused by damage to the perirhinal cortex and not the entorhinal cortex. Although, our results do not permit a direct test of this distinction, other studies have shown that the perirhinal cortex makes a more significant contribution to object recognition than the entorhinal cortex (Leonard et al., 1995; Meunier et al., 1993 but cf. Suzuki et al., 1997). We also cannot entirely rule out inadvertent damage to the posterior composite gyrus as having contributed to the DNMS deficits observed in the present study. However, in contrast to monkeys, this adjacent region to the RC in dogs is thought to be primarily unimodal auditory association cortex based on its cytoarchitecture (Kreiner, 1968), connectivity (Kosmal, 2000; Kosmal et al., 1997), and functional characteristics (Tunturi, 1950; 1970). Further, the impairments observed on the DNMS task were of similar magnitude in all four RC subjects despite differences in the extent of unintended damage.

The impairments resulting from damage to the dlPFC were considerably different from those of the RC lesions. Unlike RC subjects, all dlPFC subjects showed an impairment in their retention of a visuospatial DNMP task. This finding is consistent with several previous dog studies that found impairments in *auditory* spatial performance following dlPFC lesions, as assessed by spatial delayed response and delayed alternation tasks (Konorski and Lawicka, 1959; Lawicka and Konorski, 1959; Lawicka et al., 1966; Lawicka, 1969; Stasiak and Lawicka, 1990). Similarly, nonhuman primate lesion studies report a critical role for the dlPFC in spatial delayed (non-) matching tasks (Bauer and Fuster, 1976; Glick et al., 1969; Kowalska et al., 1991; Levy and Goldman-Rakic, 1999; Meunier et al., 1997; Mishkin and Manning, 1978; Oscar-Berman, 1978; Passingham, 1975). Also consistent with the present findings, electrophysiological studies in monkeys report spatially selective neuronal delay activity in the dlPFC (Funahashi et al., 1989; 1993; Wilson et al., 1993, and reviewed in Funahashi, 2006). For example, recordings from neurons in the dlPFC of monkeys, or Areas 46 and 8 in and around the principal sulcus, show selective activation of dlPFC cells during an oculomotor spatial delayed response task (Funahashi et al., 1989). By contrast, only weak activation of dlPFC neurons is found during performance of an object-based working memory task (Wilson et al., 1993). Similarly, several human neuroimaging studies report that the dlPFC is engaged during maintenance of spatial information in working memory (Courtney et al., 1996; Kohler et al., 1995; Kohler et al., 1998; Owen et al., 1996).

The impairment in retention of the DNMP task observed in the present study was transient; with continued training, the animals successfully relearned the task and attained maximal memory scores that were identical to their pre-surgery scores. Transient learning and memory deficits following dlPFC damage have been reported previously, especially when prolonged training at short delay intervals is administered (Glick et al., 1969; Lawicka et al., 1966; Mogensen et al., 2005; Oscar-Berman, 1978). Consistent with the present findings, dlPFC lesions in dogs produce a transient impairment on auditory delayed matching and non-matching tasks; following an increase in post-surgery errors to criterion, pre-surgery levels of performance were eventually obtained (Stasiak and Lawicka, 1990). These findings suggest a selective role for the dlPFC in retention of the rules required to perform the DNMP task versus the storage of spatial information, and are consistent with a working-with-memory model of PFC function, which has been proposed to describe the PFC's role in strategic processes during the encoding and retrieval of declarative information (Moscovitch, 1992; Moscovitch and Winocur, 1995). In this view, a strategic component mediated by the PFC coordinates, interprets and elaborates on information that is being encoded and retrieved from the MTL system. So, rather than impairing memory itself, damage to the PFC seems to disrupt the application of remembered events to the organization of behaviours in a current context (Moscovitch, 1992; Moscovitch and Winocur, 1995). Further studies are necessary to directly test this hypothesis in dogs.

Based on the results of the present study, an intact dlPFC in canines appears to be critical for representation of the non-matching rule required to relate relevant information to the selection and organization of appropriate goal-directed behaviours at least until some other compensatory brain regions or behavioural strategies are recruited with prolonged training. Damage to ventral portions of the classically defined dlPFC in the present study was limited and therefore we cannot exclude the possibility that the transient impairment observed in DNMP retention may have been due, in part, to incomplete damage to the dlPFC. However, relatively extensive tissue damage in RC animals did not impair DNMP retention or performance and selectively disrupted performance on a DNMS task, which supports our interpretation of functional specialization of the dlPFC versus RC in dogs.

Overall, the present results add to our understanding of the basis of cognitive decline in aged beagle dogs. Previous findings suggest that the accumulation of beta-amyloid plaques and cortical atrophy are associated with deficits in object memory and executive function in aged beagles (Cummings et al., 1996a; Head et al., 1998; Tapp et al., 2004). Our findings confirm and extend these results suggesting that pathological changes in the RC underlie age-related impairments in object memory, while dlPFC changes contribute to deficits in the retention of rules required to perform a spatial DNMP task.

Acknowledgments

This research was supported by the National Institute on Aging grant AG12694, awarded to C.W. Cotman (P.I.), a Natural Sciences and Engineering Research Council of Canada postgraduate scholarship awarded to L.-A. Christie, and the National Institute of Mental Health Intramural Research Program. The authors gratefully acknowledge Dr. Anna Kosmal, Dr. and for their ongoing guidance and technical support on the project, David Yu and Caroline Runyan for their assistance with histological processing, Dr. for help with statistical analyses, and numerous behavioral testers for their invaluable assistance.

Support: National Institute on Aging (Grant number: AG12694), Natural Sciences and Engineering Research Council of Canada and National Institute of Mental Health Intramural Research Program

Literature Cited

- Alvarado MC, Bachevalier J. Comparison of the effects of damage to the perirhinal and parahippocampal cortex on transverse patterning and location memory in rhesus macaques. *J Neurosci.* 2005; 25(6):1599–1609. [PubMed: 15703414]
- Bauer RH, Fuster JM. Delayed-matching and delayed-response deficit from cooling dorsolateral prefrontal cortex in monkeys. *J Comp Physiol Psychol.* 1976; 90(3):293–302. [PubMed: 819472]
- Baxter MG, Murray EA. Impairments in visual discrimination learning and recognition memory produced by neurotoxic lesions of rhinal cortex in rhesus monkeys. *Eur J Neurosci.* 2001; 13(6):1228–1238. [PubMed: 11285020]
- Buckmaster CA, Eichenbaum H, Amaral DG, Suzuki WA, Rapp PR. Entorhinal cortex lesions disrupt the relational organization of memory in monkeys. *J Neurosci.* 2004; 24(44):9811–9825. [PubMed: 15525766]
- Buffalo EA, Reber PJ, Squire LR. The human perirhinal cortex and recognition memory. *Hippocampus.* 1998; 8(4):330–339. [PubMed: 9744420]
- Callahan H, Ikeda-Douglas C, Head E, Cotman CW, Milgram NW. Development of a protocol for studying object recognition memory in the dog. *Prog Neuropsychopharmacol Biol Psychiatry.* 2000; 24(5):693–707. [PubMed: 11191709]
- Chan AD, Nippak PM, Murphey H, Ikeda-Douglas CJ, Muggenburg B, Head E, Cotman CW, Milgram NW. Visuospatial impairments in aged canines (*Canis familiaris*): the role of cognitive-behavioral flexibility. *Behav Neurosci.* 2002; 116(3):443–454. [PubMed: 12049325]
- Charles DP, Browning PG, Gaffan D. Entorhinal cortex contributes to object-in-place scene memory. *Eur J Neurosci.* 2004; 20(11):3157–3164. [PubMed: 15579170]
- Courtney SM, Ungerleider LG, Keil K, Haxby JV. Object and spatial visual working memory activate separate neural systems in human cortex. *Cereb Cortex.* 1996; 6(1):39–49. [PubMed: 8670637]
- Cummings BJ, Head E, Afagh AJ, Milgram NW, Cotman CW. Beta-amyloid accumulation correlates with cognitive dysfunction in the aged canine. *Neurobiol Learn Mem.* 1996a; 66(1):11–23. [PubMed: 8661247]
- Cummings BJ, Head E, Ruehl W, Milgram NW, Cotman CW. The canine as an animal model of human aging and dementia. *Neurobiol Aging.* 1996b; 17(2):259–268. [PubMed: 8744407]
- Cummings BJ, Satou T, Head E, Milgram NW, Cole GM, Savage MJ, Podlisny MB, Selkoe DJ, Siman R, Greenberg BD, Cotman CW. Diffuse plaques contain C-terminal A beta 42 and not A beta 40: evidence from cats and dogs. *Neurobiol Aging.* 1996c; 17(4):653–659. [PubMed: 8832640]
- Funahashi S. Prefrontal cortex and working memory processes. *Neuroscience.* 2006; 139(1):251–261. [PubMed: 16325345]
- Funahashi S, Bruce CJ, Goldman-Rakic PS. Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. *J Neurophysiol.* 1989; 61(2):331–349. [PubMed: 2918358]
- Funahashi S, Bruce CJ, Goldman-Rakic PS. Dorsolateral prefrontal lesions and oculomotor delayed-response performance: evidence for mnemonic “scotomas”. *J Neurosci.* 1993; 13(4):1479–1497. [PubMed: 8463830]
- Gaffan D. Dissociated effects of perirhinal cortex ablation, fornix transection and amygdalotomy: evidence for multiple memory systems in the primate temporal lobe. *Exp Brain Res.* 1994; 99(3):411–422. [PubMed: 7957720]
- Gearing M, Tigges J, Mori H, Mirra SS. A beta40 is a major form of beta-amyloid in nonhuman primates. *Neurobiol Aging.* 1996; 17(6):903–908. [PubMed: 9363802]
- Glick SD, Goldfarb TL, Jarvik ME. Recovery of delayed matching performance following lateral frontal lesions in monkeys. *Commun Behav Biol.* 1969; 3:299–303.
- Head E, Callahan H, Muggenburg BA, Cotman CW, Milgram NW. Visual-discrimination learning ability and beta-amyloid accumulation in the dog. *Neurobiol Aging.* 1998; 19(5):415–425. [PubMed: 9880044]

- Head E, McCleary R, Hahn FF, Milgram NW, Cotman CW. Region-specific age at onset of beta-amyloid in dogs. *Neurobiol Aging*. 2000; 21(1):89–96. [PubMed: 10794853]
- Holdstock JS, Gutnikov SA, Gaffan D, Mayes AR. Perceptual and mnemonic matching-to-sample in humans: contributions of the hippocampus, perirhinal and other medial temporal lobe cortices. *Cortex*. 2000; 36(3):301–322. [PubMed: 10921661]
- Johnstone EM, Chaney MO, Norris FH, Pascual R, Little SP. Conservation of the sequence of the Alzheimer's disease amyloid peptide in dog, polar bear and five other mammals by cross-species polymerase chain reaction analysis. *Brain Res Mol Brain Res*. 1991; 10(4):299–305. [PubMed: 1656157]
- Kohler S, Kapur S, Moscovitch M, Winocur G, Houle S. Dissociation of pathways for object and spatial vision: a PET study in humans. *Neuroreport*. 1995; 6(14):1865–1868. [PubMed: 8547586]
- Konorski J, Lawicka W. Physiological mechanism of delayed reactions: I. The analysis and classification of delayed reactions. *Acta Bio Exp*. 1959:175–197.
- Kosmal A. Subcortical connections of the prefrontal cortex in dogs: afferents to the preoreal gyrus. *Acta Neurobiol Exp(Wars)*. 1981; 41(1):69–85. [PubMed: 6267904]
- Kosmal A. Organization of connections underlying the processing of auditory information in the dog. *Prog Neuropsychopharmacol Biol Psychiatry*. 2000; 24(5):825–854. [PubMed: 11191716]
- Kosmal A, Malinowska M, Woznicka A. Diversity of connections of the temporal neocortex with amygdaloid nuclei in the dog (*Canis familiaris*). *Acta Neurobiol Exp (Wars)*. 1997; 57(4):289–314. [PubMed: 9519547]
- Kowalska DM, Bachevalier J, Mishkin M. The role of the inferior prefrontal convexity in performance of delayed nonmatching-to-sample. *Neuropsychologia*. 1991; 29(6):583–600. [PubMed: 1944863]
- Kowalska DM, Kusmierek P, Kosmal A, Mishkin M. Neither perirhinal/entorhinal nor hippocampal lesions impair short-term auditory recognition memory in dogs. *Neuroscience*. 2001; 104(4):965–978. [PubMed: 11457584]
- Kreiner J. The myeloarchitectonics of the frontal cortex of the dog. *J Comp Neurol*. 1961; 116:117–133. [PubMed: 13754214]
- Kreiner J. Homologies of the fissural and gyral pattern of the hemispheres of the dog and monkey. *Acta Anat (Basel)*. 1968; 70(2):137–167. [PubMed: 4973261]
- Lawicka W. A proposed mechanism for delayed response impairment in prefrontal animals. *Acta Biol Exp*. 1969; 29(3–4):401–414.
- Lawicka W, Konorski J. Physiological mechanism of delayed reactions: III. The effects of prefrontal ablations on delayed reactions in dogs. *Acta Biol Exp*. 1959:221–231.
- Lawicka W, Mishkin M, Kreiner J, Brutkowski S. Delayed response deficit in dogs after selective ablation of the preoreal gyrus. *Acta Biol Exp (Warsz)*. 1966; 26(3):309–322. [PubMed: 4960555]
- Lee AC, Bussey TJ, Murray EA, Saksida LM, Epstein RA, Kapur N, Hodges JR, Graham KS. Perceptual deficits in amnesia: challenging the medial temporal lobe 'mnemonic' view. *Neuropsychologia*. 2005; 43(1):1–11. [PubMed: 15488899]
- Leonard BW, Amaral DG, Squire LR, Zola-Morgan S. Transient memory impairment in monkeys with bilateral lesions of the entorhinal cortex. *J Neurosci*. 1995; 15(8):5637–5659. [PubMed: 7643207]
- Levy R, Goldman-Rakic PS. Association of storage and processing functions in the dorsolateral prefrontal cortex of the nonhuman primate. *J Neurosci*. 1999; 19(12):5149–5158. [PubMed: 10366648]
- Maclean CJ, Gaffan D, Baker HF, Ridley RM. Visual discrimination learning impairments produced by combined transections of the anterior temporal stem, amygdala and fornix in marmoset monkeys. *Brain Res*. 2001; 888(1):34–50. [PubMed: 11146050]
- Malkova L, Bachevalier J, Mishkin M, Saunders RC. Neurotoxic lesions of perirhinal cortex impair visual recognition memory in rhesus monkeys. *Neuroreport*. 2001; 12(9):1913–1917. [PubMed: 11435922]
- Markow-Rajkowska G, Kosmal A. Organization of cortical afferents to the frontal association cortex in dogs. *Acta Neurobiol Exp (Wars)*. 1987; 47(4):137–161. [PubMed: 3442268]
- Meunier M, Bachevalier J, Mishkin M. Effects of orbital frontal and anterior cingulate lesions on object and spatial memory in rhesus monkeys. *Neuropsychologia*. 1997; 35(7):999–1015. [PubMed: 9226661]

- Meunier M, Bachevalier J, Mishkin M, Murray EA. Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *J Neurosci.* 1993; 13(12):5418–5432. [PubMed: 8254384]
- Milgram NW, Head E, Weiner E, Thomas E. Cognitive functions and aging in the dog: acquisition of nonspatial visual tasks. *Behav Neurosci.* 1994; 108(1):57–68. [PubMed: 8192851]
- Milgram NW, Head E, Zicker SC, Ikeda-Douglas C, Murphey H, Muggenberg BA, Siwak CT, Tapp PD, Lowry SR, Cotman CW. Long-term treatment with antioxidants and a program of behavioral enrichment reduces age-dependent impairment in discrimination and reversal learning in beagle dogs. *Exp Gerontol.* 2004; 39(5):753–765. [PubMed: 15130670]
- Milgram NW, Head E, Zicker SC, Ikeda-Douglas CJ, Murphey H, Muggenburg B, Siwak C, Tapp D, Cotman CW. Learning ability in aged beagle dogs is preserved by behavioral enrichment and dietary fortification: a two-year longitudinal study. *Neurobiol Aging.* 2005; 26(1):77–90. [PubMed: 15585348]
- Milgram NW, Siwak CT, Gruet P, Atkinson P, Woehrle F, Callahan H. Oral administration of adrafinil improves discrimination learning in aged beagle dogs. *Pharmacol Biochem Behav.* 2000; 66(2): 301–305. [PubMed: 10880682]
- Mishkin M, Manning FJ. Non-spatial memory after selective prefrontal lesions in monkeys. *Brain Res.* 1978; 143(2):313–323. [PubMed: 415803]
- Mishkin M, Ungerleider LG, Macko KA. Object vision and spatial vision: two cortical pathways. *Trends Neurosci.* 1983; 6(10):414–417.
- Mogensen J, Moustgaard A, Khan U, Wortwein G, Nielsen KS. Egocentric spatial orientation in a water maze by rats subjected to transection of the fimbria-fornix and/or ablation of the prefrontal cortex. *Brain Res Bull.* 2005; 65(1):41–58. [PubMed: 15680544]
- Moscovitch C, Kapur S, Kohler S, Houle S. Distinct neural correlates of visual long-term memory for spatial location and object identity: a positron emission tomography study in humans. *Proc Natl Acad Sci U S A.* 1995; 92(9):3721–3725. [PubMed: 7731972]
- Moscovitch M. Memory and working-with-memory: A component process model based on modules and central systems. *J Cog Neurosci.* 1992; 4(3):257–267.
- Moscovitch M, Winocur G. Frontal lobes, memory, and aging. *Ann N Y Acad Sci.* 1995; 769:119–150. [PubMed: 8595020]
- Murray EA, Baxter MG, Gaffan D. Monkeys with rhinal cortex damage or neurotoxic hippocampal lesions are impaired on spatial scene learning and object reversals. *Behav Neurosci.* 1998; 112(6): 1291–1303. [PubMed: 9926813]
- Murray EA, Mishkin M. Visual recognition in monkeys following rhinal cortical ablations combined with either amygdectomy or hippocampectomy. *J Neurosci.* 1986; 6(7):1991–2003. [PubMed: 3734871]
- Nystrom LE, Braver TS, Sabb FW, Delgado MR, Noll DC, Cohen JD. Working memory for letters, shapes, and locations: fMRI evidence against stimulus-based regional organization in human prefrontal cortex. *Neuroimage.* 2000; 11(5 Pt 1):424–446. [PubMed: 10806029]
- Oscar-Berman M. The effects of dorsolateral-frontal and ventrolateral-orbitofrontal lesions on nonspatial test performance. *Neuropsychologia.* 1978; 16(3):259–267. [PubMed: 100717]
- Owen AM, Evans AC, Petrides M. Evidence for a two-stage model of spatial working memory processing within the lateral frontal cortex: a positron emission tomography study. *Cereb Cortex.* 1996; 6(1):31–38. [PubMed: 8670636]
- Passingham R. Delayed matching after selective prefrontal lesions in monkeys (macaca mulatta). *Brain Res.* 1975; 92(1):89–102. [PubMed: 809096]
- Pihlajamaki M, Tanila H, Kononen M, Hanninen T, Hamalainen A, Soininen H, Aronen HJ. Visual presentation of novel objects and new spatial arrangements of objects differentially activates the medial temporal lobe subareas in humans. *Eur J Neurosci.* 2004; 19(7):1939–1949. [PubMed: 15078568]
- Postle BR, Stern CE, Rosen BR, Corkin S. An fMRI investigation of cortical contributions to spatial and nonspatial visual working memory. *Neuroimage.* 2000; 11(5 Pt 1):409–423. [PubMed: 10806028]

- Rainer G, Asaad WF, Miller EK. Memory fields of neurons in the primate prefrontal cortex. *Proc Natl Acad Sci U S A*. 1998; 95(25):15008–15013. [PubMed: 9844006]
- Rajkowska G, Kosmal A. Intrinsic connections and cytoarchitectonic data of the frontal association cortex in the dog. *Acta Neurobiol Exp (Wars)*. 1988; 48(4):169–192. [PubMed: 3188998]
- Rao SC, Rainer G, Miller EK. Integration of what and where in the primate prefrontal cortex. *Science*. 1997; 276(5313):821–824. [PubMed: 9115211]
- Rosenkilde CE. Delayed alternation behavior following ablations of the medial or dorsal prefrontal cortex in dogs. *Physiol Behav*. 1978; 20(4):397–402. [PubMed: 693609]
- Rousset GA, Thorpe SJ, Fabre-Thorpe M. How parallel is visual processing in the ventral pathway? *Trends Cog Sci*. 2004; 8(8):363–370.
- Sala JB, Courtney SM. Binding of what and where during working memory maintenance. *Cortex*. 2007; 43(1):5–21. [PubMed: 17334204]
- Satou T, Cummings BJ, Head E, Nielson KA, Hahn FF, Milgram NW, Velazquez P, Cribbs DH, Tenner AJ, Cotman CW. The progression of beta-amyloid deposition in the frontal cortex of the aged canine. *Brain Res*. 1997; 774(1–2):35–43. [PubMed: 9452189]
- Selkoe DJ, Bell DS, Podlisny MB, Price DL, Cork LC. Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. *Science*. 1987; 235(4791):873–877. [PubMed: 3544219]
- Siwak-Tapp CT, Head E, Muggenburg BA, Milgram NW, Cotman CW. Neurogenesis decreases with age in the canine hippocampus and correlates with cognitive function. *Neurobiol Learn Mem*. 2007; 88(2):249–259. [PubMed: 17587610]
- Siwak-Tapp CT, Head E, Muggenburg BA, Milgram NW, Cotman CW. Region specific neuron loss in the aged canine hippocampus is reduced by enrichment. *Neurobiol Aging*. 2008; 29(1):39–50. [PubMed: 17092609]
- Sloane JA, Pietropaolo MF, Rosene DL, Moss MB, Peters A, Kemper T, Abraham CR. Lack of correlation between plaque burden and cognition in the aged monkey. *Acta Neuropathol (Berl)*. 1997; 94(5):471–478. [PubMed: 9386780]
- Stasiak M, Lawicka W. Behavioral recovery on a spatial variant of the Konorski test following prefrontal damage in dogs. *Acta Neurobiol Exp (Wars)*. 1990; 50(4–5):201–212. [PubMed: 2130642]
- Su MY, Head E, Brooks WM, Wang Z, Muggenburg BA, Adam GE, Sutherland R, Cotman CW, Nalcioğlu O. Magnetic resonance imaging of anatomic and vascular characteristics in a canine model of human aging. *Neurobiol Aging*. 1998; 19(5):479–485. [PubMed: 9880050]
- Suzuki WA, Miller EK, Desimone R. Object and place memory in the macaque entorhinal cortex. *J Neurophys*. 1997; 78(2):1062–1081.
- Tanaka D. Neostriatal projections from cytoarchitectonically defined gyri in the prefrontal cortex of the dog. *J Comp Neurol*. 1987; 261(1):48–73. [PubMed: 2442202]
- Tapp PD, Siwak CT, Gao FQ, Chiou JY, Black SE, Head E, Muggenburg BA, Cotman CW, Milgram NW, Su MY. Frontal lobe volume, function, and beta-amyloid pathology in a canine model of aging. *J Neurosci*. 2004; 24(38):8205–8213. [PubMed: 15385603]
- Tunturi AR. Physiological determination of the boundary of the acoustic area in the cerebral cortex of the dog. *Am J Physiol*. 1950; 160(2):395–401. [PubMed: 15406335]
- Tunturi AR. The pathway from the medial geniculate body to the ectosylvian auditory cortex in the dog. *J Comp Neurol*. 1970; 138(2):131–136. [PubMed: 5413317]
- Ungerleider LG, Haxby JV. 'What' and 'where' in the human brain. *Curr Opin Neurobiol*. 1994; 4(2):157–165. [PubMed: 8038571]
- Wilson FA, O'Scalaidhe SP, Goldman-Rakic PS. Dissociation of object and spatial processing domains in primate prefrontal cortex. *Science*. 1993; 260(5116):1955–1958. [PubMed: 8316836]
- Woznicka A, Kosmal A. Cytoarchitecture of the canine perirhinal and postrhinal cortex. *Acta Neurobiol Exp (Wars)*. 2003; 63(3):197–209. [PubMed: 14518511]
- Woznicka A, Malinowska M, Kosmal A. Cytoarchitectonic organization of the entorhinal cortex in the canine brain. *Brain Res Rev*. 2006; 52(2):246–367.

Figure 1.

Intended lesion, shown in grey, of the rhinal cortex. The numbers to the right of each coronal section (10 to 14) correspond to those on the left of the ventral view (A), from rostral to caudal, through the rhinal cortex. Photomicrographs of thionin-stained sections are from approximately the same level and arrowheads indicate the cytoarchitectonic boundaries of the target lesion site. A, amygdala; EC, entorhinal cortex; H, hippocampus; PC, posterior composite gyrus; PRh; perirhinal cortex; PS, parasubiculum; rc, recurrens sulcus; rha, anterior rhinal sulcus; rhp, posterior rhinal sulcus; rs, retrosplenial sulcus; ss, Sylvian sulcus.

Figure 2.

Intended lesion, shown in grey, of the dorsolateral prefrontal cortex. The numbers to the right of each coronal section (1 to 5) correspond to the levels specified to the left of the dorsal view (A), from rostral to caudal, through the prefrontal cortex. Photomicrographs of thionin-stained sections are from approximately the same level and arrowheads indicate the cytoarchitectonic boundaries of the gyri surrounding the target lesion site. CR, cruciate gyrus; crs, cruciate sulcus; cs, coronal sulcus; ORB, orbital gyrus; pcrs, postcruciate sulcus; PG, pregenual gyrus; POR, paraorbital gyrus; PR, prereal gyrus; prs, prereal sulcus; ps, presylvian sulcus; us, unnamed sulcus at border of orbital cortex.

Figure 3.

Actual lesion, indicated by horizontal lines, of the rhinal cortex for each subject. The letters on the left of each coronal section correspond to those on the left of the ventral view, from rostral to caudal, through the rhinal cortex.

Figure 4.

Photomicrographs and corresponding representations of coronal sections through the rhinal cortex lesion in RC4. The right hemisphere in this subject sustained more damage than the left, especially in more rostral sections. The horizontal lines represent tissue that was damaged, and the numbers 11, 13 and 14 correspond to levels 11, 13 and 14 shown in Figures 1 and 3.

Figure 5.

Actual lesion, indicated by horizontal lines, of the dorsolateral prefrontal cortex for each subject. The letters on the left of each coronal section correspond to those on the left of the dorsal view, from rostral to caudal, through the rhinal cortex.

Figure 6.

Photomicrographs corresponding representations of coronal sections through the dorsolateral prefrontal cortex lesion in dlPFC3. The horizontal lines represent tissue that was damaged, and the numbers 1, 2 and 3 correspond to levels 1, 2 and 3 shown in Figures 2 and 5.

Figure 7.

RC lesions disrupt retention and maximal memory performance on an object recognition (DNMS) task. Shown are the DNMS data for individual animals (dots), as well as the 25th to 75th quartile range (boxes) and median values (lines in boxes) for the three surgical groups for (A) post- versus pre-surgery percent retention at a 5-second delay, and (B) post- versus pre-surgery maximal memory scores. RC subjects show disrupted retention and reduced maximal memory of the DNMS task relative to the CON group. Arrows in panel B indicate overlapping data points: two RC subjects attained scores of -25, two CON subjects attained scores of 0 and two dlPFC subjects attained scores of 10. The dashed line at zero indicates no change from pre-surgery performance.

Figure 8.

DIPFC, but not RC, lesions disrupt retention of a spatial recognition (DNMP) task. Shown are the DNMP data for individual animals (dots), as well as the 25th to 75th quartile range (boxes) and median values (lines in boxes) for the three surgical groups for (A) post- versus pre-surgery percent retention at a 5-second delay, and (B) post- versus pre-surgery maximal memory scores. dIPFC subjects show disrupted retention of the DNMP task relative to the CON group, while maximal memory remained intact in all groups. Arrows in panel B indicate overlapping data points: two CON subjects attained scores and all three dIPFC subjects attained scores of 0. The dashed line at zero indicates no change from pre-surgery performance.

Table 1

Extent of RC damage

Subjects	Rh				PRh				EC				CP			
	L	R	Avg	W	L	R	Avg	W	L	R	Avg	W	L	R	Avg	W
RC1	53.9	63.2	58.6	34.1	95.6	88.4	92.0	84.5	31.1	49.4	40.2	15.3	84.9	89.3	87.1	75.8
RC2	63.2	76.2	69.7	48.1	60.2	69.0	64.6	41.5	64.8	80.6	72.7	52.3	52.6	54.6	53.6	28.7
RC3	47.9	81.8	64.9	39.2	48.6	74.3	61.4	36.1	47.6	86.0	66.8	40.9	9.3	28.7	19.0	2.7
RC4	37.9	80.2	59.1	30.4	39.2	79.8	59.5	31.3	37.2	80.4	58.8	29.9	7.7	26.6	17.1	2.0
Mean	50.7	75.4	63.0	38.2	60.9	77.8	69.4	47.4	45.2	74.1	59.6	33.5	38.7	49.8	44.2	19.2

Data are the estimated percentage of normal volume. Rh, rhinal cortex; PRh, perirhinal cortex; EC, entorhinal cortex; CP, posterior composite gyrus; L, left; R, right; Avg, average; W= (L X R)/100 [weighted index as defined by Hodos and Bobko, 1984].

Table 2

Extent of dlPFC damage

Subjects	dlPFC				Orb			
	L	R	Avg	W	L	R	Avg	W
dlPFC1	46.8	74.1	60.5	34.7	0.0	2.3	1.2	0.0
dlPFC2	53.0	74.1	63.6	39.3	0.0	0.0	0.0	0.0
dlPFC3	62.0	57.6	59.8	35.7	0.5	0.0	0.2	0.0
Mean	54.0	68.6	61.3	37.0	0.2	0.5	0.5	0.0

Data are the estimated percentage of normal volume. dlPFC, dorsolateral prefrontal cortex; Orb, orbitofrontal cortex; L, left; R, right; Avg, average; W= (L X R)/100 [weighted index as defined by Hodos and Bobko, 1984].

Table 3

DNMS performance

Subjects	Pre-surgery performance			Post-surgery performance		
	Errors	Performance accuracy	Maximal memory score	Errors	Retention score	Maximal memory score
RC1	18.0	72.2	30	214.5	-1091.7	0
RC2	14.0	72.2	30	67.0	-378.6	5
RC3	18.0	86.1	30	155.0	-761.1	10
RC4	64.0	72.2	30	178.0	-178.1	5
dIPFC1	35.0	75.0	70	26.0	25.7	110
dIPFC2	11.0	83.3	20	146.0	-1227.3	30
dIPFC3	70.0	83.3	20	85.0	-21.4	30
CON1	45.0	80.6	5	47.0	-4.4	30
CON2	29.0	72.2	70	16.0	44.8	70
CON3	50.0	86.1	20	8.0	84.0	20
CON4	6.0	80.6	50	8.0	-33.3	20

A summary of DNMS performance of subjects pre- and post-surgery. Errors=number of errors made until criterion performance was attained at a 5-sec delay; Performance accuracy=mean percentage of correct trials over three stabilization days at the maximal delay interval attained; Maximal memory score=maximum delay at which criterion was attained over 50 sessions; Retention score=(pre-surgery errors – post-surgery errors/pre-surgery errors) × 100.

Table 4

DNMP performance

Subjects	Pre-surgery performance			Post-surgery performance		
	Errors	Performance accuracy	Maximal memory score	Errors	Retention score	Maximal memory score
RC1	5.0	77.8	150	6.0	-20.0	180
RC2	7.0	72.2	50	6.0	14.3	30
RC3	26.0	77.8	210	4.0	84.6	210
RC4	16.0	88.9	210	5.0	68.8	180
dIPFC1	4.0	80.6	240	51.5	-1187.5	240
dIPFC2	1.0	80.6	210	14.0	-1300.0	210
dIPFC3	12.0	72.2	110	53.0	-341.7	110
CON1	8.0	80.6	30	12.0	-50.0	30
CON2	9.0	72.2	270	1.0	88.9	240
CON3	10.5	83.3	110	3.0	71.4	70
CON4	52.5	77.8	30	33.3	37.1	30

A summary of DNMP performance of subjects pre- and post-surgery. Errors=number of errors made until criterion performance was attained at a 5-sec delay; Performance accuracy=mean percentage of correct trials over three stabilization days at the maximal delay interval attained; Maximal memory score=maximum delay at which criterion was attained over 50 test sessions; Retention score=(pre-surgery errors – post-surgery errors/pre-surgery errors) × 100.