Primate Rhinal Cortex Participates in Both Visual Recognition and Working Memory Tasks: Functional Mapping With 2-DG

LILA DAVACHI AND PATRICIA S. GOLDMAN-RAKIC
Section of Neurobiology, Yale University School of Medicine, New Haven, Connecticut 06511

Received 24 November 1999; accepted in final form 22 January 2001

Davachi, Lila and Patricia S. Goldman-Rakic. Primate rhinal cortex participates in both visual recognition and working memory tasks: functional mapping with 2-DG. J Neurophysiol 85: 2590–2601, 2001. The rhinal cortex in the medial temporal lobe has been implicated in object recognition memory tasks and indeed is considered to be the critical node in a visual memory network. Previous studies using the 2-deoxyglucose method have shown that thalamic and hippocampal structures thought to be involved in visual recognition memory are also engaged by spatial and object working memory tasks in the nonhuman primate. Networks engaged in memory processing can be recognized by analysis of patterns of activation accompanying performance of specifically designed tasks. In the present study, we compared metabolic activation of the entorhinal and perirhinal cortex during the performance of three working memory tasks [delayed response (DR), delayed alternation (DA), and delayed object alternation (DOA)] to that induced by a standard recognition memory task [delayed match-to-sample (DMS)] and a sensorimotor control task in rhesus monkeys. A region-of-interest analysis revealed elevated local cerebral glucose utilization in the perirhinal cortex in animals performing the DA, DOA, and DMS tasks, and animals performing the DMS task were distinct in showing a strong focus of activation in the lateral perirhinal cortex. No significant differences were evident between groups performing memory and control tasks in the entorhinal cortex. These findings suggest that the perirhinal cortex may play a much broader role in memory processing than has been previously thought, encompassing explicit working memory as well as recognition memory.

INTRODUCTION

The role of the medial temporal lobes in various forms of memory function has been a subject of interest in both animal and human studies (Murray and Mishkin 1986; Squire and Zola-Morgan 1985, 1991). A favored approach to modeling the human amnesiac syndrome has been through lesions of medial temporal lobe structures in the nonhuman primate (Meunier et al. 1993; Mishkin 1978; Murray and Mishkin 1984; Zola-Morgan and Squire 1985). Medial temporal lobe lesions most often are associated with deficits on declarative memory tasks such as delayed alternation (DA), has also been reported (Correll and Scoville 1967; Mahut 1971; Mahut and Cordeau 1963; Zola-Morgan and Squire 1985).

Working memory has been defined as the “on-line” or “active” maintenance of information over a short period of time (Goldman-Rakic 1987). Declarative memory has been defined as the recall and/or recognition of past events (Squire and Zola 1996). These two “types” of memory function are not easily dissociable either empirically or conceptually and may instead exist on a continuum of time and circumstance. For example, the initial processing of a stimulus may involve short-term maintenance of that stimulus while related associations and functions are being accessed through long-term memories related to that stimulus. However, a distinction between these two memory systems has traditionally been based on evidence from human neuropsychology and lesion studies in animals. These studies have demonstrated that damage to the prefrontal cortex causes impairments in working memory tasks with short delays while damage to the medial temporal lobe is associated with impairments with longer delay periods (Alvarez et al. 1994). Working memory tasks also differ from recognition memory tasks in placing a greater demand on “active” maintenance of information, as indicated, for example, by the higher level of proactive interference usually observed in working memory tasks than in recognition memory tasks.

Although a distinction between frontally mediated working memory processes and medial-temporal-lobe-dependent long-term memory processes has been widely accepted, recent neuroimaging studies in humans have clearly shown that the prefrontal cortex is activated during the performance of episodic memory tasks (Kapur et al. 1994; Tulving et al. 1994). Conversely, medial temporal lobe lesions, in nonhuman primates, including selective lesions of the perirhinal cortex have been shown to impair memory performance with relatively short delays (Gaffan and Murray 1992; Meunier et al. 1993; Zola-Morgan et al. 1989). To further explore this issue of delay-dependent involvement of medial temporal lobe memory system, we set out to examine whether the rhinal cortex, consisting of the entorhinal and perirhinal cortex, is metabolically activated in animals performing different memory tasks with short delay intervals.

A major difficulty in using lesions to localize memory functions is the inevitable variations in the size of the lesion and reactive compensatory mechanisms, such as neuronal sprouting, that have been documented following lesions to medial temporal lobe structures (Leonard et al. 1995; Lynch et al. 1972). Thus there are distinct advantages to studying the intact...
brain during performance of memory tasks as is being done in studies of human brain imaging. Positron emission tomography and functional magnetic resonance imagine (MRI) techniques have shed much light on the functional anatomy of the human brain but lack the spatial resolution to differentiate between cytoarchitectonically distinct areas within the medial temporal cortex, such as the entorhinal and perirhinal cortices. The 2-deoxyglucose imaging technique (2-DG), on the other hand, has excellent spatial resolution in animal subjects and is sufficiently sensitive to differentiate cytoarchitectonic areas and cortical laminae during the performance of different memory tasks.

In the present study, we examined activation in the rhinal cortex (entorhinal and perirhinal areas) while animals performed either object or spatial working memory tasks [delayed object alternation (DOA), DA, or delayed response (DR)] or a recognition memory task (trial-unique DMS), all with similar short delay intervals (12–30 s). Given our previous findings that hippocampal and thalamic structures known to be important for recognition memory also exhibit elevated local cerebral glucose utilization (LCGU) during working memory performance (Friedman and Goldman-Rakic 1988; Friedman et al. 1990), we hypothesized that LCGU in the rhinal cortex would also be elevated in animals performing working memory tasks as well as recognition tasks. Also, based on the involvement of the perirhinal cortex in tasks that require object identification and its abundance of cortical input from visual association cortex (Buckley and Gaffan 1998b; Suzuki and Amaral 1994; Thornton et al. 1997), we expected that the perirhinal cortex would be activated preferentially in tasks requiring the memory of object features.

METHODS

Subjects

Seventeen male rhesus monkeys (Macaca mulatta, 2.0–6.0 kg) were trained to perform either one of four memory tasks (n = 14) or a control task (n = 3). Monkeys were housed individually and fed a diet of monkey chow and fruit, adjusted to maintain body weight at 90% of free-feeding weight. Monkeys were given free access to water. Water was available ad libitum. Data for the rhinal cortex of the medial temporal lobe was obtained from 11 animals that performed the DOA (n = 3), DA (n = 4), and DR (n = 3) tasks and the sensory-motor task (SMC, n = 1) in our previous 2-DG studies of LCGU in the prefrontal cortex (Friedman and Goldman-Rakic 1994) and hippocampus (Friedman and Goldman-Rakic 1988). The DMS (n = 4) group and two animals from the SMC group are new additions to this experiment. All behavioral methods used in the new cases were identical to those employed previously. The data analysis for all animals was performed de novo and in the same manner for this study.

Apparatus and testing procedures

Monkeys were seated in a primate chair while being tested in a modified Wisconsin General Test Apparatus (WGTA), which included a test tray with two or three food wells (depending on the task, see following text) where stimuli and rewards were provided to the animal and a manually manipulated screen for imposing delays. The testing room was darkened and sound attenuated. A white-noise generator supplied a constant level of background noise during testing (90 dB).

Training began after the monkeys became acclimated to the chair, testing room, and WGTA. Initially, monkeys were only required to move cardboard plaques or objects to retrieve a food reward from either of the two wells. The training on their respective tasks progressed depending on the individual monkeys’ performance from very short delays (1 or 2 s) until proficiency was demonstrated (85–100% correct within a session) to longer delays with an increased number of trials per session. This was continued over a period of weeks and months until the desired criterion (90% correct) was achieved at the prescribed delay in a 45- to 50-min continuous test session. The 2-DG test date was then scheduled. The procedures for some of the behavioral tests have been described previously (Friedman and Goldman-Rakic 1988; Pribram and Mishkin 1956) and are reviewed in the next section.

Tasks

DELAYED RESPONSE. Three monkeys were trained on this task. One food well was baited with a food reward in full view of the monkey, and then both wells were covered with identical cardboard plaques. The screen was lowered for 12 s to obscure the monkey’s view of the experimenter and the testing tray. Following the delay, the screen was raised and the monkey chose a well. For the animal to succeed, the monkey had to choose the previously baited well. The position of the food reward varied in a random order according to Gellerman (1933).

DELAYED SPATIAL ALTERNATION. Four monkeys were trained on this task. Food wells were baited with the screen lowered. On the first trial, both wells were baited with a food reward and covered with identical cardboard plaques, and the monkey was allowed to displace either plaque to obtain a reward. On the following trial, only the well that was not selected on the previous trial was baited, and subsequent trials proceeded in this fashion. Therefore the location of the previous reward had to be remembered over the delay (12 or 30 s) for the monkey to select the alternate well and obtain the reward on the new trial. Two monkeys were trained with a delay of 12 s and two with a delay of 30 s on this task.

DELAYED OBJECT ALTERNATION. Three monkeys were trained to perform this task. This paradigm required that objects as opposed to spatial locations be remembered over the delay to achieve a food reward. A blue cube (6.5 cm square × 3 cm high) and a green cylinder (6.5 cm diam × 8 cm high) were the objects used to cover the food wells. Monkeys were first taught to do a simple object discrimination task using a criterion of 90% correct in 60 trials before reversing the reward contingencies. Thus one object (either the blue cube or the green cylinder) was always rewarded until the animal reached criterion, after which the other object was rewarded. The number of trials to reversal was then gradually decreased from 60 to 30, 15, 10, and 5 trials until 1-trial alternation was achieved, and the intertrial interval was gradually increased to 12 s. The animals were now choosing the alternate object on every trial with the spatial position of the reward being randomly varied (Gellerman 1933). Once again, as in DOA, the preceding response had to be remembered over the delay for the monkey to select the correct object and obtain a reward.

DELAYED MATCH-TO-SAMPLE. Four monkeys were trained to perform this visual recognition memory task. To maximize reliance on recognition memory processes, trial unique objects were used in the administration of the DMS task. Stimuli consisted of “junk” objects varying in size, color, and shape. A sample object was presented over the middle well covering a food reward. The monkey was required to displace this object after which a delay of 12 s ensued. After this delay, the sample object and a novel object were placed over the two side wells and the correct choice (i.e., the object that was baited) was the object that “matched” the sample. The novel object was not baited. If the monkey chose the novel object, the trial was terminated, and an intertrial interval of 15 s ensued before the beginning of the next trial. Monkeys performing this task performed an average of 80 trials per session and thus were exposed to an average of 160 novel objects during the 2-DG experiment.
SENSORY-MOTOR CONTROL. Three monkeys were trained to perform this task in which both wells are baited and identical plaques were placed on the testing board. Monkeys were required to displace a plaque and retrieve a food reward. The opaque screen was lowered for a delay of 12 s between trials, but the monkey did not have to remember anything during this time because a reward was given on every trial. The sensory stimuli and motor responses used in this task were similar to those used in all other tasks.

2-DG experiment

PREPARATION. The quantitative 2-DG method developed by Sokoloff et al. (1977) was followed. Seventeen monkeys received arterial and venous catheters of the femoral vessels under halothane and nitric oxide anesthesia prior to the 2-DG test (1 monkey received a catheter into the saphorous artery). Four monkeys (2 DA and 2 DOA) were catheterized 24 h prior to the 2-DG injection to further promote alert testing performance and were therefore given ketamine (5 mg/kg) in addition to the gas anesthesia. In these cases, the catheterization was done under aseptic conditions. Sterile catheters were inserted and tied to the arteries and veins, and the exposed ends were sealed and secured about the sutured wound with bandages for protection. These monkeys were kept in their home cages overnight. The free ends of the catheters were opened prior to the 2-DG experiment. The other 13 monkeys were catheterized the morning of the 2-DG experiment at least 2 h prior to testing. They received only gas anesthesia and were allowed ample time to recover from anesthesia before beginning the experiment and receiving the 2-DG injection. Subcutaneous lidocaine was applied during the 24-h catheterization experiments, and topical anesthetics were applied liberally during both procedures. Analyses of data showed that there was no apparent correlation between the type of catheterization and subsequent task performance.

EXPERIMENTAL SESSION. About 3–5 min into the test session, 14C-2-DG (100 μCi/kg in 1 μCi/10 μl sterile saline, 50–60 mCi/mM; American Radiolabeled Chemicals) was injected followed by a saline flush. Blood samples were then taken over the 45-min test period at timed intervals. At the end of the test session, the monkey was lightly perfused through the heart with a solution of buffered 3.3% paraformaldehyde (1.5–3.0 l, pH 7.4). The brain was quickly removed and sectioned into blocks before immersion in cold isopentane (−40°C). Blocks were stored at −70°C.

TISSUE PROCESSING. Brain blocks were cut at 20-μm thickness on a cryostat (Hacker Instruments) at −22°C. In most cases, 4 of every 20 serial sections was saved throughout the entire extent of the brain. One section in this series was mounted on a glass slide for cresyl violet staining while the adjacent three sections were mounted on glass coverslips and dried on a hot plate for autoradiography. These coverslips were taped to 7 × 8-in cardboard, and the sections were then exposed to X-ray film (SB5 or Biomax, Kodak) for 4–10 days together with a set of polymethylmethacrylate 14C standards (0–1.08 mCi/g, Amersham). Films were then processed in developer and fixative (GBX, Kodak) according to packaged instructions.

BLOOD GLUCOSE AND 14C LEVELS. Blood samples collected at fixed intervals during the experiment were immediately centrifuged. Plasma samples (20 μl) were then analyzed for glucose (Beckman Glucose Analyzer 2) and for 14C concentration using a liquid scintillation counter (Beckman scintillation counter). Integrated arterial plasma specific activities were derived from the blood concentration curves, and these were used to convert tissue 14C concentrations to LCGU as described by Kennedy et al. (1978) for each monkey.

QUANTIFICATION OF AUTORADIOGRAMS. Autoradiograms of selected brain sections along with the 14C standards for every page of film were digitized using a computerized video imaging processing system (MCID, M2, Imaging Research, Ontario, Canada). This system is comprised of an NEC Express5800 computer equipped with a 200-MHz Pentium Pro processor, a high-resolution PCI graphics card, a Dage MTI CCD72 video camera, and a color video monitor. Pixel gray values were computed by optical density and were then translated into 14C radioactivity levels. These were then translated into LCGU rates using the integrated plasma specific activities obtained for each monkey (Sokoloff et al. 1977).

Data analysis

Data were analyzed using two methods. The first method was a region of interest analysis (ROI), which was employed to compare LCGU between groups over the extent of any given cortical area, in this case, the entorhinal and perirhinal cortex. This method has been used in previous 2-DG experiments (Friedman and Goldman-Rakic 1994) and is described in more detail in the next section. In addition, a local maxima analysis was employed to reveal common and disparate patterns of activation within these same regions. This method highlights areas or subregions that reach a specified criterion that can then be compared between groups (see following text).

ROI analysis

ENTORHINAL CORTEX. The entorhinal cortex (EC) is located ventral to the amygdala and hippocampal formation and is bounded medially by the parahippocampal and laterally by the fundus of the rhinal sulcus (Fig. 1). Medial EC was defined as the cortex bounded medially by the parahippocampal and laterally by the lip of the rhinal sulcus while lateral EC was defined as the cortex on the medial bank of the rhinal sulcus ending in the fundus of the rhinal sulcus (Saleem and Tanaka 1996; Suzuki and Amaral 1994). LCGU was measured in the EC of the right hemisphere starting at the appearance of the rhinal sulcus and extending throughout its rostrocaudal extent. ROIs were sampled using an adjustable sampling tool (a “ribbon” tool) that allowed measurement of the entire mediolateral extent of the cortex and layers II–V regardless of the width of the cortex. Layers I and VI were not sampled because LCGU rates were very low in these layers. Density values were summed across the length of the ribbon tool, thus one value representing the mean LCGU in the EC per section was obtained. Data from animals in the DA, DR, or DOA groups were sampled by defining ROI in three serial sections at 400-μm intervals throughout the entire extent of the area examined (Friedman and Goldman-Rakic 1994; Friedman et al. 1989). The cutting schedule was modified for the DMS and two control animals. As a consequence, data from these animals were obtained by defining regions of interest in four equally spaced sections every 400 μm. An average of 73 sections, hence 73 measures, per animal was used to determine the mean LCGU per ROI. Across groups an average of 83, 87, and 62 sections were analyzed for the DMS, SMC, and working memory groups, respectively. One mean LCGU rate was then calculated for the entire EC and these means were then transferred into SYSTAT (Sherman, IL) for statistical analysis (see following text).

PERIRHINAL CORTEX. The perirhinal cortex (PC) was analyzed beginning at the level of the rhinal sulcus as seen in coronal sections and therefore the rostral portion (area 36d and rostral 35 and 36r) of this area was not included in our analysis. Caudally, the analysis of the PC extended to the end of the rhinal sulcus. Medial PC was defined as cortex within the lateral bank of the rhinal sulcus and lateral PC was defined as PC lateral to medial PC and bordering inferotemporal cortex. The lateral border of PC was defined as the midpoint between the lateral lip of the rhinal sulcus and the medial lip of the anterior temporal medial sulcus (AMTS). This border was obtained by applying a straight-edge ruler to the screen displaying the image to be sampled. It should be noted that this border is largely in keeping with Saleem and Tanaka’s (1996) designations. In anterior sections where the AMTS ended, the lateral border was defined as a third of the distance from the lateral lip of the rhinal sulcus to the medial lip of the
local maxima analysis

To obtain a more detailed view of focal regions activated within the EC and the PC and to be certain that regional variations were not being diluted by the ROI analysis, a local maxima analysis was performed. This consisted of highlighting pixels on each section that reached a specific density and size criterion. First, mean LCGU for the rhinal cortex within a single section was determined. Pixels that were at least 1.5 SD above this mean were then highlighted. This resulted in hundreds of contiguous pixels being highlighted (Fig. 2B). A size filter was then applied such that only activations that were at least 2 SD above the average size of activated pixels were included. This cutoff was used to eliminate small activations that may be artifactual. This yielded the most informative and manageable picture of activity within a section (Fig. 2C). This thresholding procedure was applied to each section independently. Thus variations in tissue thickness did not affect the outcome. The same sections that were used for the region of interest analysis were used for the local maxima analysis. Thus in all an average of 73 sections per animal was analyzed for local maxima.

Local maxima were then characterized according to where they were located, their intensity (measured by the LCGU rates), and their size (measured by the number of consecutive sections that contained that activation). All animals displayed activations in the fundus of the rhinal sulcus, and these activations often crossed the cytoarchitectonic borders of the EC and the PC, both within a section and across sections. Since these activations spanned both the entorhinal region of the fundus as well as the perirhinal region of the fundus, they will be referred to as “fundus” activations instead of belonging to one or another cortical region. The size and intensity of local maxima were compared between groups. The peak level of glucose utilization within local maxima was compared using an analysis of covariance.

statistical analysis

An analysis of covariance was used to control for individual differences in general brain metabolism (Friedman and Goldman-Rakic 1988). The medial geniculate body was used as the covariate for reasons described in the preceding text. Adjusted mean LCGU was then derived from the analysis of covariance and is the dependent variable used in all data presentations. The analysis was done using a computer-based statistics program (SYSTAT, Sherman, IL).

RESULTS

General results

Performance on the 2-DG test date ranged from 73 to 100% correct. The animals that performed memory tasks obtained a mean of 88% correct. Within tasks, mean performance was 80% or better over the 45-min test period (Table 1). Tasks could be ranked in order of difficulty based on the number of trials to criterion in initial learning. By this criterion, the DOA task was judged to be the most difficult since training required more sessions on average than any other task and percent correct performance was lower than any other task. By the same criterion, the DA task was the penultimate in difficulty followed by the DMS, DR, and SMC tasks, in that order.

General pattern of glucose uptake in the rhinal cortex

The general pattern of glucose uptake in the rhinal cortex is illustrated in Fig. 3. Glucose uptake was always visibly lower in the medial temporal regions compared with neighboring cortical areas (Fig. 3, A–C). The EC was characterized by less activation in medial portions compared with lateral portions and LCGU increased in the fundus of the rhinal sulcus possibly.
due to the compression of cortical layers. Laminar patterns of uptake in medial EC changed somewhat in the antero-posterior (AP) axis. Specifically, the middle cortical layers of medial EC were more evenly. activated across layers in anterior portions of the EC, while a superficial and deep band of activity emerge at more caudal levels. Conversely, a uniform pattern of LCGU across cortical layers was seen in lateral EC at both anterior and posterior levels.

Within the medial PC, the highest glucose utilization was localized to deep layer III/IV, whereas in the lateral PC, it spanned layers II–V. Local maxima activations (see Fig. 7 for examples) were usually located within the middle layers of cortex where LCGU always appeared to be the strongest. For example, lateral PC activations were usually localized within layers III–V in all animals.

**TABLE 1.** Trials to criterion (85% correct in 100 trials) in initial training percent correct and number of trials performed on the 2-DG test day.

<table>
<thead>
<tr>
<th>Task Monkey</th>
<th>Performance 2-DG Test Day</th>
<th>Initial Training (Trials to Criterion)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent correct</td>
<td>Trials</td>
</tr>
<tr>
<td><strong>DOA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOA1</td>
<td>82</td>
<td>127</td>
</tr>
<tr>
<td>DOA2</td>
<td>86</td>
<td>140</td>
</tr>
<tr>
<td>DOA3</td>
<td>73</td>
<td>169</td>
</tr>
<tr>
<td>Mean DOA animals</td>
<td>80</td>
<td>145.33</td>
</tr>
<tr>
<td><strong>DA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA1</td>
<td>89</td>
<td>160</td>
</tr>
<tr>
<td>DA2</td>
<td>91</td>
<td>128</td>
</tr>
<tr>
<td>DA3</td>
<td>76</td>
<td>62</td>
</tr>
<tr>
<td>DA4</td>
<td>97</td>
<td>87</td>
</tr>
<tr>
<td>Mean DA animals</td>
<td>88</td>
<td>109.25</td>
</tr>
<tr>
<td><strong>DMS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMS1</td>
<td>93</td>
<td>80</td>
</tr>
<tr>
<td>DMS2</td>
<td>91</td>
<td>81</td>
</tr>
<tr>
<td>DMS3</td>
<td>87</td>
<td>81</td>
</tr>
<tr>
<td>DMS4</td>
<td>88</td>
<td>80</td>
</tr>
<tr>
<td>Mean DMS animals</td>
<td>90</td>
<td>80.50</td>
</tr>
<tr>
<td><strong>DR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR1</td>
<td>95</td>
<td>131</td>
</tr>
<tr>
<td>DR2</td>
<td>84</td>
<td>128</td>
</tr>
<tr>
<td>DR3</td>
<td>96</td>
<td>143</td>
</tr>
<tr>
<td>Mean DR animals</td>
<td>92</td>
<td>134.00</td>
</tr>
<tr>
<td><strong>SMC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMC1</td>
<td>N/A</td>
<td>130</td>
</tr>
<tr>
<td>SMC2</td>
<td>N/A</td>
<td>140</td>
</tr>
<tr>
<td>SMC3</td>
<td>N/A</td>
<td>149</td>
</tr>
<tr>
<td>Mean SMC animals</td>
<td>139.67</td>
<td></td>
</tr>
</tbody>
</table>

The only exceptions to this were in the fundus of the rhinal sulcus where activations often spanned across more than three cortical layers. An example of the general pattern of activation in a magnified image of the fundus of the rhinal sulcus is displayed in Fig. 4. The pattern seen in an anterior section (where the fundus predominantly lies in perirhinal region area 35) and a posterior section (where the fundus lies within the boundaries of the EC) demonstrates that regardless of whether the fundus lies within the entorhinal or perirhinal region, it emerges as a region of elevated LCGU. Again, it can be seen more clearly from this view that the lateral entorhinal region is more uniformly activated than the medial perirhinal region (lining the rhinal sulcus) where deep layer III/IV is activated more than the surrounding layers.

The general pattern of activity is also displayed on a flattened map of LCGU through the anterior posterior extent of the EC and PC in a representative case (Fig. 3D). This twodimensional flattened map through the middle layers of cortex (layers III–V) is based on 78 different AP levels to demonstrate the pattern of glucose uptake across the anterior-posterior dimension. Again, this figure demonstrates that anterior regions of EC were less metabolically active than posterior portions and lateral EC is more active than medial regions across most AP levels. Furthermore these regional differences within the EC withstood statistical analyses showing that, across all animals, lateral EC was more active than medial EC ($F = 5.935, df = 1, P < 0.05$) and that within the lateral EC, posterior regions were more active than anterior regions ($F = 7.252, df = 1, P < 0.02$). Also evident in this representative image are peaks of activity (shown in red) localized around the fundus (in area 28 or 35 depending on which peak). Such peaks, which were observed in all animals, are analyzed further in the following text.

It is important to note that the general patterns of glucose utilization displayed in Figs. 3 and 4 were observed in all animals regardless of the type of task they were performing. The striking similarity between control and experimental groups in patterns of glucose uptake has been noted in previous studies of 2-DG imaging (Friedman and Goldman-Rakic 1988, 1994). As in prior studies, group differences in glucose uptake emerged only when we applied quantitative autoradiographic methods to areas of interest (ROI analysis) and local maxima analyses (see METHODS).
FIG. 3. General pattern of LCGU observed in rhinal cortex. A: an example of an autoradiographic image from 1 animal showing that overall LCGU is lower in medial temporal lobe regions compared with surrounding cortical areas. Inset: higher power view of this image showing details of the pattern of LCGU within the rhinal cortex (see RESULTS). B: pseudo-colored (according to calibration of LCGU in top right corner) image of same section shown in A. C: cresyl-violet stained section adjacent to that seen in A. Areas are labeled in inset. D: a flattened map through the entorhinal and perirhinal cortices of a representative animal. Sections are flattened and stacked from most anterior (bottom) to most posterior (top) with medial to left and lateral to the right. The map is pseudo-colored according to the calibration bar at the right representing LCGU values. Anatomical borders corresponding to medial and lateral EC (BA 28) and medial (BA 35) and lateral (BA 36) PC are drawn on the map with white dotted lines, labeled at the top of the map. This map was drawn based on the cytoarchitectonic borders described by Suzuki and Amaral (1994). Distance calibrations are included at bottom and left sides of the map. Three autoradiographic sections are included at the left to give an idea of the AP level of sections. A, amygdala; rs, rhinal sulcus; AMTS, anterior medial temporal sulcus.
LCGU rates in the rhinal cortex: ROI analysis

Figure 5 displays LCGU for all groups in the rhinal cortex. The ROI analysis revealed that animals performing the DMS, DA, and DOA tasks exhibited significantly elevated LCGU in the rhinal cortex as a whole compared with controls. A 2 (area) x 5 (task) analysis of covariance (ANCOVA) revealed a significant task effect ($F = 5.367$, $df = 4$, $P = 0.001$) and Fisher’s LSD post hoc comparisons confirmed DMS, SMC, $P = 0.02$; DA, SMC, $P = 0.01$; DOA, SMC, $P = 0.01$). The difference between the DR and SMC groups was not significant.

Separate analyses performed on the EC and PC revealed that the differences noted in the overall rhinal analysis arose out of task differences in the PC, not the EC (Fig. 6). A one-way ANCOVA performed on the perirhinal data showed a significant main effect of task ($F = 4.705$, $df = 4$, $P < 0.01$) and post hoc comparisons confirmed that LCGU in the DMS, DA, and DOA groups was significantly higher than the SMC group (Fisher’s LSD, $P < 0.01$, 0.01, 0.02, respectively).

Although a significant task effect did not emerge in the EC ($F = 1.433$, $df = 4$, $P = 0.249$), the pattern of LCGU was similar to that observed in the PC except that the DMS group now displayed a conspicuously low LCGU in the EC compared with the PC, a finding confirmed statistically, i.e., a one-way ANCOVA revealed a significant area effect: $F = 5.449$, $df = 1$, $P < 0.04$. In addition, the DOA group was the only other group to exhibit lower LCGU in the EC over that in the PC although at a marginal level of significance ($F = 4.41$, $df = 1$, $P = 0.06$). Interestingly, there was no significant difference in LCGU between perirhinal and EC in the animals performing the DA and DR spatial memory tasks.

Given the heterogeneity of activation within EC (see preceding text), with lateral regions exhibiting stronger activation than medial regions and posterolateral regions exhibiting more activation than anterolateral regions, separate analyses were...
conducted to determine if task differences were concealed by collapsing across EC regions. These analyses confirmed that there were no significant differences between groups in these regions (medial, lateral, anterolateral and posterolateral EC). However, there was a trend for the animals performing the DA task to show increased activation in the lateral EC compared with controls ($F = 3.318, df = 2, P = 0.069$) when collapsing across both anterior and posterior regions of the lateral EC. Implications for this trend are discussed in the following text.

**Local maxima analysis**

Figure 7 displays examples of local maxima in four animals [DMS (A1–A4), DOA (B1–B4), and 2 DA (C1–C4 and D1–D4)] at similar rostrocaudal levels throughout the extent of the rhinal cortex. All animals displayed peak activations in the fundus of the rhinal sulcus and in the lateral PC. These activations were evident along a considerable portion of the anterior-posterior axis of the rhinal cortex in all animals (see Fig. 8).

**DISCUSSION**

Our results confirm that the PC is activated during the performance of both working and recognition memory tasks, whether spatial or object based. LCGU was significantly elevated in comparison to controls in the PC in animals performing the DA, DOA and DMS tasks. Our data also demonstrate that animals performing the object tasks (DMS and DOA) activated the PC significantly more than the EC. Furthermore, within the lateral PC, animals performing the DMS task exhibited a distinct focus of enhanced LCGU that was greater than that seen in controls and other memory tasks. In contrast, the only group difference in the EC was a trend for the animals performing a spatial working memory task to show enhanced LCGU in the lateral EC (but not the medial EC). Finally, the fundus of the rhinal sulcus was elevated in all animals including controls, indicating that areas within the rhinal cortex may be activated by nonmnemonic factors common to all behavioral tasks, i.e., visual processing, attention or motivation. These findings reveal that the rhinal cortex is functionally heterogeneous and add to the increasing number of studies implicating the rhinal cortex not only in visual recognition memory but also in a broad range of other tasks including visual–visual associations, tactile delayed nonmatch to sample, spatial scene learning and configural learning (Murray et al. 1993, 1998; Suzuki et al. 1993). The present study adds explicit spatial working memory tasks to this list.

**Correspondence with lesion studies**

Although studies of postoperative performance on delayed matching tasks following lesions to the rhinal cortex report deficits at delays longer than those used in the present study, impaired acquisition of these tasks with a shorter delay (8 s) has been reported (Gaffan and Murray 1992; Zola-Morgan and Squire 1985), and acquisition of delayed matching tasks with short delay intervals correlates strongly with performance at 3D). The local maxima observed in the lateral PC were significantly stronger in the animals performing the DMS task relative to controls and the DR and DOA working memory groups (main effect: $F = 4.05$, df = 4, $P = 0.03$; Fisher’s LSD post hoc comparisons revealed DMS > SMC, $P < 0.01$; DMS > DR, $P = 0.05$, DMS > DOA, $P < 0.02$; see Fig. 8). In contrast, group differences were not evident for local maxima in the fundus of the rhinal sulcus (main effect: $F = 1.054$, df = 4, $P = 0.428$; see Fig. 8).
longer delay intervals (Murray and Mishkin 1984). The present data support these findings in showing that the rhinal cortex is activated in animals performing the DMS with short delay intervals (12 s). Our study further reveals that LCGU is relatively more enhanced in the perirhinal region than the entorhinal region for this task. These findings are also consistent with lesion studies showing that lesions restricted to the PC cause as severe a deficit in memory performance as lesions following combined entorhinal and perirhinal lesions (Meunier et al. 1993).

Increases in LCGU above control levels were observed in the PC in the animals performing the DA task but not the animals performing the DR task. This result may in part explain why a number of previous studies involving medial temporal lobe ablations have failed to show deficits on DR tasks (Mahut 1971; Murray and Mishkin 1986; Zola-Morgan and Squire 1985), whereas such lesions produce impairments on spatial DA tasks (Correll and Scoville 1967; Mahut 1971; Mahut and Cordeau 1963; Zola-Morgan and Squire 1985). The differential activations observed between the DA and DR tasks suggest that the two tasks may differ in ways beyond reliance on spatial working memory processes. In the present study, many more trials were required to reach criterion on the DA task than on DR (see Table 1). In contrast to DR, performance on both DA and DOA requires continuous updating of the location or identity of the previous stimulus similar to n-back tasks used in humans (Smith and Jonides 1998). Their continuous nature introduces a higher level of proactive interference than is present in DR, where each trial is independent of the previous trial. Another significant parameter that may differentiate DA, DOA, and DMS from DR is the requirement in the former tasks to suppress movement toward the location or object where the food reward was last seen. In DMS, monkeys have to overcome their strong predisposition to approach novel objects (Mishkin and Delacour 1975). Alternatively, activation of the PC by the working memory tasks may be modulated by increased processing demands while activation of the PC by the DMS task may be related to the process of object recognition as indicated by recent lesion and electrophysiological studies (Fahy et al. 1993; Gaffan and Murray 1992; Meunier et al. 1993; Xiang and Brown 1998; Zhu et al. 1995; Zola-Morgan et al. 1989). The importance of task parameters for adjusting processing demands is evident in lesion studies, where, for example, deficits following perirhinal ablations in monkeys can be revealed by increasing processing demands, either by lengthening delays, using foils, increasing the number

FIG. 7. Example of local maxima in the rhinal cortex at 4 similar rostrocaudal levels in four animals performing memory tasks. A1–A4: DMS animal; B1–B4: DOA animal; C1–C4: DA animal; D1–D4: DA animal. sts, superior temporal sulcus; amyg, amygdala.
Functional heterogeneity within EC

One of the most striking results emerging from this study is the heterogeneity of glucose uptake within the EC. Specifically, medial regions of EC show strikingly lower LCGU values than lateral regions. In fact, the lateral regions of EC appear more similar to perirhinal regions than to medial EC regions in terms of the general levels and patterns of glucose uptake seen across all animals. This is interesting in light of previous anatomical investigations that have demarcated the region medial to the rhinal sulcus [referred to here as lateral EC] based on divisions by Suzuki and Amaral (1994) as the “prorhinal” cortex based on connectional and cytoarchitectonic criteria. For example, Insauti et al. (1987) have shown that the lateral regions of EC receive a much stronger cortical innervation than the medial EC. Nevertheless, they still include this region as part of the EC (see Insauti et al. 1987, p. 391). The present data, however, may renew the debate as to whether this region is to be considered part of the EC or a transitional zone between EC and PC based on physiological grounds of differential glucose uptake. Further evidence for a heterogeneity within the EC comes from the present trend (P = 0.069) for the animals performing the DA task, a spatial working memory task, to show greater activation in lateral regions of EC. Specifically, it may imply that the lateral EC, which receives a stronger input from area TF of the parahippocampal cortex (Insauti et al. 1987), as well as PC may be activated by spatial memoranda while the lateral perirhinal region, which receives a stronger innervation from inferotemporal cortex, may be preferentially activated by object information (Suzuki et al. 1997). In light of the heterogeneity of the rhinal cortex observed in the present study of memory-guided behavior, further heterogeneity in its functional architecture can be expected and deserves further investigation.

Local maxima within the EC and PC

The local maxima analysis revealed that all animals exhibited local maxima within the fundus of the rhinal sulcus and the lateral PC. However, although the local maxima in the fundus was prominent in all animals, there were no group differences in the quantitative estimates of LCGU within this region. The area in the fundus of the rhinal sulcus is often included in lesions of either the entorhinal or perirhinal cortices and it is possible that damage to this region may cause nonspecific deficits since we have shown that it is equally active in all animals, including controls.

In contrast to the activations observed in the fundus, the local maxima in the lateral perirhinal region revealed that the animals performing the DMS task exhibited the highest levels of LCGU in this region. As the PC receives substantial cortical input from inferotemporal cortex (Suzuki and Amaral 1994), it is possible that this increase reflects neuronal activation resulting from the greater number of novel visual objects seen by the DMS animals compared with animals in the other groups. However, it seems unlikely that this activation is purely perceptual since damage to PC has been shown to leave perception intact while altering memory abilities (Buffalo et al. 1998; Gutnikov et al. 1997). Thus given the wealth of data supporting the crucial role of the PC in memory, these focal activations in lateral PC may reflect the involvement of the neural tissue most responsible for supporting stimulus memory within this region, and, arguably, within the medial temporal lobe.

While an overall significant increase in LCGU in the PC was observed in the monkeys performing the DA and DOA tasks, no evidence of focal activity comparable to that observed in the DMS monkeys was observed. It is possible that such a focus would be found outside the regions of interest analyzed in the present study. Indeed the dorsolateral prefrontal cortex, which has been shown to support performance of the DA and DOA tasks, has stronger connections with the hippocampal formation through the parahippocampal cortex and the caudomedial lobule than through the perirhinal cortices (Goldman-Rakic et al. 1984). Future studies could be directed toward examining the role of these posterior structures in the battery of tasks employed here. It also remains to be determined whether the focal activation exhibited by the DMS group is directly or multisynaptically connected to the ventral/orbital regions of the prefrontal cortex that have been implicated in the performance of DMS tasks (Bachevalier and Mishkin 1986; Kowalska et al. 1991; Meunier et al. 1997; Parker and Gaffan 1998; Passingham 1975) distinct from dorsal lateral regions engaged in the performance of working memory tasks (Goldman and Rosvold 1970; Wilson et al. 1993).

We thank Dr. Harriet Friedman for valuable contributions to the earlier stages of this investigation. We also thank J. Coburn, M. Pappy, and H. Findlay for help in cryostat sectioning of the large primate brains; J. Hickey for help in training animals; T. Beattie for help in all phases of the experiment; and L. Romanski, S. O’Scalaidhe, and N. Vae for helpful comments on the manuscript.
REFERENCES


GAFFAN D and MURRAY EA. Monkeys (Macaca fascicularis) with rhinal cortex ablations succeed in object discrimination learning despite 24-hr intertrial intervals and fail at matching to sample despite double sample presentations. Behav Neurosci 106: 30–38, 1992.

GELLERMAN LW. Chance orders of alternating stimuli in visual discrimination experiments. J Gen Psychol 42: 207–208, 1933.


GUTNIKOV SA, MA YY, BUCKLEY MJ, and GAFFAN D. Monkeys can associate visual stimuli with reward delayed by 1 s even after perirhinal cortex ablation, uncinate fascicule section or amygdalectomy. Behav Brain Res 87: 85–96, 1997.


MURRAY EA, BAXTER MG, and GAFFAN D. Monkeys with rhinal cortex damage or neurotoxic hippocampal lesions are impaired on spatial scene learning and object reversals. Behav Neurosci 112: 1291–1303, 1998.


