The Journal of Neuroscience, December 1988, 8(12): 4693–4706

Activation of the Hippocampus and Dentate Gyrus by Working-Memory: A 2-Deoxyglucose Study of Behaving Rhesus Monkeys

Harriet R. Friedman and Patricia S. Goldman-Rakic

Section of Neuroanatomy, Yale University School of Medicine, New Haven, Connecticut 06510

The 2-deoxyglucose method was used to examine metabolic activity in the hippocampus, dentate gyrus, and amygdala of rhesus monkeys performing working-memory and control tasks. A working-memory group was tested on 1 of 3 tasks requiring trial-by-trial updating of information: delayed spatial response, delayed spatial alternation, or delayed object alternation. A control group was tested either on an associative memory problem, visual pattern discrimination, or a sensory-motor task that did not have an explicit mnemonic component. Local cerebral glucose utilization (LCGU) in specific layers of the dentate gyrus and the CA1 and CA3 sectors of the hippocampus, as well as in 7 distinct nuclei of the amygdala, was measured and compared across groups.

Metabolic rate in specific layers of the dentate gyrus and the CA3 and CA1 fields of the hippocampus was enhanced in the working-memory compared with the control group: LCGU was between 18 and 24% higher in the granule cell and molecular layers of the dentate gyrus and in the molecular and radiatum layers of CA1 and CA3 in the hippocampus. In contrast, no significant group differences in LCGU were found for any of the 7 amygdaloid nuclei examined: the lateral, lateral basal, medial basal, accessory basal, cortical, central, and medial nuclei.

These results are consistent with previous evidence showing that lesions of the hippocampus affect memory selectively, producing deficits on some memory problems while sparing others. Our findings further suggest that working-memory may be a common denominator among those tasks that are sensitive to hippocampal damage in monkeys. The contribution of the amygdala to performance on memory tasks, on the other hand, appears to be independent of the specific type of memory process that is engaged.

Although damage to the hippocampus has long been associated with memory loss in both humans (Scoville and Milner, 1957) and animals (Mishkin and Pribram, 1954; Pribram et al., 1962; Correll and Scoville, 1967; O'Keefe and Nadel, 1978; Olton et al., 1979), recent studies have offered new insights concerning the nature of the mnemonic processes affected (Mishkin et al., 1984; Mahut and Moss, 1984; Squire and Zola-Morgan, 1985) and the contribution of specific sectors of the hippocampus to these processes (Zola-Morgan et al., 1986). The character of medial temporal lobe amnesia has recently been delimited by reports showing that memory for tasks that tap perceptual and motor skills is not impaired, even in the case of H.M., the exemplar for human temporal lobe dysfunction (Milner et al., 1968; Milner, 1970; Cohen et al., 1985; Corkin et al., 1985). Medial temporal lobe damage, therefore, appears to leave "procedural" (Squire and Cohen, 1984; Cohen et al., 1985) or "habit" (Mishkin et al., 1984) memory intact. By contrast, the performance of these patients on a variety of verbal and spatial cognitive tasks (Scoville and Milner, 1957; Milner, 1970; Corkin et al., 1985) has indicated profound impairments on what has been termed "declarative" memory (Squire and Zola-Morgan, 1983; Squire and Cohen, 1984).

Selective memory deficits also are produced by hippocampal lesions in the monkey (Mishkin and Pribram, 1954; Orbach et al., 1960; Pribram et al., 1962; Mahut, 1971; Zola-Morgan and Squire, 1986) and in the rat (Olton et al., 1979; Jarrard, 1980; Kesner, 1985). As in the clinical literature, a dichotomy exists with respect to the kinds of memory tasks that are and are not affected by damage to the hippocampal formation. Generally, tasks in which information must be remembered for only a limited period of time, that is, working-memory tasks (Honig, 1978; Olton et al., 1979; Baddeley, 1982), are particularly sensitive to hippocampal damage. For example, rats with hippocampal lesions are impaired on radial maze paradigms that specifically require working-memory (Olton et al., 1979; Olton, 1983; Kesner, 1985). Similarly, monkeys with hippocampal lesions display poor performance on delayed nonmatching-to-sample tests (Mahut et al., 1982; Zola-Morgan and Squire, 1986) and on spatial mnemonic tests (Pribram et al., 1962; Mahut, 1971), both of which also engage working-memory. In contrast, hippocampal damage does not seriously impair the performance of monkeys on relatively simple associative memory tasks in which the stimulus–response contingencies remain constant across trials (Zola-Morgan and Squire, 1984, 1986). Therefore, it is possible that working-memory is a common process engaged by tasks sensitive to hippocampal damage in monkeys and is one among other mnemonic processes such as memory consolidation (e.g., Mahut and Moss, 1984) that involve the hippocampus.

Just as the memory processes mediated by the hippocampal formation are becoming specified, so also are the neural com-

Received Nov. 10, 1987; revised Apr. 18, 1988; accepted Apr. 22, 1988.

Preliminary data from this report were presented at the Dallas, Texas meeting of the Society for Neuroscience, 1985. This work was supported by USPHS Grants MH38546, MH00298 and NS22807. We wish to thank L. Adel, J. Coburn, L. Ladewig, J. Mazer, J. Musco, M. Pappy, and L. Yao for expert technical assistance. We especially thank N. B. Riley for her invaluable contribution to early stages of this project.

Correspondence should be addressed to H. R. Friedman, Section of Neuroanatomy, C-303, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510.

ponents that contribute to this function. In their seminal analysis, Scoville and Milner (1957) emphasized the relationship between the extent of hippocampal damage in humans and the severity of consequent amnesia. However, the contribution of other regions, particularly the amygdala, to the amnesic syndrome has not yet been fully dissociated from that of the hippocampus because the kinds of traumatic brain injury that produce amnesia in humans, for example, anoxia and stroke, usually damage multiple brain sites. Similarly, the components of the medial temporal lobe lesion that produces amnesia in the nonhuman primate are still being assessed (Horel, 1978; Moss et al., 1981; Zola-Morgan et al., 1982; Murray and Mishkin, 1986). While damage to the hippocampal formation is sufficient to impair performance on some memory tasks (Mahut et al., 1982; Zola-Morgan and Squire, 1986), additional damage to the amygdala may be necessary to produce a substantial deficit in recognition memory (Mishkin, 1978; Murray and Mishkin, 1984; but see Squire and Zola-Morgan, 1988).

To provide further insight into the role of the hippocampus, dentate gyrus, and amygdala in memory processes, we used the ^{14}C-2-deoxyglucose (2-DG) method (Sokoloff et al., 1977) to study functional metabolic activity in these brain areas of monkeys performing working-memory tasks. This study is a departure from many previous experiments on memory in the nonhuman primate and from previous 2-DG experiments because intact, rather than brain-damaged, monkeys were used and because the experimental variable was performance on a cognitive task. We measured glucose utilization in specific components of the hippocampus: the CA3 and the CA1 sectors of Ammon's horn, in the dentate gyrus and in specific amygdaloid nuclei to determine whether these areas are selectively or differentially engaged by tasks with working-memory requirements.

Three different working-memory tasks were used in the present study: two were tests of spatial memory (delayed response and delayed alternation) and one was a test of nonspatial memory (delayed object alternation). The delayed spatial response and delayed spatial alternation tasks both require trial-by-trial updating of information about the spatial position of the reward. The delayed object alternation test, like its spatial analog, also requires that the monkey remember the events of the preceding trial because the correct response requires an accurate memory of the object previously selected. Therefore, the 3 paradigms were alike in their reliance upon working-memory processes but differed in the nature of the information to be remembered. We compared glucose utilization rates in the dentate gyrus, in the CA1 and the CA3 sectors of the hippocampus, and in the amygdala of monkeys performing working-memory tasks with rates in the same brain regions of a control group of monkeys who performed tasks that did not engage working-memory. Monkeys in this control group were tested on either a well-learned visual pattern discrimination task in which the association between the stimulus and the correct response was invariant across trials or on a sensory-motor task that had no explicit mnemonic component but was similar to all the other tasks in its sensory, motor, and motivational aspects.

Materials and Methods

Subjects

Fifteen male rhesus monkeys (*Macaca mulatta*) weighing 2.0–6.0 kg were trained on 1 of the 5 different behavioral paradigms used: delayed spatial response, delayed spatial alternation, delayed object alternation, visual pattern discrimination, and a sensory-motor control task. All monkeys were housed individually, and their diet was adjusted to maintain a stable level of motivation.

Apparatus and test procedures

Monkeys were restrained in a primate chair and tested in a modified Wisconsin General Test Apparatus (WGTA) containing a test tray with 2 recessed food wells and an opaque screen that could be lowered and raised to limit access to the tray. The WGTA was inside a darkened, sound-shielded room that also housed a white-noise generator that supplied a constant level (90 dB) of background noise during testing.

Behavioral training was preceded by several sessions in which monkeys were chair-adapted in the WGTA and were taught to displace 3-dimensional objects (delayed object alternation) or square (8 cm) cardboard plaques (all other test paradigms) in order to retrieve a reward (peanut quarters, raisins, or small pieces of apple). Monkeys were initially trained using short delay and intertrial intervals (0–5 sec). After achieving proficiency (85% correct in 100 trials) on this preliminary version of a given task, the intertrial delay, the number of trials per session, and the duration of each test session were gradually increased over a period of weeks and months until criterion (90%) was achieved at the prescribed delay in a 45–50 min daily test session. The 2-DG test was scheduled thereafter. The procedures of these behavioral tests have been previously described (Pribram and Mishkin, 1956; Goldman, 1971) and are reviewed briefly below.

Delayed spatial response (Fig. 1). Three monkeys were trained on this task. Monkeys observed as one well was baited (cue phase) and then both wells were covered with identical cardboard plaques. A 12 sec delay period followed, during which visual contact with the wells was prevented by the lowered screen. After the delay, the screen was raised and the appropriate plaque could be displaced to retrieve the reward (response phase). The position of the reward was randomly varied on each trial (Gellerman, 1933).

Delayed spatial alternation (Fig. 1). In this task, wells were baited on each trial with the screen lowered. On the first trial of each session both wells were baited and covered with identical cards; thereafter, only the well not selected on the preceding trial was baited. Thus, the position of the previous correct response must be remembered to select the alternate well and obtain the reward on each new trial. Four monkeys were trained. Two were assigned to a 12 sec, and 2 to a 30 sec delay condition. One of these (DA3) had previously received training in the delayed object alternation paradigm.

Delayed object alternation (Fig. 1). Two monkeys were trained on this paradigm in which features of objects rather than spatial location (as in delayed spatial response and delayed spatial alternation) provided the relevant information for correct performance. A blue cube (6.5 cm square × 3 cm high) and a green cylinder (6.5 cm diameter × 8 cm high) were used to cover the wells. Training occurred in stages. Monkeys first were taught a simple object discrimination reversal task using a criterion of 90% correct in 60 trials before reversing the reward contingencies. The number of trials to reversal was decreased in stages from 60 to 30, 15, 10, and 5 trials until 1-trial alternation was achieved, and the intertrial interval was gradually increased to 12 sec. The spatial position of the objects, and hence the reward, was randomly varied (Gellerman, 1933). Therefore, as in spatial alternation, information about the immediately preceding response must be used to guide the correct response on each successive trial.

Visual pattern discrimination (Fig. 2). In this paradigm, 4 monkeys were trained to discriminate between 2 visual stimuli that were shown simultaneously on each trial. Both stimuli were white cut-outs (5 × 5 cm) pasted on black cardboard; the S+ was a plus sign and the S− was an outline of a square. The same stimuli were used on all trials across all test sessions. A 10 sec delay separated all trials. The spatial position of the stimuli was randomly varied (Gellerman, 1933).

Sensory-motor control (Fig. 2). Two monkeys were assigned to this condition in which one or both of the wells were baited and identical cards were placed on the testing board, sometimes covering the wells, sometimes not. The bait always was retrieved on each trial. Thus, the sensory stimuli and motor responses used in this paradigm were similar to those present in all other tasks. Although the screen was lowered for 12 sec between trials, the monkey did not have to remember anything during this delay because the reward was automatically provided on each trial. Monkeys were trained on this paradigm until they were adapted to the testing situation and reliably retrieved rewards throughout a 45 min session.

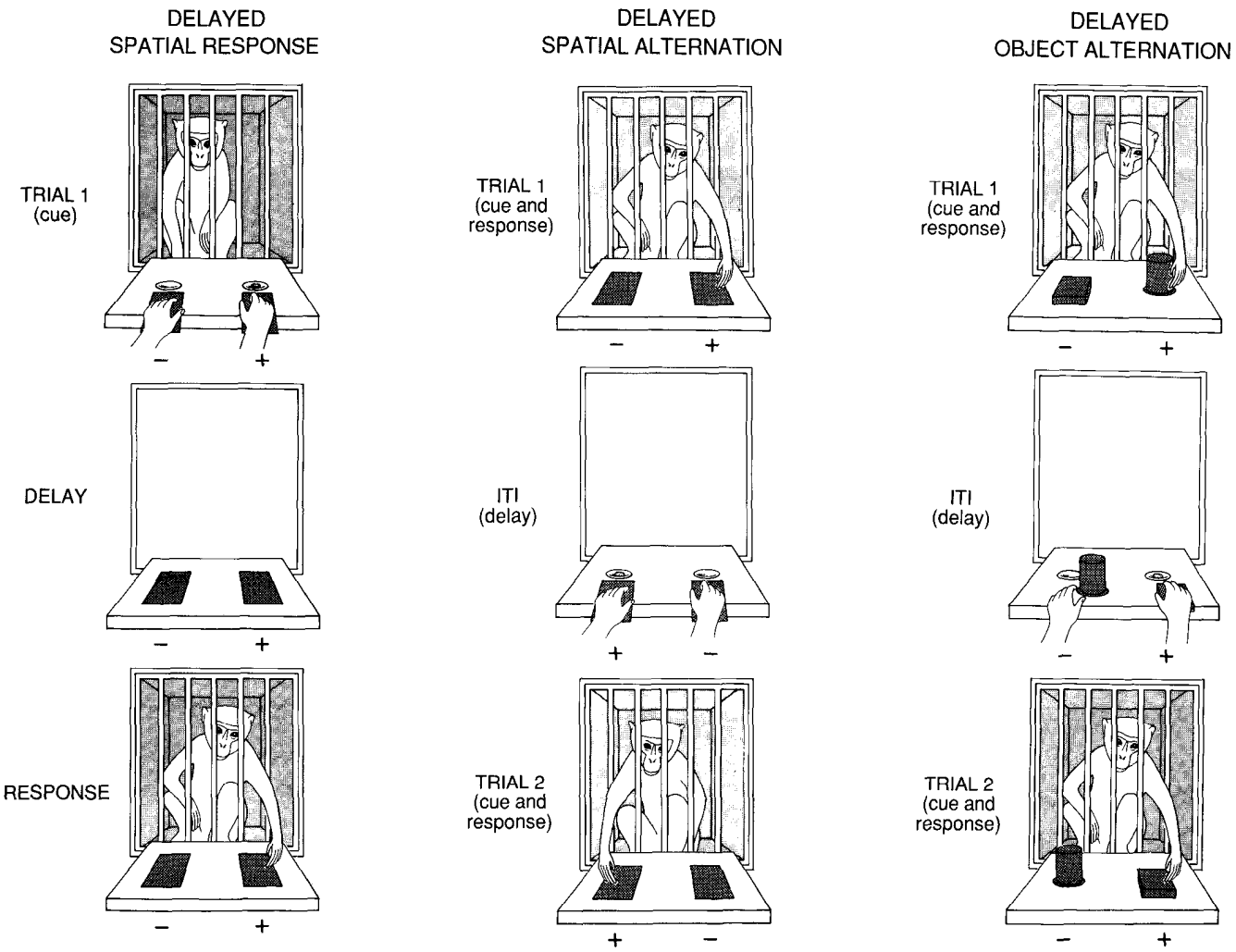

Figure 1. The working-memory tasks. *Left,* In the *delayed spatial response* task, the monkey watched as one well was baited (*cue*) and both wells were covered by identical cards. The screen was lowered for the delay and then raised to permit a response. *Middle,* In the *delayed spatial alternation* task, wells were baited and covered with the screen down during the intertrial interval (*ITI*). On successive trials (e.g., Trials 1 and 2) alternate wells were baited. *Right,* In the *delayed object alternation* task, wells were baited and covered with the screen down during the intertrial interval (*ITI*). Alternate objects covered the baited well on successive trials (e.g., Trials 1 and 2).

2-DG

Preparation. The quantitative DG method described by Sokoloff et al. (1977) was followed. Monkeys were anesthetized using a mixture of nitrous oxide and halothane gas in conjunction with local anesthetics for the insertion of catheters into the femoral artery and vein of one leg prior to the 2-DG test. These animals sat in the primate chair for at least 2 hr to insure recovery from the effects of anesthesia prior to behavioral testing. In 5 cases, however, the catheterization was performed about 24 hr prior to the 2-DG injection to further promote alert testing performance. In these instances, monkeys received ketamine (10 mg/kg) in addition to the anesthetic gas mixture and catheters were inserted under aseptic conditions. Sterile catheters were inserted and tied to the femoral vessels, 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 exposed and opened prior to the 2-DG experiment. Subcutaneous lidocaine and topical anesthetics were applied liberally during surgery and before the 2-DG experiment for both procedures.

Experimental session. Five minutes into the test session, monkeys were injected with ^{14}C-2-DG (100 μCi/kg in 1 μCi/10 μl sterile saline, 50–60 mCi/mM; American Radiolabeled Chemicals) followed by a saline flush. Timed arterial blood samples were taken over the next 45 min, after which the monkey was killed with an overdose of sodium pentobarbital. In 5 cases, the brain was rapidly removed, sectioned into blocks, and immersed in cold (−40°C) isopentane. In all other cases, monkeys were first perfused through the heart using a buffered 3.3% paraformaldehyde solution (1.5–2.0 liter, pH 7.4). This procedure was adopted because it consistently improved the quality of the sectioned tissue. All brain tissue was stored at −70°C.

Tissue processing. Tissue sections 20 μm thick were cut at −22°C on a cryostat (Bright Instruments). Four serial sections were saved every 400 μm throughout the brain; one section was picked up on a glass slide and stained with cresyl violet, and the remaining 3 adjacent sections were picked up on cold coverslips and rapidly dried on a hot plate. Coverslips were taped to cardboards and exposed to X-ray film (SB5, Kodak) for 8–10 d together with a set of polymethylmethacrylate ^{14}C standards (0–1.08 μCi/g, Amersham). Films were processed using developer and fixative (GBX, Kodak) according to packaged instructions.

Blood glucose and ^{14}C levels. Blood samples taken during the experiment were centrifuged after collection, and 20 μl plasma samples were analyzed immediately after the experiment for glucose (Beckman Glucose Analyzer 2) and for ^{14}C concentration using a liquid scintillation counter. Figure 3 shows a representative set of concentration curves for one monkey during the course of the 2-DG experiment. Integrated arterial plasma specific activities were derived from the blood concen-

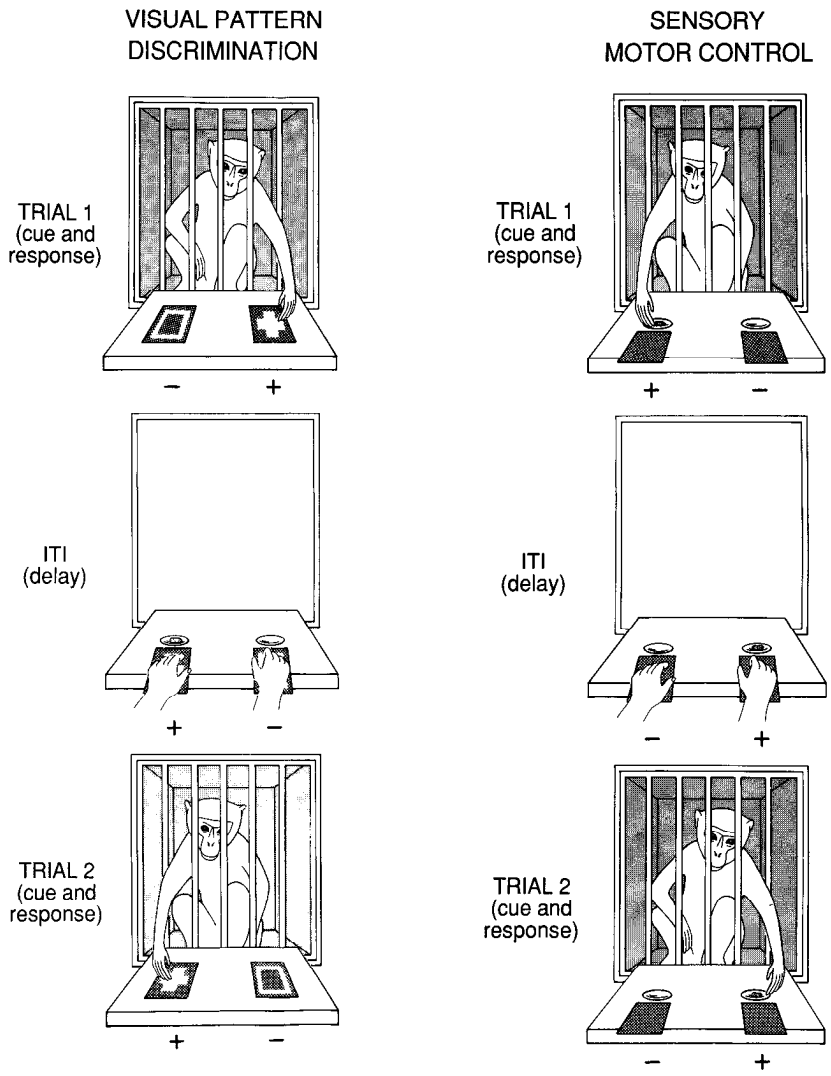

Figure 2. The control tasks. *Left,* In the *visual pattern discrimination* task, wells were baited and covered with patterned cards during the ITI (screen down). One pattern, a plus sign, always covered the baited well. *Right,* In the *sensory motor* task, the wells were baited during the ITI (screen down) and the rewards were retrieved by the monkey on each trial.

tration curves, and these were used to convert tissue ^{14}C concentrations to local cerebral glucose utilization (LCGU) as described by Kennedy et al. (1978) for the monkey.

Quantification of autoradiograms

Autoradiograms were digitized using an image-processing system comprised of a PDP-11 computer equipped with a Datacube graphics board (768 × 512 pixels, 256 gray levels), a Dage MCI video camera, and a color video monitor. The set of ^{14}C standards that were coexposed on each film with the tissue sections were digitized and used to quantify the ^{14}C radioactivity in the brain images. Gray-values were translated to ^{14}C radioactivity concentrations, and these were converted to LCGU using the integrated plasma specific activities (see above).

To measure LCGU in anatomically defined regions of the brain, we developed a computer program in which the brain regions of interest were first defined on a cresyl violet-stained tissue section and then LCGU was automatically determined for corresponding areas in the adjacent autoradiogram. The procedure was to designate regions of interest by placing an array of computer-generated box outlines on the digitized image of the cresyl violet section which was visible on the video monitor; the autoradiogram of the adjacent section was then aligned to this image. The computer digitized the autoradiogram image and transposed the box array onto this new image, and LCGU was subsequently given for each area outlined by these boxes. An array of boxes was used so that all regions of interest in a given autoradiogram were analyzed at the same time. The individual boxes which outlined each layer or nucleus were adjusted to accommodate the size, shape, and orientation of these areas across the anterior–posterior extent of the temporal lobe; however, the box arrays used were quite comparable for all monkeys.

Sampling LCGU in the dentate gyrus and hippocampus. Both the dentate gyrus and the hippocampus consist of a superficial portion, a deeper polymorphic cell portion, and a middle principal cell layer (Lorente de No, 1934). Sample measurements of LCGU were taken separately from these characteristic laminae (Fig. 4). In the dentate gyrus, 3 box outlines were used to sample LCGU in the molecular layer, the granule cell layer, and the polymorphic layer (hilus). The CA3 and CA1 sectors were selected to represent the hippocampus. In both CA3 and CA1, LCGU was measured in samples from the pyramidal cell layer and the polymorphic layer, termed oriens/alveus. In the superficial portion of CA1, we could distinguish 2 layers, the molecular and the radiatum, in the cresyl violet-stained sections, and these areas were separately measured. The curvature of the hippocampus made it difficult to similarly differentiate the superficial portion of CA3 in the digitized cresyl violet sections. Therefore, only one measurement was made superficial to the pyramidal layer, which was termed molecular/radiatum as it overlapped both of these layers. Therefore, LCGU was determined for 10 laminar subdivisions of the hippocampus and dentate gyrus in each autoradiogram.

In each monkey, a uniform area through 5–6 mm of the anterior–posterior extent of the hippocampus and dentate gyrus was analyzed. This sample area represented ⅓ of the hippocampus and dentate gyrus beginning with the postuncal portion of the anterior hippocampus and extending through the dentate gyrus and main body of the hippocampus to the posterior limit of the medial pulvinar. Throughout this sample region, the hippocampus is fully developed, and the envelope of the dentate gyrus in the CA fields is most recognizable. One side was ar-

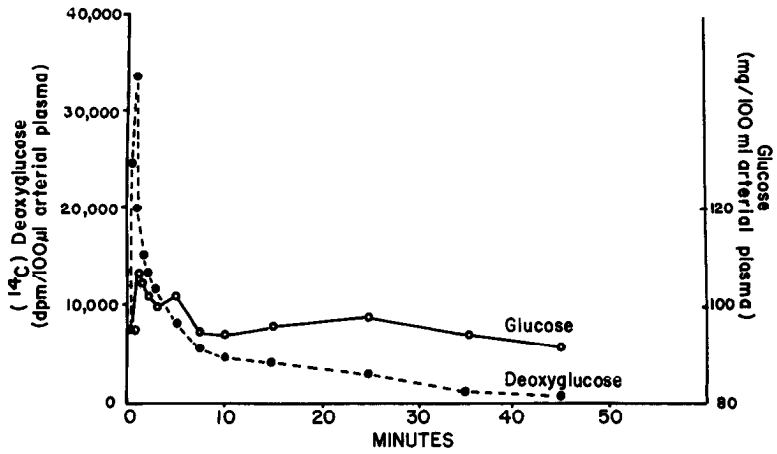

Figure 3. Representative blood glucose and ^{14}C radioactivity concentration curves for the 45 min 2-DG test session. Blood samples from each monkey were collected at 14 time points during the 2-DG test session. Note that blood radioactivity concentration rapidly peaks and declines, whereas blood glucose levels remain relatively stable during the course of the experiment.

bitrarily selected for measurement, although in 3 monkeys, the other hemisphere was substituted because the tissue was better. In total, LCGU measurements were taken from the hippocampus and dentate gyrus of 13 monkeys. In 2 additional monkeys (DR1 and VD1), cutting artifacts precluded measurement of the hippocampus and dentate gyrus but not the amygdala.

Each hippocampus and dentate gyrus was sampled at 10–15 different levels (at least 400 μm apart) through the anterior–posterior sample area. We digitized 2–3 adjacent sections at each level. The median value across adjacent sections for a given layer was taken as the LCGU value for that layer at each specific level, and the arithmetic average of these medians through the entire sample region was used as the summary LCGU value for a given layer. Thus, each summary value represented 30–45 separate measurements of a given layer. These summary values were used in the statistical analysis of individual layers. As a general index of LCGU for the entire dentate gyrus, CA3 and CA1 sectors, we used the arithmetic average of these summary values across the sampled layers in each structure.

Amygdaloid nuclei. We used the cytoarchitectonic study of Crosby and Humphrey (1941) and later elaboration of this work (Aggleton and Mishkin, 1984) as a guide for outlining specific nuclei in the amygdala. In the amygdala, boxes were placed to measure LCGU in the nuclei of the basal-lateral group: the lateral, lateral basal, medial basal, and accessory basal nuclei, as well in the medial, central, and cortical nuclei (Fig. 5). A 2–2.5 mm anterior–posterior region of the amygdala through which the basal nuclear group is most expansive and well-defined was selected as the sample region. This region was marked anteriorly by the crossing of the anterior commissure and posteriorly by the insinuation of the pole of the hippocampus in the medial temporal lobe. The amygdala was sampled at 5 levels (each separated by at least 400 μm) through this anterior–posterior extent. Adjacent sections (usually 3) at each level were measured. The median measurement for a given nucleus across adjacent sections was taken as the LCGU value for that nucleus at each level. The arithmetic average of these medians was used to summarize the LCGU of a specific nucleus, and these values were statistically analyzed.

Statistical analysis

The major goal of this study was to determine whether working-memory differentially influences glucose utilization rates in the hippocampus, the dentate gyrus, and in the amygdala. Monkeys performing delayed spatial response, delayed spatial alternation, and delayed object alternation tasks comprised a working-memory group, and their LCGU data were compared with the LCGU data from the control group comprising monkeys tested in the visual pattern discrimination and sensory-motor tasks. These groups were compared by an analysis of covariance (see below and Winer, 1971, pp. 752–812) using a computer-based statistical package (SYSTAT; Wilkinson, 1986).

Analysis of covariance. Individual differences in overall brain metabolism coupled with minor differences in the 2-DG protocol can contribute undesirable variance in 2-DG experiments (Gallistel et al., 1982; Eilbert, 1986). Because we were examining between-group differences in LCGU, a model of the analysis of variance (ANOVA) was selected in which a covariate is used to factor-out such unwanted individual effects. The covariate needed is the LCGU of a brain region unlikely to be differentially affected by the task manipulation. The medial geniculate body (MGB) was selected as this control region because there was no reason to suspect that this thalamic auditory nucleus would be differentially influenced by the different tasks as the same white masking noise was present during experiments. Indeed, the average LCGU (in μmol/100 gm/min) for the MGB (measured bilaterally in 3 adjacent autoradiograms at 4–5 different anterior–posterior levels in each monkey) was nearly identical for both groups: 75.59 for the working-memory group and 75.45 for the control group, despite a large range of values within both groups. This group difference in medial geniculate LCGU was highly insignificant by ANOVA [$F(1, 13) = <0.001$, ns]. Whereas LCGU for MGB did not differentiate the working-memory and control groups, these data were highly significant predictors of each monkey's individual LCGU for the dentate gyrus, CA3, and CA1 sectors of the hippocampus [$F(1, 10) = 11.14$, 13.73, and 6.66, $p \leq 0.05$, for these areas respectively in 13 monkeys] and for all the nuclei measured in the amygdala [$F(1, 12) = 10.33$, 10.63, 15.48, 17.52, 19.55, 25.24 and 27.31; $p < 0.01$ for the 7 amygdaloid nuclei in 15 monkeys]. The high correlation between individual medial geniculate LCGU and LCGU in these other regions of interest indicates the importance of individual differences in LCGU and validates the use of the MGB as a covariate factor in the analysis of the data.

The ANOVA model used—LCGU (per layer or nucleus) = $LCGU_{Norm}$ + ($LCGU_{MGB}$) + Behavioral Paradigm—essentially hypothesized that the LCGU of a particular structure is a linear function of the characteristic glucose utilization rate ($LCGU_{Norm}$) of that structure, the characteristic glucose utilization rate of the individual monkey (the covariate: $LCGU_{MGB}$), and an additional metabolic amount attributable to the specific behavioral paradigm (working-memory vs control). The significance of this last variable, the contribution of working-memory, was the focus of our study.

Results

Behavioral training

The goal of training was to insure reliable criterion performance on each task for the 45 min 2-DG session, and we individually tailored the training regimen of each monkey to facilitate learning. The 2-DG session followed 1 to 8 months of training, depending upon task difficulty. Because the sensory-motor control task had no explicit mnemonic requirement, training was brief. At the other extreme, the delayed object alternation task was the most difficult, requiring an average of 6.5 months and 7400 trials to bring monkeys through the object discrimination reversal stage to the final criterion on 12 sec object alternation. A high failure rate on this task has been reported (Pribram and Mishkin, 1956; Mishkin and Manning, 1978), and monkey DA3 in our study was reassigned from object alternation because he

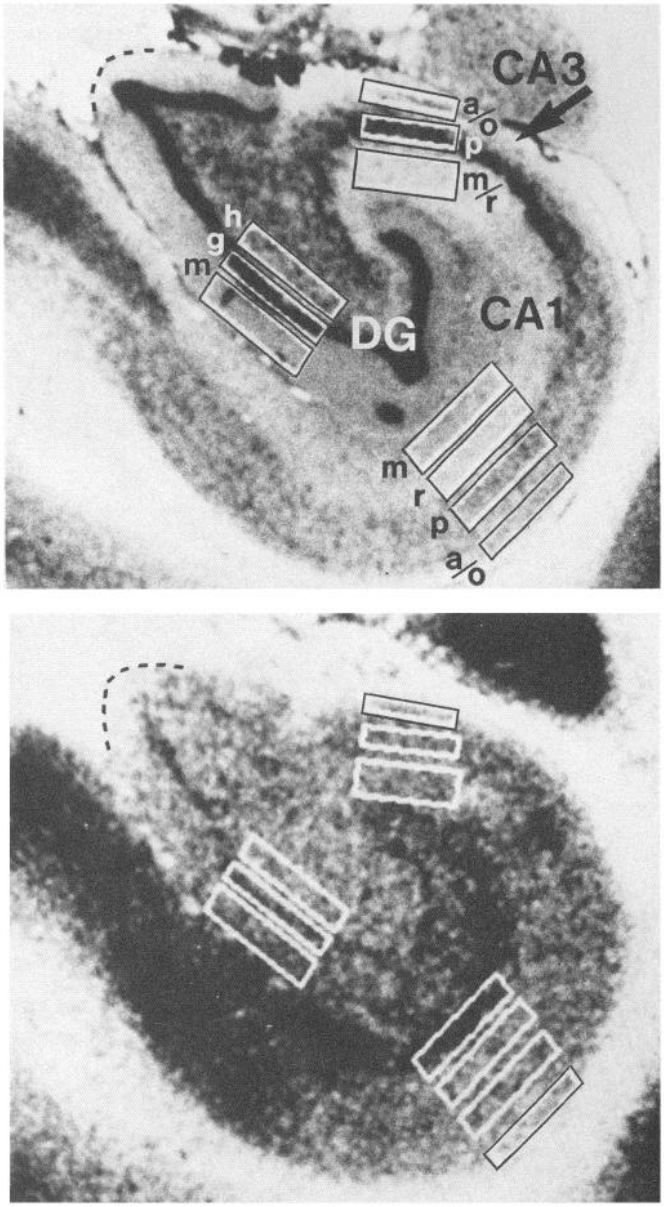

Figure 4. Photographs of an adjacent pair of digitized images through the hippocampus and dentate gyrus sample area. *Top,* A cresyl violet-stained section showing the laminar organization of the dentate gyrus and hippocampus. A representative array of measurement boxes defining the laminae that were measured is superimposed. (The white borders of boxes are outlined for contrast). Boxes are labeled to identify the laminae measured. *Bottom,* The autoradiogram image of the adjacent section. This image was aligned to the cresyl violet image and the box array was superimposed automatically to measure LCGU (see Materials and Methods). Abbreviations: *DG,* dentate gyrus; *g,* granule cell layer; *h,* hilus layer; *m,* molecular layer; *m/r,* molecular/radiatum layers; *r,* radiatum layer; *o/a,* oriens/alveus layer; and *p,* pyramidal cell layer.

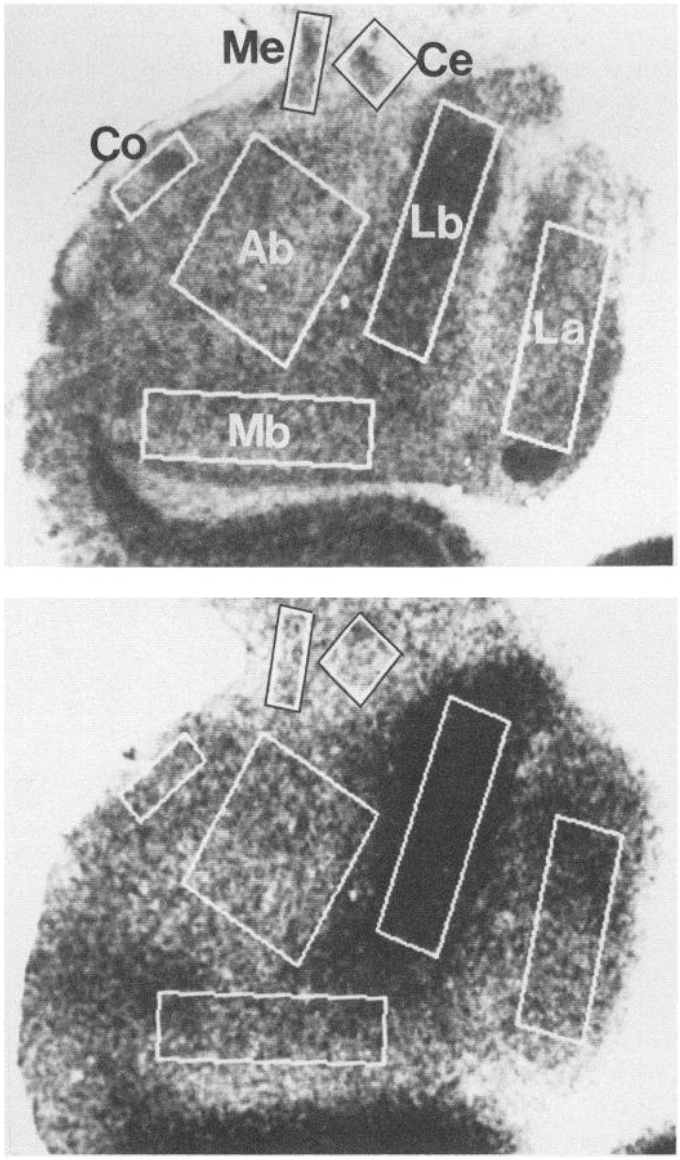

Figure 5. Photographs of an adjacent pair of digitized images through the amygdala sample area. *Top,* A cresyl violet-stained section of the amygdala. A representative array of measurement boxes defining the 7 nuclei measured is superimposed. The labels identify these nuclei. *Bottom,* The autoradiogram image of the adjacent section was aligned to the cresyl violet image and the box array was superimposed automatically to measure LCGU in these nuclei (see Materials and Methods). Abbreviations: *Lat,* lateral; *Lb,* lateral basal; *Mb,* medial basal; *Ab,* accessory basal; *Me,* medial; *Ce,* central; and *Co,* cortical.

did not progress through the stages of the task with sufficient speed. Training averaged 3.5 months on delayed spatial alternation, 3.7 months for delayed spatial response, and 2.0 months for visual pattern discrimination.

Whereas the total duration of training provides one index of task difficulty, these data are not strictly comparable because of differences in the delays and intertrial intervals and because no attempt was made to keep the number of trials per session or the number of sessions constant. The data from an earlier stage of training are more instructive because uniformly short delay and intertrial intervals (0–5 sec) were used. Table 1 shows the number of trials and sessions required for each monkey to achieve initial proficiency (85% correct in 100 trials) on this preliminary version of each task. The mean data shown in Table 1 again indicate that object alternation was most difficult followed by, in order of relative difficulty, delayed spatial alternation, delayed spatial response, and the visual pattern discrimination task. These data show that the initial learning of delayed spatial alternation required more sessions and trials than delayed spatial delayed response, although on the whole, as indicated above, the total duration of training was comparable for both. What is also brought out in Table 1 is the similarity of delayed spatial

response and visual pattern discrimination with respect to initial learning in contrast to their differences with respect to the extent of the complete training regimen: 3.7 vs 1.7 months for delayed spatial response and visual pattern discrimination, respectively. Increments in the temporal delay, between the cue and the response in delayed response and between trials in the discrimination paradigm, differentially affected the difficulty of these 2 tasks. There were no parallel behavioral results for the sensory-motor control task; the preliminary (and final) criterion in this paradigm was met upon completion of sessions (given in Table 1) in which monkeys steadily retrieved the rewards for 45 min in the WGTA.

Experimental performance

Prior to the 2-DG experimental session, monkeys were performing at 90% accuracy on their respective tasks except for delayed object alternation, where 85% correct was acceptable owing to the difficulty of the task. This level of performance was approximated by 11/13 monkeys on the critical test day (Table 1). Response accuracy deteriorated toward the end of the 45 min 2-DG test period for 2 monkeys, DR2 and DA1; the performance of these monkeys was, however, at criterion in the critical early stages of the period during which most ^{14}C-2-DG is incorporated (Sokoloff et al., 1977). The average number of responses (trials) during the 2-DG test session was relatively uniform across tasks in which the delay period was 10–12 sec (Table 1). Thus, if responding is equated with work, the magnitude of work exerted by monkeys was comparable across tasks. On the 30 sec delayed spatial alternation test, however, the average number of trials was roughly half that for the 12 sec spatial alternation task.

Qualitative profile of the hippocampus and dentate gyrus in autoradiograms

The pattern of 2-DG uptake in autoradiograms varied across the layers of the dentate gyrus, the CA3 and the CA1 sectors (see Fig. 4). The pattern observed in each structure was similar across all monkeys. In the dentate gyrus, a band of label corresponding to the granule cell layer was apparent in autoradiograms across monkeys; its width in autoradiograms appeared slightly smaller than in the adjacent cresyl violet-stained sections. The superficial layers of the dentate gyrus and CA1 and CA3 also were dark in the autoradiograms relative to the deeper layers of these sectors.

LCGU in the dentate gyrus, CA3, and CA1

Figure 6 shows LCGU for each layer in CA3, CA1, and the dentate gyrus across the anterior–posterior sample area for 13 monkeys. In CA1 and CA3, LCGU was graded across the layers, being highest in the superficial layers and lowest in the deeper layers of these sectors. In the dentate gyrus, however, LCGU was highest in the granule cell layer, although group differences were most pronounced in the molecular layer.

In every layer measured, average glucose utilization rates were higher for the working-memory group compared with the control group (Table 2). Thus, in the dentate gyrus, LCGU was enhanced in the molecular layer, the granule cell layer, and the hilus. The maximum between-group difference in LCGU was in the molecular layer, where the mean glucose utilization rate was 24% higher in the working-memory group [$F(1, 10) = 13.99$, $p < 0.005$]. The average LCGU in the granule cell layer was 19% greater in the working-memory group, a difference that also

Table 1. Trials and sessions required to achieve proficiency (85% in 100 trials) on preliminary versions of each task and behavioral scores for the 2-DG session

	Preliminary training		2-DG test	
Task	Trials	Sessions	Trials	% Correct
Spatial response				
DR1[a]	240	8	143	96
DR2	150	1	128	84
DR3	240	8	131	95
Mean	210	6	134	92
Spatial alternation				
DA1 (30 sec)	619	8	62	76
DA2 (30 sec)	1208	13	87	97
Mean	—	—	75	87
DA3 (12 sec)	410	5	160	89
DA4 (12 sec)	1205	23	128	91
Mean	860[b]	12[b]	144	90
Object alternation				
OA1	1560	22	127	82
OA2	3100	56	140	86
Mean	2330	39	138	84
Visual discrimination				
VD1[a]	240	8	129	100
VD2	351	9	160	97
VD3	290	9	92	94
VD4	270	5	180	100
Mean	288	8	140	98
Sensory-motor				
SC1[c]	766	6	130	—
SC2[c]	1830	12	180	—
Mean	1298	9	155	—

[a] Monkeys used only in the amygdala analysis.

[b] Mean for all 4 monkeys tested on delayed spatial alternation.

[c] These are for 45 min sessions that preceded the 2-DG test.

was significant [$F(1, 10) = 5.77$, $p < 0.05$]. In the hilus region as well, mean LCGU was enhanced by 15% in the working-memory group relative to the control group [$F(1, 10) = 3.97$, ns].

The average LCGU in the CA3 sector (over all layers) was generally lower than LCGU in CA1 or in the dentate gyrus (Table 2). Nevertheless, the mean LCGU for layers in this region still was enhanced in the working-memory group relative to the control monkeys, although these differences were not statistically significant. As was the case for the dentate gyrus, the largest between-group difference was in the superficial, molecular-radiatum partition, where mean LCGU was 18% higher for the working-memory group [$F(1, 10) = 3.57$, ns]. The average glucose utilization rates in the pyramidal cell [$F(1, 10) = 1.82$, ns] and oriens/alveus layers [$F(1, 10) = 1.34$, ns] were 11 and 9% higher, respectively, in the working-memory group.

The average LCGU was significantly higher for the working-memory group in 3 of the 4 layers measured in CA1. As was the case for the other sectors, the largest between-group difference in LCGU was in the molecular layer, where the mean glucose utilization rate was 21% higher in the working-memory group [$F(1, 10) = 16.36$, $p < 0.005$]. In the radiatum [$F(1, 10) = 12.61$, $p < 0.005$] and the pyramidal layers [$F(1, 10) = 7.75$, $p < 0.02$], mean LCGU also was significantly higher in the working-memory group being, respectively, 19 and 16% higher

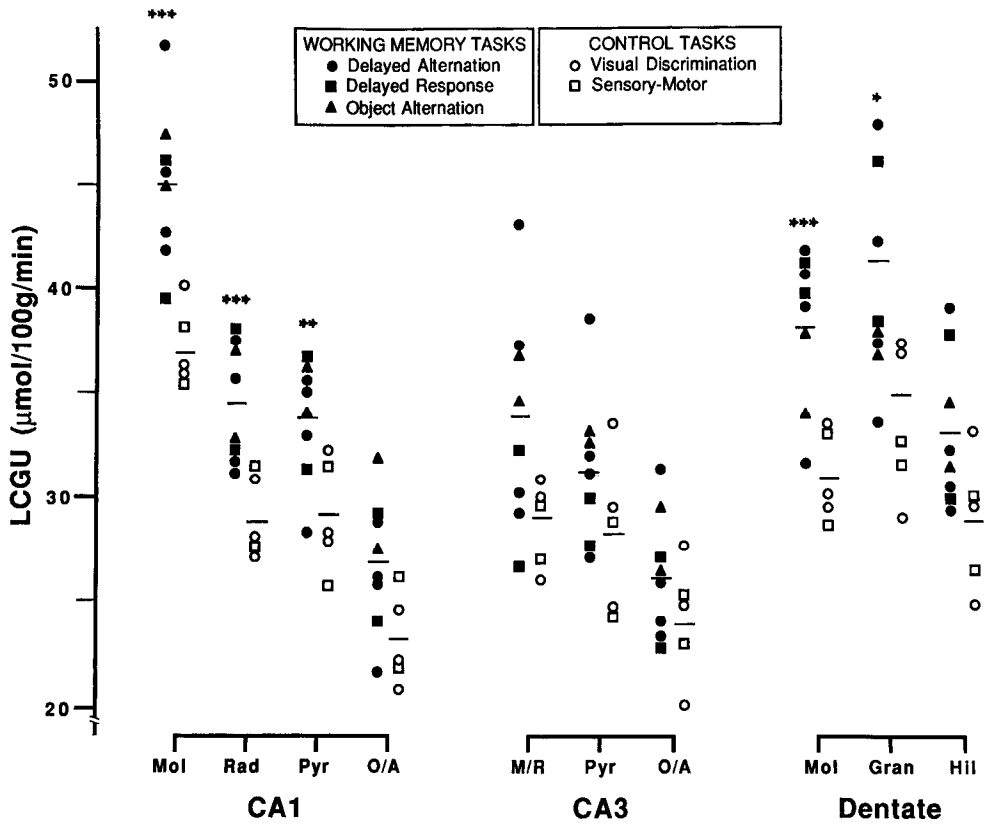

Figure 6. Local cerebral glucose utilization (LCGU) in the hippocampus and dentate gyrus of individual monkeys performing working-memory and control tasks. The *solid horizontal line* in each column is the mean LCGU for each group. Significant differences in LCGU as a function of working-memory are indicated (*, **, *** = $p < 0.05$, 0.02, and 0.005, respectively). Abbreviations: *gran,* granule cell layer; *hil,* hilus; *mol,* molecular layer; *m/r,* molecular/radiatum layers; *o/a,* oriens/alveus; *pyr,* pyramidal cell layer.

for these monkeys. Although the average LCGU in the oriens/alveus area was 16% higher in the working-memory group, this was not a significant enhancement of LCGU [$F(1,10) = 4.54$, ns].

Anterior–posterior differences in LCGU

Anterior and posterior lesions of the hippocampal formation may not be equipotent in producing behavior deficits (Squire and Zola-Morgan, 1983). Therefore, we evenly divided our 6 mm sample area into 3 mm anterior and posterior regions to determine whether glucose utilization values in CA1, CA3, and the dentate gyrus differed along this dimension. The anterior region included the hippocampus and the dentate gyrus from the level of the posterior uncus through the anterior portion of the body of the hippocampus. The posterior region included the body of the hippocampus through the level of the posterior medial pulvinar.

We found that mean LCGU in the posterior sample region was higher than in the anterior sample region (see Table 3). This difference in glucose utilization rate across the anterior–posterior sample area was significant by ANOVAs performed on the anterior–posterior difference scores in the dentate gyrus, CA1, and CA3 [$F(1, 10) = 7.60$, 6.21, and 15.80, all $p < 0.05$, respectively). This enhancement of LCGU in the posterior region relative to the anterior region was not differentially influenced by working-memory [$F(1, 10) = 2.40$, 2.17, 0.34, all ns, for the dentate gyrus, CA1, and CA3, respectively]. Instead, working-memory performance enhanced glucose utilization rates in both the anterior and posterior regions of CA3, CA1, and the dentate gyrus (Table 3). Thus, LCGU was significantly greater for the working-memory group relative to the control group in the anterior region of the dentate gyrus [$F(1, 10) = 6.82$, $p < 0.05$] and CA1 [$F(1, 10) = 7.854$, $p < 0.02$], as well as in the posterior regions of both structures [for the dentate gyrus: $F(1, 10) = 10.14$, $p < 0.02$; for CA1: $F(1, 10) = 20.38$, $p < 0.005$]. The enhancement of LCGU in the anterior and posterior regions of CA3 in the working-memory group was not statistically significant.

Within-group task differences

Although the number of subjects in each particular task was small, the within-group data were compared to evaluate whether participation in one specific working-memory or control task differentially contributed to the glucose utilization rates. In the control group, the average LCGU for all layers combined was similar for monkeys tested on the sensory-motor routine relative to monkeys tested on the visual pattern discrimination task [$F(1, 2) < 1.0$, ns]. For the working-memory group as well, there was no significant between-task difference in the average LCGU over all layers [$F(2, 4) < 1.0$, ns], even though these tasks appeared to vary in their relative difficulty (Table 1). The length of the delay interval also had no independent effect upon LCGU, at least with respect to the delayed alternation task, because no significant differences in the mean LCGU for all layers combined were measured in monkeys performing the 30 sec delayed spatial alternation task relative to the 12 sec version of this task [$F(1, 1) < 1.0$, ns].

LCGU in amygdaloid nuclei

The nuclear organization of the amygdala was characterized by a distinctive pattern in the autoradiograms. The outstanding feature of these autoradiograms was the lateral basal nucleus (Fig. 5) because it was the darkest region in the amygdala. The pattern of 2-DG uptake within the lateral basal nucleus was,

Table 2. Mean LCGU ± SEM in layers of the dentate gyrus and hippocampal CA3 and CA1 sectors in 8 monkeys of the working-memory group (WM) and 5 monkeys of the control group (CONT)

Sector	LCGU (μmol/100 gm/min)
Dentate gyrus	
Molecular	
WM	38.28 ± 1.86[c]
CONT	30.93 ± 2.34
Granule	
WM	41.54 ± 2.07[a]
CONT	34.93 ± 2.69
Hilus	
WM	33.13 ± 1.58
CONT	28.81 ± 2.45
CA3	
Molecular/radiatum	
WM	33.90 ± 2.21
CONT	28.80 ± 2.02
Pyramidal	
WM	31.28 ± 1.50
CONT	28.19 ± 2.45
Oriens/alveus	
WM	26.13 ± 1.32
CONT	23.99 ± 2.22
CA1	
Molecular	
WM	44.94 ± 2.16[c]
CONT	37.10 ± 3.43
Radiatum	
WM	34.53 ± 1.43[c]
CONT	28.94 ± 1.82
Pyramidal	
WM	33.71 ± 1.37[b]
CONT	29.08 ± 1.93
Oriens/alveus	
WM	26.91 ± 1.40
CONT	23.21 ± 1.43

[a] $p \leq 0.05$, [b] $p < 0.02$, and [c] $p < 0.005$ for group differences.

itself, not homogeneous; the dorsal-most part of the lateral basal nucleus was more darkly labeled than the ventromedial part. At some anterior–posterior levels, labeling in the lateral nucleus also appeared to be graded. Glucose utilization rates in the lateral basal nucleus were overwhelmingly higher than the rates measured in any of the other amygdaloid nuclei; it was nearly 40% greater than the combined (over all the nuclei) average LCGU across all monkeys. LCGU was quite similar across the remaining nuclei, although activity was slightly higher in the lateral nucleus compared with the central, medial, and cortical nuclei (Table 4). The central nucleus could not be measured in monkey SC1.

Unlike the preceding analysis for the hippocampus and the dentate gyrus, there was no group effect on LCGU in the amygdala (see Table 4). Although mean LCGU in most of the amygdaloid nuclei was slightly higher for the working-memory group, these between-group differences were small, ranging from 4–9% across 6 of the 7 nuclei examined. The largest between-group differences (~9%) were measured in the medial and medial basal nuclei. Interestingly, mean LCGU in the lateral basal nucleus was slightly decreased (3%) in the working-memory group relative to the control group. LCGU in these nuclei are plotted for individual monkeys in Figure 7.

Table 3. Mean LCGU ± SEM for anterior and posterior sectors of the dentate gyrus, CA3, and CA1 in 8 monkeys in the working-memory group (WM) and 5 monkeys of the control group (CONT)

Sector	LCGU (μmol/100 gm/min) Anterior	Posterior
Dentate gyrus		
WM	35.99 ± 1.75	39.58 ± 1.73
CONT	30.72 ± 2.74	32.59 ± 2.00
CA3		
WM	29.85 ± 1.81	31.14 ± 1.42
CONT	26.69 ± 2.80	27.50 ± 1.47
CA1		
WM	33.43 ± 1.67	36.78 ± 1.27
CONT	28.81 ± 2.35	30.51 ± 1.83

Discussion

The results of the foregoing 2-DG analysis have shown that functional activity in the CA1 and CA3 regions of the hippocampus and the dentate gyrus is enhanced by the mnemonic demands of the working-memory tasks. Glucose utilization rates were increased by as much as 24% in monkeys performing these working-memory tasks relative to the rates measured in monkeys tested on the control tasks. Functional activity was particularly magnified in 2 regions measured: the dentate gyrus and

Table 4. Mean LCGU ± SEM in 7 amygdaloid nuclei for 9 monkeys in the working-memory group (WM) and 6 monkeys in the control (CONT) group

Nucleus	LCGU	ANOVA
Lateral		
WM	31.80 ± 2.04	1.35, ns
CONT	29.55 ± 2.61	
Lateral basal		
WM	39.75 ± 2.63	0.20, ns
CONT	41.04 ± 3.95	
Medial basal		
WM	29.67 ± 2.11	1.23, ns
CONT	27.35 ± 2.80	
Accessory basal		
WM	28.28 ± 1.75	0.82, ns
CONT	26.34 ± 3.01	
Central		
WM	25.13 ± 1.60	0.57, ns
CONT	24.06 ± 3.45	
Medial		
WM	25.39 ± 1.71	0.87, ns
CONT	23.39 ± 2.72	
Cortical		
WM	27.90 ± 1.82	0.25, ns
CONT	26.44 ± 3.70	

The analysis of variance (ANOVA) scores also are given (degrees of freedom = 1,12 except for the central nucleus where it was 1,11; ns = not significant).

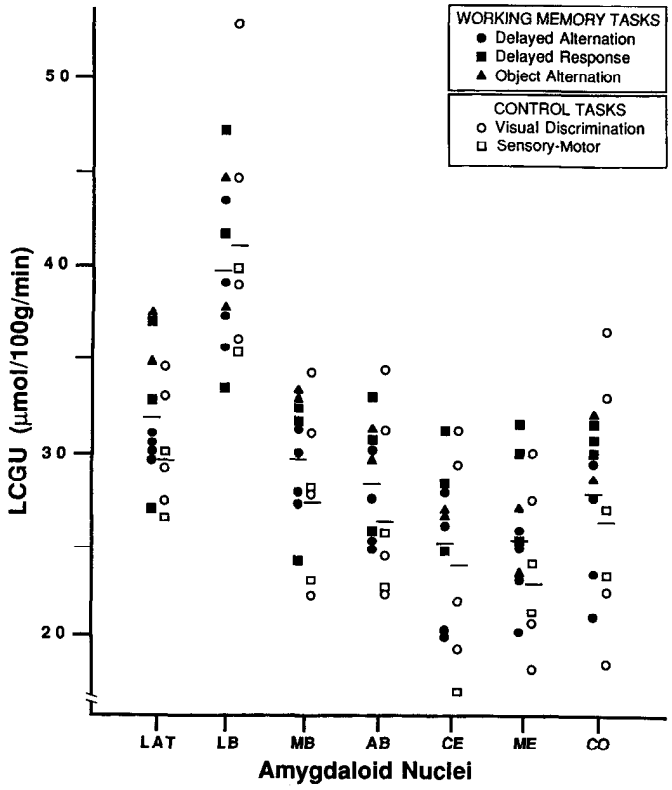

Figure 7 Local cerebral glucose utilization (LCGU) in the amygdala of individual monkeys performing working-memory and control tasks. The *solid horizontal line* in each column is the mean LCGU for each group. Abbreviations as in Figure 5.

the CA1 field of the hippocampus. By contrast with these data, LCGU in 7 amygdaloid nuclei did not vary significantly as a function of working-memory.

Working-memory

Following Honig (1978), Olton et al. (1979), and others (Baddeley, 1986; Roitblat, 1987), we have invoked the concept of working-memory to describe the processing of the context-specific or trial-dependent information in memory tasks by experimental animals. The delayed spatial alternation, delayed spatial response and delayed object alternation paradigms used in this experiment were operationally defined as working-memory tests. Whereas the particular performance rule, for example, "alternate responses," was invariant across trials, the relevance of the explicit stimulus–response contingency was of short tenure—one trial only. Therefore, correct performance always involved on-line processing because old information must be supplanted in memory by the outcome of each succeeding trial. By contrast, the visual pattern discrimination and the sensory motor control tasks have an invariant relationship between the informational stimulus and the response which was well learned before the 2-DG session. These paradigms are based on the mechanism of associative learning and have previously been characterized as reference memory (Olton et al., 1979), procedural, or skill learning tests (Zola-Morgan and Squire, 1984, 1985).

LCGU as a function of mnemonic demand

The 2-DG results show that the operationally distinct mnemonic processes engaged by the working-memory and control group paradigms can be accompanied by quantitative differences in "brain work" (e.g., Ingvar, 1975). In the CA1 and CA3 areas of the hippocampus proper and in the dentate gyrus, the effect of performance on the working-memory paradigms was to increase glucose utilization rates relative to the control group. The magnitude of this enhancement ranged from nearly 10% in the oriens/alveus layers of the hippocampus to 24% in the molecular layer of CA1. These differences in LCGU values during behavioral performance are of the same order of magnitude as the changes in brain activity reported for humans during cognitive processing (Ingvar, 1975; Roland and Friberg, 1985; Roland et al., 1987). Therefore, working-memory performance appears to be more effective in driving functional activity in the hippocampus and the dentate gyrus than performance on the associative tasks of the control conditions.

The facilitation of metabolic activity in the hippocampus and dentate gyrus of the working-memory group parallels the findings of previous hippocampal lesion studies. With respect to the delayed spatial alternation task, lesions of the hippocampal formation, as well as lesions which more extensively involve the medial temporal lobe, have been shown to impair the performance of monkeys on this task when delays of 5 sec or more are used, although individual variability due to differences in testing history, lesion size, or learning strategies has been noted (Orbach et al., 1960; Pribram et al., 1962; Mahut and Cordeau, 1963; Correll and Scoville, 1967; Waxler and Rosvold, 1970; Mahut, 1971). The length of the delay, beyond a critical interval, however, may not potentiate the adverse effects of the lesion (Correll and Scoville, 1967). The data of the present experiment indicated that the converse also may hold because LCGU in CA1, CA3, and dentate gyrus for monkeys performing a 12 sec delayed alternation task was comparable to that for monkeys performing the version of this task that used 30 sec delays.

The evidence for a delayed spatial response impairment after hippocampal damage, however, is more equivocal because differences in the extent of the lesion, the duration of the delay, and the testing history of the monkey are all critical for obtaining a deficit. For example, Zola-Morgan and Squire (1985) reported that lesions of the hippocampus (including the amygdala) seriously impaired the performance of monkeys on this task when the delay was 15 sec but not when the delay was 8 sec, and in a study by Mishkin and Pribram (1954), hippocampal damage was shown to have an adverse affect on delayed response learning when there was no preoperative testing history but not when monkeys had extensive preoperative training. Conversely, in their studies of the effect of hippocampal or medial temporal lesions, Orbach et al. (1960), Mahut and Cordeau (1963), Correll and Scoville (1967), and Mahut (1971) appeared to indicate no deficit on the delayed spatial response task with 0–15 sec delays. However, careful reexamination of these results reveals that some of their subjects required more training and performed more poorly on the delayed response paradigm than did the control-lesioned monkeys. Finally, task-related changes in unit activity in the hippocampus have been reported for monkeys performing the delayed spatial response task (Watanabe and Niki, 1985). The differential activity of a large proportion of units was correlated with the delay period of the task, and importantly, the majority of these units were located in the CA1 sector of the hippocampus.

The delayed object alternation task has not yet been studied in monkeys with hippocampal lesions; however, some evidence suggests that performance on this task might be sensitive to such damage. For example, hippocampal damage impairs perfor-

mance on delayed nonmatching-to-sample (Mahut et al., 1982; Zola-Morgan and Squire, 1986), and this test is similar to delayed object alternation because both are working-memory tasks in which the correct object alternates between trials and accurate performance relies upon the use of information from the preceding trial. Furthermore, electrophysiological studies of the hippocampus have shown that some units are preferentially driven by the conjunction of an object in a relevant context (Brown, 1982; Wilson et al., 1986). These data suggest that hippocampal lesions may deleteriously affect performance on delayed object alternation paradigms, but this remains to be experimentally studied.

Two of the three tasks in the working-memory group required response alternation, and this specific requirement may be important independent from the particular mnemonic requirements of the task. However, delayed spatial response, which does not explicitly require alternation, also enhanced metabolic rate in the hippocampus and dentate gyrus. Thus, alternation was not an obligatory feature of the working-memory tasks, although this kind of performance may have contributed to the facilitation of LCGU in the dentate gyrus and hippocampus.

Finally, hippocampal lesions that spare the anterior hippocampus have been associated with only minimal impairments in performance on cognitive tasks (Mahut et al., 1981; Squire and Zola-Morgan, 1983). In our analysis comparing anterior and posterior portions of the dentate gyrus, CA3, and CA1, LCGU values were significantly different across the anterior–posterior extent of the sampled area. The enhancement of LCGU by working-memory, however, was independent of the anterior or posterior extent of the sample area. As the area sampled in this study did not include the most anterior extent of the hippocampus and dentate gyrus, however, the possibility of functional differences in the participation of these anterior and posterior regions in working-memory cannot be ruled out.

The control tasks used were association or skill learning problems, and the hippocampus is not essential either for the acquisition or retention of such learning. In monkeys, neither specific damage to the hippocampus nor more extensive medial temporal lesions seriously impair performance on motor-skill (Zola-Morgan and Squire, 1984, 1986), visual pattern discrimination (Orbach et al., 1960; Zola-Morgan and Squire, 1985, 1986), size discrimination (Orbach et al., 1960), or object discrimination tasks (Mahut, 1971). Performance may be impaired, however, when difficult concurrent discrimination problems are used (Mahut et al., 1982; but see Malamut et al., 1984).

Spatial and nonspatial memory

Whereas the delayed spatial alternation, delayed spatial response, and delayed object alternation all engaged working-memory processing, these tasks differ in the type of trial-dependent information to be remembered. For the delayed response and delayed alternation tasks, this information was about the spatial position of the reward. For the object alternation task, however, the nature of this information was not spatial but instead referred to objects. Despite this difference, LCGU in CA3, CA1, and the dentate gyrus did not differ as a function of the spatial versus nonspatial characteristics of the working-memory tasks. These data suggest that the essential feature of these tasks that facilitated LCGU was the general process of working-memory rather than the specific type of information that was remembered. Thus, the present LCGU data may provide some rapprochement of current theories of hippocampal function in the nonhuman primate that have focused on recognition memory (e.g., Mishkin et al., 1984; Squire and Zola-Morgan, 1985) to historically earlier experiments that examined spatial memory (e.g., Mishkin and Pribram, 1954; Orbach et al., 1960; Correll and Scoville, 1967; Mahut, 1971). These 2-DG data also serve to reinforce the connecting link between hippocampal function in the monkey and in the rat because the importance of the hippocampus for the processing of spatial information in the rat is indisputable (O'Keefe and Nadel, 1978; Olton et al., 1979, 1983; Jarrard, 1980; Kesner, 1985).

LCGU in the amygdala

In the 7 amygdaloid nuclei that were examined, LCGU was not significantly different in the working-memory group relative to the control group. This finding is consistent with experimental lesion studies showing that amygdalectomy does not seriously impair the performance of monkeys on simple visual pattern discrimination problems (Schwartzbaum, 1965) or on delayed spatial alternation tests (Orbach et al., 1960; Barrett, 1969, for a similar task). Importantly, these results also are supported by recent work (Squire and Zola-Morgan, 1988) showing that circumscribed lesions of the amygdala in monkeys do not impair performance on delayed spatial response tasks using various delay periods, on nonmatching-to-sample tasks, nor on discrimination problems.

Whether the LCGU results reflect a lack of essential relevance of the memory process itself for amygdala function or bespeak a common role for the amygdala in all of the tasks examined is not clarified by our data. The latter possibility, however, is supported by previous studies suggesting that the amygdala is important for encoding stimuli that have motivational and reinforcement-related significance (Sanghera et al., 1979; Mishkin and Aggleton, 1981; Spiegler and Mishkin, 1981; Sarter and Markowitsch, 1985; Gaffan and Harrison, 1987). At the least, however, the LCGU data provide another basis for distinguishing the contribution of the amygdala from that of the hippocampus and dentate gyrus. Many lesion studies in the primate have suggested that the amygdala and hippocampus have an additive effect on memory (Mishkin, 1978; Murray and Mishkin, 1984, 1986). The present study dissociates the contribution of these areas to mnemonic function because the negative findings in the amygdala were drawn from the same monkeys for whom working-memory performance facilitated LCGU in the CA3 and CA1 sectors of the hippocampus and the dentate gyrus.

An outstanding feature of the 2-DG data for the amygdala in monkeys of both groups was high LCGU in the lateral basal nucleus relative to the other nuclei. The anatomical basis for this effect may be differences in the intrinsic (Aggleton, 1985), cortical (Turner et al., 1980; Porrino et al., 1981) or hippocampal (Aggleton, 1986) connectivity of these nuclei. However, the relevance of our finding is not clear because evidence for a functional dissociation of individual amygdaloid nuclei in the monkey is weak (Aggleton and Passingham, 1981; Sarter and Markowitsch, 1985), although recent electrophysiological studies indicate that neuronal activities to sensory stimuli may be topographically organized in the amygdala (Nishijo et al., 1988a, b).

Relevance of cortical connectivity

Metabolic activity in monkeys performing working-memory tasks was enhanced in regions that contain critical parts of the

trisynaptic pathway that first links the hippocampus and dentate gyrus to their main source of cortical afferents, entorhinal cortex, and then allows the flow of information from the dentate gyrus through the CA sectors of the hippocampus (Rosene and Van Hoesen, 1977; and 1987, for review). For example, group differences in metabolic activity were largest in the superficial portion of the hippocampus and dentate gyrus, and the molecular layer is the target zone for entorhinal efferents, the perforant pathway, to the hippocampus (Van Hoesen and Pandya, 1975). The contribution of this projection to CA3, however, appears to be reduced relative to other sectors (Van Hoesen and Pandya, 1975). Such topographical differences may be one basis for our finding that enhanced LCGU in the CA3 sector was less pronounced than in the dentate gyrus and CA1. CA3, on the other hand, is both a terminal region for dentate gyrus fibers and a source of intrinsic hippocampal projections to CA1. In this intermediate position, CA3 may play a particularly important role in the processing of information streaming through the trisynaptic circuit (Rolls, 1987, for discussion of the role of CA3 in such processing). CA1, as the third synaptic link in the intrinsic connections of the hippocampus, can be considered the recipient of highly processed hippocampal information. Working-memory significantly enhanced activity in 3 of the 4 layers measured in this sector. The importance of this region for memory function has recently been reemphasized by a clinical report correlating anterograde amnesia and circumscribed damage to this sector (Zola-Morgan et al., 1986). Furthermore, projections of the CA fields to the subiculum and other cortical areas appear to arise exclusively from CA1 (Rosene and Van Hoesen, 1987); this sector, therefore, represents a means by which the hippocampus can exert a direct and widespread influence on the cortex.

Emphasis on the cortical connectivity of the hippocampus is particularly relevant with respect to the specific working-memory tasks used in this experiment. Several lines of evidence suggest that the hippocampus, in conjunction with the prefrontal cortex, cooperatively mediates performance on the working-memory tasks. Anatomically, the entorhinal and subicular cortices are convergence sites for direct projections from prefrontal as well as other association cortices in the monkey (Van Hoesen et al., 1979; Van Hoesen, 1982; Goldman-Rakic et al., 1984; Insausti et al., 1987). Indeed, the entorhinal and subicular cortices appeared dark in autoradiograms, and whether functional metabolic activity in these medial temporal regions also is facilitated by working-memory is currently being examined. Behaviorally, the critical importance of the dorsolateral prefrontal cortex for spatial working-memory has been well established (Goldman-Rakic, 1987, for review), and the inferior lateral convexity of the prefrontal cortex appears crucial for performance on delayed object alternation problems (Mishkin and Manning, 1978). Preliminary evidence from this laboratory also suggests that these frontal cortical areas are activated by working-memory tasks in monkeys (Bugbee and Goldman-Rakic, 1984), as are the thalamic regions that are connected with prefrontal cortex (Friedman et al., 1987). Interestingly, in the rat, glucose utilization in the hippocampus and frontal cortex also correlates with performance on spatial tests (Gage et al., 1984).

The 2-DG method and cognitive behavior

The 2-DG method has proven to be a powerful means for evaluating metabolic activity underlying a variety of behavioral and physiologically relevant events. In many reports, the technique has been used in conjunction with experimental manipulations such as brain lesions (Kennedy et al., 1976; Macko et al., 1982) and stimulation (or deprivation) of specific sensory or motor systems (Kennedy et al., 1976; Juliano et al., 1981) which produce dramatic changes in metabolic activity. The experimental design of the present 2-DG study differs considerably from these more usual protocols; both the working-memory and the control tasks were similar in nearly every respect except for the nature of the *psychological* process that was guiding performance. Although we predicted that performance on cognitive tasks with different mnemonic demands would differentially affect metabolic activity in the brain, we did not know what to expect with respect to the kind and magnitude of these effects given the subtle differences among the paradigms employed. This demonstration that working-memory selectively enhances the pattern of functional metabolic activity in the hippocampus not only emphasizes the importance of the hippocampus for such mnemonic processing, but also indicates that the 2-DG method is sufficiently sensitive to detect differences in psychological processing.

References

Aggleton, J. P. (1985) A description of intra-amygdaloid connections in old world monkeys. Exp. Brain Res. *57:* 390–399.

Aggleton, J. P. (1986) A description of the amygdalohippocampal interconnections in the macaque monkey. Exp. Brain Res. *64:* 515–526.

Aggleton, J. P., and M. Mishkin (1984) Projections of the amygdala to the thalamus in the cynomolgus monkey. J. Comp. Neurol. *222:* 56–68.

Aggleton, J. P., and R. E. Passingham (1981) Syndrome produced by lesions of the amygdala in monkeys. J. Comp. Physiol. Psychol. *95:* 961–977.

Baddeley, A. D. (1982) Implications of neuropsychological evidence for theories of normal memory. Phil. Trans. R. Soc. [Biol.] *298:* 59–72.

Baddeley, A. (1986) *Working Memory,* Clarendon, Oxford, UK.

Barrett, T. W. (1969) Studies of the function of the amygdaloid complex in *Macaca mulatta.* Neuropsychologia *7:* 1–12.

Brown, M. W. (1982) Effect of context on the response of single units recorded from the hippocampal region of behaviourally trained monkeys. In *Neuronal Plasticity and Memory Formation,* C. A. Marsan and H. Matthies, eds., pp. 557–573, Raven, New York.

Bugbee, N. M., and P. S. Goldman-Rakic (1981) Functional 2-deoxyglucose mapping in association cortex: Prefrontal activation in monkeys performing a cognitive task. Soc. Neurosci. Abstr. *7:* 416.

Cohen, N. J., H. Eichenbaum, B. S. Deacedo, and S. Corkin (1985) Different memory systems underlying acquisition of procedural and declarative knowledge. Ann. NY Acad. Sci. *444:* 54–71.

Corkin, S., N. J. Cohen, E. V. Sullivan, R. A. Clegg, T. J. Rosen, and R. H. Ackerman (1985) Analysis of global memory impairments of different etiologies. Ann. NY Acad. Sci. *444:* 10–40.

Correll, R. E., and W. B. Scoville (1967) Significance of delay in the performance of monkeys with medial temporal lobe resections. Exp. Brain Res. *4:* 85–96.

Crosby, E. C., and T. Humphrey (1941) Studies of the vertebrate telencephalon. II. The nuclear pattern of the anterior olfactory nuclei, tuberculum olfactorium, and the amygdaloid complex in adult man. J. Comp Neurol. *74:* 309–352.

Eilbert, J. L. (1986) Quantitative analysis of autoradiographs. Exp. Biol. Med. *10:* 122–142.

Friedman, H. R., J. Janas, and P. S. Goldman-Rakic (1987) Metabolic activity in the thalamus and mammillary bodies of the monkey during spatial memory performance. Soc. Neurosci. Abstr. *13:* 206.

Gaffan, D., and S. Harrison (1987) Amygdalectomy and disconnection in visual learning for auditory reinforcement by monkeys. J. Neurosci. *7:* 2285–2292.

Gage, F. H., P. A. T. Kelly, and A. Bjorklund (1984) Regional changes in brain glucose metabolism reflect cognitive impairments in aged rats. J. Neurosci. *4:* 2856–2865.

Gallistel, C. R., C. T. Piner, T. O. Allen, N. T. Adler, E. Yadin, and

M. Negin (1982) Computer assisted analysis of 2-DG autoradiographs. Neurosci. Biobehav. Rev. *6:* 409–420.
Gellerman, L. W. (1933) Chance orders of alternating stimuli in visual discrimination experiments. J. Gen. Psychol. *42:* 207–208.
Goldman, P. S. (1971) Functional development of the prefrontal cortex in early life and the problem of neuronal plasticity. Exp. Neurol. *32:* 366–387.
Goldman-Rakic, P. S. (1987) Circuitry of primate prefrontal cortex and regulation of behavior by representational memory. In *Handbook of Physiology, The Nervous System,* Vol. 9, F. Plum, ed., pp. 373–417, American Physiological Society, Bethesda, MD.
Goldman-Rakic, P. S., L. D. Selemon, and M. L. Schwartz (1984) Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and the parahippocampal cortex in the rhesus monkey. Neuroscience *12:* 719–743.
Honig, W. K. (1978) Studies of working memory in the pigeon. In *Cognitive Processes in Animal Behavior,* S. H. Hulse, H. Fowler, and W. K. Honig, eds., pp. 211–248, Erlbaum, Hillsdale, NJ.
Horel, J. A. (1978) The neuroanatomy of amnesia. A critique of the hippocampal memory hypothesis. Brain *101:* 403–445.
Ingvar, D. H. (1975) Patterns of brain activity revealed by measurements of regional cerebral blood flow. In *Brain Work,* D. H. Ingvar and N. A. Lassen, eds., pp. 397–413, Academic, New York.
Insausti, R., D. G. Amaral, and W. M. Cowan (1987) The entorhinal cortex of the monkey: II. Cortical afferents. J. Comp. Neurol. *264:* 356–395.
Jarrard, L. E. (1980) Selective hippocampal lesions and behavior. Physiol. Psychol. *8:* 198–206.
Juliano, S. L., P. J. Hand, and B. Whitsel (1981) Patterns of increased metabolic activity in somatosensory cortex of monkeys, *Macaca fascicularis,* subjected to controlled cutaneous stimulation: A 2-deoxyglucose study. J. Neurophysiol. *46:* 1260–1284.
Kennedy, C., M. H. Des Rosiers, O. Sakurada, M. Shinohara, M. Reivich, J. Jehle, and L. Sokoloff (1976) Metabolic mapping of the primary visual system of the monkey by means of the autoradiographic [^{14}C] deoxyglucose technique. Proc. Natl. Acad. Sci. USA *73:* 4230–4234.
Kennedy, C., O. Sakurada, M. Shinohara, J. Jehle, and L. Sokoloff (1978) Local cerebral glucose utilization in the normal conscious macaque monkey. Ann. Neurol. *4:* 293–301.
Kesner, R. P. (1985) Correspondence between humans and animals in coding of temporal attributes: Role of hippocampus and prefrontal cortex. Ann. NY Acad. Sci. *444:* 122–136.
Lorente de No, R. (1934) Studies on the structure of the cerebral cortex. II. Continuation of the study of the Ammonic system. J. Psychol. Neurol. *46:* 113–177.
Macko, K. A., C. D. Jarvis, C. Kennedy, M. Miyaoka, M. Shinohara, L. Sokoloff, and M. Mishkin (1982) Mapping the primate visual system with [2-^{14}C]deoxyglucose. Science *218:* 394–397.
Mahut, H. (1971) Spatial and object reversal learning in monkeys with partial temporal lobe ablations. Neuropsychologia *9:* 409–424.
Mahut, H., and J.-P. Cordeau (1963) Spatial deficit in monkeys with amygdalohippocampal ablations. Exp. Neurol. *7:* 426–434.
Mahut, H., and M. Moss (1984) Consolidation of memory: The hippocampus revisited. In *Neuropsychology of Learning,* L. R. Squire and N. Butters, eds., pp. 65–77, 297–315, Guilford, New York.
Mahut, H., M. Moss, and S. Zola-Morgan (1981) Retention deficits after combined amygdalohippocampal and selective hippocampal resections in the monkey. Neuropsychologia *19:* 201–225.
Mahut, H., S. Zola-Morgan, and M. Moss (1982) Hippocampal resections impair associative learning and recognition in the monkey. J. Neurosci. *2:* 1214–1229.
Malamut, B. L., R. C. Saunders, and M. Mishkin (1984) Monkeys with combined amygdalohippocampal lesions succeed in object discrimination learning despite 24-hour intertrial intervals. Behav. Neurosci. *98:* 759–769.
Milner, B. (1970) Memory and the medial temporal regions of the brain. In *Biology of Memory,* K. H. Pribram and D. E. Broadbent, eds., pp. 29–50, Academic, New York.
Milner, B., S. Corkin, and H.-L. Teuber (1968) Further analysis of the hippocampal amnesic syndrome: 14-yr follow-up study of H.M. Neuropsychologia *6:* 215–234.
Mishkin, M. (1978) Memory in monkeys severely impaired by combined but not separate removal of amygdala and hippocampus. Nature *273:* 297–298.
Mishkin, M., and J. Aggleton (1981) Multiple functional contributions of the amygdala in the monkey. In *The Amygdaloid Complex,* Y. Ben Ari, ed., pp. 409–420, Elsevier, New York.
Mishkin, M., and F. J. Manning (1978) Non-spatial memory after selective prefrontal lesions in monkeys. Brain Res. *143:* 313–323.
Mishkin, M., and K. H. Pribram (1954) Visual discrimination performance following partial ablations of the temporal lobe: I. Ventral vs. lateral. J. Comp. Physiol. Psychol. *47:* 14–20.
Mishkin, M., B. Malamut, and J. Bachevalier (1984) Memories and habits: Two neural systems. In *Neurobiology of Learning and Memory,* G. Lynch, J. L. McGaugh, and N. M. Weinberger, eds., pp. 65–77, Guilford, New York.
Moss, M., H. Mahut, and S. Zola-Morgan (1981) Concurrent discrimination learning of monkeys after hippocampal, entorhinal or fornix lesions. J. Neurosci. *1:* 227–240.
Murray, E. A., and M. Mishkin (1984) Severe tactual as well as visual memory deficits follow combined removal of the amygdala and hippocampus in monkeys. J. Neurosci. *4:* 2565–2580.
Murray, E. A., and M. Mishkin (1986) Visual recognition in monkeys following rhinal cortical ablations combined with either amygdalectomy or hippocampectomy. J. Neurosci. *6:* 1991–2003.
Nishijo, H., T. Ono, and H. Nishino (1988a) Topographic distribution of modality-specific amygdala neurons in alert monkey. J. Neurosci. *8:* 3556–3569.
Nishijo, H., T. Ono, and H. Nishino (1988b) Single neuron responses in amygdala of alert monkey during complex sensory stimulation with affective significance. J. Neurosci. *8:* 3570–3583.
O'Keefe, J., and L. Nadel (1978) *The Hippocampus as a Cognitive Map,* Claredon, Oxford, UK.
Olton, D. S. (1983) Memory functions and the hippocampus. In *Neurobiology of the Hippocampus,* W. Seifert, ed., pp. 335–373, Academic, New York.
Olton, D. S., J. T. Becker, and G. E. Handelmann (1979) Hippocampus, space and memory. Behav. Brain Sci. *2:* 352–359.
Orbach, J., B. Milner, and T. Rasmussen (1960) Learning and retention in monkeys after amygdala-hippocampal resection. Arch. Neurol. *3:* 230–251.
Porrino, L. J., A. M. Crane, and P. S. Goldman-Rakic (1981) Direct and indirect pathways from the amygdala to the frontal lobe in the rhesus monkey. J. Comp. Neurol. *198:* 121–136.
Pribram, K. H., and M. Mishkin (1956) Analysis of the effects of frontal lesions in monkey: III. Object alternation. J. Comp. Physiol. Psychol. *49:* 41–45.
Pribram, K. H., W. A. Wilson, and J. Connors (1962) Effects of lesions of the medial forebrain on alternation behavior of rhesus monkeys. Exp. Neurol. *6:* 36–47.
Roitblat, H. L. (1987) *Introduction to Comparative Cognition,* Freeman, New York.
Roland, P. E., and L. Friberg (1985) Localization of cortical areas activated by thinking. J. Neurophysiol. *53:* 1219–1243.
Roland, P. E., L. Eriksson, S. Stone-Elander, and L. Widen (1987) Does mental activity change the oxidative metabolism of the brain? J. Neurosci. *7:* 2373–2389.
Rolls, E. T. (1987) Information representation, processing and storage in the brain: Analysis at the single neuron level. In *The Neural and Molecular Mechanisms of Learning,* J.-P. Changeux and M. Konishi, eds., pp. 503–540, Springer, Berlin.
Rosene, D. L., and G. W. Van Hoesen (1977) Hippocampal efferents reach widespread areas of the cerebral cortex and amygdala in the rhesus monkey. Science *198:* 315–317.
Rosene, D. L., and G. W. Van Hoesen (1987) The hippocampal formation of the primate brain. In *Cerebral Cortex,* E. G. Jones and A. Peters, eds., pp. 345–456, Plenum, New York.
Sanghera, M. K., E. T. Rolls, and A. Roper-Hall (1979) Visual responses of neurons in the dorsolateral amygdala of the alert monkey. Exp. Neurol. *63:* 610–626.
Sarter, M., and H. J. Markowitsch (1985) Involvement of the amygdala in learning and memory: A critical review with emphasis on anatomical relations. Behav. Neurosci. *99:* 342–380.
Schwartzbaum, J. S. (1965) Discrimination behavior after amygdalectomy in monkeys. Visual and somesthetic learning and perceptual capacity. J. Comp. Physiol. Psychol. *60:* 314–319.
Scoville, W. B., and B. Milner (1957) Loss of recent memory after bilateral hippocampal lesions. J. Neurol. Neurosurg. Psychiatry *20:* 11–21.
Sokoloff, L., M. Reivich, C. Kennedy, M. H. Des Rosiers, C. S. Patlak, K. D. Pettigrew, O. Sakurada, and M. Shinohara (1977) The

[^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure and normal values in the conscious and anesthetized albino rat. J. Neurochem. *28:* 897–916.

Spiegler, B. J., and M. Mishkin (1981) Evidence for the sequential participation of inferior temporal cortex and amygdala in the acquisition of stimulus-reward associations. Behav. Brain. Res. *3:* 303–317.

Squire, L. R., and N. J. Cohen (1984) Human memory and amnesia. In *Neurobiology of Learning and Memory,* G. Lynch, J. L. McGaugh, and N. M. Weinberger, eds., pp. 3–64, Guilford, New York.

Squire, L. R., and S. Zola-Morgan (1983) The neurology of memory: The case for correspondence between the findings for human and nonhuman primate. In *The Physiological Basis of Memory,* J. A. Deutsch, ed., pp. 199–268, Academic, New York.

Squire, L. R., and S. Zola-Morgan (1985) The neuropsychology of memory: New links between humans and animals. Ann. NY Acad. Sci. *444:* 137–149.

Squire, L. R., and S. Zola-Morgan (1988) Memory: Brain systems and behavior. Trends Neurosci. *11:* 170–175.

Turner, B. H., M. Mishkin, and M. Knapp (1980) Organization of amygdalopetal projections from modality specific cortical association areas in the monkey. J. Comp. Neurol. *191:* 515–543.

Van Hoesen, G. W. (1982) The parahippocampal gyrus: New observations regarding its cortical connections in the monkey. Trends Neurosci. *5:* 345–350.

Van Hoesen, G. W., and D. N. Pandya (1975) Some connections of the entorhinal (area 28) and perirhinal (area 35) cortices of the rhesus monkey. III. Efferent connections. Brain Res. *95:* 35–59.

Van Hoesen, G. W., D. L. Rosene, and M.-M. Mesulam (1979) Subicular input from temporal cortex in the rhesus monkey. Science *205:* 608–610.

Watanabe, T., and H. Niki (1985) Hippocampal unit activity and delayed response in the monkey. Brain Res. *325:* 241–254.

Waxler, M., and H. E. Rosvold (1970) Delayed alternation in monkeys after removal of the hippocampus. Neuropsychologia *8:* 137–146.

Wilkinson, L. (1986) *Systat: The System for Statistics,* Systat, Evanston, IL.

Wilson, F. A. W., M. W. Brown, and I. P. Riches (1986) Sensory and motor related unit activity in the monkey hippocampal formation. Soc. Neurosci. Abstr. *12:* 556.

Winer, B. J. (1971) *Statistical Principles in Experimental Design,* McGraw-Hill, New York.

Zola-Morgan, S., and L. R. Squire (1984) Preserved learning in monkeys with medial temporal lesions: Sparing of motor and cognitive skills. J. Neurosci. *4:* 1072–1085.

Zola-Morgan, S., and L. R. Squire (1985) Medial temporal lesions in monkeys impair memory on a variety of tasks sensitive to human amnesia. Behav. Neurosci. *99:* 22–34.

Zola-Morgan, S., and L. R. Squire (1986) Memory impairment in monkeys following lesions limited to the hippocampus. Behav. Neurosci. *100:* 155–160.

Zola-Morgan, S., L. R. Squire, and M. Mishkin (1982) The neuroanatomy of amnesia: Amygdalohippocampus versus temporal stem. Science *218:* 1337–1339.

Zola-Morgan, S., L. R. Squire, and D. G. Amaral (1986) Human amnesia and the medial temporal region: Enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J. Neurosci. *6:* 2950–2967.