Behavioral/Systems/Cognitive

Impaired Recency Judgments and Intact Novelty Judgments after Fornix Transection in Monkeys

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Over four experiments based on the delayed matching-to-sample task, fornix-transected and normal control monkeys were presented with a sequence of five sample stimuli and then received intermixed within-session recency (WSR) and between-session recency (BSR) tests in experiment 1, only BSR tests in experiment 2, only absolute novelty (AN) tests in experiment 3, or only WSR tests in experiment 4. In WSR tests, monkeys chose which of two samples had occurred more recently in the immediately preceding sequence. In BSR and AN tests, monkeys were required to choose one sample from the immediately preceding sequence in preference to a foil unseen in the present session (BSR) or an AN foil that had never been presented before. When tests of WSR and BSR were intermixed (experiment 1), fornix monkeys performed below the level of the control monkeys in both types of test, although this difference was not statistically significant. In experiment 2, fornix monkeys were significantly impaired on tests of BSR alone, in which memory for a stimulus presented in an immediately preceding sequence could compete with memory for a foil presented in an earlier training session. In tests of AN (experiment 3), fornix monkeys performed at the same level as control animals in distinguishing a previously experienced stimulus from a previously unseen foil. In experiment 4, fornix transection significantly impaired tests of WSR alone. Taken together, these results suggest that one specialized role of the fornix is to process temporal information.

Key words: **memory; hippocampus; fornix; monkey; recency; recognition**

Introduction

Bilateral removal of the human medial temporal lobes results in dense amnesia (Scoville and Milner, 1957), a crucial feature of which is a deficit in recognition memory. Attempts to replicate these recognition deficits in monkeys using delayed nonmatching-to-sample (DNMS) or delayed matching-to-sample (DMS) have produced disparate accounts of the specific medial temporal structures implicated. Accumulating evidence from electrophysiological investigations (Fahy et al., 1993; Brown and Xiang, 1998) and ablation experiments (Meunier et al., 1993; Buckley and Gaffan, 1998) suggests that the perirhinal cortex is crucial for recognition memory. However, the role of the hippocampus in recognition memory is unclear. Selective neurotoxic lesions of the hippocampus that aim to spare nontarget regions have produced no impairments in DNMS (Murray and Mishkin, 1998), mild impairments (Zola et al., 2000), or more substantial impairments (Beason-Held et al., 1999), for which a number of possible contributing factors have been proposed (Baxter and Murray, 2001a,b; Zola and Squire, 2001).

When hippocampal contribution to recognition memory is assessed by transecting one of its major input–output pathways, the fornix, a similar pattern of results emerges. In tests of DNMS, these discrepant findings range from no impairment (Mahut et al., 1982), small impairments (Bachevalier et al., 1985a,b; Zola-Morgan et al., 1989), or more substantial impairments (Gaffan and Weiskrantz, 1980; Owen and Butler, 1981, 1984; Gaffan et al., 1984a). The same pattern of inconsistencies arises in tests of DMS, which show no impairment (Gaffan et al., 1984b), small impairments (Bachevalier et al., 1985a), or substantial impairments (Gaffan, 1974).

One factor complicating interpretation of the hippocampal contribution to recognition memory relates to the size of the stimulus pool used in DNMS and DMS tasks. Unlike tests that use absolute novel (AN) stimuli, those using small stimulus pools may repeatedly present stimuli either within or between training episodes, such that putative impairments in recognition might best be considered as impairments in the judgment of the relative recency of familiar repetitious stimuli. If stimulus pool size is a contributory factor, then it is notable that experiments using either fewer than 400 objects (Gaffan, 1974; Gaffan and Weiskrantz, 1980; Gaffan et al., 1984a; Owen and Butler, 1984; Zola-Morgan et al., 1989) or at least 400 objects (Beason-Held et al., 1999; Zola et al., 2000) report recognition impairments. However, when pool size is much larger, exceeding 1000, impairments are either transient or nonexistent (Bachevalier et al., 1985b; Murray and Mishkin, 1998).

The present set of experiments compared different conditions of recognition performance within animals. We used a between-session recency (BSR) paradigm to establish whether recognition memory impairments after fornix transection are attributable to a requirement to make temporal judgments regarding the relative recency of stimuli arising from their repeated presentations

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across training sessions. Second, we tested the effect of fornix transection on recognition memory using AN foils that had never been presented before. Third, we assessed the contribution made by the fornix to judgments of within-session recency (WSR).

Materials and Methods

Subjects

Six rhesus monkeys (*Macaca mulatta*) acted as the subjects in experiments 1–4. Before the current experiment, all animals had performed as subjects in the same series of visual discrimination learning tasks (Buckley et al., 2004), in which the monkeys were assigned to the two surgical groups so as to equate their preoperative learning ability. During an average period of 17 months that elapsed between surgery and commencement of the current experiment, the same subjects also had identical experience in an additional visual object discrimination learning task. Three subjects (FNX 1–3) received bilateral fornix transection, and another three subjects (CON 1–3) acted as unoperated controls. One monkey (CON 1) was male, and the others were female. The animals' mean weight was 4.35 kg, and their mean age was 4 years, 10 months. All were housed either individually or in pairs in their home cages with water provided *ad libitum*.

Surgery

Three animals received bilateral fornix transection (group FNX) and the remaining animals acted as unoperated controls (group CON). All procedures were performed under license in compliance with the United Kingdom Animals (Scientific Procedures) Act 1986. The operations were performed in sterile conditions with the aid of an operating microscope, and the monkeys were anesthetized throughout surgery with barbiturate (5% thiopentone sodium solution) administered through an intravenous cannula. A D-shaped bone flap was raised over the midline and the left hemisphere. The dura mater was cut to expose the hemisphere up to the midline. Veins draining into the saggital sinus were cauterized and cut. The left hemisphere was retracted from the falx with a brain spoon. A glass aspirator was used to make a sagittal incision no more than 5 mm in length in the corpus callosum at the level of the interventricular foramen. The fornix was sectioned transversely by electrocautery and aspirated with a 20 gauge metal aspirator that was insulated to the tip. The dura mater was drawn back but not sewn, the bone flap was replaced, and the wound was closed in layers

Histology

At the conclusion of the experiments, animals with fornix transection were anesthetized deeply, then perfused through the heart with saline followed by formol–saline solution. The brains were blocked in the coronal stereotaxic plane posterior to the lunate sulcus, removed from the skull, and allowed to sink in sucrose–formalin solution. The brains were cut in 50 μm sections on a freezing microtome. Every fifth section was retained and stained with cresyl violet. Microscopic examination of the stained sections revealed, in every case, a complete section of the fornix (Fig. 1) with no damage outside the fornix, except for the most ventral part of cingulate gyrus in one hemisphere in one animal (FNX 3), illustrated in the right section in Figure 1, and the incision in the corpus callosum of each animal, as described in the surgical procedures.

Apparatus

Each experiment was conducted in the same automated testing apparatus contained within an experimental cubicle that was dark, except for the display background illumination. Monkeys were brought to the apparatus in a wheeled transport cage that was secured opposite a touchscreen 380 mm wide and 280 mm high with a display resolution of 800 × 600 pixels, on which the experimental stimuli (described below) were presented. Animals could reach through the horizontal bars at the front of the cage and interface with the touchscreen. An automated pellet-dispensing device made an audible click when delivering banana-flavored reward pellets (190 mg) into a well positioned to the right at the foot of the screen. An automated spring-loaded lunch box (length, 200 mm; width, 100 mm; height, 100 mm) positioned to the left at the foot of the touchscreen opened immediately after the end of each daily session to deliver the animals' daily diet of wet primate chow, pieces of fruit, and

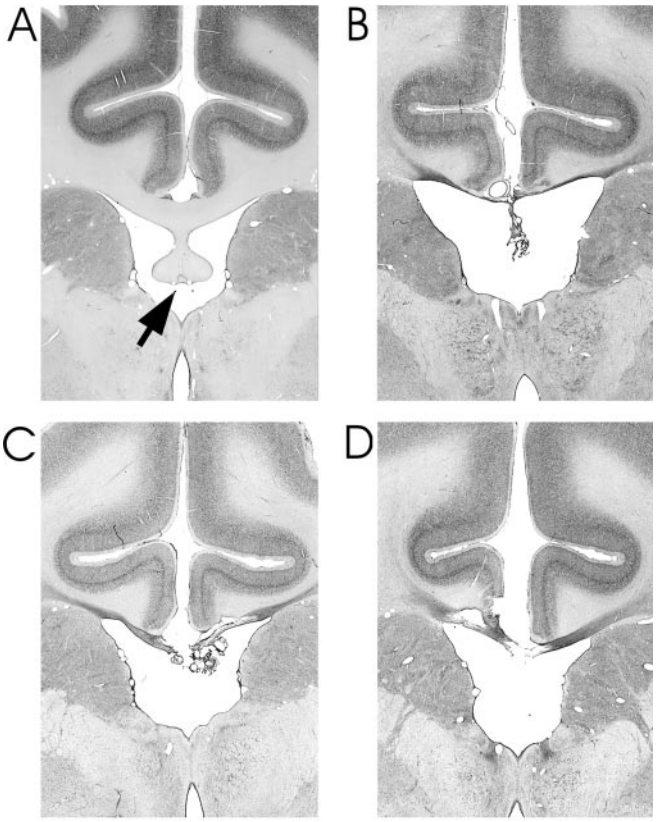

Figure 1. Coronal sections from the brains of a normal monkey (*A*), where the arrow indicates the intact fornix, and subjects FNX 1–3 (*B–D*), where the fornix has been completely transected at the level of the interventricular foramen.

dates. A closed circuit TV monitor positioned above the touchscreen and in front of the subject was used for observation from another room from where the stimulus display, food delivery, and experimental contingencies were computer controlled.

Stimulus material

The visual stimuli presented on the touchscreen were individual clipart images obtained from commercially available internet sources. Each clipart image was 128 × 128 pixels in size and comprised a unique foreground colored image, imposed on a white-colored screen-congruent background. All stimuli were presented against a white background that occupied the whole touchscreen, and a touch-sensitive area extending 50 pixels beyond each side of each image was used to ensure the detection of stimulus-directed responses. A single pool of 600 images was used for all stages of behavioral testing in experiments 1 and 2. Stimuli were used from the pool in a random order without replacement within each daily test session and were then reused in a new random order in the next session.

In experiments 3 and 4, a novel pool of 1500 individual clipart images obtained from freely available internet sources were used as stimuli. These stimuli were not dissimilar to the set described above in terms of their image size, color, and dimensions. In experiment 3 only, 500 of these stimuli were chosen randomly to constitute the pool of samples, which were used once every day. The remaining 1000 stimuli constituted the pool of foils, each of which were used once only in the entire experiment. In experiment 4, stimuli were used from the pool in a random order without replacement within each daily test session and were then reused in a new random order in the next session.

Behavioral testing

Summary. The monkeys were tested on four main experimental tasks. In each task, monkeys were presented with a sequence of five sample stimuli, followed by a choice trial. In WSR choice trials, monkeys were re-

quired to choose which of two samples had occurred more recently in the immediately preceding sequence. In BSR and AN trials, monkeys were required to choose the sample from the immediately preceding sequence in preference to a foil unseen in the present session (BSR) or an AN foil that had never been presented before. Before the first experiment, monkeys were pretrained on DMS (pretraining stage 1) and two additional variants of this task. In the latter two tasks, as in the main experiments, monkeys were presented with a sequence of five sample stimuli, followed by a choice trial. Choice trials consisted of the first and final samples (pretraining stage 2), then the first and fourth samples (pretraining stage 3) from the sequence, and in each type of trial, monkeys were required to choose the sample that had occurred more recently in the sequence. After completion of pretraining, monkeys received intermixed WSR and BSR trials in experiment 1, BSR trials alone in experiment 2, AN trials in experiment 3, and WSR trials alone in experiment 4.

Pretraining stage 1: DMS. All subjects were trained on a DMS task presented on the automated touchscreen. Each trial procedure consisted of a sample and a choice stage separated by a delay. In the sample stage, subjects were required to touch a stimulus positioned in the center of the screen that caused it to disappear. After a 2 sec delay, subjects received a choice test in which the previous sample stimulus and a foil were presented in the horizontal midline of the screen positioned equidistant on opposite sides of the central point. The foil was a stimulus that had not been seen in the present session but had been presented in previous sessions. Choosing the sample caused both stimuli to disappear from the screen and caused the immediate production of a food reward. After a 5 sec intertrial interval, the trial procedure was repeated. Touching the foil caused both stimuli to disappear immediately without food reward and caused the commencement of a 10 sec intertrial interval before repeating the procedure. Touching the screen during the intertrial interval reset this interval. Over a mean period of 10 d, the number of trials in each daily session was increased until all subjects were making 100 correct responses daily. This stage of training ended when a criterion of 90% correct responses was attained in one session. The session terminated only when monkeys had made the final correct response, which triggered an automated spring-loaded lunch box to open immediately and deliver the animals' daily diet (see apparatus above).

Pretraining stage 2: "1 versus 5." The procedure adopted was similar to stage 1, except that now a sequence of five samples preceded a choice trial. Subjects were required to touch a sample that caused it to disappear from the screen. After a 1 sec interstimulus interval, the next sample in the sequence appeared and subjects were required to touch it, and so on until the final sample had been touched. A 1 sec delay preceded presentation of the choice trial in which subjects were required to choose between the first and fifth samples in the sequence. Choosing the fifth sample caused both stimuli to disappear from the screen and caused the immediate production of a food reward. After a 5 sec intertrial interval, the procedure was repeated. Choosing the first sample in the choice trial produced no food reward and caused both stimuli to disappear from the screen, followed by a 10 sec intertrial interval. Touching the screen during the intertrial interval reset this interval. Subjects received 50 within-session unique trials daily, with each session terminating on completion of the final trial. At this point, the automated spring-loaded lunch box opened immediately to deliver the animals' daily diet. Training sessions ceased when animals attained a criterion of 90% correct trials in one session.

Pretraining stage 3: "1 versus 4." This stage was the same in all respects to stage 2, with the exception that in choice trials, subjects were required to choose between the first and fourth samples in the sequence. Choosing the fourth sample produced a food reward. Subjects received up to 75 trials during each daily session, and the session ceased immediately after the final trial, with the lunch box opening to deliver the animals' diet. Training sessions ceased when animals attained a criterion of 80% correct trials in one session.

Experiment 1: intermixed WSR and BSR retention trials. Each type of trial consisted of a sample stage, as in pretraining stage 2, in which a sequence of five samples preceded a choice trial. Touching each sample when presented caused it to disappear from the screen. After a 1 sec interstimulus interval, the next sample appeared, and so on until the final sample had been touched. A 1 sec delay preceded presentation of the retention trial. Retention trials (Table 1) were composed of two possible types: WSR trial types consisted of two items selected from the previously experienced sequence, and the rewarded choice was the item that occurred more recently in the sequence. For example, in a choice trial of samples 3 and 5, choosing the latter would produce a food reward. BSR trial types consisted of one item in the list of samples presented before the choice stage and a foil randomly selected from the stimulus pool that had not appeared previously in the session of the current day but that had been presented in the preceding session. Choosing the sample stimulus caused both stimuli to disappear from the screen and caused the immediate production of a food reward.

Table 1. WSR trial types using all combinations of the five samples

WSR trials				BSR and AN trials
1− versus 2+	2− versus 3+	3− versus 4+	4− versus 5+	1+ versus foil−
1− versus 3+	2− versus 4+	3− versus 5+		2+ versus foil−
1− versus 4+	2− versus 5+			3+ versus foil−
1− versus 5+				4+ versus foil−
				5+ versus foil−

BSR and AN trial types are composed of one sample from the list of five, together with either a foil from a familiar stimulus pool (BSR) or an AN trial-unique foil. +, Rewarded cue; −, foil.

Table 1 illustrates all possible trial types that each subject received. The 10 WSR trial types are composed of all combinations of any two samples from the sequence. The five BSR trial types are composed of a sample from each position in the sequence paired with a foil. Because the sample stage of each was identical, subjects had no idea of the trial type until the retention test stage. Each trial was constructed from the stimulus pool, randomly ordered into a list of 600 at the beginning of each daily session. For each trial, the stimuli were taken in order from the start of the list and were not replaced during a session. For example, WSR trial types required five stimuli to represent the sequence and the retention trial stimuli. BSR trial types required six stimuli, with the first five representing the sample sequence from which one was selected for the retention trial and the sixth stimulus representing the foil. Exactly one trial of each of the 15 trial types made a block, and six blocks of trials completed the daily training session. Within each block, the order of trials was randomized. The total of 90 trials consisted of 60 WSR trial types and 30 BSR trial types. Completion of the final trial triggered the lunch box to open immediately and deliver the animals' daily diet. Animals were trained daily until each had completed 2250 trials consisting of 150 trials of each trial type.

Experiment 2: BSR trials alone. The apparatus and general procedures for this and the following experiments were the same as those described for experiment 1, unless stated otherwise.

This task was the same as in experiment 1, with the exception that no WSR trials were included. After presentation of a sequence of five sample stimuli, monkeys received a retention trial in which they were required to select the sample that appeared in the sequence, in preference to a foil that had not appeared previously in the session of the current day but that had been presented in the preceding session. Daily training sessions consisted of 20 blocks of five randomized trial types, totaling 100 trials. Training continued for 10 d until the subjects completed a total of 200 trials of each trial type.

Experiment 3: AN trials. In this task, monkeys were presented with a sequence of five sample stimuli and then were required to choose a familiar stimulus that had appeared in the sequence in preference to an AN foil that had not been presented before. The task was the same as described above for experiment 2, with the exception that 500 of the stimuli were assigned as samples and assorted randomly into a list at the beginning of each session. Sample stimuli for each trial were taken in order from the start of the list and were not replaced during a session. The additional 1000 stimuli were used as AN foils. Each foil was truly trial unique and assigned to only one retention trial. Daily sessions consisted of 20 blocks of five randomized trial types, totaling 100 trials. Training continued for 10 d until the subjects completed a total of 200 trials of each trial type.

Experiment 4: WSR trials alone. The experimental task was the same as in experiment 1, with the exception that only WSR trial types were pre-

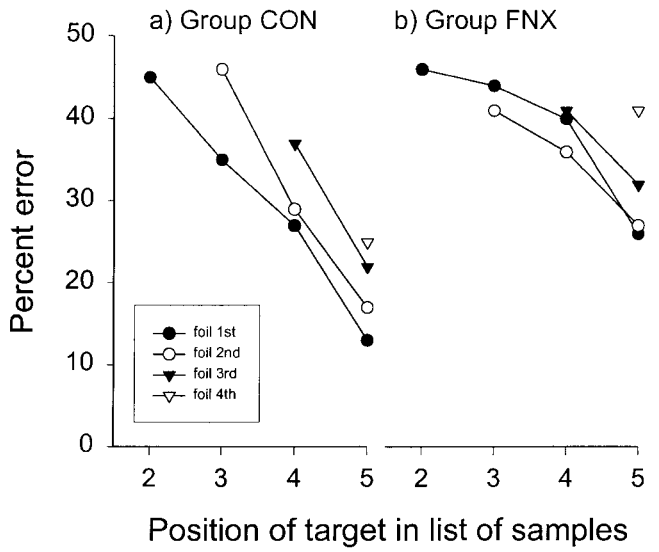

Figure 2. Mean percentage of error scores in intermixed WSR trials (experiment 1) as a position of the target in the list of samples for control (*a*) and fornix-transected (*b*) animals.

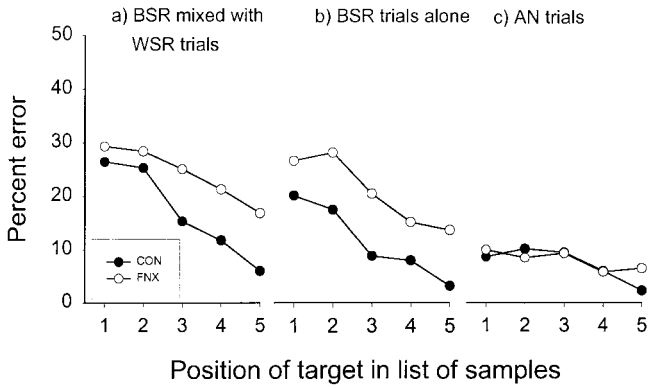

Figure 3. Mean percentage of error scores as a position of the target in the list of samples for intermixed BSR trials in experiment 1 (*a*), BSR trials alone in experiment 2 (*b*), and AN trials in experiment 3 (*c*).

sented. After presentation of a sequence of five samples, monkeys received retention trials that required them to choose which of two items selected from the previously experienced sequence had occurred more recently. A daily session consisted of 10 blocks of 10 randomized trial types, totaling 100 trials. Training continued for 15 d until the subjects completed a total of 150 trials of each trial type.

Results

Pretraining stages

Although, on average, group FNX attained criterion more slowly than group CON in tests of DMS and the subsequent two stages of pretraining in recency judgments, the difference in terms of both errors (largest $t = 1.141$; df = 4; $p = 0.317$) and trials (largest $t = 1.841$; df = 4; $p = 0.140$) was not statistically significant at any of these three stages.

Experiment 1

This task was designed to investigate whether BSR and WSR were differentially affected by fornix transection and, therefore, we analyzed the data to assess whether there was a group task interaction. The total errors accrued over trials for both WSR and BSR trial types were analyzed together by a parametric ANOVA with one between-subjects factor (group: FNX vs CON) and one within-subjects factor (trial type). There was a nonsignificant effect of group on error score ($F = 2.80$; df = 1.4; $p = 0.17$), and the interaction term was nonsignificant ($F < 1$). The between-group difference for WSR and BSR percentage of error scores are similar [WSR: (CON) 70.5 − (FNX) 62.8 = 7.7; BSR: (CON) 83 − (FNX) 75.8 = 7.2]. A separate analysis undertaken on WSR trials only showed an effect approaching the level of statistical significance ($F = 5.520$; df = 4; $p = .079$). Thus, in this task, in which WSR and BSR trials were intermixed, the difference between the FNX and CON groups did not reach significance.

The mean percentage of error scores in WSR retention trials for groups CON and FNX are presented in Figure 2. Performance accuracy reported as a function of the position of the target within the sequence is plotted against the position of the foil within the sequence, represented by the curves. Because the target stimuli were items occurring more recent in the sequence, only the final four locations are relevant, and these are numbered 2–5. A foil stimulus could, therefore, be either the first, second, third, or fourth item in the sequence, and each plot represents the foil item that was paired with a particular target item in choice trials. For example, in a choice trial, 1 versus 2, 1 would be the foil that is plotted against the target, 2, which is the item in this choice trial that occurred more recently in the sequence. Figure 2*a* shows the performance accuracy for group CON. Two effects are observed. First, a decrease in the error rate score is shown as you move from the left to the right of the graph, reflecting the position of the target gradually occurring later in the sequence. For target items occurring later in the sequence, the percentage of error scores were lower than for target items occurring earlier, demonstrating a clear recency effect. Second, there is a foil–target separation pattern; when the target and foil occur close together in a sequence, the percentage of error scores were higher, and this is most evident for neighboring items in the sequence. The percentage of error scores on retention trials 1 versus 2 and 2 versus 3 exceed the scores for retention trials 4 versus 5, suggesting that in trials in which neighboring items occurred later in the sequence, the target sample was more accurately identified. In contrast, retention trials in which the target sample and foil were separated by an increasing number of intervening items in the sequence were performed with a greater degree of accuracy. Performance accuracy was greatest for the trials in which the target sample and foil were separated by the greatest number of intervening items, 1 versus 5, with performance accuracy exceeding all other trials.

Figure 2*b* shows the mean percentage of error scores for group FNX. The mean percentage of correct scores across WSR retention trials is lower for group FNX (group FNX, 62.8; group CON, 70.5) than for group CON, although this failed to reach significance (Fig. 2*a*). Although a similar pattern of recency effects is evident, the mean performance of group FNX was poorer than that of group CON. In contrast to the pattern of performance observed for group CON in Figure 2*a*, the order of the foil–target separation pattern for group FNX differs on trials in which the foil is the first item in the sequence. In these trials, performance accuracy is worse, on average, than trials in which the foil is the second item. Within these trials, a greater separation within the sequence of the foil and target did not result in improved performance accuracy for group FNX.

The performance of groups CON and FNX in BSR retention trials is presented in Figure 3*a*, in which the percentage of error scores are plotted against the position of the target item within the sequence. For group CON, the performance accuracy across all trials exceeded that for group FNX. For each group, scores for

trials in which target items occurred earlier in the sequence were lower than for target items occurring later.

Experiment 2: BSR trials alone

When WSR and BSR trials were intermixed in experiment 1, the difference between control and fornix-transected groups did not reach significance. It is possible that BSR test performance could be adversely affected by the requirement to make WSR judgments and vice versa. To examine this possibility, each requires testing separately. Experiment 2 was designed to investigate whether BSR retention trials tested alone are impaired by fornix transection. Figure 3*b* shows the mean percentage of error scores in BSR retention trials for groups CON and FNX. Performance accuracy is reported as a function of the position of the target item within the sequence. Across all trial types, group CON performed more accurately than group FNX. Across groups, error scores were higher for retention trials in which the target items occurred earlier in the list than for those occurring later. When compared with the performance attained across intermixed BSR trial types in experiment 1, the present results show that the difference in the mean percentage of correct scores is greater [experiment 1, CON 83 vs FNX 75.8 (difference, 7.2); experiment 2, CON 88.5 vs FNX 79.2 (difference, 9.3)]. A *t* test conducted on the mean error scores in the current experiment showed that group FNX was significantly worse than group CON ($t = 2.223$; $df = 4$; $p = 0.045$; one tailed).

Experiment 3: AN trials

This task was designed to investigate the effect of fornix transection on the monkeys' ability to discriminate a familiar stimulus that had appeared in the sequence in preference to an AN foil that had not been presented before. In Figure 3*c*, the mean percentage of error scores obtained by groups CON and FNX are plotted as a function of the position of target items within the sequence. In each group, a general trend was toward target items occurring later in the sequence remembered better than items occurring earlier. In contrast to the findings from tests of intermixed BSR judgments in experiment 1 and tests of BSR alone in experiment 2, the present results show a greater overlap in performance between groups. With the exception of target positions at the beginning and end of the sequence, group FNX scored a lower number of errors than group CON. The mean percentage of correct scores for group FNX was 91.2 compared with 92.4 for group CON. A *t* test conducted on the mean error scores did not approach significance ($t < 1$; $df = 4$).

Experiment 4: WSR trials alone

When WSR and BSR trials were intermixed in experiment 1, the difference between fornix-transected and control groups failed to reach significance. However, experiment 2 showed that fornix transection impairs tests of BSR alone. This task was designed to investigate whether WSR retention trials, when tested alone, are impaired by fornix transection. In Figure 4, WSR performance accuracy is reported as a function of the position of the target within the sequence, plotted against the sequential position of the foil represented by the curves. For target stimuli, only the final four locations in the sequence are relevant, and these are numbered 2–5. A foil stimulus could, therefore, be the first, second, third, or fourth item in the sequence, and each plot represents the foil item that was paired with a particular target item in choice trials. For example, in a choice trial, 1 versus 2, 1 would be the foil that is plotted against the target, 2, which is the item in this choice trial that occurred more recently in the sequence. Performance

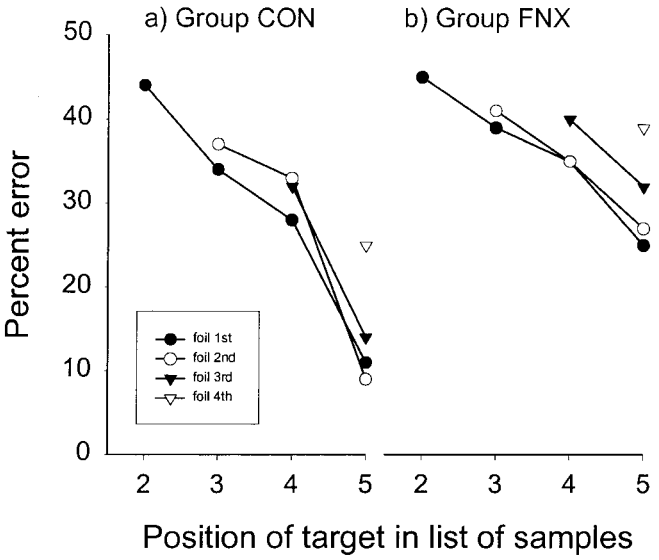

Figure 4. Mean percentage of error scores in WSR trials alone (experiment 4) as a position of the target in the list of samples for control (*a*) and fornix-transected (*b*) animals.

accuracy for group CON is illustrated in Figure 4*a*. A recency effect is demonstrated by the lower performance accuracy for target items occurring earlier in the sequence than for those occurring later. Two effects are observed. First, a decrease in the error rate score is shown as you move from the left to the right of the graph, reflecting the position of the target gradually occurring later in the sequence. For target items occurring later in the sequence, the percentage of error scores were lower than for target items occurring earlier. An additional effect shows a higher percentage of error scores for retention trials in which the target and foil occur close together in a sequence, especially for neighboring items. Performance accuracy was greatest for the trials in which the target sample and foil were separated by a greater number of intervening items, in this case 2 versus 5, followed by 1 versus 5, in which accuracy exceeded all other trials. The pattern of effects observed is strikingly similar to that observed for WSR trials in experiment 1. A comparison between the percentage of correct scores for intermixed WSR trials (experiment 1) and the present results shows that when WSR is tested alone, a higher degree of performance accuracy is observed [group CON: 70.5 (experiment 1) vs 73.3 (experiment 2)].

Figure 4*b* shows the performance of group FNX, plotted exactly as described above for Figure 4*a*. The pattern of effects observed is similar to group CON, although the mean performance of group FNX was poorer than that of group CON. In contrast to the results obtained for WSR trials in experiment 1, a greater separation within the sequence of the foil and target produced an improvement in performance accuracy [group FNX, 62.8 (experiment 1) vs 64.2 (experiment 2)]. A *t* test conducted on the mean error scores demonstrated a significant effect of fornix transection on tests of WSR alone ($t = 3.379$; $df = 4$; $p = 0.014$; one tailed).

Summary of results

In experiment 1, when tests of WSR and BSR were intermixed, the difference between the control and fornix-transected groups did not reach significance. However, when a separate analysis of WSR trials was undertaken, this showed an effect approaching the level of statistical significance. Fornix transection impaired

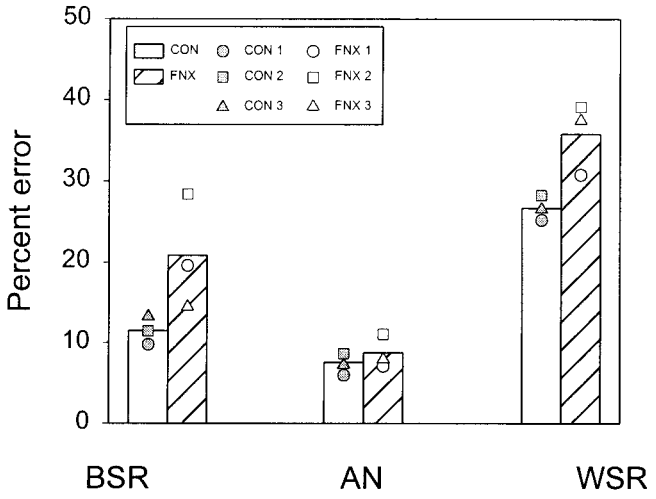

Figure 5. Mean percentage of errors across trial types for tests of BSR alone (experiment 2), AN (experiment 3), and WSR (experiment 4).

both BSR trials alone (experiment 2) and WSR trials alone (experiment 4) when tested separately and failed to impair AN judgments (experiment 3).

Figure 5 shows the mean percentage of errors across trial types for tests of BSR (experiment 2), AN (experiment 3), and WSR (experiment 4). Across tasks, judgments of AN are performed more accurately than judgments of BSR, and performance accuracy in each of these tasks is higher than that reported for judgments of WSR. An examination within tasks shows that although AN judgments are performed at the same level of accuracy for both groups, across all trial types, BSR judgments are performed less accurately by group FNX than by group CON. To assess the possibility that performance difference across tasks could be explained by unseen within-task variation instead of effects attributable to fornix transection, two designed comparisons were conducted. A designed comparison (using the pooled error term from the data obtained in experiments 2–4) comparing the effects of fornix transection on tests of AN in experiment 3 and tests of BSR alone in experiment 2 showed a significant difference ($t = 2.533$; df = 8; $p = 0.018$; one tailed). Similarly, a designed comparison (using the above pooled error term) comparing the effect of fornix transection in tests of AN and WSR alone showed a significant difference ($t = 2.474$; df = 8; $p = 0.019$; one tailed). Together with the initial comparison, this latter test confirms the observed contrast in performance between judgments of BSR, AN, and WSR.

Discussion

The present experiments were designed to investigate whether tests of WSR, BSR, and AN place different mnemonic demands on monkeys. Although we initially gave intermixed tests of WSR and BSR in experiment 1, subsequent testing of each task individually showed that intermixing these trials acts to distort our performance measure. Greater performance accuracy was achieved when BSR and WSR were each tested alone in experiments 2 and 4, respectively, than when they were intermixed in experiment 1. Experiment 2 showed that fornix transection impairs tests of BSR alone. In experiment 3, we removed this relative recency factor so that tests now consisted of distinguishing a previously experienced stimulus from a novel foil that had never been presented before. This experiment showed that fornix transection produces no impairment in judgments of AN. In experiment 4, we showed an impairment in tests of WSR alone (Fig. 5). This selective impairment in memory for recency judgments contributes to evidence showing that, in monkeys, the fornix performs a role in memory beyond the spatial domain.

The impairment in judgments of BSR alone observed in experiment 2 is consistent with previous findings in monkeys (Gaffan, 1974; Gaffan and Weiskrantz, 1980; Owen and Butler, 1981; Bachevalier et al., 1985a), which show that fornix transection impairs judgments of relative recency within the context of DNMS. In these tasks, memory for a previously presented sample can compete with memory for foils appearing in earlier testing sessions. Despite inconsistencies in the pattern of impairments shown by humans with amnesia attributable to fornix damage (Gaffan et al., 1991) when stimulus familiarity is increased because of repeated presentation of stimuli, subjects with fornix damage also show impairments in tests of DNMS (Aggleton et al., 2000). In contrast, Owen and Butler (1981) failed to observe an effect of fornix transection in a test of DNMS using a pool of only two stimuli presented repeatedly. However, this finding is limited by evidence of floor effects, particularly the failure of one control to obtain criterion. In rats, Shaw and Aggleton (1993) showed that fornix transection failed to impair performance on a test of relative recency in which a decreasing number of stimulus pairs were presented repeatedly within a session. Although clearly tests of recency memory, the procedures adopted in these tasks differ from those we used. In our acquisition procedure, a sequence of five stimuli preceded the choice trial, and any one of these stimuli could be the target, so the mnemonic demands of our task differed from those in which only a single sample stimulus precedes the choice trial (Owen and Butler, 1981, 1984; Shaw and Aggleton, 1993). Moreover, by presenting each of our five sample stimuli in the same place, our procedure limits the possibility of confusion suggested to arise from the use of an incidental spatial cue that distinguishes the acquisition and retention stages in both DMS and DNMS. This possibility of spatial confusion was suggested by Gaffan (1992) to account for previous inconsistencies in findings after fornix transection in the context of DMS and DNMS (Gaffan, 1974; Gaffan and Weiskrantz, 1980; Owen and Butler 1981, 1984). Briefly, in acquisition trials the sample is presented on a central well in a three-well testing board, whereas in retention trials the sample and a foil are presented on the lateral wells. In support of our finding in tests of WSR alone and BSR alone, recent evidence shows that radiofrequency lesions of the hippocampus selectively impair the rats' memory for items appearing earliest in a sequence of odors while leaving intact novelty recognition (Fortin et al., 2002).

In contrast, tasks that require a subject to select a previously presented sample in preference to a previously unseen AN foil do not require a judgment of the relative recency of each of these items at the retention test. The lack of impairment in AN judgments after fornix transection in experiment 3, although consistent with previous findings in tests of DNMS that use AN stimuli (Owen and Butler, 1984), contrasts with mounting evidence showing the perirhinal cortex is a crucial structure serving AN judgments. In tests of DNMS using truly trial-unique stimuli, impairments in recognition follow bilateral perirhinal cortex ablation alone (Meunier et al., 1993). Electrophysiological evidence suggestive of recognition processes in the perirhinal cortex reflect the mnemonic demands placed by tests of AN, BSR, and WSR. In recordings from monkeys viewing large stimulus sets, subvarieties of reduced neuronal response patterns in anterior inferior temporal cortex, including the perirhinal cortex, reflect information about the novelty, relative familiarity, and relative

recency of visual stimuli (Brown and Xiang, 1998; Xiang and Brown, 1998). Neuronal responses elicited by the initial presentation of a novel stimulus become weaker on its second and subsequent presentations. Familiarity responses characterized by stimuli that elicit reduced responses on their subsequent presentation do not distinguish how recently a stimulus was seen. Relative recency responses also elicit reduced responding on exposure to a previously experienced stimulus but do not distinguish whether or not the stimulus is familiar. Nevertheless, where recency monitoring is required when small sets of repeating stimuli are used in DMS, rhinal cortex ablation fails to impair performance (Eacott et al., 1994).

There is sparse evidence that within the hippocampus some (~1–2%) cells show activity that reflects the relative recency and familiarity of visual stimuli alone (Brown et al., 1987; Rolls et al., 1993; Xiang and Brown, 1998) or when combined with spatial information (Rolls et al., 1989). Although this evidence seems inconsistent with the results obtained in tests of WSR in experiment 4, any subsequent disruption in entorhinal cortex function that follows fornix transection could account for the results we obtained. Anatomically, efferent projections from the subiculum of the hippocampus to the medial mamilliary nuclei pass through the fornix (Swanson and Cowan, 1975), and the entorhinal cortex is reciprocally connected with both the subiculum and the perirhinal cortex. Thus, disruption in entorhinal cortex function may compromise mnemonic coding that depends on activity exchanged between different medial temporal cortical structures. Recently, it has been shown that mnemonic activity in the perirhinal cortex can subsequently backproject and activate cells in inferotemporal area TE. Naya et al. (2001) used a paired-associate task to show that although stimulus-specific activation occurs initially in TE, the subsequent retrieval-related activation to one member of the paired associate that occurs in the perirhinal cortex backprojects to these TE cells. This intrahemispheric backward-projecting mnemonic signal is disrupted after lesions of the rhinal (entorhinal and perirhinal) cortex. Postoperatively recorded inferotemporal cortical activity correlating with sets of paired associates learned either preoperatively or postoperatively failed to reflect associations between members of each pair after rhinal lesions (Higuchi and Miyashita, 1996). Moreover, lesions of the rhinal cortex have been shown to impair the ability of monkeys to associate a visual cue with sequential information relevant to a reward schedule that informs the state of progress toward a particular rewarded stimulus (Liu et al., 2000). These latter two findings suggest that lesions that include the entorhinal cortex disrupt task relevant mnemonic coding that, in the case of the former example, is dependent on exchange of information via reciprocal connections between medial temporal cortical structures.

Task differences do not permit us to compare directly the results we obtained in tests of WSR, with the findings of reduced neural activity suggestive of the relative recency of a stimulus reported by Brown and Xiang (1998). In our task, monkeys were explicitly trained to maintain in memory a unique sequence of five stimuli and to correctly identify in retention tests the item that occurred most recently. In contrast, the conditions in which electrophysiological studies have elicited reduced neural responding have not included a training requirement to recognize in a sequence of items, the subsequent presentation of a previously viewed stimulus (Fahy et al., 1993). In other tasks, monkeys are required to remember either only one stimulus at a time or an undetermined number in serial recognition experiments (Brown and Xiang, 1998). These procedures differ from those used in our task, in which within-session trial-unique sequences of five stimuli were presented and monkeys were explicitly required to remember a previously presented sample in the sequence (BSR and AN) or the sample that had occurred more recently in the sequence (WSR).

In addition to the evidence that shows a role for the fornix in tasks such as scene memory (Gaffan, 1994), place memory (Gaffan and Harrison, 1989) and, more recently, in explicitly spatial tasks such as concurrent spatial discrimination learning (Buckley et al., 2004), the findings from our series of experiments extend the role of the fornix to the processing of temporal information. Consistent with this, Brasted et al., (2003) reported that associations between stimuli and temporally differentiated responses are impaired after fornix transection. Taken together, the evidence supporting a role in the processing of both spatial and temporal information suggests a supramodal processing function for the fornix.

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