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Fornix Transection Impairs Visuospatial Memory Acquisition More Than Retrieval

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It has been hypothesized that some fornical fibres may instantiate a neuromodulatory reinforcement signal supporting memory acquisition in medial temporal cortical regions. This suggests that fornix transection should impair postoperative new learning more severely than the recall of preoperatively acquired information. Here, postoperative recall of 288 concurrent visuo-spatial discrimination problems acquired preoperatively was unaffected after fornix transection in the macaque, whereas new postoperative learning of 72 problems was impaired. This and other recent evidence supports the idea that the main function of the fornix in macaque monkeys is to support new learning about spatio-temporal context.

Keywords: medial temporal lobe, hippocampus, diencephalon, learning, macaque

Early studies investigating the effects of fornix transection in the macaque monkey reported impairments in place discrimination reversal but not impairments in object discrimination reversal (Mahut, 1972; Mahut & Zola, 1973; Zola & Mahut, 1973). Postoperative acquisition of trial-unique delayed object matching-to-sample (Gaffan, 1974; Owen & Butler, 1981, 1984) and concurrent object discrimination learning were also observed to be unimpaired (Moss, Mahut, & Zola-Morgan, 1981). That the fornix may have a more important role in learning about spatial relationships than about objects was taken forward in subsequent work investigating the effects of fornix transection on memory for complex naturalistic scenes (Gaffan, 1977, 1992, 1993b). The detrimental effects of fornix transection upon scene learning have also been investigated in detail in paradigms in which algorithmically generated computer scenes could be generated and presented on a touch-screen; both human patients with fornix transection and nonhuman primates are impaired on this task (Aggleton et al., 2000; Gaffan, 1994b). Nevertheless, one cannot infer from those studies that fornix transection affects spatial memory per se, as scene learning is not a purely spatial task. Previously, observations of spatial learning impairments after fornix transection were limited to tasks in which macaques learned about the spatial position of food reward in the Wisconsin General Test Apparatus or in a T-maze (Gaffan, 1994a; Gaffan & Harrison, 1989; Murray, Davidson, Gaffan, Olton, & Suomi, 1989). However, a more recent study (Buckley, Charles, Browning, & Gaffan, 2004) confirmed that deficits after fornix transection also extended to tasks in which animals had to learn about the spatial positions of stimuli presented upon a touch-screen in an automated apparatus.

Buckley et al. (2004) showed that the deficit after fornix transection was selective in that postoperative retention of 40 preoperatively learned problems were unaffected whereas the learning of new postoperative problems was impaired. This is consistent with the view (Gaffan, Parker, & Easton, 2001) that some fornical fibres may instantiate a neuromodulatory reinforcement signal that supports learning in the medial temporal lobe (MTL) cortex. To evaluate postoperative retention abilities without confounding retention with postoperative re-learning it is necessary to conduct 1-trial retention tests in which the performance on each problem is assessed only once postoperatively (see Buckley, 2005). The powers of such analyses are therefore limited by the numbers of problems that the animals are able to acquire preoperatively. Moreover, concurrent discrimination paradigms (in which animals are repeatedly tested on lists of discrimination problems until they learn the whole set) have traditionally employed relatively few problems per set (typically between 8 and 20) that facilitates acquisition of the set. In a recent review of MTL lesion studies, Buckley and Gaffan (2006) concluded that adopting concurrent object discrimination learning tasks with larger set sizes acts to increases the demands that these tasks place upon stimulus identification in macaques.

In the present study then, to facilitate a more robust assessment of postoperative retention compared to new postoperative acquisition, and to place higher demands upon spatial learning, we preoperatively trained macaques on a much larger set of 288 concurrent spatial discrimination problems (compared to 40 problems in Buckley et al., 2004). These 288 problems were subdivided into four successively acquired sub-sets of 72 concurrently learned problems to assist acquisition of such a large number of total problems. However, this design feature had a dual purpose; it also facilitated our investigation into whether fornix transection might produce a gradient of retrograde amnesia over the course of the acquisition period in which each of the sub-sets were successively acquired. It was our expectation that retention would not be impaired after fornix transection; further, to strengthen this finding with respect to confirming whether it may generalize across different levels of stimulus familiarity, we further divided the problems in each set into three problem types that differed in their

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relative exposure levels during the learning phase (some problems were seen once per session, some twice per session, and some three times per session in the preoperative acquisition phase). Subsequently, to assess the selectivity of the effect of fornix transection with respect to new postoperative learning we required the animals to acquire new postoperative concurrent spatial discrimination problems that we expected to be impaired.

Materials and Method

Subjects

Six experimentally naïve male cynomolgus monkeys (*Macaca fascicularis*) took part in this experiment. Their mean weight at the start of behavioral testing was 5.8 kg (range 3.9–5.6 kg), and their mean age was 3 years and 4 months. They were housed together in a group enclosure (except for two who were housed together as a pair) in an enriched environment in which they were also able to forage for small food items (seeds, etc.). All had automatically regulated lighting and water was available ad libitum.

Surgery

After completing preoperative training, three animals received bilateral fornix transection (group FNX) and the other three remained unoperated controls (group CON). All licensed procedures were carried out in compliance with the United Kingdom Animals (Scientific Procedures) Act of 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 sagittal 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 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. The operated monkeys rested for 11 to 14 days after surgery before beginning postoperative training. Unoperated control monkeys rested for the same period of time between preoperative and postoperative training. In common with most ablation experiments using macaque monkeys, ours did not have an operated control group. This means that we cannot completely rule out the possibility that the effects seen in the animals with fornix transection were because of the callosal incision, the anesthesia, or some other component of the surgical operation other than the fornix transection itself. However, in earlier experiments with fornix transection an operated control group was sometimes included, and a change from the control group's preoperative ability was never reported (Gaffan & Saunders, 1985; Gaffan, Shields, & Harrison, 1984). Similarly in the human brain, patients with fornix transection in the course of colloid cyst removal showed memory impairments, but those patients in which a similar surgical operation was performed while leaving the fornix intact showed no memory impairments (Aggleton et al., 2000).

Histology

At the conclusion of this, and a following series of experiments, the animals with fornix transection were deeply anaesthetized, 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 (see Figure 1, panels B, C, and D) with no damage outside the fornix except for the incision in the corpus callosum as described in the surgical procedures and at most, only slight damage to the most ventral part of the cingulate gyrus at the same anterior-posterior level in only one hemisphere of one animal (see Figure 1, panel B).

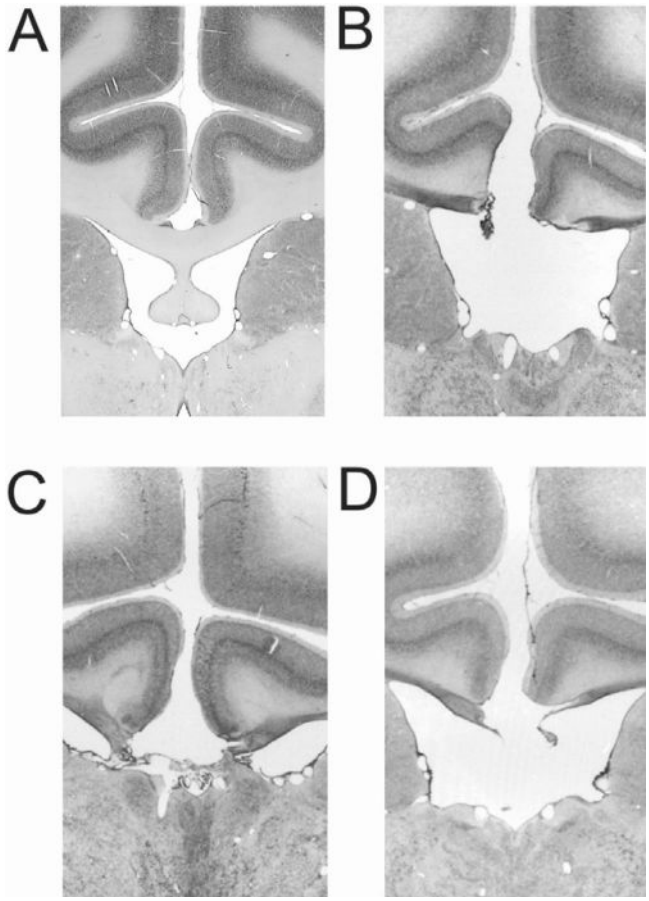


Figure 1. (A) Coronal section from the brain of a normal unoperated macaque brain just posterior to the level of the interventricular foramen; (B–D) coronal sections from the brains of three fornix transected subjects showing that the fornix transection was complete (the anterior-posterior level of the fornix transection varies between these subjects depending at which level the fornix was cut through a small hole made in the corpus callosum at that level).

Apparatus

The task was performed in an automated test apparatus. The subject sat in a wheeled transport cage fixed in position in front of a touch-sensitive screen (380 mm × 280 mm) on which the stimuli were displayed. The animals were unrestrained within this transport cage and could reach out between the horizontal bars (spaced approx. 50 mm apart) of the otherwise open frontage of the transport cage to touch the screen. An automated pellet delivery system, controlled by the computer, delivered reward pellets into a food well (approx. 80 mm in diameter) positioned beneath and to one side of the screen. Banana flavored reward pellets (190 mg supplied by Noyes Company Inc.) were delivered in response to correct choices made by the subject to the touch-screen. Pellet delivery was accompanied by an audible click. An automated lunchbox (approx. dimensions were: length 200 mm, width 100 mm, height 100 mm) was also positioned beneath and to one side of the subject. The lunchbox was operated by a spring-loaded solenoid allowing it to be opened immediately with a loud crack on completion of the whole task. The lunch box contained the subject's daily diet of wet monkey chow, proprietary primate pellets, nuts, raisins, and slices of apple, banana, and orange. An infrared camera was positioned to look down into the transport cage from above to allow the macaque to be observed from a separate room while it was engaged in the task. The whole automated apparatus was housed in an experimental cubicle that was dark apart from the background illumination from the touch-screen. The presentation of visual stimuli on the screen was controlled by a computer that also recorded the touches that subjects made to the screen and controlled the delivery of reward pellets after correct responses and the opening of the lunchbox after completion of the session.

Stimuli

The visual stimuli presented on the touch-screen were taken from a large library of individual clipart images obtained from commercially available Internet sources. Each clipart bitmap image was 128 × 128 pixels in dimension and comprised a distinct foreground multi-colored cartoon-like image on a white background. The background of the whole touch-screen was set so as to match this color, with the effect that the visible borders of our stimuli matched the outlines of their actual shapes and not the rectangular border of each clipart image. The particular stimuli assigned to each problem set were chosen at random (without replacement) from a library of over 6,000 clipart stimuli. The resolution of the visual display on the touch-screen was set at 800 × 600 pixels with the effect that each visual stimulus on the screen subtended approximately 12 to 15 degrees of visual angle from the typical viewpoint and perspective of a macaque in its transport cage. Some examples of the stimuli used in this study are shown in Figure 2.

Behavioral Task

Pretraining stage. The subjects were first accustomed to feeding in the apparatus (described above) and were then taught in stages to touch patterns appearing on the touch screen to obtain food reward. After a standard shaping process was completed, they began a preliminary training stage (see later) before commencing the preoperative testing stage described in the next paragraph.

Preoperative testing stage. The task in the current study was a concurrent visuo-spatial conditional discrimination task. In each trial two identical visual stimuli appeared on the screen, one in

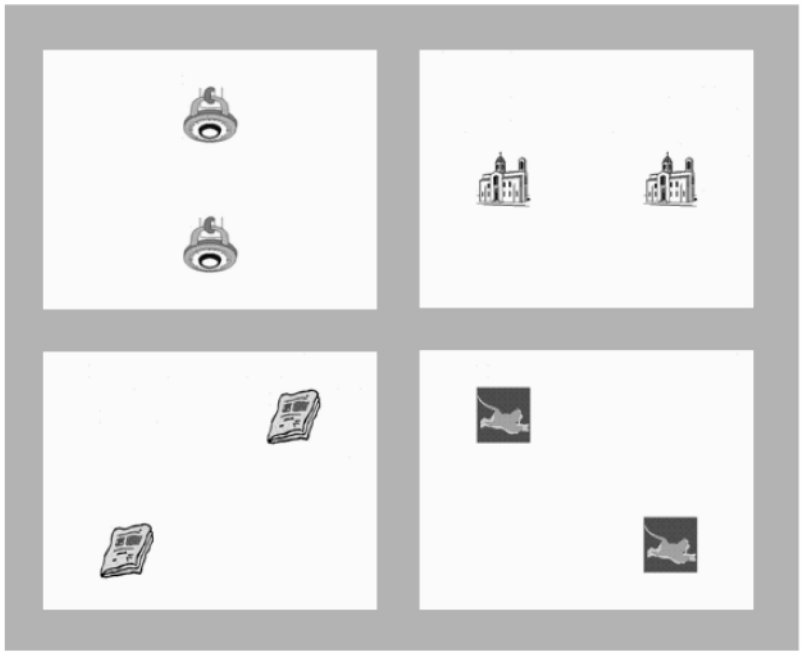


Figure 2. The four panels illustrate four examples of problems from the visuo-spatial conditional discrimination task showing the four possible arrangements of the pairs of identical stimuli that appear in each trial. In each problem the animal has to learn by trial and error which of the two positions is correct; for each of the four arrangements equal numbers of each position are rewarded across the entire problem set.

each of two 'slots' positioned opposite each other, and the monkey was required to chose, by touching whichever of the two positions occupied by the stimuli in that trial was reinforced for that particular stimulus. There were 72 problems in each set (and so each problem set comprised 72 unique visual stimuli) and the problems in each set were learned concurrently. Of these 72 problems, 18 were displayed on the screen in such a way that the two identical copies of the visual stimulus in those problems occupied 'high-center' and 'low-center' slots on the touch screen, respectively; another 18 problems had visual stimuli that occupied 'mid-left' and 'mid-right' slots, 18 problems used 'high-right' and 'low-left' slots, and the remaining 18 used 'high-left' and 'low-right' slots. Figure 2 shows one example of a problem from each of the four possible stimulus arrangements. Across the whole set of 72 problems, each of 8 slots on the screen used were associated with reward in 9 of the 18 problems that used that slot and were associated with nonreward in the other 9 problems, thereby ensuring that the reward-values of each slot were counterbalanced and rewarded exactly half of the time. Further, within each problem set there were equal numbers of three different types of problems. The first problem type (Type I) were problems that were shown only once per daily session, the second problem type (Type II) were problems that were shown twice within in each daily session, and the third type (Type III) were problems that were shown three times within in each daily session. Each daily session was therefore comprised of exactly 144 trials [$(1 \times 24) + (2 \times 24) + (3 \times 24)$].

Each session proceeded as follows. The order of the 144 trials that comprised the entire session was randomized before the session began and then each trial was given in the random order of the day (subsequent days had a different random order). The two identical stimuli for the current trial were presented at the same time in their respective slots and remained on the screen until the monkey touched one or other of these stimuli. If the monkey touched the area in which the correct stimulus was positioned for that trial then the monkey immediately received one 190 mg banana pellet as reinforcement food reward. At the same time, the incorrect stimulus was removed from the screen leaving just the correctly positioned stimulus on the screen as further visual feedback for a correct response. This was followed by a blanking of the entire screen for an inter-trial interval of 5 s that preceded commencement of the next trial. If instead, the monkey touched the area of the screen in which the incorrect stimulus was positioned for that trial then both stimuli were immediately removed form the screen, no reward pellet was delivered, and a longer inter-trial interval of 20 s was commenced. There were no correction trials. The monkeys were trained on this task daily until they attained performance levels of 90% correct or better in a single daily session on Type III problems in this first problem set (Set A). On the day after attaining criterion on Set A the monkeys commenced training on a new set of 72 problems (Set B) in exactly the same manner until they again attained the same criterion on Set B, and this was followed by two more sets (Sets C and D) in the same manner until all four sets (and therefore 288 problems in total) were acquired to criterion.

To familiarize the animals with the demands of the task, we preceded training on the first set (Set A) by a preliminary practice stage that consisted of only 16 problems in total (none of these problems appeared in any of the four test sets A–D). The practice sessions introduced these 16 problems gradually in six stages: (1) new learning of 2 problems (problems 1–2), (2) new learning of 2 problems (problems 3–4), (3) concurrent testing with all 4 problems (problems 1–4), (4) new concurrent learning of 4 problems (problems 5–8), (5) concurrent testing of all 8 problems (problems 1–8), (6) new concurrent learning of 8 problems (problems 9–16). Unlike in the task proper (i.e., Sets A-D) repetition-correction trials were employed in these practice sessions as a further aid to task acquisition. Each new stage (1–6) commenced when performance reached 90% or greater on the preceding stage. Thus, before the animals commenced training on the first full set (Set A) they were already well practiced at acquiring concurrent visuo-spatial conditional problems.

To summarize, we trained six animals preoperatively on 288 test problems that were divided into four problem sets (A–D) comprised of 72 problems each. Each of the four sets were learned in turn so that relative to the time of surgery, Set D was the most recently acquired, and Sets C, B, and A were each acquired successively further back in time relative to the date of surgery (see Table 1). This design feature was incorporated into our study to allow us to determine whether or not there might be any postoperative patterns or gradients in retrograde memory loss. Additionally, the inclusion of the three types (Types I–III) of problems was a design feature that allowed us to examine whether fornix transection would differentially affect the recall of memories acquired to different levels of proficiency preoperatively.

Table 1
The Number of Errors-to-Criterion That Each Animal Accrued While Learning Each of Four Preoperative Sets (A–D) and the One Postoperative Set (E)

	Set A	Set B	Set C	Set D	Set E
CON1	860 (19)	1026 (22)	575 (13)	545 (12)	259 (6)
CON2	1379 (30)	1089 (22)	918 (19)	559 (12)	517 (12)
CON3	137 (4)	433 (9)	255 (6)	275 (5)	164 (4)
Mean CON	**792 (17.7)**	**849 (17.7)**	**583 (12.7)**	**460 (9.7)**	**313 (7.3)**
FNX1	473 (10)	444 (8)	404 (9)	350 (7)	447 (10)
FNX2	237 (6)	319 (8)	298 (6)	315 (9)	482 (12)
FNX3	740 (15)	659 (14)	530 (10)	463 (11)	457 (10)
Mean FNX	**483 (10.3)**	**474 (10)**	**411 (8.3)**	**376 (9)**	**462 (10.7)**

Note. The number of sessions-to-criterion taken in each case are indicated in parentheses.

After the animals attained criterion on the last set they were given a retention test (Retention Test 1) that started on the next day. In the retention test, each of the 288 problems was tested once. The retention test was split over three successive days in such a way that 96 problems were given in each of three successive sessions (the 288 problems were tested in a random order across these 3 days).

On the following day they recommenced re-training (again by the method described in preoperative testing stage in which the number of times each problem was presented in a session was determined by its type; i.e., Types I–III) on all of the four sets of stimuli, with Sets A through D presented for 1 day per set over the course of four successive days. This was repeated four times (so that there were 16 days of re-training in total).

On the next day they commenced a second preoperative retention test (Retention Test 2) using the same method as described for Retention Test 1. Based on their performance on this Retention Test 2 the animals were divided into two groups in which the mean performance in each was equated. One group then received bilateral fornix transection (Group FNX) and the other group (Group CON) remained unoperated controls. Approximately 2 weeks later, both the CON and FNX groups commenced the postoperative testing phase.

Postoperative testing stage. The animals were given a postoperative retention test 2 weeks after surgery. The format of this test was identical to the preoperative retention tests described earlier in which the 288 preoperatively learned problems were tested once each over the course of three successive days. On the day following completion of the postoperative retention test, the animals commenced learning of a new postoperative set of problems (Set E). We adopted an identical procedure for acquisition of these new postoperative sets as was done for the preoperatively acquired sets and the animals continued daily testing on Set E until they attained criterion of 90% or better performance in a single session at which point their participation in the current study was completed.

Results

Preoperative Training

The six animals made on average 638 errors while learning the first set of 72 problems (Set A) to criterion. Sets B to D were subsequently learned to criterion in an average of 662, 497, and 418 errors, respectively. In the first retention test, the animals' performances averaged across all four sets was 63% correct for Type I problems, 67% correct for Type II problems, and 75% correct for Type III problems. The animals then progressed to the re-training stage (see Method) after which they were tested on a second retention test identical in format to the first (see Method). In Retention Test 2, the animals' performances averaged across all sets was 72% correct for Type I problems, 79% correct for Type II problems, and 87% correct for Type III problems.

Preoperative testing stage. The animals were assigned to CON and FNX groups based on their relative performance on the Retention Test 2 that immediately preceded surgery. The mean performance of the animals assigned to the CON and FNX groups across all 288 problems in Retention Test 2 was 77.3% and 81.7%, respectively and there was no significant difference between the performance levels of the two groups at retention ($t < 1$). A repeated measures ANOVA also confirmed that there were no significant differences between the two groups in preoperative learning rate across the four preoperative sets either in terms of errors-to-criterion ($F = 1.15$, $df = 1,4$, $p > 0$) or days-to-criterion ($F = 1.06$, $df = 1,4$, $p > 0$).

Postoperative retention. Postoperatively the mean performance of the CON group and FNX group on the postoperative retention test for the 288 problems was 77.7 and 76.3%, respectively. A repeated measures ANOVA with one between-subject factor 'Group' (with two levels: FNX and CON) and one within-subject factor 'Condition' (with two levels: Preop and Postop) showed that there was no significant Group × Condition interaction ($F = 2.43$, $df = 1,4$, $p > .1$). We conclude that the FNX group was not impaired at their overall retention of 288 preoperatively learned concurrent spatial discriminations (Figure 3, panel A).

We also analyzed the pre- and postoperative retention data of these 288 problems with respect to their relative performance on each problem type (Type I-III) and on each set (Set A–D). Table 2 shows the individual animals' and group mean preoperative and postoperative performance levels with respect to the measures. A repeated measures ANOVA with one between-subject factor 'Group' (with two levels: FNX and CON) and three within-subject measures, 'Condition' (with two levels: Preop and Postop), 'Set' (with four levels: Sets A–D), and 'Type' (with three levels: Types I–III) confirmed that there no interaction between 'Group' × 'Condition' ($F < 1$) and also showed that there was no significant three way interactions between 'Group' × 'Condition' × 'Set' ($F < 1$), nor was there a significant three way interaction between 'Group' × 'Condition' × 'Type' ($F = 1.72$, $df = 2,8$, $p > 0.1$). Thus, we conclude that fornix transection did not affect any of the four sets of problems (acquired over different periods of time; see Figure 3, panel B) or any of the three types of problems (with different degrees of familiarity; see Figure 3, panel C) differentially. In short, we found that the performance of the FNX group was not statistically different from the CON group on all of our retention test measures and we therefore conclude that fornix transection does not affect retention of large numbers of preoperatively learned concurrent visuo-spatial conditional discrimination problems.

On average, although animals showed a trend towards acquisition of a learning set preoperatively, a repeated measures ANOVA showed that they did not as a group make statistically fewer errors in learning successively presented sets ($F = 3.41$, $df = 1,5$, $p > .1$ for linear trend across sets). Instead, there was found to be some variation in which particular sets (A–D) individual animals learned at the slowest and fastest rate (see Table 1). To verify that fornix transection did not have a differential, even if subtle, effect upon sets learned at different rates we determined for each animal that of the four sets was learned at the fastest and slowest rate preoperatively (from the data in Table 1) and then calculated the difference in retention test scores for those two sets (from the data in Table 2) both pre- and postoperatively. A repeated measures ANOVA with one between-subject factor 'Group' (CON vs. FNX), and one within-subject factor 'Condition' (Preop vs. Postop) showed that there was no 'Group' × 'Condition' interaction ($F = 1.98$, $df = 1,4$, $p > .1$) in this measure. Therefore, we conclude that within the range of learning-rates observed in this study, fornix transection does not have any effect upon retention.

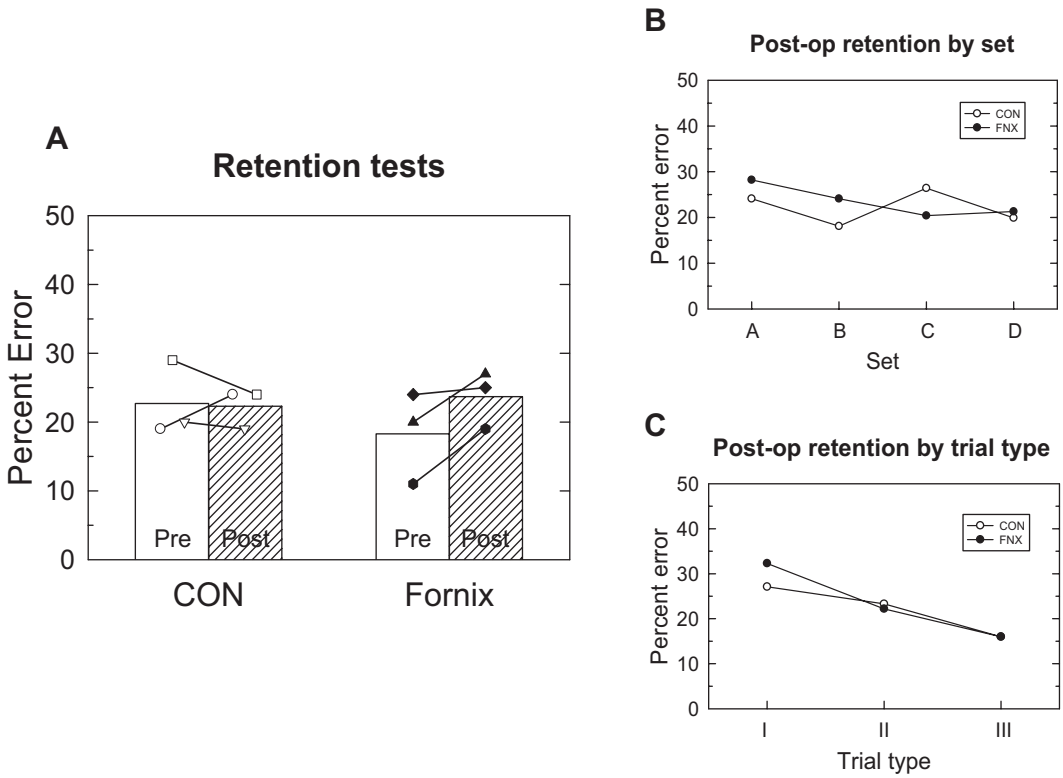


Figure 3. Panel A depicts, for the unoperated control group (CON) and for the fornix transected group (FNX), the mean percent error across all 288 visuo-spatial conditional discrimination problems in the last preoperative retention test (Pre) compared to the postoperative rentention test (Post). The changes in pre- and postoperative performance of individual animals in each group are indicated by symbols connected by lines. Panel B indicates the mean percent error made by both groups on the problems in the postoperative retention test that were learned as parts of Sets A through D, respectively. Panel C indicates the mean percent error made by both groups on the problems in the postoperative retention test according to whether the problems were presented once (trial Type I), twice (trial Type II) or three times (trial Type III) per session during the preoperative learning phase.

Postoperative new learning. The CON and FNX groups acquired the new postoperative set (Set E) while accruing on average of 313 and 462 errors-to-criterion respectively (Table 1 and Figure 4). On the immediately preceding preoperative set (Set D) the CON and FNX groups accrued a mean of 460 and 376 errors-to-criterion, respectively (Table 1 and Figure 4). As the distribution of the data in this errors-to criterion measure appeared to depart from normality we log transformed the data before analysis, an approach that is recommended to better equate variance in such circumstances (Kirk, 1982). A repeated measures ANOVA with one between-subject factor 'Group' (with two levels: FNX and CON) and one within-subject factor 'Condition' (with two levels: Last Preop Set and Postop Set) showed that there was a significant Group $\times$ Condition interaction ($F = 8.123$, $df = 1,4$, $p = .046$). A similar analysis that considered the mean errors to criterion made by both groups on all four preoperative sets (Sets A–D) compared to the postoperative set (Set E) likewise showed that there was a significant Group $\times$ Condition interaction ($F = 9.178$, $df = 1,4$, $p = .039$). Although Levene's tests did not indicate that any of the groups in these analyses suffered from significant inhomogeneity of variance (all $p > .05$), in light of the small set size we also ran nonparametric test on the nontransformed data to further investigate the robustness of this deficit. Although there was overlap between groups in the raw new postoperative learning scores, it is important to take into account preoperative learning abilities of each animal before interpreting postoperative learning scores. Therefore, as in our parametric tests, our first analysis considered the most recent preoperative learning scores (Set D) as the preoperative measure to which postoperative performance would be compared. A comparison of the differences between groups in the pre- versus postoperative errors-to-criterion learning scores showed that there was no overlap between the groups (see Table 1) in this comparative measure (Mann-Whitney U, $p = .05$) that provides further confirmatory evidence that fornix transection does impair new postoperative learning of concurrent visuo-spatial conditional discriminations (Figure 4). Although Set D was not arbitrarily chosen in the analysis above, we also proceeded to examine whether there would be any overlap between groups on this measure if any of the other preoperative sets were chosen as the measure of preoperative ability. Apart from Set A (the first set to be acquired, and one in which there might be expected to be stronger task acquisition effects), all other sets (i.e., the last three preoperatively acquired sets) either considered individually or averaged together also showed no overlap in the preoperative

Table 2
Individual and Group Mean Percent Correct Scores for the Second Preoperative Retention Test (PRE-OP) and for the Postoperative Retention Test (POST-OP)

PRE-OP	AI	AII	AIII	BI	BII	BIII	CI	CII	CIII	DI	DII	DIII
CON1	62.5	58.3	87.5	79.2	87.5	91.7	66.7	79.2	87.5	75.0	95.8	100.0
CON2	58.3	70.8	70.8	58.3	70.8	91.7	66.7	62.5	79.2	70.8	70.8	83.3
CON3	66.7	70.8	100.0	70.8	91.7	87.5	75.0	75.0	83.3	70.8	95.8	70.8
Mean CON	**62.5**	**66.6**	**86.1**	**69.4**	**83.3**	**90.3**	**69.5**	**72.2**	**83.3**	**72.2**	**87.5**	**84.7**
FNX1	50.0	66.7	83.3	79.2	83.3	87.5	66.7	66.7	87.5	70.8	79.2	91.7
FNX2	91.7	83.3	95.8	75.0	91.7	79.2	83.3	83.3	100.0	83.3	95.8	100.0
FNX3	79.2	70.8	79.2	75.0	91.7	79.2	75.0	66.7	91.7	70.8	87.5	87.5
Mean FNX	**73.6**	**73.6**	**86.1**	**76.4**	**88.9**	**82.0**	**75.0**	**72.2**	**93.1**	**75.0**	**87.5**	**93.1**
POST-OP	AI	AII	AIII	BI	BII	BIII	CI	CII	CIII	DI	DII	DIII
CON1	58.3	70.8	83.3	62.5	83.3	83.3	62.5	83.3	87.5	79.2	79.2	83.3
CON2	83.3	83.3	75.0	79.2	83.3	91.7	62.5	58.3	75.0	75.0	66.7	79.2
CON3	62.5	83.3	83.3	87.5	75.0	91.7	75.0	70.8	87.5	87.5	83.3	87.5
Mean CON	**68.0**	**79.1**	**80.5**	**76.4**	**80.5**	**88.9**	**66.7**	**70.8**	**83.3**	**80.6**	**76.4**	**83.3**
FNX1	62.5	70.8	79.2	62.5	75.0	87.5	75.0	87.5	83.3	50.0	79.2	91.7
FNX2	75.0	83.3	75.0	79.2	75.0	75.0	75.0	79.2	91.7	87.5	87.5	91.7
FNX3	54.2	70.8	75.0	66.7	79.2	83.3	75.0	66.7	83.3	50.0	79.2	91.7
Mean FNX	**63.9**	**75.0**	**76.4**	**69.5**	**76.4**	**81.9**	**75.0**	**77.8**	**86.1**	**62.5**	**82.0**	**91.7**

Note. Animals in the control (CON) and fornix transected (FNX) groups according to problem set (Set A–D) with A being the first set learnt preoperatively and D being the last set learnt pre-operatively and according to type of problem (Type I–III) in which problems were presented either once, twice, or three times per session during training.

versus postoperative comparison measure (all Mann-Whitney U, $p = .05$). Taken together with the parametric tests above, these non-parametric tests confirm that there is good evidence that the FNX group produces a significant impairment in new postoperative learning.

Discussion

This study confirmed that bilateral fornix transection in the macaque produced an impairment in a concurrent visuo-spatial conditional discrimination-learning task that was selective to new postoperative learning. Postoperative recall of 288 preoperatively learned problems remained completely intact. This dissociation between learning and retrieval is not something that has been assessed by most previous studies that have examined the effects of fornix transection on associative learning, because most earlier studies have confounded retention with re-learning (Gaffan, 1994a; Gaffan, Saunders et al., 1984). This confound was avoided here by employing 1-trial postoperative retention tests (1TPORT) of large numbers of preoperatively learned problems. A recent study (Buckley et al., 2004) also used a similar 1TPORT procedure to examine concurrent discrimination learning in the spatial domain, albeit with different kinds of stimuli and fewer problems than in the current study. This earlier study also showed a selective deficit in acquisition but not in retention of concurrent spatial discriminations after fornix transection. The current study with its more extensive and sensitive test of retention (288 problems tested in 1TPORT conditions) allows us to conclude that fornix transection does indeed produce a robust anterograde deficit in concurrent spatial discrimination learning while retention of spatial discriminations is not affected. As the pattern of results in the earlier study (Buckley et al., 2004) that used predominantly female macaques and this study that used all male macaques were so similar we do not consider it very likely that the lesion effects are different across sexes even though there has been some evidence that in other spatial tasks, primarily working memory, males exhibit superior spatial performance than females (Lacreuse et al., 2005).

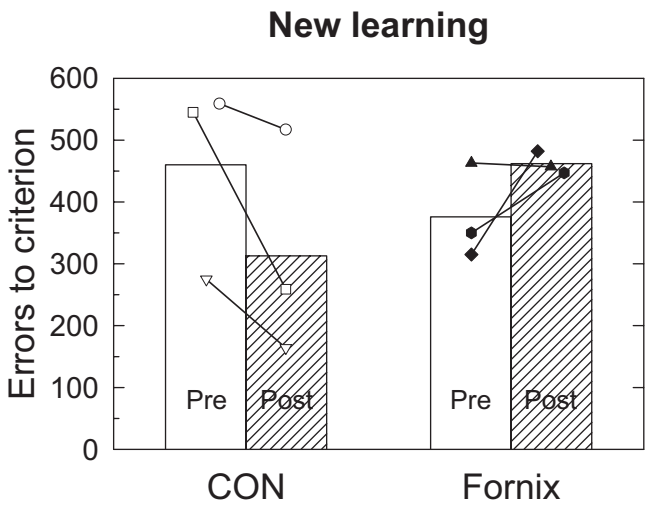


Figure 4. Errors to criterion accrued by the unoperated control group (CON) and the fornix transected group (FNX) while learning a new postoperative set of 72 visuo-spatial conditional discrimination problems. The changes in pre- and postoperative performance of individual animals in each group are indicated by symbols connected by lines.

Comparison With Earlier Studies

An earlier study by Gaffan (1993a) investigated the effects of fornix transection upon retention of preoperatively acquired memories of a different kind, namely memories for complex naturalistic scenes, and likewise avoided the re-learning/retrieval confound

described above by adopting a 1TPORT paradigm. Although that study did find a significant impairment in retention, it was of a very small magnitude (approximately 2% and 7% more errors depending upon stimulus set) compared to large magnitude of impairments observed (Gaffan, 1992) in new postoperative learning of complex naturalistic scenes (where compared to unoperated controls there was an approximate doubling or tripling of errors accrued, depending upon set). Another study which looked at the effects of fornix transection upon algorithmically generated complex scenes also employed 1TPORT and found that although retention was unimpaired, new learning was significantly impeded (Gaffan, 1994b). Unlike the present task, neither the complex naturalistic scene task nor the algorithmically generated complex scene task necessarily require spatial memory per se (as memories for foreground objects or background detail can aid performance of each) so it is not possible to infer from these studies that retrieval of spatial memory is unaffected by fornix transection. Nevertheless, the observations of a very mild or lack of impairment in each of these studies respectively, is certainly consistent with the idea that fornix transection affects new learning far more that it affects memory retrieval in a range of different paradigms. The study by Buckley et al (2004) and the current study allow this conclusion to be applied definitively to problems in the spatial domain.

The current study confirmed that fornix transection did not impair retrograde memory for visuo-spatial discriminations. Our previous study (Buckley et al., 2004) led us to predict that this would be the case and in this study we sought to strengthen the finding to apply across as wide a possible range of rate, strengths, and temporal factors of acquisition. The absence of retrieval deficits in the current study were found across three different levels of strength of memory trace (Type I, II, or III problems) and four different time periods in the past (successively learned Sets A–D) and we found that that retrieval deficits were absent across a range of learning-rates too. There has been renewed interest in theories of memory reconsolidation in recent years as evidenced by a number of recent reviews (Dudai, 2004; Dudai, 2006; Riccio, Millin, & Bogart, 2006; Sara, 2000); the theory that re-activated memories are liable to be disrupted by amnesia-inducing events raises the possibility that the re-training stage (on all problems in all sets) incorporated in the current study after successive set acquisition was completed might obliterate the observation of an otherwise retrograde gradient of memory loss after amnesic events. An alternative argument is that reconsolidation and consolidation are not equivalent processes (Alberini, 2005) and that reconsolidation does not erase old memory traces but rather lays down a new trace that (in the absence of amnesic events) acts to strengthen or update the memory. The partial persistence of old memories is perhaps best captured by Dudai's (2004) Lady Macbeth analogy, '*What is done, cannot be undone*'; furthermore, some studies have shown that in certain circumstances older memories have actually found to be *more* resistant to reconsolidation (see Dudai [2006] for a discussion). It is impossible at this stage to make a confident prediction as to the pattern and extent of retrograde amnesia when reconsolidation occurs, but fortunately this is not an important issue in the current context as the flat retention gradient in the current study was because of the complete *absence* of any retrograde deficit across all problems of all types in all sets. In short, fornix transection does not produce retrograde memory loss, at least for spatial learning. With respect to object learning, postoperative acquisition of concurrent object discriminations has repeatedly been shown to not be affected by fornix transection except in cases where there is deemed to be considerable interference from previously acquired memories (Gaffan, 1992; Mahut, Zola-Morgan, & Moss, 1982; Moss et al., 1981; Zola-Morgan, Squire, & Amaral, 1989a). Therefore, consistent with the view outlined in the introduction, the new learning impairments after fornix transection appear to lie mainly within the spatial domain.

Cortical and Subcortical Contributions to Learning and Retention

Retention and new learning of concurrent object discriminations have also been assessed after cortical lesions within the temporal lobe. It is established that perirhinal cortex ablations produce more severe deficits in reacquiring preoperatively learned sets of problems than in learning new sets of discriminations (Buckley & Gaffan, 1997). Furthermore, some authors have used the 1TPORT procedure to confirm that perirhinal lesions do produce deficits in retention per se (Hampton & Murray, 2002) as do combined perirhinal and entorhinal cortical lesions (Thornton, Rothblat, & Murray, 1997). Additionally, a new analysis from our own laboratory (Buckley & Gaffan, unpublished data) also used 1TPORT to confirm that perirhinal lesions alone are sufficient to impair retention of a small set of concurrent object discriminations whereas leaving new postoperative learning of the same small number of problems unimpaired. New learning deficits have also been observed to be absent after combined perirhinal and entorhinal cortical lesions (Thornton et al., 1997). This is not to deny that new learning deficits never follow cortical lesions in this region, as it is know that they do when the demands placed upon stimulus identification are sufficiently high (Buckley & Gaffan, 1997, 1998), rather, we merely wish to emphasize the point that when retention is explicitly contrasted with new learning, then retention is consistently found be impaired to a greater extent after such cortical lesions. Moreover, this effect, namely a greater retrograde than anterograde amnesic deficit, is not limited to cortical lesions in the medial temporal lobe. Dean and Weiskrantz (1974) found that lesions to lateral temporal lobe cortex outside of the medial temporal lobe, namely to area TE within the inferior temporal cortex, also impair 1TPORT of preoperatively learned object discriminations more than new learning.

Thus, a contrast can be made between the effects of lesions to temporal lobe neocortical regions both within (rhinal cortex) and external to the MTL (area TE) that produce stronger retrograde memory than anterograde learning deficits, as compared to the effects of disconnecting some of the cortical-subcortical connections of the temporal lobe (via fornix transection) that has the reverse effect in that it results in a stronger anterograde learning than retrograde memory deficit. The fact that densely amnesic human subjects such as H.M. typically show both anterograde and retrograde amnesia is therefore consistent with their sustaining lesions both to cortical (including hippocampal) neurons and to cortical-subcortical connections (Corkin, Amaral, Gonzalez, Johnson, & Hyman, 1997).

In macaque monkeys, combined lesions to the fornix, amygdala and anterior temporal stem also produces dense anterograde amnesia (Gaffan et al., 2001). This particular combination of lesions

was introduced as a means to disconnect all three of the routes by which fibres originating from the basal forebrain can innervate the temporal lobe cortex. Gaffan et al. (2001) have accordingly argued that disconnection of the temporal lobe from the basal forebrain cortex may be the explanation for dense organic anterograde amnesia in humans, as humans patients who have acquired substantial medial temporal lobe lesions such as H.M. have, in addition to their cortical lesions, a similarly extensive disconnection of temporal lobe from the basal forebrain.

An alternative theory is that anterograde amnesia is due to hippocampal damage instead. Although the precise role of the hippocampus is not universally agreed, one prominent theory asserts that hippocampal allocortex subserves the process of consolidating hippocampal-dependent short-term memory into more robust and extra-hippocampal long-term memories (Squire & Alvarez, 1995). According to this theory, hippocampal damage should impair new learning more so than the retrieval of established long-term memories, whereas neocortical lesions should produce more severe retrograde than anterograde amnesic effects. Indeed, amnesic patients whose medial temporal lobe brain lesions appear on histological examination to be limited to the CA fields of the hippocampus have been reported to have moderate anterograde amnesia in the absence of retrograde amnesia (Rempel-Clower, Zola, Squire, & Amaral, 1996). However, cerebral ischemia in animals can also result in neuronal loss limited to the hippocampus, but the memory impairments are not explicable by the hippocampal damage observed, because they are more severe than those that follow surgical removal of the hippocampus (Mumby et al., 1996). Ischemia leads to cerebral edema and the mechanical pressure on axons during edema could produce axonal damage while at the same time allowing the axons' distant cell bodies to survive. Thus, one cannot rule out that patients who have become amnesic through ischemic accidents may be amnesic because of damage sustained to subcortical-cortical pathways as opposed to neuronal loss in the hippocampus itself.

This study has shown that fornix transection in macaques impairs learning in the spatial domain. Previous studies have shown that fornix transection in macaques also impairs memory for the relative order in which stimuli appear (Charles, Gaffan, & Buckley, 2004) and in learning to associate stimuli with temporally differentiated responses (Brasted, Bussey, Murray, & Wise, 2003). However, the effects of fornix transection upon object recognition memory are inconsistent (Bachevalier, Parkinson, & Mishkin, 1985; Bachevalier, Saunders, & Mishkin, 1985; Charles et al., 2004; Gaffan, 1974, 1994a; Gaffan, Shields et al., 1984; Mahut et al., 1982; Murray et al., 1989; Owen & Butler, 1981, 1984; Zola-Morgan, Squire, & Amaral, 1989b) and fornix transection has been shown not to impair associative learning about objects when the possibility of interference from previously acquired memories is ruled out (Moss et al., 1981). We conclude that cortical-subcortical connections via the fornix are not important for all forms of new learning but are selectively concerned with learning about the spatial and temporal relationships of stimuli and responses. Further research with the 1TPORT paradigm will be necessary to dissociate the contribution that other cortical regions and cortical-subcortical connections, both within and beyond the temporal lobe, make to learning and retention of different kinds of stimulus material.

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