DOI: 10.1093/brain/awg103 *Brain* (2003), **126,** 1202–1223

Role of the hippocampal system in associative learning beyond the spatial domain

P. J. Brasted,[1] T. J. Bussey,[3] E. A. Murray[2] and S. P. Wise[1]

[1]*Laboratory of Systems Neuroscience and* [2]*Laboratory of Neuropsychology, National Institute of Mental Health, Bethesda, MD, USA*

[3]*Present address: Department of Experimental Psychology, University of Cambridge, Cambridge, UK*

Correspondence to: Dr P. J. Brasted, Laboratory of Systems Neuroscience, National Institute of Mental Health, Building 49, Room B1EE17, 49 Convent Drive, MSC 4401, Bethesda, MD 20892-4401, USA
E-mail: peter_brasted@nih.gov

Summary

Expert opinion remains divided on the issue of whether the hippocampal system functions exclusively in spatial information processing, e.g. in navigation or in understanding spatial relations, or whether it plays a more general role in higher brain function. Previous work on monkeys and rats has tended to support the former view, whereas observations in the clinic point to the latter, including functions as diverse as declarative knowledge, episodic memory, word learning, and understanding relations among objects. One influential theory posits a general role for the hippocampal system in associative learning, with emphasis on associations learned rapidly and recently. The results presented here are consistent with this theory, along with previous clinical and theoretical studies indicating that the hippocampal system is necessary for associative learning even if no component of the association relies on spatial information. In the study reported here, rhesus monkeys learned a series of conditional stimulus–response associations involving complex visual stimuli presented on a video monitor. Each stimulus instructed one of three responses: tapping the stimulus with the hand, steady hand contact with the stimulus for a brief period of time, or steady contact for a longer time. Fornix transection impaired the learning of these associations, even though both the stimuli and the responses were nonspatially differentiated, and this deficit persisted for at least 2 years. This finding indicates that the hippocampal system plays an important role in associative learning regardless of the relevance of spatial information to any aspect of the association. Fornix-transected monkeys were impaired in learning new stimulus–response associations even when the stimuli were highly familiar. Thus, the deficit was one of associating each stimulus with a response, as opposed to problems in distinguishing the stimuli from each other. In contrast to these effects, fornix transection did not impair performance when familiar stimuli instructed a response according to an already-learned association, which shows that the deficit was one of learning new associations rather than one of retention or retrieval of previously learned ones. Taken together, these results show that fornix transection causes a long-lasting impairment in associative learning outside of the spatial domain, in a manner consistent with theories of hippocampal-system function that stress a general role in the rapid acquisition of associative knowledge.

Keywords: fornix; conditional motor learning; conditional discrimination; declarative memory

Abbreviations: HS = hippocampal system

Introduction

The hippocampal system (HS) consists of a number of cortical fields—specifically CA1–CA4, the dentate gyrus, and a group of cortical areas collectively termed the subicular complex—as well as the fibres of the fornix and fimbria, which provide inputs to and outputs from these parts of the cerebral cortex. Because lesions of these structures result in severe anterograde amnesia, which profoundly disrupts the ability to record the 'what', 'where' and 'when' of everyday experience, a view emerging from clinical studies is that the HS provides the critical substrate for episodic memory

Published by Oxford University Press

(Schacter and Tulving, 1994; Vargha-Khadem *et al*., 1997; Tulving and Markowitsch, 1998; Aggleton *et al*., 2000; Spiers *et al*., 2001).

By contrast, the prevailing view of HS function that comes from research on experimental animals focuses primarily on its role in spatial localization and navigation (O'Keefe and Nadel, 1978). This outlook is based upon an extensive series of neurophysiological (e.g. O'Keefe, 1976; O'Keefe and Speakman, 1987; Wilson and McNaughton, 1993) and neuropsychological (e.g. Morris *et al*., 1982; Aggleton *et al*., 1986; Jarrard, 1993, 1995; Deacon *et al*., 2001) studies, mainly in rodent species (for reviews see O'Keefe, 1999; Redish, 1999). Whilst it is conceivable that the focus on spatial or idiothetic information processing in rodents might reflect the fossorial habit common to most laboratory rodents, compatible results have been obtained from other experimental animals, including nonhuman primates. In macaque monkeys, HS lesions impair performance on many tasks dependent on information processing in the spatial domain (Mahut and Moss, 1986; Murray *et al*., 1989; Parkinson *et al*., 1988; Angeli *et al*., 1993; Gaffan, 1998), but not on several tasks lacking such features, such as learning object–object associations (Murray *et al*., 1993; Gaffan *et al*., 1984*a*, Gaffan and Harrison, 1989) or object–reward associations, i.e. learning which of two objects should be approached to produce reward (Moss *et al*., 1981; Gaffan *et al*., 1984*a*). A particularly clear-cut example of spatial specificity in HS function was reported in the late 1980s by Gaffan and colleagues. They studied the learning of conditional associations between visual stimuli—differentiated on the basis of colour and shape—and a motor response, often termed 'conditional motor learning' (Passingham, 1993). In one form of the task, the stimuli instructed a spatial response, either approaching the stimulus or withdrawing from it (Rupniak and Gaffan, 1987). In another form of the task, the same type of stimuli instructed a nonspatial response, specifically whether to repeatedly tap or to make sustained contact with the stimulus (Gaffan and Harrison, 1988). Fornix transections impaired learning of the spatial version of the task, but not the nonspatial version.

Thus, one could make the case for a profound species difference between humans and experimental animals, with the HS specialized for spatial information processing in nonhuman animals but playing a more general role in our species. However, it has been argued that the spatial properties of the HS revealed in nonhuman animals can be extended to account for episodic memory function in humans (Gaffan, 1994*b*; O'Keefe, 1999). Moreover, there has been increased interest recently in the contribution of the HS of nonhuman animals to nonspatial information processing. Experimental results from a highly diverse set of animals—including rodents (Bunsey and Eichenbaum, 1996; Dusek and Eichenbaum, 1998; Hampson *et al*., 1999; Alvarez *et al*., 2001; Fortin *et al*., 2002; Kennedy and Shapiro, 2002), lizards (Day *et al*., 2001) and monkeys (Gaffan, 1994*b*; Ridley and Baker, 1997; Hampson *et al*., 2002)—point to an important role of the HS beyond the spatial domain. Along the same lines, a variety of connectionist models of HS function suggest that it is well-suited for general, associative learning (Alvarez and Squire, 1994; McClelland *et al*., 1995; Murré, 1996; Murré *et al*., 2001; O'Reilly and Rudy, 2000). These ideas encompass the view that HS function has profound importance in spatial information processing, but incorporate a broader range of findings (Gaffan, 1994*a*, *b*; Aggleton and Brown, 1999; Aggleton *et al*., 2000).

However, this broader view of HS function makes it more difficult to account for findings suggesting spatial specificity, such as those summarized above involving conditional visuomotor associations (Rupniak and Gaffan, 1987; Gaffan and Harrison, 1988). Indeed, several authors have concluded that all of the reliable experimental data—from both humans and experimental animals—can be accounted for in terms of the role of the HS in spatial information processing (O'Keefe, 1999; Redish, 1999; Gaffan, 2001). These views acknowledge the importance of nonspatial information processing in the HS, but hold that to involve HS in the performance of some task or behaviour, spatial information must also be critical. Because the more general conception of HS function predicts a deficit in learning conditional visuomotor associations regardless of whether the stimuli and responses are spatially or nonspatially differentiated, we re-examined the effects of fornix transection on such behaviour in monkeys. Some of these data have been reported previously in preliminary form (Brasted *et al*., 2001, 2002*a*, *b*).

Methods

Subjects

The final group of subjects comprised eight naive male rhesus monkeys (*Macaca mulatta*) ranging in weight from 5.4 to 8.1 kg at the time of surgery or rest. These are the same subjects as those studied by Brasted *et al*. (2002*a*). Two additional animals were removed from the study after initial attempts to shape their behaviour indicated their unsuitability. The monkeys were housed socially and were fed a diet of Purina Primate Chow (Purina Mills, St Louis, MO, USA) supplemented with fruit. This food was restricted during testing such that the weight of each animal was between 95 and 85% of its free feeding weight. Water was available *ad libitum*. All procedures conformed with the *Guide for the Care and Use of Laboratory Animals* (revised 1996, ISBN 0-309-05377-3) and complied with an institutionally approved animal study proposal.

Apparatus

Training was conducted in a sound-attenuating test cubicle. Monkeys were seated in a primate chair immediately in front of a 13-inch video monitor fitted with a touch-sensitive screen. An automated reward dispenser was used to deliver

190 mg, banana-flavoured food pellets (Noyes, Lancaster, NH, USA) to a food cup immediately below the touchscreen.

Stimuli

Each visual stimulus consisted of two differently coloured ASCII characters, superimposed, one ~5.0 cm in height and the other ~3.5 cm in height (Gaffan and Harrison, 1988). The use of 62 distinct ASCII characters, taken two at a time, and eight different colours meant that $>2 \times 10^5$ novel stimuli could be generated that were, by visual inspection, discriminable both from each other and from previously learned, highly familiar stimuli (see below for a definition of familiar stimuli).

Initial training

Initial shaping

Monkeys were initially shaped to touch the visual stimuli on the monitor. In phase 1, a stimulus appeared on the screen for 10 s, after which the stimulus was removed and a reward pellet was delivered. If the subject touched the stimulus during the 10 s display period, the stimulus disappeared immediately and a pellet was delivered at the same time. Sessions consisted of 30 such trials presented at a variable interval, with a mean of 120 s. Criterion was set at two or more touches in each of two consecutive sessions. In phase 2 of shaping, a stimulus would appear for 30 s, and reward was contingent on the monkey touching the stimulus (i.e. contacting the image on the monitor). As in phase 1 of shaping, a touch response led to the removal of the stimulus from the screen and reward delivery. Sessions consisted of 50 such trials, and criterion was set at 49 of 50 correct responses for two consecutive sessions.

Establishing the response repertoire

Subsequent sessions were designed to teach the subjects three distinct nonspatially differentiated responses, termed 'tap', 'long hold' and 'short hold'. Throughout this part of training, sessions consisted of 50 trials and monkeys were trained at the rate of two to three sessions per day. Subjects were first required to learn the tap response. A stimulus appeared on the monitor screen, and the monkey was required to tap the screen a requisite number of times, at which point the stimulus was removed and reward was delivered. The tap requirement was initially set at two and there was no limit on response time. Each individual tap was registered when the touchscreen detected not only definitive contact but also a subsequent period of no contact. When the criterion of 45 correct responses in 50 trials was achieved during a single session, the tap requirement was increased by one tap. The tap requirement was incremented in this fashion until subjects were able to respond with a tap requirement of eight at a minimum of 45 of 50 trials correct for two consecutive sessions. The tap response thus required the subject to contact the screen eight times, each contact lasting up to 2 s. In practice, however, the monkeys tapped the touchscreen much more quickly, with few, if any, contacts lasting more than 0.2 s. Frequent visual examination of each animal's tap response confirmed that all individual taps had approximately the same contact time throughout training and testing.

Subjects were next required to learn the long-hold response. A stimulus was presented on the monitor screen, and the subject was required to touch and maintain contact with the screen for a period slightly longer than the subject's contact period for a tap response. Once the contact time exceeded the required limit, reward was delivered and the stimulus was removed. Once the monkeys achieved the criterion of 45 correct responses in 50 trials, the duration required was incremented by a few hundred milliseconds. As was the case for the tap response, the response requirement was gradually increased across sessions until the subject performed consistently above criterion with a period of contact greater than 4 s. Reward was delivered and the stimulus removed either when the subject removed its hand after 4 s or when contact exceeded 8 s regardless of hand withdrawal. With one or two exceptions, monkeys broke contact with the touchscreen between 4 and 8 s, as opposed to holding until the 8 s had expired. When this phase of training took a particularly long time, sessions of tap-response training were interleaved with sessions of long-hold training, each response being associated with a different stimulus.

Following long-hold training, monkeys received sessions that contained both tap trials and long-hold trials, each associated with a different stimulus. When performance on both trial types in these sessions was adjudged to be consistently good, monkeys were trained on the short-hold response in separate sessions.

The short-hold stimulus appeared on the monitor screen, and monkeys were required to touch the screen and maintain contact for a short period. Reward was contingent on the monkey removing its hand from the screen within a defined time period, which was less than the period used for long-hold responses but longer than the contact period defined as a tap. This time range was gradually incremented each session until the subject was maintaining contact for >2 s but <4 s. During short-hold training, the monkeys received occasional sessions of interleaved tap and long-hold trials, so that they would retain these responses in their repertoire. If necessary to maintain an animal's performance level, we implemented a slightly shorter lower limit to the short-hold response, 1.6 s instead of 2.0 s. In all instances, the lower limit for the short-hold response also served as the upper limit for each component of the tap response.

Once a subject had mastered each of the three response types in this way, the main task was introduced. Now the sessions consisted of three trial types, one for each of the three stimulus–response mappings. If necessary, animals were given 'remedial' training using the previous training

Table 1 *Order and duration of postoperative behavioural testing*

Row	Experiment	Testing programme	C1	C2	C3	C4	L1	L2	L3	L4
1	Visuomotor learning I	Novel mappings	4	6	6	5	5	7	7	3
2	Fast learning and retention	Novel mappings	7	9	10	11	16	21	11	9
3	Fast learning and retention	Familiar (remote) mappings*	11	12	12	23	21	28	18	19
4	Fast learning and retention	Novel and familiar (remote) mappings	15	15	13	26	27	32	24	25
5	Visuomotor learning II	Novel mappings	30	34	35	38	37	37	33	34
6	Fast learning and retention	Novel and familiar (remote) mappings	31	37	38	38	39	47	35	36
7	Stimulus features**		38	44	45	43	45	55	42	42
8	Larger and smaller sets	Novel 2:2 mappings: standard response parameters	48	47	48	48	51	60	47	44
9	Control conditions	Novel and 3 kinds of familiar mappings: remote, recent-remapped, recent-fixed	56	56	57	58	59	68	56	65
10	Control conditions	Visual discrimination	65	63	64	67	66	76	64	72
11	Control conditions	Transverse patterning	69	64	65	69	67	77	67	73
12	Visuomotor learning III	Novel mappings	77	76	77	73	77	82	71	76
13	Larger and smaller sets	Novel 6:3 mappings	78	79	78	75	80	83	75	78
14	Larger and smaller sets	Novel 9:3 mappings	81	81	80	76	82	87	77	80
15	Larger and smaller sets	Novel 2:2 mappings: modified long-hold	102	105	103	101	104	110	99	104
16	Larger and smaller sets	Novel 2:2 and 6:2*** mappings: modified short-hold	104	109	105	103	106	112	106	106

For each testing programme, designated by row, the numerals in the rightmost eight columns indicate when testing commenced, in terms of the number of weeks after the date each monkey completed preoperative training. C1–C4 = the four control subjects; L1–L4 = the four monkeys in the lesion group. Unless otherwise stated, all conditional visuomotor associations involved 3:3 mappings (i.e. three stimuli, each uniquely instructing three responses). *All testing with familiar stimuli in 'fast learning and retention' used the familiar (remote) set of stimuli; **a testing programme that yielded unremarkable results, which are omitted from this report; ***L1 and C1 were not tested on 6:2 mappings.

paradigms (e.g. long-hold training only), in addition to the sessions that contained all three responses.

Baseline performance and group assignment

Preoperatively, each monkey learned seven sets of visuomotor associations, each set comprising three problems and each problem consisting of a single visuomotor association. Because the problem set consisted of three stimuli instructing three different responses, we refer to this kind of problem as a 'three-on-three' mapping, abbreviated 3:3. The last five sets were used as the measure of preoperative, baseline learning rates. Two groups, approximately matched for their learning rates, were formed.

One subject (L1) initially received a bilateral aspiration lesion of the dorsal prefrontal cortex. Since the dorsal prefrontal lesion had no effect on subsequent performance, this animal completed postoperative testing and then received a fornix transection. Thus, for this subject, baseline performance was derived from the five problem sets learned immediately before fornix transection. No result reported here depended for statistical significance upon this animal's data, unless explicitly noted.

Overview of experimental design

The main study examined the role of the fornix in the learning of novel associations between stimuli and responses that were non-spatially differentiated. Monkeys were trained preoperatively on 3:3 visuomotor mappings, as described above. Both groups were then tested postoperatively on their ability to learn new 3:3 mappings at three distinct time points up to 18 months after surgery. These data are presented in the section entitled Non-spatial visuomotor learning (3:3 mappings). In the intervening periods, the monkeys of both groups were trained and tested on other, related tasks and additional tasks designed to serve as control conditions. Table 1 gives the order and duration of each testing stage for each monkey.

As the experiment progressed, the monkeys were tested periodically on a set of highly familiar associations, made up of five separate 3:3 problem sets. The monkeys performed one session weekly with each of these problem sets in order to maintain the three non-spatially differentiated responses and to maintain the memory of these mappings. Performance on these familiar sets was compared with data from other behavioural tasks that probed the ability of both groups of monkeys to learn novel conditional 3:3 visuomotor mappings quickly. These data from the 'fast learning' task and the comparison with performance using familiar problem sets are presented in a later section (see Fast learning and retention).

Another set of tasks explored the monkeys' capabilities in learning mappings with fewer stimuli and responses (2:2 mappings) or with more stimuli (6:3 and 9:3 mappings) than were used in the main mapping task (3:3) described above. These data are presented below (see Larger and smaller problem sets).

Finally, a series of different tasks was presented to both groups of monkeys in order to establish control conditions. Each of these tasks was intended to alleviate some of the interpretational problems inherent in the main task. These tasks examined whether the deficits caused by fornix

transection on learning the conditional associations between stimuli and responses might instead be due to difficulty in discriminating each stimulus from the others in a problem set, or to nonspecific effects of task difficulty. These data are presented below (see Control conditions).

Nonspatial visuomotor learning (3:3 mappings)

Experimental design

The main experiment involved the learning of novel associations between a stimulus that was differentiated from alternative stimuli by nonspatial features (mainly colour and shape) and a response that was differentiated from alternative responses by a different nonspatial factor (the number of contacts or the duration of contact with the touchscreen). Eight rhesus monkeys were trained preoperatively on 3:3 visuomotor mappings, as described above. The eight monkeys were then assigned to two groups (n = 4) matched for learning rate. The subjects in one group received fornix transections (see below for surgical methods). Then both groups were evaluated postoperatively for their rate of learning 3:3 mappings. Each monkey received five novel problem sets in each of three postoperative testing periods. The first of these tests was conducted within the first 2 months after surgery, the second was conducted about 8 months after surgery, and the third was performed about 18 months after surgery.

Each trial began with the appearance of a stimulus on the monitor screen, selected pseudorandomly from a problem set, as defined above. The task required animals to learn that, for a problem set of three novel stimuli, a tap response was instructed by one of the three stimuli, a short-hold response by a different member of the set, and a long-hold response by yet another stimulus. Thus, each stimulus instructed one and only one response. The stimulus was displayed continuously until one of the three responses had been completed. As soon as a response was registered, the stimulus was removed from the screen, and a food pellet was delivered immediately if the correct response had been made. The screen was blank throughout the 8 s intertrial interval. Trials that ended with an incorrect response were followed by correction trials, i.e. repeated presentation of the same stimulus. Correction trials were repeated without limitation until the correct response was emitted. Correct responses on correction trials were reinforced in the same way as on noncorrection trials (termed 'first trials').

Testing procedure

Sessions typically contained 100 trials (not including correction trials), in which the three trial types were presented in random order under computer control. A given problem set was presented for several sessions, until subjects attained the performance criterion of at least 90 correct responses in a 100-trial session. Occasionally, sessions of 50 trials were given early in training, but in those relatively rare instances performance was still judged over 100 trials (i.e. over two 50-trial sessions). Only first trials were scored; performance on correction trials was not considered in assessing criterion. No restrictions were placed on how the 10 allowed errors in a session could be distributed across the three possible trial types, but performance was monitored periodically to determine whether there might be large response biases. After a subject had attained criterion, a new problem set, involving three novel stimuli, was presented in the next session. Animals typically performed two sessions each day, with the exception that new problem sets would only be introduced at the beginning of a day's testing.

Initial postoperative testing and visuomotor learning I

For each group, a mean of 27.5 days intervened between the end of preoperative testing and the start of postoperative testing. Control animals underwent no surgical procedures, but had a rest (nontesting) period that matched that of the operated monkeys. Subjects were first tested on a 'retention' set (the last stimulus set learned prior to surgery or rest) until they attained criterion. These data were reported by Brasted *et al*. (2002*a*) and will not be repeated here. The monkeys were then tested with five novel problem sets; criterion was the same as for preoperative, baseline testing. With few exceptions, the same problem sets were presented to all monkeys in the same sequence, both for baseline and subsequent testing.

To assess the effect of fornix transection on the rate of learning new visuomotor associations, we compared the average number of errors to criterion for the five problem sets learned preoperatively (or before rest for the control group) with the number for the five sets learned postoperatively, or after rest (Table 1, row 1). The score included errors made in the session in which the subject attained criterion, but excluded all correction trials and trials in incomplete sessions. Sessions were defined as incomplete if they contained <40 trials, and such sessions, which were rare, typically contained far fewer than 40 trials.

Visuomotor learning II

All monkeys were required to learn another five new problem sets to criterion ~8 months after surgery (Table 1, row 5) in order to determine the stability of the deficit. The testing parameters were identical to those outlined for the initial postoperative testing on novel 3:3 mappings.

Visuomotor learning III

Because intervening tasks might have interfered with performance of the visuomotor learning rules, the monkeys were first required to complete 5 days of testing using stimulus sets

they had experienced previously [specifically, what are defined below as 'familiar (remote)' problem sets]. All monkeys demonstrated retention of the main task, and therefore all progressed to the testing stage. Roughly 18 months after operation or rest, all monkeys were required to learn another five new problem sets to a criterion of 90% correct responses in a 100-trial session, exactly as they had in the initial postoperative testing, with the exception that the intertrial interval was 3 s rather than 8 s (Table 1, row 12).

Fast learning and retention

To promote the development of a learning set, the monkeys were given additional problem sets, on which they were trained for a fixed number of trials rather than to a performance criterion, as was the case for the main task. Initially, subjects were presented with five stimulus sets, similar to the main task, and each stimulus set was presented for a total of only 200 trials. On subsequent days, animals were presented with another 10 novel stimulus sets (comprising altogether 30 different problems), and each stimulus set was presented for 100 trials. Then, 15 novel stimulus sets were presented for 50 trials each (Table 1, row 2).

At this stage, five familiar stimulus sets were established, so that the performance of subjects using novel and familiar stimuli could eventually be compared directly (Table 1, row 3). The five stimulus sets that were presented during initial postoperative testing were used for this purpose. These problem sets and the stimuli in those sets are termed 'familiar (remote)', to contrast them with other familiar stimuli presented in later stages of testing. Each of these five sets was presented for 50 trials, and this cycle was repeated three times. After this procedure novel sets were reintroduced, such that every day monkeys were given three 50-trial sessions; the first two used two novel stimulus sets and the third used one of the five familiar sets. Subjects completed 130 novel sets in this fashion (Table 1, rows 4 and 6): 80 sets in one block of testing and 50 sets in a subsequent block (the second half of which was used for analysis; Table 1, rows 9 and 12 were used for Fig. 6). The intertrial interval was 8 s in the first block of within-session learning and 3 s in the second. This reduction in the intertrial interval was introduced in an attempt to encourage the adoption of certain problem-solving strategies that have been observed previously (Wise and Murray, 1999; Bussey *et al*., 2001), but no such strategies were adopted convincingly by the present subjects.

Larger and smaller problem sets

2:2 mappings using standard response parameters

In part to assess the impact of task difficulty, and to facilitate comparison of the lesion effect with that observed in previous studies, monkeys were tested on problem sets involving two response choices, rather than three as in the standard task described above. In the first phase of this testing, monkeys were given a series of five novel problem sets in which each set comprised only two stimuli and two responses. The two stimuli always mapped to the same two response types for each animal: the tap response and either the short-hold (three lesion and three control monkeys) or the long-hold response (one lesion and one control), whichever was executed more accurately by a given subject when using familiar stimuli sets. In this phase of testing, the response parameters were the same as described above for the main task. The intertrial interval was 3 s; otherwise the task was identical to that given in initial postoperative testing, meaning that chance performance was still 33.3% correct. Criterion was set at $\geqslant$90 correct responses in a single 100-trial session.

6:3 mappings

Subjects were required to learn three problems sets, each of which comprised six novel stimuli. All three responses were used, and the response parameters were as defined in the main task. Thus, each response was associated with two different stimuli. Testing procedures and criterion were the same as those used for 2:2 mappings.

9:3 mappings

After testing on 6:3 mappings was completed, monkeys were presented with two problem sets, each of which comprised nine novel stimuli. Thus, each response was associated with three different stimuli. Response parameters were as defined in the main task. Testing procedures and criterion were the same as those used for 2:2 mappings.

2:2 mappings using modified long-hold parameters and different response combinations

The tap and short-hold responses were as described for initial postoperative testing, but the long-hold response was modified so that subjects had to maintain contact with the screen until the end of the long-hold period (8 s for seven subjects, 6 s for one lesion subject). After this interval had elapsed, the stimulus was removed from the screen and a reward was delivered if appropriate. Monkeys were required to learn 15 problems sets, each of which comprised two novel stimuli. For five of the sets, the two stimuli mapped to a tap response and a short-hold response, respectively, as originally defined; for another five sets, the two stimuli mapped to a tap response and a long-hold response, as modified to require screen contact until the end of the long-hold period; for the last five sets, the two stimuli mapped to a short-hold response and a long-hold response, the latter modified as described above. The combination of responses was changed after the completion of each set. The intertrial interval was 3 s, chance performance was 33.3% correct, and criterion was set at $\geqslant$90 correct responses in a single 100-trial session.

2:2 Mappings using modified short-hold parameters

The tap response was as originally defined, but in this phase of testing we used a different modification of the hold response: animals had to maintain contact with the screen only until the end of the short-hold period of 2 s (or 1.6 s, if that was their original short-hold response limit). After that period elapsed, the stimulus was removed from the screen and reward was delivered if appropriate. Monkeys were required to learn five problems sets, each of which comprised two novel stimuli mapping onto tap and short-hold responses. The intertrial interval was 8 s, chance performance was 50% correct, and the criterion was $\geqslant$90 correct responses in a single 100-trial session.

6:2 Mappings using modified short-hold parameters

This phase of testing used the modified hold response, as described in the section immediately above. Monkeys were presented with five problem sets, each of which comprised six novel stimuli, half the stimuli being associated with the tap response and half with the short-hold response. Neither the animal with a pre-existing prefrontal lesion (L1) nor the control that was best matched preoperatively (C1) was included in this phase of testing. The intertrial interval was 8 s, chance performance was 50% correct, and the criterion was $\geqslant$90 correct responses in a single 100-trial session.

Control conditions

Mappings versus discrimination

An impairment in learning the associations between novel stimuli and responses could potentially be confounded with an impairment in the ability to discriminate among novel visual stimuli. To address this problem, we trained subjects to learn novel conditional visuomotor associations using familiar stimuli that were associated with different responses on different days (Table 1, row 9). In other words, monkeys were required to remap familiar stimuli onto familiar responses. This was carried out on the assumption that, because the monkeys had already discriminated the stimuli from each other, learning under these conditions would more accurately reflect learning of the associations, uncontaminated by any difficulty in discriminating the visual stimuli.

Initially monkeys learned a series of 10 novel problems sets, each consisting of 3:3 mappings, to a criterion of $\geqslant$90 correct responses within a single 100-trial session, using the parameters outlined for initial postoperative testing and a 3 s intertrial interval. Once a monkey had learned five of these 10 problem sets to criterion, it was presented with each of these five sets for 50 trials each. Monkeys continued to cycle through these newly learned sets until they achieved at least eight correct responses in the first 10 trials of a session for each of the five sets, and then completed an additional five cycles of testing. Monkeys then learned an additional five problem sets in this manner, initially learning them serially to a criterion of 90%, then cycling through them in 50-trial sessions until the stimuli were highly familiar. Four of these 10 familiar sets were designated 'familiar (recent–remapped)' sets, in which the stimulus–response associations would change from day to day, and another four were designated 'familiar (recent–fixed)' sets, in which the stimulus–response associations would never change. The remaining two problem sets were discarded. The eight problem sets detailed above were termed 'familiar (recent)' in order to differentiate them from the remotely acquired familiar problem sets first learned during initial postoperative testing and periodically presented during testing to maintain consolidation (Table 1), which were termed 'familiar (remote)' problem sets.

Monkeys then began four 5-day testing cycles for each of the four remapped and four fixed problem sets. On the first day of each testing cycle, the monkeys performed two 50-trial sessions, one for each of the designated pair of familiar (recent–remapped) and familiar (recent–fixed) problem sets. On each of following 4 days, monkeys were given four daily sessions of 50 trials each: (i) one with a novel problem set; (ii) one with a familiar (recent–remapped) set; (iii) one with a familiar (recent–fixed) set; and (iv) one with a familiar (remote) set. The first two sets (i and ii) required the learning of new mappings; the last two (iii and iv) had fixed mappings. The order of these sets was varied, with the exception that the familiar (remote) set was always performed last. The visuomotor mappings instructed by the familiar (recent–remapped) set was constrained such that each of the three stimuli was associated with a different response compared with the previous testing session. This 5-day testing cycle was repeated for each of the four pairs of familiar (recent–remapped) and familiar (recent–fixed) sets. Testing for this final pair was extended for an extra 9 days, and behavioural data for these 9 days of testing were used for analysis because, by this stage, the monkeys had had extensive experience with these stimuli and remappings.

Visual discrimination learning

To examine the effects of fornix transection on the ability to discriminate visual stimuli, monkeys were trained to perform a series of concurrent visual discriminations. In view of the monkeys' exclusive training in nonspatially differentiated responses, they were first trained simply to touch stimuli on the monitor screen, using stimulus material of the same type used for the visuomotor mappings. Stimuli appeared on either the left or the right side of the screen and the monkey learned to touch it. A correct response led to the delivery of food reward and the removal of the stimulus from the screen; all other responses were without effect. These shaping sessions consisted of 50 trials.

In the next phase of training, the monkeys were required to discriminate between two stimuli, one of which was

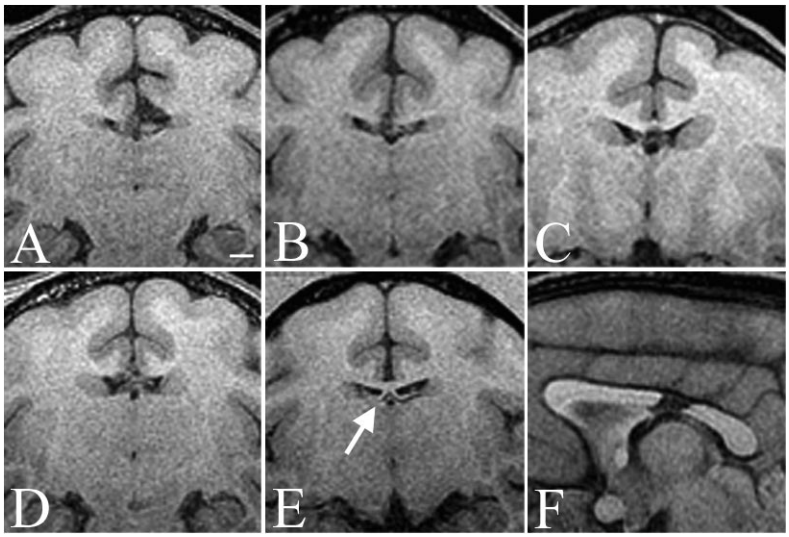

Fig. 1 (**A**–**D**) Coronal images from the four animals that received fornix transections. Note that the fornix has been transected bilaterally. Slight unilateral damage to the cingulate gyrus can be seen in one monkey (**A**). (**E**) Coronal image from a control subject from a rostrocaudal level comparable to that in **A**–**D**. The intact fornix is indicated with an arrow. (**F**) Sagittal view of fornix transection (from the same subject as **C**). Scale bar in **A** = 4 mm. Note the limited extent of the damage to the corpus callosum and the absence of any damage to adjacent structures, such as the thalamus and the septum. Images **A**–**D** correspond to monkeys L4, L1, L2 and L3, respectively.

arbitrarily designated correct and the other incorrect (S^+ and S^-, respectively). On each trial, the two stimuli were presented simultaneously on the screen, one to the left and one to the right of the centre of the screen; the correct stimulus was as likely to appear on the left as on the right. If the monkey touched the correct stimulus, then both stimuli disappeared and a food pellet was delivered. If the incorrect stimulus was touched, both stimuli disappeared and no pellet was delivered. These incorrect trials were repeated until the monkey made the correct response, but these correction trials were not used to assess accuracy. The intertrial interval was 7 s, and each session comprised 96 trials. Sessions continued using this stimulus pair until the subject reached a criterion of 90% correct responses within one session. Once criterion was reached, subjects performed the same task using two novel stimulus pairs, each consisting of a correct and incorrect stimulus. Once subjects had learned these visual discrimination problems to a criterion of 90% correct responses, they then performed the task using four stimulus pairs (i.e. a four-pair 'concurrent discrimination learning task'). The monkeys were required to solve a total of 5 four-pair concurrent discrimination problems, serially, again to a criterion of 90% correct responses (and a minimum of 75% correct for each pair). In addition to this testing, one day a week was set aside for all monkeys to perform the main task using each of the five familiar (remote) problem sets.

Transverse patterning

To examine whether fornix transection might affect acquisition of *any* difficult task, monkeys were trained on transverse patterning. The transverse patterning task used the same parameters as in concurrent visual discrimination, except that now subjects were required to solve three overlapping discrimination problems, A^+B^-, B^+C^- and C^+A^-, where $^+$ indicates the correct stimulus. The correct stimulus was as likely to appear on the left as on the right. Initially, subjects were trained using one of the discrimination problems (A^+B^-) until a criterion of 90% correct responses (i.e. $\geqslant$29 correct in 32 trials) was attained. Then, subjects were presented with two of the three discrimination problems (A^+B^-, B^+C^-) until they attained the same criterion as before (i.e. $\geqslant$58 correct responses in 64 trials). Only then did subjects progress to the full task, in which the three discrimination problems were presented concurrently. In the full task, each monkey received a total of 32 sessions, each session containing 32 trials of each problem type. In addition to this testing, one day a week was set aside for all monkeys to perform the main task for each of the five familiar (remote) problem sets.

Surgery

As indicated earlier, monkeys were allocated to their respective groups on the basis of their preoperative learning rates. In four subjects, bilateral transection of the fornix was performed with sterile procedures under visual control with the aid of an operating microscope. Dexamethasone sodium phosphate (0.4 mg/kg, i.m.) and an antibiotic (Di-Trim®, sulfadiazine/trimethoprim 0.1 ml/kg, 24% w/v solution, i.m.; Syntex Animal Health, West Des Moines, IA, USA) were administered for 1 day before surgery to reduce swelling and to prevent infection, respectively. On the day of surgery the monkeys were immobilized with ketamine hydrochloride (10 mg/kg, i.m.) and were anaesthetized with isoflurane

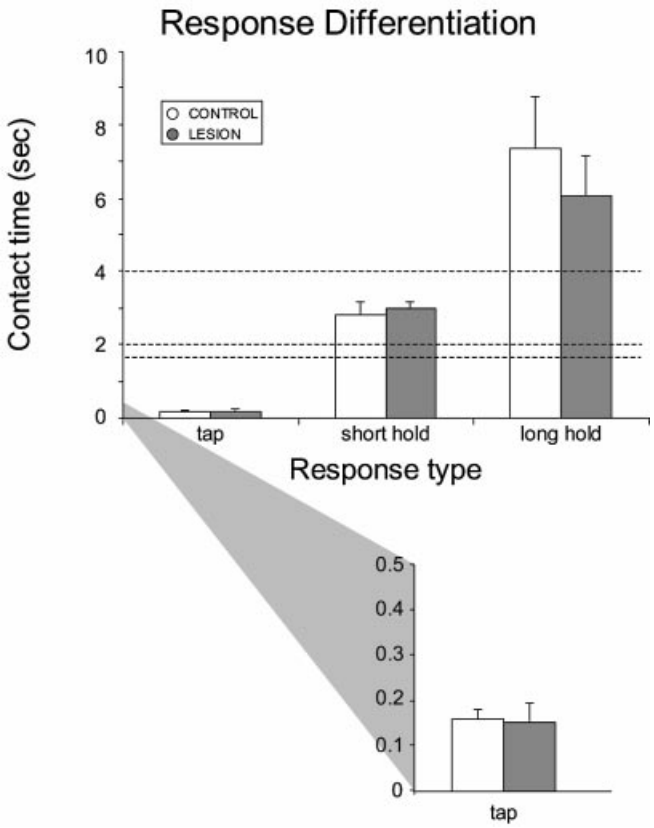

Fig. 2 Response differentiation. The mean latencies (+ standard deviation) for each group and each response type. Horizontal dashed lines show the time limits of the short-hold response. Note that for some animals the lower limit of the short-hold response was 2.0 s, whereas for others it was 1.6 s. Note also that the tap response represents the mean for each of the eight taps in a tap response. All taps were of approximately equal duration and, as illustrated in the inset in the lower panel, were an order of magnitude smaller than the shortest short-hold response.

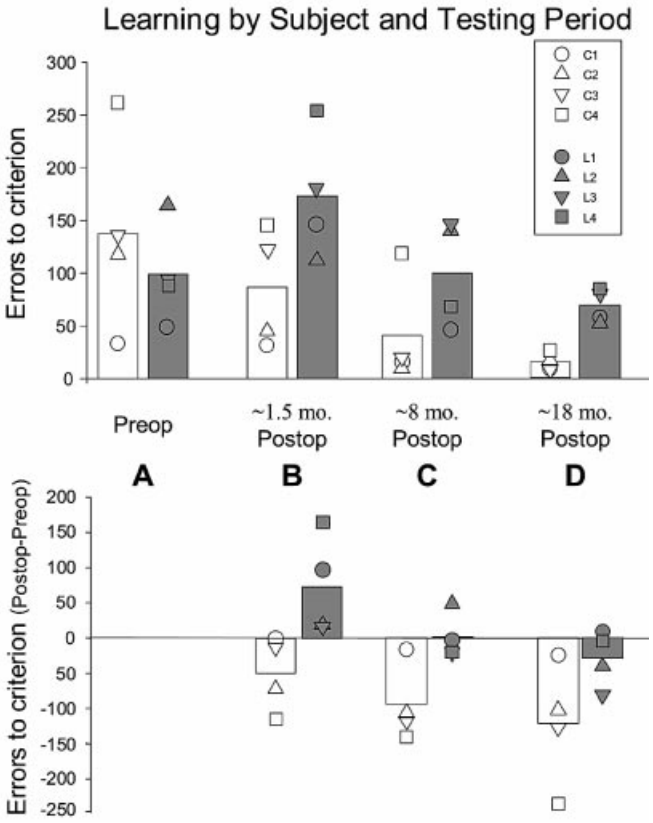

Fig. 3 Total number of errors to criterion (*top*) and difference (postoperative performance minus preoperative performance) in mean number of errors to criterion (*bottom*), by group, for the learning of five novel problem sets at each of three postoperative testing periods: (**B**) within the first 2 months after surgery in the lesion group; (**C**) ~8 months after surgery; and (**D**) ~18 months after surgery. Each monkey's score is noted for the control (C1–C4) and lesion (L1–L4) subjects. The subject with a pre-existing lesion in the prefrontal cortex (see Methods) was L1.

(1–3%, to effect). They received an i.v. drip of isotonic fluids containing an antibiotic (Cefazolin®, Cefazoline Sodium, American Pharmaceutical Partners, Los Angeles, CA, USA), and heart rate, respiration rate, body temperature, blood pressure and expired CO_2 were monitored closely throughout the procedure. The fornix was transected in the following manner. Access to the midline was achieved by turning a unilateral bone flap that extended just over the midline and reflecting the dura mater towards the midline. One hemisphere was then retracted slightly and the corpus callosum was sectioned for a short distance with a glass aspirator. The descending columns of the fornix were identified and then transected using a small-gauge aspirator with cautery. Using suction, the fornix was lifted slightly and then cauterized; this procedure was repeated until the transection was complete.

At the completion of surgery, the bone flap was replaced and the wound was closed in anatomical layers. Dexamethasone sodium phosphate (0.4 mg/kg, i.m.) and an antibiotic (Di-Trim®, sulfadiazine/trimethoprim, 0.1 ml/kg, 24% w/v solution, i.m.; Syntex Animal Health) were administered for 1 week following surgery to reduce swelling and to prevent infection, respectively. Monkeys also received acetaminophen (paracetamol) (40 mg) or Banamine® (flunixin meglumine, 5 mg) (Schering-Plough, Kenilworth, NJ, USA) as an analgesic for 3 days following surgery.

Lesion verification

Lesion accuracy was assessed using MRI techniques. A 1.5 T whole body scanner (Signa; General Electric Medical Systems, Milwaukee, WI, USA) was used to obtain 60 coronal and 60 sagittal MRIs, derived from T_1-weighted structural MRI. Coronal images were at 1 mm intervals; sagittal images were at 1.5 mm intervals. Images were prepared for publication using Scion Image for Windows 4.02 and Adobe Photoshop 6.0.

Examination of the MRIs confirmed that the lesions were as intended and revealed the transection of the fornix to be complete in all four animals that had undergone surgery (Fig. 1A–D). The fornix was clearly seen to be intact in the one control monkey that was also scanned (Fig. 1E). In

addition to the intended minor damage to the corpus callosum (Fig. 1F), two monkeys sustained slight inadvertent damage to the cingulate gyrus in one hemisphere; the damage extended ~3 mm above the corpus callosum in one monkey (L4) and <2 mm in the other (L2). There was no damage evident in adjacent structures, including the thalamus, which lies immediately ventral to the fornix, to the anteriorly located septal nuclei, or to the laterally located caudate nuclei.

Results

Nonspatial differentiation of responses

Figure 2 illustrates the screen-contact times for the three response types, for correct trials executed during postoperative performance of familiar (remote) stimulus sets, averaged over all monkeys. There was no significant difference in these values between groups [group, $F(1,6) = 2.0$, n.s.; response type × group, $F(2,12) = 2.2$, n.s.]. The monkeys' responses were similar for all stimulus types, including both familiar and novel stimuli, throughout the testing period. Note that the screen-contact time for the tap response was an order of magnitude less than that for the shortest hold response.

Visuomotor learning (3:3 mappings)

Preoperative learning: baseline scores

For the final five problem sets presented prior to surgery (baseline), the control group scored slightly more errors in attaining criterion than did the lesion group. Control subjects averaged 138 ± 47 (mean ± SEM) errors to criterion, whereas monkeys assigned to receive fornix transections averaged 99 ± 24 errors to criterion. This difference was not statistically significant by the *t*-test.

Postoperative testing

An analysis of covariance assessed the effect of fornix transection for the three postoperative blocks of testing illustrated in Fig. 3, accounting for individual differences in preoperative performance. This analysis confirmed that the fornix transection affected performance in all three postoperative testing blocks [group, $F(1,5) = 45.9$, $P < 0.01$; block × group, $F(2,10) < 1$, n.s.]. Excluding L1 from the analysis yielded a similar group effect [group, $F(2,4) = 22.5$, $P < 0.01$; block × group, $F(4,8) < 1$, n.s.]. Figure 4 illustrates the group means for each of the preoperative problem sets (Fig. 4A), for the five novel problem sets presented in initial postoperative testing (Fig. 4B), and for later testing (Fig. 4C, D).

The difference between the groups was reflected in all three response types (Fig. 5). The group effect revealed by the analysis of covariance remained highly significant even if one of the three responses was omitted from the analysis: short-hold versus long-hold response [group, $F(1,5) = 28.0$, $P < 0.01$; block × group, $F(2,10) < 1$, n.s]; tap versus long-hold response [group, $F(1,5) = 21.38$, $P < 0.01$; block × group, $F(2,10) < 1$, n.s]; and tap versus short-hold response [group, $F(1,5) = 37.23$, $P < 0.01$; block × group, $F(2,10) < 1$, n.s.].

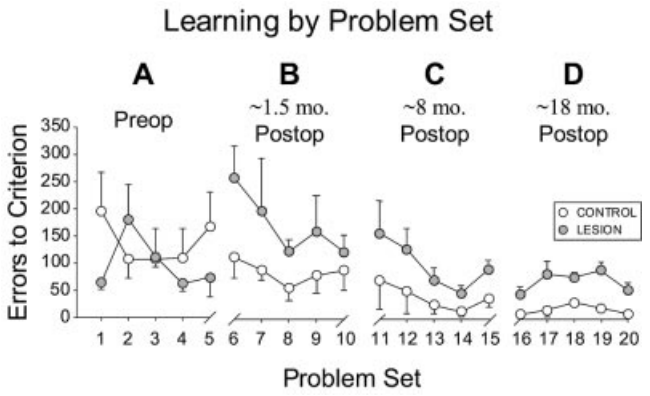

Fig. 4 The mean number of errors to criterion (+ standard error of the mean), by group, for each of five novel problem sets for the preoperative testing period (**A**) and for each of three postoperative testing periods: (**B**) within the first 2 months after surgery in the lesion group; (**C**) ~8 months after surgery; and (**D**) ~18 months after surgery. Note that as testing continued the control animals had progressively less room for improvement (known as a 'floor effect'). Nevertheless, there was still a significant difference deficit between the groups 18 months after surgery.

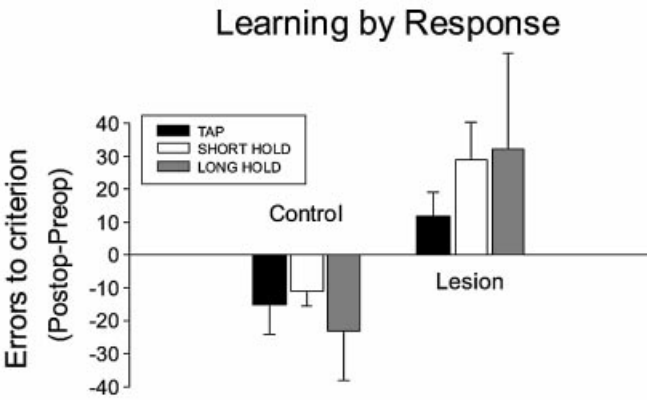

Fig. 5 Difference in mean number of errors to criterion (postoperative performance minus preoperative performance), by group, for each response type. Data are means for the five novel problem sets presented in the first postoperative testing session (<2 months after surgery). Note that, relative to preoperative learning rates, both the reduction in errors to criterion for the controls and the increase in errors for the operated group held for all three response types.

With these basic results in mind, it is instructive to examine the individual postoperative testing blocks. In the initial postoperative testing period (Figs 3B and 4B), the operated monkeys made more errors than controls. Monkeys with fornix transections scored 173 ± 31 errors to criterion postoperatively compared with 86 ± 28 for the controls. As shown in Fig. 3B (bottom panel), all four fornix-transected monkeys made more errors in attaining criterion postoperatively compared with their preoperative baseline scores,

whilst all four controls made fewer errors. The mean difference between baseline and postoperative testing was +74 errors for the fornix-transected group (indicating more errors to criterion than preoperatively) versus –51 errors for the control group (indicating fewer errors with added experience). This group difference was statistically significant [$t(6) = 2.8$, $P = 0.03$].

When monkeys were presented with an additional five novel problem sets ~8 months after surgery, the fornix-transected monkeys continued to perform poorly relative to controls. On average, the operated monkeys returned to their preoperative levels of performance over the 8 month period of training on similar material (Fig. 3C, bottom panel, and Table 1), as the controls continued to improve. Operated monkeys scored 100 ± 25 errors in achieving criterion, whilst controls scored 41 ± 26. Compared with baseline testing, the operated monkeys thus made an average of +1 errors versus –97 errors for the controls, a group difference that was statistically significant [$t(6) = 3.1$, $P < 0.03$].

When monkeys were presented with an additional five novel problem sets ~18 months after surgery, operated animals showed a persistent deficit (Figs 3D and 4D). Fornix-transected monkeys averaged 70 ± 8 errors to criterion, whilst control animals averaged only 15 ± 4 errors (Fig. 3D, top panel), a difference that was statistically significant [$t(6) = 5.8$, $P < 0.01$]. By this stage in testing (Table 1), the comparison with baseline failed to attain significance [$t(6) = 1.9$, n.s.] (Fig. 3D, bottom panel) due to a floor effect on control group performance (Fig. 4D).

Fast learning and retention

Within-session learning

Preoperatively, the control and lesion groups performed comparably during the first 200 trials (67 ± 5 and 72 ± 2% correct, respectively), the first 100 trials (62 ± 5 and 69 ± 3%) and the first 50 trials (55 ± 4 and 62 ± 2%) of novel problem sets [all $t(6) < 1.5$, n.s.]. Postoperatively, when a given stimulus set was presented for a predetermined number of trials, controls performed better than fornix-transected monkeys on problem sets presented for 200 trials [79 ± 5% correct versus 64 ± 2%; $t(6) = 2.8$, $P < 0.05$] and for 100 trials [76 ± 6 versus 58 ± 3%; $t(6) = 2.6$, $P < 0.05$]. When problem sets were first limited to 50 trials, the group difference initially failed to reach significance [68 ± 9 versus 50 ± 2% for 15 sets; $t(6) = 2.1$, n.s.], but later did so as the monkeys gained more experience [78 ± 6 versus 59 ± 4% for 50 sets; $t(6) = 2.8$, $P < 0.05$]. Figure 6 presents learning curves, which show that, compared with fornix-transected monkeys, control animals learned at about a three-fold faster rate (learning constants of 6.2 versus 19.8 trials). Note that this does not imply that the controls made one-third the number of errors to criterion; rather, this measure reflects the speed with which they eliminated errors (for data source see rows 9 and 12 of Table 1).

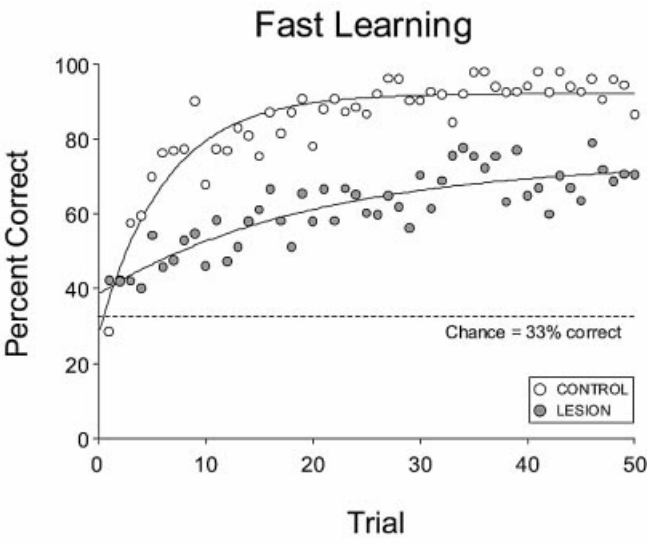

Fig. 6 Learning curves for novel three-choice (3:3) problem sets. Data points show the average per cent correct performance for each group (all >12 months after surgery). For controls, the performance rate fitted a three-factor exponential function with an initial state of 26.3% correct, a limit of 92.2% correct and a learning rate (defined as the number of trials to reach e^{-1} errors) of 6.2 trials ($R^2 = 0.86$). For the lesion group, the initial state was 38.5% correct, the limit was 74.0% correct and the learning rate was 19.8 trials ($R^2 = 0.74$). For both groups, the fit to an exponential function was better than for a linear regression. Note that the number of times the monkeys had seen a given problem (i.e. a given stimulus in the problem set) was one-third of the total number of trials.

Retention of remotely acquired mappings

Data from the latter half of the second block of within-session learning (Table 1, row 6) were used to compare the performances of the operated and control monkeys for both the familiar (remote) stimulus sets and the novel stimulus sets. These data were subjected to ANOVA (analysis of variance) with two within-subject factors [stimulus type (novel or familiar) and response type (tap, short hold, or long hold)] and one between-subject factor [group (lesion or control)]. There was a significant group × stimulus-type interaction [$F(1,6) = 20.1$, $P < 0.01$]. As illustrated in Fig. 7, *post hoc* analysis (Newman–Keuls) revealed that whilst the control group performed better than the fornix-transected group when novel stimuli were used [$q(2,6) = 7.2$, $P < 0.01$], there was no significant difference between the groups when they performed the task using the familiar (remote) problem set [$q(2,6) = 2.1$, n.s.]. Thus, there was intact retention and retrieval of practised, familiar visuomotor associations after fornix transection.

Strategy analyses

Previous studies of visuomotor conditional learning have identified the use of certain strategies (Wise and Murray, 1999). For example, if after a correctly performed trial the stimulus changes, some monkeys are more likely to choose a

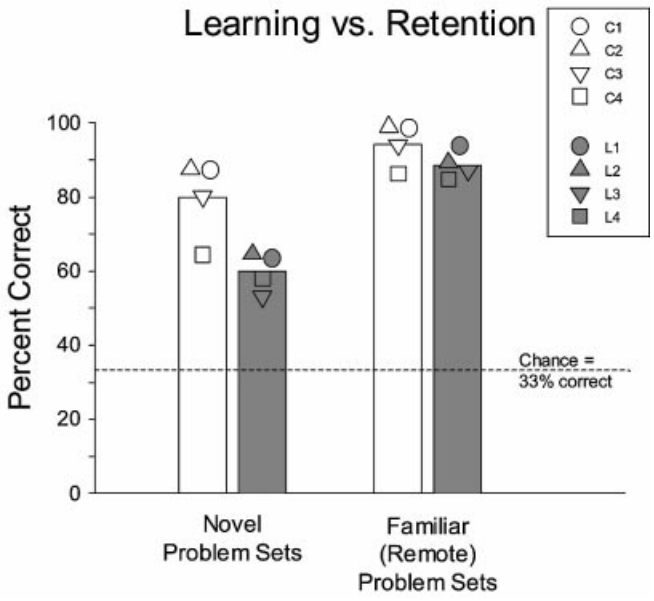

Fig. 7 Percentage of correct responses during sessions limited to 50 trials when performing the task with novel (*left*) versus familiar (*right*) problem sets. Fornix transection impaired the ability to learn novel visuomotor associations but did not impair performance for well-learned associations.

different response (termed a change-shift strategy), but if the stimulus does not change they are likely to repeat a previous response (termed a repeat-stay strategy). Neither of those strategies was adopted unambiguously by the subjects in the present experiment.

Larger and smaller problem sets

Figure 8 shows the errors to criterion for comparably tested problem sets of different sizes. When problem sets comprised only two novel stimuli and two responses (2:2 mappings, standard response parameters), fornix-transected monkeys scored 14 ± 5 errors in attaining criterion, whereas controls made only 3 ± 1 errors. The data for 3:3 mappings are given above for the main experiment (~18 months after surgery). For novel 6:3 mappings, fornix-transected monkeys scored 191 ± 28 errors in achieving criterion, compared with 49 ± 6 errors for controls. For novel 9:3 mappings, operated animals averaged 217 ± 62 errors in achieving criterion, whereas controls scored 66 ± 11 errors. The average number of errors per problem (Fig. 8B) was subjected to ANOVA with one within-subjects factor [set size (2, 3, 6, or 9)] and one between-subjects factor [group (control or lesion)]. There was a significant interaction between group and set size [$F(3,18) = 3.37$, $P < 0.05$]. *Post hoc* analysis (Newman–Keuls) revealed that the difference between the fornix-transected monkeys and controls was not significant for the 2:2 mappings [$q(2,18) = 1.1$, n.s.], but that the difference between the groups was significant for 3:3 mappings [$q(2,18) = 3.8$], 6:3 mappings [$q(2,18) = 4.9$] and 9:3 mappings [$q(2,18) = 3.5$, all $P < 0.05$]. Group differences were significant, however, for 2:2 mappings using the modified hold responses (see Methods; Wilcoxon rank-order test, $P < 0.05$). The number

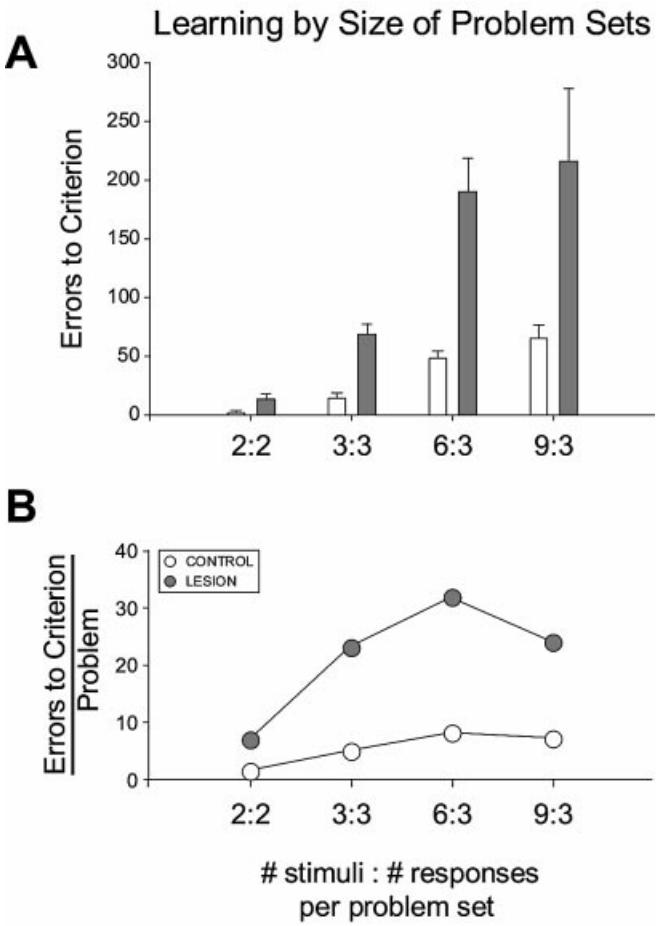

Fig. 8 (**A**) Average number of errors to criterion (+ standard error of the mean) for novel problem sets containing different numbers of stimuli. The number of stimuli per problem set *a* and the number of responses *b* are labelled on the abscissa as *a*:*b*. (**B**) Average number of errors to criterion per problem for each group.

of errors was small for 2:2 mappings using the modified short-hold response (control, 1.6 ± 0.1 errors to criterion; lesion, 3.4 ± 0.9), but larger for 6:2 mappings using the same responses (control, 9.6 ± 1.6; lesion 49.7 ± 19.5). For 2:2 mappings using the modified long-hold response (control, 3.9 ± 2.6; lesion, 14.8 ± 8.3), analysis of variance revealed a group effect [$F(1,6) = 6.29$, $P < 0.05$] and *post hoc* analysis (Newman–Keuls) showed that fornix-transected monkeys made significantly more errors than controls for each of the three response combinations.

Control conditions

The possibility that poor stimulus-discrimination abilities contributed to the impairment in visuomotor learning was tested in three ways. In the one described first, which we call 'mapping versus discrimination', the monkeys became familiar with several problem sets. Half of these were tested

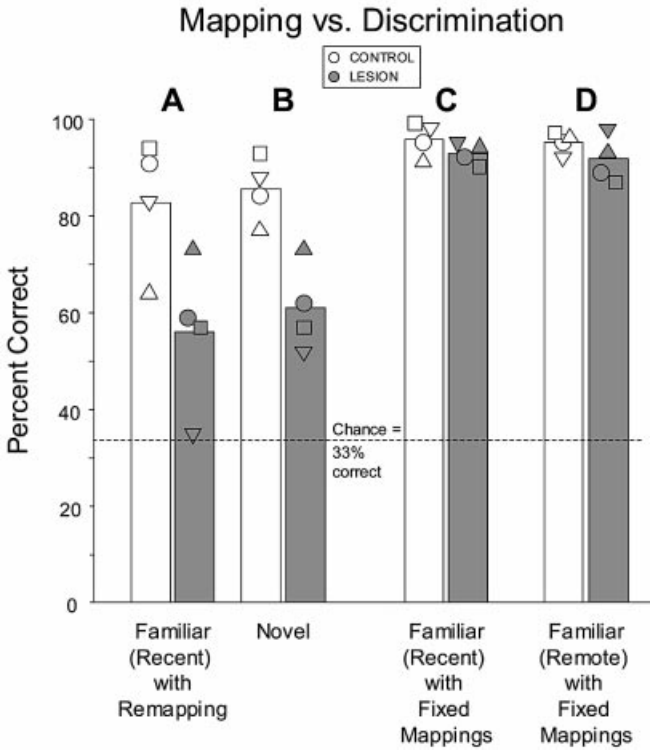

Fig. 9 Percentage of correct responses during 50-trial sessions when subjects were required to (left to right) (**A**) learn novel associations with familiar (recent–remapped) stimuli (see text for a definition of these terms), (**B**) learn novel associations with novel stimuli, (**C**) perform the task using recently established familiar stimuli and associations [familiar (recent–fixed)], or (**D**) perform the task using remotely established stimuli and associations [familiar (remote)]. Fornix transection impaired the ability to learn conditional associations regardless of whether the stimuli used were novel or familiar, indicating that the deficit is one of associative learning rather than of discriminating novel stimuli. Note that all exposure to all the familiar stimuli occurred after surgery.

every day with fixed mappings [familiar (recent–fixed)] and the other half were tested with visuomotor mappings that changed every day [familiar (recent–remapped)], as described in Methods. On the assumption that familiar stimuli have already been discriminated from one another, this procedure could distinguish a deficit in associative learning from one in visual discrimination. In the control condition described second, visual discrimination learning was tested with a traditional, concurrent-pair design. The control condition described third—transverse patterning—proved so difficult that it served not only as a test of discrimination abilities but also as a control for generalized learning deficits due to task difficulty (Beylin *et al*., 2001).

Mapping versus discrimination

The last 9 days of testing mapping versus discrimination performance (Table 1 row 9 and Fig. 9) were subjected to ANOVA with one within-subjects factor [stimulus type, with four levels: novel, familiar (recent–remapped), familiar (recent–fixed) and familiar (remote)] and one between-subjects factor [group (control or lesion)]. There was a significant group × stimulus-type interaction [$F(3,18) = 7.8$, $P < 0.01$]. *Post hoc* analysis (Newman-Keuls) revealed that the fornix-transected monkeys were impaired relative to controls on the two stimulus types that required the learning of new visuomotor associations each day: novel and familiar (recent–remapped). For novel stimuli, learning was significantly impaired relative to both familiar (recent–fixed) stimuli [$q(3,18) = 5.9$, $P < 0.05$] and the familiar (remote) stimuli [$q(2,18) = 5.7$, $P < 0.05$]. For familiar (recent–remapped) stimuli, learning was significantly impaired relative to both familiar (recent–fixed) stimuli [$q(4,18) = 6.8$, $P < 0.05$] and familiar (remote) stimuli [$q(3,18) = 6.6$, $P < 0.05$]. Thus, fornix-transected monkeys were impaired when visuomotor associations needed to be learned, whether the stimuli were novel or familiar.

Importantly, there was no difference between the operated group's performance with novel stimuli and familiar (recent–remapped) stimuli, both of which required the learning of novel visuomotor mappings each day [$q(2,18) = 0.9$, n.s.].

The effect of stimulus type on the control group, as shown by the unfilled bars in Fig. 9, was not significant. This reflected both the conservative nature of the *post hoc* test and the fast rate of learning with novel stimuli that was evident in the controls (Fig. 6).

An additional analysis examined whether these group differences reflected perseveration. For familiar (recent–remapped) stimuli, errors were categorized with respect to the mappings used for the previous session. If the monkey made the same response as a given stimulus had instructed during the previous session, that error was classed as perseverative, otherwise it was classed as random. A two-way analysis of variance revealed that neither group made more perseverative errors than random errors [error type, $F(1,6) < 1$, n.s; error type × group, $F(1,6) < 1$, n.s].

Visual discrimination learning

As illustrated in Fig. 10, the average number of errors made in achieving criterion for the five sets of four concurrent visual discrimination problems showed no group difference [$t(6) = 0.3$, n.s.].

Transverse patterning

Data such as those presented in Fig. 8 might suggest that the deficit observed in the learning of non-spatial visuomotor mappings resulted from a non-specific effect of task difficulty. Although not designed for this purpose, the transverse patterning task gave us the opportunity to examine whether the lesion would yield a deficit on another difficult, non-spatial task. Figure 11 illustrates the data from the 32 sessions of the transverse patterning task, split into an early stage

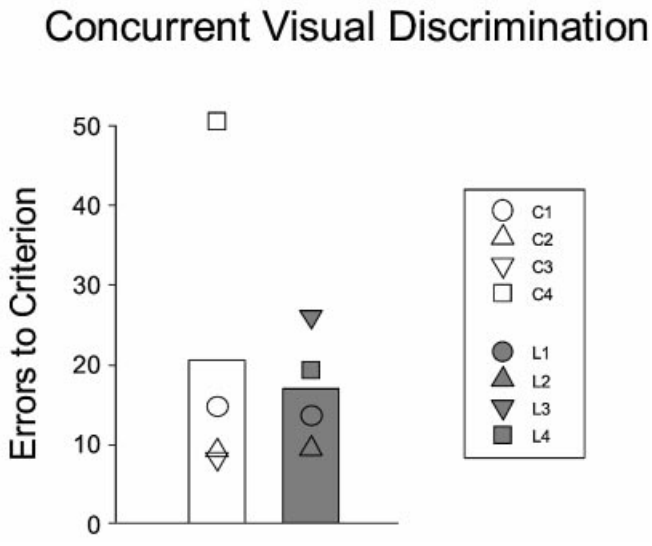

Fig. 10 Average number of errors to acquire five consecutive four-pair concurrent visual discriminations. Stimuli, although novel, were of the same type as those used in conditional visuomotor learning.

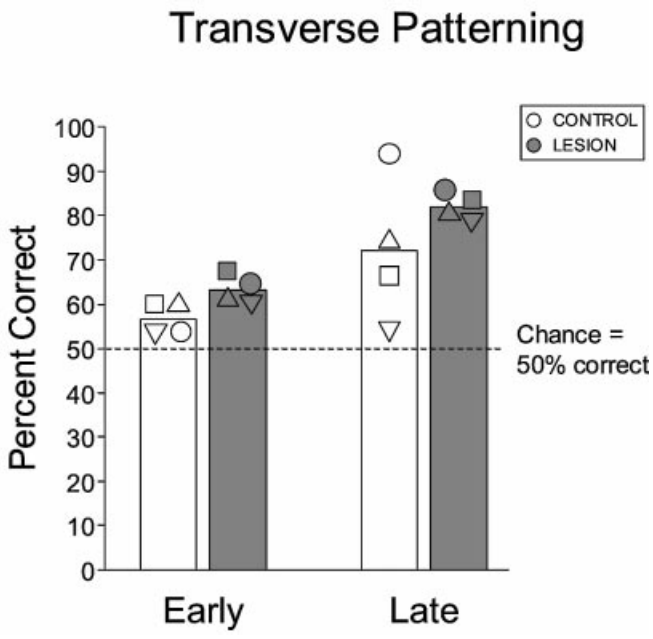

Fig. 11 Average percentage of correct responses on the transverse patterning task for each group for sessions 1–16 (Early) and sessions 17–32 (Late). All 32 of the 96-trial sessions used the same three stimuli which, although initially novel, were of the same type as used in conditional visuomotor learning. Monkeys with fornix transection showed no impairment at any stage.

(sessions 1–16) and a late stage (sessions 17–32). Performance was examined by ANOVA, with one within-subjects factor [stage (early or late)] and one between-subjects factor [group (control or lesion)]. Performance was better for the late stage [stage, $F(1,6) = 15.1$, $P < 0.01$], i.e. the animals learned the task to a significant degree, but there was no effect of fornix transection on learning the transverse patterning problem [stage × group, $F(1,6) = 0.15$, n.s.].

Discussion

The present results show that damage to the HS induced a significant and long-lasting impairment in the ability of monkeys to learn visuomotor associations, notwithstanding the fact that both the stimuli and the responses were differentiated by their nonspatial attributes.

Previous demonstrations of impairments in the learning of conditional visuomotor associations following HS damage—by fornix transection (Gaffan *et al*., 1984*a*; Rupniak and Gaffan, 1987), lesions of the CA1 field (Ridley *et al*., 1992) or removal of the hippocampus plus subjacent cortex (Murray and Wise, 1996)—have always involved responses that are differentiated in terms of their spatial targets. (In this discussion, we mainly use the phrases 'conditional visuomotor association' and 'stimulus–response learning' to describe the main task of this study. However, readers should note that this same kind of behaviour has also been called 'conditional visual discrimination', 'stimulus–response conditioning', 'arbitrary visuomotor mapping' and 'conditional motor learning'.) The previously published data thus support the hypothesis that at least one component of a conditional association must involve spatial information processing in order for HS damage to cause a deficit (Rupniak and Gaffan, 1987; Gaffan and Harrison, 1988; Murray and Wise, 1996). The results presented here, however, indicate that the impairments following damage to the HS are not confined to learning conditional associations with spatially differentiated responses or spatially differentiated stimuli. The present report confirms and extends previous findings from these subjects (Brasted *et al*., 2002*a*) by providing additional information on the rate of learning and the persistence of the deficit >1 year after surgery, as well as by reporting the results of control conditions that rule out accounts for the deficit in terms of visual discrimination ability or general task difficulty.

The present findings lead to rather different conclusions about HS function than those that arise from previous research on non-human animals. Most research on HS function in rodents (for review see O'Keefe 1999), and much of what has been reported for non-human primates (Gaffan *et al*., 1984*a*; Mahut and Moss, 1986; Murray *et al*., 1989, 1993; Parkinson *et al*., 1988; Murray and Mishkin, 1998; Gaffan, 1998, 2001), as well, has suggested that HS function is specific for spatial information, such as that involved in navigation (see Introduction). The present results are consistent, by contrast, with evidence from both the clinic and the laboratory indicating that the HS also functions in tasks that do not seem to involve the obligatory use of spatial information (Hampson *et al*., 1999; Alvarez *et al*., 2001), such as tasks involving relational (Bunsey and Eichenbaum, 1996; Dusek and Eichenbaum, 1998; Fortin *et al*., 2002) and

declarative memory (Squire and Zola, 1997). The results also appear compatible with accounts of HS function in terms of episodic memory (Gaffan, 1994*a*, *b*; Vargha-Khadem *et al*., 1997; Aggleton and Brown, 1999; Aggleton *et al*., 2000), which incorporate, but extend beyond, the processing of spatial information (see also Tulving, 2001). In accordance with these ideas, we view the present results as providing support for theories of HS function in terms of rapid associative learning of non-spatial as well as spatial information (e.g. Alvarez and Squire, 1994; McClelland *et al*., 1995; Murré, 1996; Murré *et al*., 2001; O'Reilly and Rudy, 2000).

Nature of the deficit

To support the conclusion that the HS functions in learning arbitrary associations between nonspatial stimuli and non-spatial responses, one should demonstrate that the ability to discriminate among nonspatial stimuli is unimpaired. This obligation is particularly important for the first few trials of learning, given that there is evidence to suggest that the HS is involved in the encoding of novel stimuli (Knight, 1996; Tulving *et al*., 1996; Dolan and Fletcher, 1997; Strange *et al*., 1999; Dolan and Strange, 2002).

Our results show that the impairment is unlikely to be the result of an impairment in discriminating non-spatial stimuli, for two reasons. First, the fornix-transected monkeys were not impaired on a concurrent visual discrimination learning task that used the same type of visual stimuli as used to assess visuomotor learning abilities (Fig. 10). This finding is consistent with previous studies that have shown fornix transection to impair neither object discrimination learning (Moss *et al*., 1981; Gaffan and Harrison, 1989; Zola-Morgan *et al*., 1989) nor object recognition memory (Gaffan *et al*., 1984*b*; Bachevalier *et al*., 1985*a*, *b*; Shaw and Aggleton, 1993). Furthermore, as shown in Fig. 11, fornix transection did not impair performance on the transverse patterning task (see also Bussey *et al*., 1998; but cf. Alvarado *et al*., 2002). Secondly, we controlled for object-discrimination and other stimulus-novelty effects by requiring monkeys to learn novel visuomotor associations using familiar stimuli: a set of highly familiar stimuli was associated with different responses each day. Fornix-transected monkeys were impaired relative to controls in learning new associations, regardless of whether the stimuli were novel or familiar (Fig. 9). Taken together, these results rule out an account of the deficit described here in terms of difficulty in distinguishing among novel, object-like stimuli.

It could be argued, however, that fornix transection decreased the discriminability of the competing motor responses or the ability to perform those responses. Two of the three responses in the present study, the short-hold and the long-hold responses, differ primarily in terms of screen-contact time. The role of the HS in trace conditioning (Moyer *et al*., 1990; Thompson *et al*., 1992; Clark and Squire, 1998; McEchron and Disterhoft, 1999), along with other published results (Meck *et al*., 1984; Olton *et al*., 1987), suggest a role of the HS in processing temporal information (see also Rawlins, 1985). But such an explanation cannot account for the present results because, although a temporal deficit would predict impairments for associations involving the short-hold and long-hold responses, fornix transection also significantly impaired associations involving the tap response (Fig. 5). Tapping is a very different response, and, as can be seen from Fig. 2, each tap is nearly an order of magnitude shorter than the briefest short-hold response. Thus, the deficit on tap responses is inconsistent with an interpretation of the deficit on conditional visuomotor mappings in terms of a simple and subtle timing deficit. Moreover, there was no difference between the groups when the conditional visuomotor task was performed with familiar stimuli. These results suggest that the associative-learning deficit is not the result of motor impairments, an inability to execute the three discrete responses properly, or a temporal information processing deficit *per se*.

The data are consistent, however, with a deficit—and therefore a role for the HS—in associating temporal information, such as that related to the motor response, with other information, in this case visual information pertaining to the stimulus. Such a role for HS might suggest that it plays a general role in processing information outside the domain of any single sensory modality. Like spatial information, temporal information does not derive uniquely from any single sensory receptor system. For example, there are visual–spatial maps as well as auditory–spatial maps and those involving other sensory modalities. Perhaps a general role of the HS is to process and associate information that can be derived from multiple sensory systems. Note that this concept is distinctly different from the idea of cross-modal association. In cross-modal association, each information packet that is associated with another derives from a single sensory modality. The idea presented here more resembles the concept of supramodal information processing, of which space and time are paradigmatic examples. We discuss below the possibility that the HS functions as a general-purpose pattern-associator network. However, if the HS is held to have a more limited role in associative memory, perhaps the concept of supramodal information processing will be useful in characterizing its specialization.

But are the responses and stimuli truly nonspatially differentiated? There is, of course, a spatial component to all of the responses, in that they all take place on a touchscreen, which has a certain location, and the monkeys' hands moved through space to reach the touchscreen and make their responses. However, these spatial factors did not differentiate one response from another, and reward was not contingent on these components of the response. Moreover, an increase in errors was seen in all response types (Fig. 5), so the spatial details of the response provide an unlikely account for the deficit. However, as suggested by D. Gaffan (personal communication), it is possible that monkeys approach some aspects of the response through a higher-order spatial

strategy, one involving approach towards versus withdrawal from the stimuli. One can envisage that the monkeys performed the tap response as analogous to a spatial 'approach'. At first glance this seems unlikely because the monkeys repeatedly approach and withdraw from the stimulus in performing a tap response. However, it is possible that the monkeys nevertheless view the response as one performed on the stimulus, which they must approach in order to accomplish their goal. It is further possible that the hold response can be viewed as a spatial withdrawal, on the grounds that the monkeys withdrew their hands from the stimuli at some point during the response, and withdrawal was the event leading to reward delivery. Withdrawal in this sense was obligatory for the short-hold response, but also occurred for all but one of the monkeys for the long-hold response. (Another monkey inconsistently maintained contact until the end of the 8-s period.) This approach–withdrawal account is not supported by one aspect of our results in the main task: there remains a significant deficit for conditional associations involving the short-hold and long-hold responses, alone, i.e. when tap responses are eliminated from the analysis. Both responses would be classified as withdrawal responses, but the fornix-transected monkeys were nevertheless impaired at learning the context for these two responses. Nevertheless, the approach–withdrawal interpretation could explain the differences between the present results and those of Gaffan and Harrison (1988), which precluded this strategy in their version of the tap–hold task. Whilst further work is needed to resolve this issue, our results on versions of the task with modified hold responses appear to rule out this account. In those phases of testing, monkeys had to maintain contact with the screen until the expiration of the hold period, as in the experiment of Gaffan and Harrison (1988). In all versions of the task a deficit was observed, although it was very small in the version of the task most closely matching the parameters of Gaffan and Harrison (2:2 mappings with modified short-hold responses). We consider other accounts of the difference with their results below (see Relation to previous studies on conditional visuomotor associations).

As for the nonspatial nature of the stimuli, it is certainly the case that shape cues have a spatial character and, furthermore, we know from certain probe tests (not reported here) that the monkeys used the shape cues in learning the associations. However, the stimuli did not differ in any appreciable way in their location, which was always the centre of the screen, and in this sense they were nonspatial.

Another possibility, that these learning deficits were the result of corpus callosum damage, can be ruled out. Eacott and Gaffan (1990), for example, showed that animals with complete transections of the corpus callosum (as well as the anterior commissure) can learn nonspatial visuomotor associations using similar stimulus material and nonspatially differentiated tap–hold responses similar to those of the present study. Monkeys with commissurotomies averaged fewer errors to criterion than the intact monkeys studied by Gaffan and Harrison (1988) on the same task. In addition, Ridley and colleagues showed that control monkeys, which received ablations of the corpus callosum dorsal to the fornix, were unimpaired on fornix-dependent visual conditional tasks (Ridley *et al*., 1992).

Accordingly, we conclude that fornix-transected monkeys were impaired on the present version of the conditional visuomotor task because they were slower to learn the association between the nonspatial stimulus and the nonspatial response.

Fornix transection versus hippocampal lesions

We discuss our results in terms of the HS because we recognize the important difference between damage to the hippocampus and the fibre system, the fimbria–fornix, that provides many of the afferents and efferents of the HS. Transection of the fornix disrupts both hippocampal efferents, such as subicular outputs to the diencephalon, and hippocampal afferents, such as cholinergic projections from the vertical limb of the diagonal band and septal nuclei. In addition, fornix transection disrupts fibres arising from the entorhinal and perirhinal cortex en route to the medial thalamus (Aggleton and Saunders, 1997), as well as cholinergic fibres targeting the subiculum and entorhinal cortex (Alonso *et al*., 1996). The impairment in conditional visuomotor learning observed in the present study could be due to the disruption of any of these projections. Thus, one should not assume the involvement of the hippocampus proper (dentate gyrus, CA1–CA3 and the hilar region, sometimes known as CA4) in the deficits herein attributed to the HS.

Outputs from the HS to the diencephalon, which depend upon the integrity of the fornix, could be especially important for normal rates of visuomotor learning, as with many other forms of memory. Much contemporary thinking about amnesia stresses the importance of the anterior thalamic and mamillary nuclei (Gaffan and Gaffan, 1991; Aggleton and Brown, 1999, 2002), and the deficit reported here may reflect the disruption of these diencephalic projections. There is, however, little evidence as yet that diencephalic structures such as the mamillary bodies play any role in conditional visuomotor learning, at least in rodents (Sziklas *et al*., 1996). Another efferent pathway involves the outputs from the HS to the nucleus accumbens of the striatum (Groenewegen *et al*., 1987). Lesions of the nucleus accumbens were at first reported to disrupt conditional visuomotor learning in rats (Reading *et al*., 1991). More recent work from the same laboratory (R. Quirk and T. Robbins, personal communication), however, suggests that lesions of the nucleus accumbens have little or no effect on the learning of conditional visuomotor associations, although there is a profound effect on the extinction of previously learned (i.e. familiar) ones, as originally reported (Reading *et al*., 1991).

In addition, the impairment reported here could reflect the interruption of hippocampal afferents, such as cholinergic projections from the basal forebrain. Consistent with this

possibility are studies by Ridley and colleagues, who found that lesions of the fornix (Ridley *et al*., 1992) and of the vertical limb of the diagonal band (Ridley *et al*., 1989), which provide cholinergic inputs to the hippocampus and nearby cortex, impaired the acquisition of conditional visuospatial associations in common marmosets, as did unilateral asymmetrical lesions of the diagonal band and the CA1/subiculum (Barefoot *et al*., 2002). Moreover, the deficits in conditional visuospatial learning induced by fornix transection were reversed by ectopic cholinergic basal forebrain grafts placed in the hippocampus (Ridley *et al*., 1992). Thus, it is conceivable that the learning deficits seen after fornix transection are a consequence of disrupting cholinergic hippocampal afferents. Alternatively, given that fornix transection deprives the subiculum and entorhinal cortex of cholinergic inputs as well as the hippocampus (Alonso *et al*., 1996), it is possible that the major effect of the lesion in the present study is external to the hippocampus. Gaffan (2002) has also argued that a key factor in disrupting new learning is the disconnection of the medial (and lateral) temporal cortex from cholinergic and other inputs from the basal forebrain.

Relation to previous studies on conditional visuomotor associations

The present data appear to be at variance with those of Gaffan and Harrison (1988), who found that fornix transection had no significant effect on the learning of conditional visuomotor associations involving non-spatially differentiated responses, tap and hold, and non-spatially differentiated stimuli. Their task was very similar to the present one, and the stimulus material was virtually identical.

At least two procedural differences between the two studies provide the most likely accounts for the discrepancy. First, the main task in the present study required subjects to map three distinct stimuli to three distinct responses, whereas the task used by Gaffan and Harrison (1988) used only two stimuli and two responses for each problem set. The difference in results, therefore, might be due to the increase in task difficulty, including the increased memory load, associated with larger problem sets. The data presented in Fig. 8 appear to support this idea: the group difference for the 2:2 mappings illustrated in the figure was not significant, unlike that for the 3:3, 6:3 and 9:3 mappings. However, task difficulty *per se* does not account for the deficit, as shown by the lack of impairment of fornix-transected monkeys on the transverse-patterning task (Fig. 11), a task that took many hundreds of trials to learn. Instead, the increased memory load or some other factor associated with larger problem sets appears to be responsible for the deficit. Regardless of the basis of the set-size effect, the current data (Fig. 8) demonstrate that, in experienced monkeys, the effects of fornix transection are likely to be small if, as in the study by Gaffan and Harrison, only two problems need to be learned concurrently.

Secondly, the degree of training given prior to fornix transection differed dramatically between the two studies. The monkeys in the Gaffan and Harrison (1988) study received no preoperative training on the task; all conditional visuomotor learning was postoperative. By contrast, monkeys in the present study were required to learn seven problem sets preoperatively, and other studies reporting conditional visuomotor learning deficits after HS damage involved even more extensive preoperative training (Rupniak and Gaffan, 1987; Murray and Wise, 1996). For example, the monkeys studied by Rupniak and Gaffan (1987)—on the spatial version of the task—had solved forty 2:2 problem sets prior to surgery. It is possible that disruption of a preoperatively established 'response set' may account for the fornix-transection effect. This issue is taken up again below (see Relation to episodic memory).

A distinction between fast and slow learning mechanisms might also provide an alternative account of the previous fornix-transection results in monkeys. Although the nature of responses differed between the tap–hold task of Gaffan and Harrison (1988), which yielded no deficit, and the approach–withdrawal task of Rupniak and Gaffan (1987), which did yield an impairment, there was also a dramatic difference in the rate of learning. The monkeys studied by Rupniak and Gaffan (1987) learned postoperative problems very fast, the control group averaging ~8 errors to a criterion of 90% correct responses. The monkeys studied by Gaffan and Harrison (1988) learned much more slowly, control subjects averaging ~90 errors to criterion for the first five post-operative problem sets and ~55 errors to criterion for the second five problem sets. Taking into account the increased task difficulty in our 3:3 mapping task, the learning rate of the monkeys in the present study was more akin to the fast learning rates seen in the study of Rupniak and Gaffan (1987) than the slower rates seen in the study of Gaffan and Harrison (1988). Thus, it is possible that, because of their fast learning, the monkeys in this study and the monkeys studied by Rupniak and Gaffan (1987) were more susceptible to a slowing of their learning rate by fornix transection than the slow-learning monkeys of Gaffan and Harrison (1988). This issue is taken up again below (see Fast versus slow learning systems).

Relation to episodic memory

A current view of HS function is that it is central to episodic memory (Schacter and Tulving, 1994; Tulving and Markowitsch, 1998). Could disruptions of such a memory system cause the deficits observed here?

Although the evidence is somewhat indirect in monkeys and remains controversial in humans, it has been proposed that the unique contribution of the HS involves the recording of episodic memories—the 'where', 'what' and 'when' of everyday experience (Cohen and Eichenbaum, 1993). For example, examination of patients with surgical interruptions of the fornix (to remove colloid cysts in the third ventricle)

led Aggleton *et al*. (2000) to conclude that the HS is important 'for integrating discrete item or object information with simultaneous scene or spatial information'. Episodic memory could support conditional visuomotor learning, indirectly, by recording the combinations of stimuli, responses and outcomes that constitute such trials. If this is a function of the HS, then disruption of episodic memory by damaging the HS might have the indirect effect of slowing conditional visuomotor learning.

The idea that HS functions in episodic memory might also lend credibility to the idea that naive monkeys with fornix transections approach visuomotor (and other) problems differently from naive unoperated subjects. Preoperative problem-solving experience with an intact episodic-memory system might cause a deficit because, after surgery, monkeys will presumably need to solve the same sort of problem a different way, one less reliant on an intact episodic-memory system. If monkeys encounter such problems for the first time with an already-damaged episodic memory system, as in the experiments of Gaffan and Harrison (1988), then perhaps they will not be predisposed to solve such problems using episodic memory, and fornix transection will be less likely to cause a deficit.

Fast versus slow learning systems

Expert opinion also holds that the HS underlies declarative, explicit memory generally (Squire, 1992) and that this system underlies rapid learning which is by no means restricted to spatial information. Indeed, lesions supposedly confined to the CA1 field of the hippocampus lead to deficits in acquiring declarative knowledge outside the spatial domain (Rempel-Clower *et al*., 1996), and a recent study in which CA1 NMDA (*N*-methyl-D-aspartate) receptors were genetically knocked out in mice led to the same conclusion (Rondi-Reig *et al*., 2001). By contrast, an implicit memory system is thought to acquire stimulus–response associations slowly. It is thought that this implicit memory system depends on structures other than the HS, either through the interaction of the neocortex with those parts of the basal ganglia that receive inputs from the neocortex (Mishkin and Petri, 1984; Fernandez-Ruiz *et al*., 2001) or through the neocortex acting through sensorimotor, corticocortical connections (Houk and Wise, 1995; McClelland *et al*., 1995), or both mechanisms. On the latter view, the HS functions as a rapid acquisition, pattern-associator network and the slower, non-HS system acquires similar information, but more slowly (McClelland *et al*., 1995). This idea is consistent with the present observation that, after fornix transection, the deficit was not an all-or-none inability to learn the conditional visuomotor associations, but rather a slowing in the learning rate (see also Rupniak and Gaffan, 1987).

We have discussed previously results of HS lesions that appear to be inconsistent with the idea that these structures function as a general-purpose pattern-associator network for fast learning (Wise and Murray, 1999). At one level, this theory predicts that HS manipulations should affect all kinds of associative learning. There is evidence that the HS is unnecessary for learning visual–visual associations (Murray *et al*., 1993; see also Gaffan and Harrison, 1989), also known as paired-associate learning. In the monkeys studied by Murray *et al*. (1993), paired-associate learning was unaffected by complete removal of the hippocampus. This could imply that the HS is uninvolved in associative learning when neither component of the association has a relevant spatial attribute. However, monkeys learned the paired-associate learning task slowly, and it remains an open question whether HS damage would cause deficits on rapid learning of this type. Gaffan *et al*. (1984*a*) reported unimpaired single-trial learning of object–reward associations after fornix transection, as well as unimpaired learning on a context–object association task. Although some deficits are observed in marmosets on a similar context–object association task after fornix transection, CA1 lesions or damage to the nucleus of the diagonal band (Ridley and Baker 1997), the degree of generality of HS function in associative memory and the concept of associative memory as a unitary process remain issues that will require further investigation.

Whilst the two mechanisms normally act in concert, a distinction between fast- and slow-acquisition mechanisms for conditional visuomotor learning might also explain some differences between rodent and primate studies. There have been a number of studies in rats which have shown that neither hippocampal (Winocur, 1991; Marston *et al*., 1993) nor fornix (Sziklas *et al*., 1998; Bussey *et al*., 2000) lesions have a discernible effect on the learning of conditional visuomotor associations. Most of these studies typically required hundreds, if not thousands, of trials for rats to learn two conditional associations, an experimental feature that could enhance the contribution of the putative slow-acquisition system (Marston *et al*., 1993) and might account for the lack of sensitivity to HS damage under certain conditions. It should be noted that electrolytic hippocampal lesions have been reported to impair conditional visuomotor learning in rats (Sziklas *et al*., 1996, 1998). The reasons for these discrepancies remain unresolved, although it could reflect differences either in lesion methodology or in the use of learning criteria that were differentially sensitive to fast-learning deficits.

The finding that fornix-transected monkeys in the present study were not impaired in learning the transverse patterning task may also reflect the speed of learning, among other factors. Although our results in transverse patterning appear to conflict with other recent findings in monkeys (Alvarado *et al*., 2002), the two studies differ in so many respects—including different lesion approaches, the manner in which the task was administered, the age of the animals at the time of surgery, and differences in prior training experiences—that meaningful conclusions would appear to be precluded. Performance on the transverse patterning task has also been reported to be impaired by hippocampal or fornix lesions in rats (Alvarado and Rudy, 1995; Dusek and Eichenbaum,

1998; cf. Bussey *et al*., 1998), and this result has been interpreted in terms of relational (Bunsey and Eichenbaum, 1996; Dusek and Eichenbaum, 1998) or configural (Rudy and Sutherland, 1995) memory. The lack of a deficit on the transverse patterning task in the present study could be viewed as being at variance with these theories of HS function, but given the slow rate at which the transverse patterning task is learned, we note only that the lack of impairment in the present study is consistent with the idea that the HS plays a less important role in slow learning than in rapid learning. This idea does not, however, account for the results in rodents, because they also learn the transverse patterning task relatively slowly. Clearly, further work will be required to resolve this issue.

Conclusion

The present results indicate that at least one form of damage to the HS, fornix transection, impairs conditional visuomotor learning in the absence of either spatially differentiated stimuli or spatially differentiated responses. This finding poses a challenge to theories of hippocampal function that focus exclusively on the spatial domain (O'Keefe, 1999; Gaffan, 2001) and serves to bring experimental data from non-human primates closer to the views about HS function that have developed from clinical (Schacter and Tulving, 1994; Kopelman *et al*., 1997; Vargha-Khadem *et al*., 1997; Spiers *et al*., 2001) and brain-imaging (Gabrieli *et al*., 1997; Lepage *et al*., 1998; Schacter and Wagner, 1999; Maguire *et al*., 2000) studies. Further work is needed to ascertain whether the effects reported here result from damage to the hippocampus proper, and whether HS damage affects rapidly acquired associations generally or is instead restricted to associations involving the spatial and temporal domains.

Acknowledgements

We wish to thank Mr Kevin Blomstrom for assistance with the behavioural testing, Dr Andrew R. Mitz for computer programming and Dr Richard Saunders for performing the surgery. Drs A. David Redish, David Gaffan and Mark Buckley kindly provided us with comments on a previous version of this paper. This research was supported by the Division of Intramural Research Programs of the National Institute of Mental Health.

References

Aggleton JP, Brown MW. Episodic memory, amnesia, and the hippocampal–anterior thalamic axis. Behav Brain Sci 1999; 22: 425–44.

Aggleton JP, Brown MW. Integrating systems for event memory. In: Squire LR, Schacter DL, editors. Neuropsychology of memory. 3rd ed. New York: Guilford Press; 2002. p. 377–94.

Aggleton JP, Saunders RC. The relationships between temporal lobe and diencephalic structures implicated in anterograde amnesia. Memory 1997; 5: 49–71.

Aggleton JP, Hunt PR, Rawlins JN. The effects of hippocampal lesions upon spatial and non-spatial tests of working memory. Behav Brain Res 1986; 19: 133–46.

Aggleton JP, McMackin D, Carpenter K, Hornak J, Kapur N, Halpin S, et al. Differential cognitive effects of colloid cysts in the third ventricle that spare or compromise the fornix. Brain 2000; 123: 800–15.

Alonso JR, U HS, Amaral DG. Cholinergic innervation of the primate hippocampal formation: II. Effects of fimbria/fornix transection. J Comp Neurol 1996; 375: 527–51.

Alvarado MC, Rudy JW. Comparison of 'configural' discrimination problems: implications for understanding the role of the hippocampal formation in learning and memory. Psychobiology 1995; 23: 178–84.

Alvarado MC, Wright AA, Bachevalier J. Object and spatial relational memory in adult rhesus monkeys is impaired by neonatal lesions of the hippocampal formation but not the amygdaloid complex. Hippocampus 2002; 12: 421–33.

Alvarez P, Squire LR. Memory consolidation and the medial temporal lobe: a simple network model. Proc Natl Acad Sci USA 1994; 91: 7041–45.

Alvarez P, Lipton PA, Melrose R, Eichenbaum H. Differential effects of damage within the hippocampal region on memory for a natural, nonspatial Odor–Odor Association. Learn Mem 2001; 8: 79–86.

Angeli SJ, Murray EA, Mishkin M. Hippocampectomized monkeys can remember one place but not two. Neuropsychologia 1993; 31: 1021–30.

Bachevalier J, Parkinson JK, Mishkin M. Visual recognition in monkeys: effects of separate vs. combined transection of fornix and amygdalofugal pathways. Exp Brain Res 1985a; 57: 554–61.

Bachevalier J, Saunders RC, Mishkin M. Visual recognition in monkeys: effects of transection of fornix. Exp Brain Res 1985b; 57: 547–53.

Barefoot HC, Baker HF, Ridley RM. Crossed unilateral lesions of temporal lobe structures and cholinergic cell bodies impair visual conditional and object discrimination learning in monkeys. Eur J Neurosci 2002; 15: 507–16.

Beylin AV, Gandhi CC, Wood GE, Talk AC, Matzel LD, Shors TJ. The role of the hippocampus in trace conditioning: temporal discontinuity or task difficulty? Neurobiol Learn Mem 2001; 76: 447–61.

Brasted PJ, Bussey TJ, Wise SP, Murray EA. Fornix transection impairs conditional visuomotor learning of nonspatially differentiated responses. Program No 455.8 Abstracts Viewer/ Itinerary Planner. CD-ROM. Washington (DC): Soc Neurosci Abstr 2001; 27.

Brasted PJ, Bussey TJ, Murray EA, Wise SP. Fornix transection impairs conditional visuomotor learning in tasks involving nonspatially differentiated responses. J Neurophysiol 2002a; 87: 631–3.

Brasted PJ, Bussey TJ, Murray EA, Wise SP. Impairment in learning arbitrary associations after fornix transection in monkeys: mapping vs. discrimination. Program No 8.5 Abstracts Viewer/ Itinerary Planner. CD-ROM. Washington (DC): Soc Neurosci Abstr. In press 2002b.

Bunsey M, Eichenbaum H. Conservation of hippocampal memory function in rats and humans. Nature 1996; 379: 255–7.

Bussey TJ, Warburton EC, Aggleton JP, Muir JL. Fornix lesions can facilitate acquisition of the transverse patterning task: a challenge for 'configural' theories of hippocampal function. J Neurosci 1998; 18: 1622–31.

Bussey TJ, Duck J, Muir JL, Aggleton JP. Distinct patterns of behavioural impairments resulting from fornix transection or neurotoxic lesions of the perirhinal and postrhinal cortices in the rat. Behav Brain Res 2000; 111: 187–202.

Bussey TJ, Wise SP, Murray EA. The role of ventral and orbital prefrontal cortex in conditional visuomotor learning and strategy use in rhesus monkeys (Macaca mulatta). Behav Neurosci 2001; 115: 971–82.

Clark RE, Squire LR. Classical conditioning and brain systems: the role of awareness. Science 1998; 280: 77–81.

Cohen NJ, Eichenbaum H. Memory, amnesia, and the hippocampal system. Cambridge (MA): MIT Press; 1993.

Day LB, Crews D, Wilczynski W. Effects of medial and dorsal cortex lesions on spatial memory in lizards. Behav Brain Res 2001; 118: 27–42.

Deacon RMJ, Bannerman DM, Rawlins NP. Conditional discriminations based on external and internal cues in rats with cytotoxic hippocampal lesions. Behav Neurosci 2001; 115: 43–57.

Dolan RJ, Fletcher PC. Dissociating prefrontal and hippocampal function in episodic memory encoding. Nature 1997; 388: 582–5.

Dolan RJ, Strange BA. Hippocampal novelty responses studied with functional neuroimaging. In: Squire LR, Schacter DL, editors. Neuropsychology of memory. New York: Guilford Press; 2002. p. 204–14.

Dusek JA, Eichenbaum H. The hippocampus and transverse patterning guided by olfactory cues. Behav Neurosci 1998; 112: 762–71.

Eacott MJ, Gaffan D. Interhemispheric transfer of visuomotor conditional learning via the anterior corpus callosum of monkeys. Behav Brain Res 1990; 38: 109–16.

Fernandez-Ruiz J, Wang J, Aigner TG, Mishkin M. Visual habit formation in monkeys with neurotoxic lesions of the ventrocaudal neostriatum. Proc Natl Acad Sci USA 2001; 98: 4196–201.

Fortin NJ, Agster KL, Eichenbaum HB. Critical role of the hippocampus in memory for sequences of events. Nat Neurosci 2002; 5: 458–62.

Gabrieli JD, Brewer JB, Desmond JE, Glover GH. Separate neural bases of two fundamental memory processes in the human medial temporal lobe. Science 1997; 276: 264–6.

Gaffan D. Dissociated effects of perirhinal cortex ablation, fornix transection and amygdalectomy: evidence for multiple memory systems in the primate temporal lobe. Exp Brain Res 1994a; 99: 411–22.

Gaffan D. Scene-specific memory for objects: a model of episodic memory impairment in monkeys with fornix transection. J Cogn Neurosci 1994b; 6: 305–20.

Gaffan D. Idiothetic input into object-place configuration as the contribution to memory of the monkey and human hippocampus: a review. Exp Brain Res 1998; 123: 201–9.

Gaffan D. What is a memory system? Horel's critique revisited. Behav Brain Res 2001; 127: 5–11.

Gaffan D. Against memory systems. Philos Trans R Soc Lond B Biol Sci 2002; 357: 1111–21.

Gaffan D, Gaffan EA. Amnesia in man following transection of the fornix. Brain 1991; 114: 2611–8.

Gaffan D, Harrison S. Inferotemporal-frontal disconnection and fornix transection in visuomotor conditional learning by monkeys. Behav Brain Res 1988; 31: 149–63.

Gaffan D, Harrison S. A comparison of the effects of fornix transection and sulcus principalis ablation upon spatial learning by monkeys. Behav Brain Res 1989; 31: 207–20.

Gaffan D, Saunders RC, Gaffan EA, Harrison S, Shields C, Owen MJ. Effects of fornix transection upon associative memory in monkeys: role of the hippocampus in learned action. Q J Exp Psychol B 1984a; 36: 173–221.

Gaffan D, Shields C, Harrison S. Delayed matching by fornix-transected monkeys: the sample, the push and the bait. Q J Exp Psychol B 1984b; 36: 305–17.

Groenewegen HJ, Vermeulen-Van der Zee E, te Kortschot A, Witter MP. Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris leucoagglutinin. Neuroscience 1987; 23: 103–20.

Hampson RE, Simeral JD, Deadwyler SA. Distribution of spatial and nonspatial information in dorsal hippocampus. Nature 1999; 402: 610–4.

Hampson RE, Hodge SR, West CL, Deadwyler SA. Hippocampal neural activity underlying delayed match-to-sample behavioral responses to stimulus categories in nonhuman primate. Program No. 477.3 2002 Abstracts Viewer/Itinerary Planner. CD-ROM. Washington (DC): Society for Neuroscience; 2002.

Houk JC, Wise SP. Distributed modular architectures linking basal ganglia, cerebellum, and cerebral cortex: their role in planning and controlling action. Cereb Cortex 1995; 5: 95–110.

Jarrard LE. On the role of the hippocampus in learning and memory in the rat. Behav Neural Biol 1993; 60: 9–26.

Jarrard LE. What does the hippocampus really do? Behav Brain Res 1995; 71: 1–10.

Kennedy PJ, Shapiro ML. The hippocampal system and the expression of differentially motivated responses in a non-spatial task. Program No. 183.8 2002 Abstracts Viewer/Itinerary Planner. CD-ROM. Washington (DC): Society for Neuroscience; 2002.

Knight R. Contribution of human hippocampal region to novelty detection. Nature 1996; 383: 256–9.

Kopelman MD, Stanhope N, Kingsley D. Temporal and spatial context memory in patients with focal frontal, temporal lobe, and diencephalic lesions. Neuropsychologia 1997; 35: 1533–45.

Lepage M, Habib R, Tulving E. Hippocampal PET activations of memory encoding and retrieval: the HIPER model. Hippocampus 1998; 8: 313–22.

Maguire EA, Mummery CJ, Buchel C. Patterns of hippocampal–cortical interaction dissociate temporal lobe memory subsystems. Hippocampus 2000; 10: 475–82.

Mahut H, Moss M. The monkey and the sea horse. In: Isaacson RL, Pribram KH, editors. The hippocampus. New York: Plenum Press; 1986. p. 241–79.

Marston HM, Everitt BJ, Robbins TW. Comparative effects of excitotoxic lesions of the hippocampus and septum/diagonal band on conditional visual discrimination and spatial learning. Neuropsychologia 1993; 31: 1099–18.

McClelland JL, McNaughton BL, O'Reilly RC. Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. Psychol Rev 1995; 102: 419–57.

McEchron MD, Disterhoft JF. Hippocampal encoding of nonspatial trace conditioning. Hippocampus 1999; 9: 385–96.

Meck WH, Church RM, Olton DS. Hippocampus, time, and memory. Behav Neurosci 1984; 98: 3–22.

Mishkin M, Petri HL. Memories and habits: some implications for the analysis of learning and retention. In: Squire LR, Butters N, editors. Neuropsychology of memory. New York: Guilford Press; 1984. p. 287–96.

Morris RG, Garrud P, Rawlins JN, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. Nature 1982; 297: 681–3.

Moss M, Mahut H, Zola-Morgan S. Concurrent discrimination learning of monkeys after hippocampal, entorhinal, or fornix lesions. J Neurosci 1981; 1: 227–40.

Moyer JR Jr, Deyo RA, Disterhoft JF. Hippocampectomy disrupts trace eye-blink conditioning in rabbits. Behav Neurosci 1990; 104: 243–52.

Murray EA, Mishkin M. Object recognition and location memory in monkeys with excitotoxic lesions of the amygdala and hippocampus. J Neurosci 1998; 18: 6568–82.

Murray EA, Wise SP. Role of the hippocampus plus subjacent cortex but not amygdala in visuomotor conditional learning in rhesus monkeys. Behav Neurosci 1996; 110: 1261–70.

Murray EA, Davidson M, Gaffan D, Olton DS, Suomi S. Effects of fornix transection and cingulate cortical ablation on spatial memory in rhesus monkeys. Exp Brain Res 1989; 74: 173–86.

Murray EA, Gaffan D, Mishkin M. Neural substrates of visual stimulus–stimulus association in rhesus monkeys. J Neurosci 1993; 13: 4549–61.

Murré JM. TraceLink: a model of amnesia and consolidation of memory. Hippocampus 1996; 6: 675–84.

Murré JM, Graham KS, Hodges JR. Semantic dementia: relevance to connectionist models of long-term memory. Brain 2001; 124: 647–75.

O'Keefe J. Place units in the hippocampus of the freely moving rat. Exp Neurol 1976; 51: 78–109.

O'Keefe J. Do hippocampal pyramidal cells signal non-spatial as well as spatial information? Hippocampus 1999; 9: 352–64.

O'Keefe J, Nadel L. The hippocampus as a cognitive map. Oxford: Clarendon Press; 1978.

O'Keefe J, Speakman A. Single unit activity in the rat hippocampus during a spatial memory task. Exp Brain Res 1987; 68: 1–27.

O'Reilly RC, Rudy JW. Computational principles of learning in the neocortex and hippocampus. Hippocampus 2000; 10: 389–97.

Olton DS, Meck WH, Church RM. Separation of hippocampal and amygdaloid involvement in temporal memory dysfunctions. Brain Res 1987; 404: 180–8.

Parkinson JK, Murray EA, Mishkin M. A selective mnemonic role for the hippocampus in monkeys: memory for the location of objects. J Neurosci 1988; 8: 4159–67.

Passingham RE. The frontal lobes and voluntary action. Oxford: Oxford University Press; 1993.

Rawlins JNP. Associations across time: the hippocampus as a temporary memory store. Behav Brain Sci 1985; 8: 479–96.

Reading PJ, Dunnett SB, Robbins TW. Dissociable roles of the ventral, medial and lateral striatum on the acquisition and performance of a complex visual stimulus–response habit. Behav Brain Res 1991; 45: 147–61.

Redish AD. Beyond the cognitive map. Cambridge (MA): MIT Press; 1999.

Rempel-Clower NL, Zola SM, Squire LR, Amaral DG. Three cases of enduring memory impairment after bilateral damage limited to the hippocampal formation. J Neurosci 1996; 16: 5233–55.

Ridley RM, Baker HF. Evidence for a specific information processing deficit in monkeys with lesions of the septo-hippocampal system. Cortex 1997; 33: 167–76.

Ridley RM, Aitken DM, Baker HF. Learning about rules but not about reward is impaired following lesions of the cholinergic projection to the hippocampus. Brain Res 1989; 502: 306–18.

Ridley RM, Gribble S, Clark B, Baker HF, Fine A. Restoration of learning ability in fornix-transected monkeys after fetal basal forebrain but not fetal hippocampal tissue transplantation. Neuroscience 1992; 48: 779–92.

Rondi-Reig L, Libbey M, Eichenbaum H, Tonegawa S. CA1-specific N-methyl-D-aspartate receptor knockout mice are deficient in solving a nonspatial transverse patterning task. Proc Natl Acad Sci USA 2001; 98: 3543–8.

Rudy JW, Sutherland RJ. Configural association theory and the hippocampal formation: an appraisal and reconfiguration. Hippocampus 1995; 5: 375–89.

Rupniak NMJ, Gaffan D. Monkey hippocampus and learning about spatially directed movements. J Neurosci 1987; 7: 2331–7.

Schacter DL, Tulving E. What are the memory systems of 1994? In: Schacter DL, Tulving E, editors. Memory systems 1994. Cambridge (MA): MIT Press; 1994. p. 1–38.

Schacter DL, Wagner AD. Medial temporal lobe activations in fMRI and PET studies of episodic encoding and retrieval. Hippocampus 1999; 9: 7–24.

Shaw C, Aggleton JP. The effects of fornix and medial prefrontal lesions on delayed non-matching-to-sample by rats. Behav Brain Res 1993; 54: 91–102.

Spiers HJ, Maguire EA, Burgess N. Hippocampal amnesia. Neurocase 2001; 7: 357–82.

Squire LR. Memory and the hippocampus: a synthesis from findings with rats, monkeys and humans. Psychol Rev 1992; 99: 195–231.

Squire LR, Zola SM. Amnesia, memory and brain systems. Philos Trans R Soc Lond B Biol Sci 1997; 352: 1663–73.

Strange BA, Fletcher PC, Henson RN, Friston KJ, Dolan RJ. Segregating the functions of human hippocampus. Proc Natl Acad Sci USA 1999; 96: 4034–9.

Sziklas V, Petrides M, Leri F. The effects of lesions to the mammillary region and the hippocampus on conditional associative learning by rats. Eur J Neurosci 1996; 8: 106–15.

Sziklas V, Lebel S, Petrides M. Conditional associative learning and the hippocampal system. Hippocampus 1998; 8: 131–7.

Thompson LT, Moskal JR, Disterhoft JF. Hippocampus-dependent learning facilitated by a monoclonal antibody or D-cycloserine. Nature 1992; 359: 638–41.

Tulving E. Episodic memory and common sense: how far apart? Philos Trans R Soc Lond B Biol Sci 2001; 356; 1505–15.

Tulving E, Markowitsch HJ. Episodic and declarative memory: role of the hippocampus. Hippocampus 1998; 8: 198–204.

Tulving E, Markowitsch HJ, Craik FEM, Habib R, Houle S. Novelty and familiarity activations in PET studies of memory encoding and retrieval. Cereb Cortex 1996; 6: 71–9.

Vargha-Khadem F, Gadian DG, Watkins KE, Connelly A, Van Paesschen W, Mishkin M. Differential effects of early hippocampal pathology on episodic and semantic memory. Science 1997; 277: 376–80.

Wilson MA, McNaughton BL. Dynamics of the hippocampal ensemble code for space. Science 1993; 261: 1055–8.

Winocur G. Functional dissociation of the hippocampus and prefrontal cortex in learning and memory. Psychobiology 1991; 19: 11–20.

Wise SP, Murray EA. Role of the hippocampal system in conditional motor learning: mapping antecedents to action. Hippocampus 1999; 9: 101–17.

Zola-Morgan S, Squire LR, Amaral DG. Lesions of the hippocampal formation but not lesions of the fornix or the mamillary nuclei produce long-lasting memory impairment in monkeys. J Neurosci 1989; 9: 898–913.

Received August 21, 2002. Revised December 9, 2002.
Accepted December 16, 2002