For my parents,
Amy Bik Wah and Chack Kwan Kwok

Mnemonic functions in the macaque monkey: further insight into the role of the fornix

Sze Chai Kwok

Department of Experimental Psychology
and
St Hugh's College

University of Oxford

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MNEMONIC FUNCTIONS IN THE MACAQUE MONKEY: FURTHER INSIGHT INTO THE ROLE OF THE FORNIX

Sze Chai Kwok

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ABSTRACT

The fornical tract, a major input-output pathway of the hippocampus, of the primate brain makes crucial contributions to visual memory, as effects after surgical or aetiological lesions of this tract are widely documented in the monkey and human literature. Here, a series of experiments sought to further elucidate the functions of this structure with a battery of novel tasks in macaque monkeys, conducted either on a touchscreen or in an ambulatory chamber, so as to offer a more global view of the mnemonic role accomplished by it. After receiving bilateral transection of the fornix, monkeys are impeded in the 'fast learning' phase of a large number of new visuospatial conditional problems, with major impairments seen in eliminating non-perseverative errors. These fornix transected monkeys are however facilitated in the initial acquisition of a visuovisual conditional task, with facilitation seen in their improved ability in eliminating perseverative errors. It is also demonstrated in an ambulatory apparatus, in comparison to control monkeys, these monkeys are impaired in the new learning of visuospatial context of environments, albeit still displaying intact locomotor and exploratory behaviour patterns. Contrary to the relatively clear role in new learning, the involvement of the fornix in memory retention over the very long-term is unknown. It is shown here that once some visuospatial information is learnt; the fornix is no longer implicated in the retention of the material. The effects of

fornix transection are also found to be detrimental on a spatial recognition task, with impairments observed in acquisition of the more demanding stages of the task. The overall results covered in this thesis support previous work suggesting that the fornix mediates the new learning of visual information, and I further propose that this fornical involvement lies primarily in the learning of spatio-temporal contexts, particularly during ‘fast learning’, as well as in task-sets acquisition. I also argue for dissociation in the contributions of the fornix and hippocampus to some memory processes in the macaque.

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PREFACE

This thesis is presented as a set of experimental chapters intended for publication. Each of the chapters has either been published in a peer-reviewed journal, or is in the process of being assessed by reviewers at various journals. Chapter 1 provides an introduction to the fornix and its known functions in primates and serves to act as a context within which to view the experimental chapters which follow. Chapter 7 provides a general discussion and conclusions of the work undertaken in Chapters 2 – 6 to constitute a continuous theme. In addition to specific abstracts provided at the beginning of each of the experimental Chapters 2 – 6, a more detailed abstract of this thesis is also included.

Each of Chapters 2 – 6 describes a separate piece of work undertaken during the course of my DPhil. In all chapters, I am the principal author and have analysed the behavioural data. In addition I have tested all the monkeys involved, been a major contributor to the design of the experiments in Chapters 2 – 4 and 6, and written the computer programmes to analyse the behavioural data in Chapters 2, 3 and 6. As each chapter was submitted for publication, a separate list of references is contained in that chapter. For readers' convenience, all references have been gathered in a consolidated reference list placed at the back of this thesis. The full list of authors for each paper, along with the publication status of each, is presented below.

Chapter 2
Fornix transection selectively impairs fast learning of visuospatial discriminations
Sze Chai Kwok and Mark J. Buckley
Presented here in the format for submission to Hippocampus

Chapter 3
Fornix transection selectively facilitates initial acquisition of concurrent visuovisual discriminations
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Chapter 4
Forgetting over the very long-term is unaffected by fornix transection
Sze Chai Kwok and Mark J. Buckley
Presented here in the format for submission to European Journal of Neuroscience

Chapter 5
Fornix transection impairs spatial recognition memory in macaques
Sze Chai Kwok and Mark J. Buckley
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Chapter 6
Fornix transection impairs exploration but not locomotion in ambulatory macaque monkeys
Sze Chai Kwok and Mark J. Buckley
Published, August 2006, Hippocampus

EXTENDED ABSTRACT

This thesis considers the role of the fornical tract (the fornix) – a major input/output pathway of the hippocampus – in visual memory in the macaque monkey. The experiments were either conducted on an automated computer touchscreen (Chapters 2 – 5) or in a purposely built chamber that allows ambulatory movements of monkeys (Chapter 6). All five studies were carried out postoperatively.

Chapter 2 assesses the effects of fornix transection on the acquisition of rapidly learnt conditional visuospatial discrimination problems. Macaque monkeys with and without fornix transection were trained on three sets of problems in a total of 104 problems (8, 32 and 64 problems in each set) on a touchscreen. Each problem consisted of four identical visual stimuli and animals had to learn which stimulus position was rewarded. The lesioned animals made significantly more errors-to-criterion on the smallest set of problems, consistent with the idea that the most rapidly acquired sets would be more vulnerable to fornical damage. During the early stages of acquisition, fornix transection also selectively impaired monkeys' abilities to eliminate non-perseverative errors in correction trials, consistent with an inability to monitor or correct erroneous spatial responses made further back in time than the last trial. Neither one-trial learning nor errorless learning (facilitation of performance) was dependent on the fornix. The data indicate that the fornix is not important for all forms of new learning; rather it is selectively concerned with the relatively rapid acquisition of spatial and temporal relationships between stimuli and responses.

Chapter 3 investigates whether monkeys with fornix transection would also be impaired in the rapid acquisition of visuovisual conditional associations, given the established impairments in both spatial (visuospatial) and non-spatial (visuomotor) associative tasks after fornix transection. I trained monkeys, postoperatively, on three sets of concurrent conditional stimulus-stimulus associations. Fornix transection did not impair early learning of these associations; rather, animals with fornix transection demonstrated facilitations in performance and accrued significantly fewer perseverative errors during the initial acquisition stages. These results challenge the idea that the hippocampal system plays a general role in the rapid acquisition of all kinds of associative knowledge. I suggest that the lesion induced facilitation of this non-spatial task may be secondary to an impairment in visuospatial processing, which might act to bias animals away from attempts to learn about spatial strategies for solving novel tasks. Both fornix and control monkeys were observed to adopt a change-shift response strategy on successive trials that were different; the use of this strategy was also fornix independent.

Chapter 4 is an extension of the experiment reported in Chapter 2 in which the macaque monkeys learnt postoperatively to discriminate a total of 104 new concurrent visuospatial conditional problems to criterion. Chapter 4 measures and compares long-term retention of these problems with two 1-trial postoperative retention tests administered 3 and 15 months respectively after acquisition. All animals showed some forgetting of these problems but still remembered well above chance after 15 months. The amount forgotten by each group did not differ significantly at either time point. These results show that long-term retention of visuospatial information and the reliability in retention between tests are independent of the fornix. These present

findings suggest fornix transected monkeys learnt the task differently from the control monkeys, perhaps with less emphasis placed on contextual cues; and that forgetting could be attributable to either interference or memory decay, or both. A similarity in resistance to forgetting was drawn between fornix transected macaques and patients with anterograde amnesia. I also discussed some implications on clinical rehabilitation.

Chapter 5 aims to elucidate the role of the fornix in spatial recognition memory. I tested the ability of macaque monkeys with and without fornix transection to perform an automated delayed matching to position task presented on a touchscreen. The fornix transected group was unimpaired in acquiring the basic task but showed pronounced impairment when the delays between sample and choice phases were varied in Experiment 1; however the fornix transected group remained unimpaired when the spatial separation between choice stimuli was varied in Experiment 2. The unimpaired performance of the fornix transected group in the acquisition phase and in Experiment 2 appears to rule out explanations based simply in terms of motor, perceptual or motivational deficits. The absence of delay-dependent deficit in Experiment 1 suggests that the deficit is not necessarily in memory per se. Rather, I propose that fornix transection leads to an impairment in acquiring new task-sets in certain circumstances. Our data, combined with previous experiments, also suggest that the contributions of the fornix and hippocampus to recognition memory are dissociable.

Chapter 6 examines exploratory movements in monkeys with or without fornix transection in a novel octagonal chamber in which the monkeys were introduced to

explore freely for six daily sessions each lasting 20min. Both groups visited, punctuated by stops, the majority of the floor-space of the environment in each of the sessions. The exploratory movements of both groups were not significantly different on most of the measures taken over 6 consecutive days. These measures included cumulative distance traveled, number and duration of stops, travelling patterns, and proportion of time spent in each of 12 designated zones of floor-space. The high degree of similarity in behaviour between control and fornix transected groups suggests that an intact hippocampal system is not necessary for the display of normal exploratory movement per se. On the other hand, the two groups did behave differently on two measures. First, the control group exhibited a decrement in distance traversed over consecutive epochs within the first test session whereas fornix transected animals did not. Second, on those days in which the chamber was made visually asymmetrical, the control animals tended to show a predilection for spending proportionally more time within one particular quadrant of the chamber. These observations are consistent with the idea that interrupting normal hippocampal system function by means of fornix transection is detrimental to learning about the spatial layout of environments. We therefore suggest that while monkeys with fornix transection still display intact locomotor and exploratory behaviour patterns, their new learning of visuospatial context is impaired.

Taken together, the results presented in this thesis provide evidence for the following. Whilst extending the existing body of evidence which shows that the fornix performs a role in the processing of and new learning of spatial and temporal information, it strengthens the notion that the fornix is particularly important for learning contextual information. This thesis also provides a new contribution by demonstrating that the

fornix is important for ‘fast learning’, or the initial phases of acquisition. In addition, it suggests the fornix is not critical for all kinds of conditional learning, plays no role in the retention of information once material is learnt, and has negligible involvement in motor, perceptual, or motivational capabilities. Finally, I argue that the contributions of the fornix and hippocampus to some memory processes are dissociable.

CHAPTER 1

GENERAL INTRODUCTION

CHAPTER 1: GENERAL INTRODUCTION

1.1 A brief history of contemporary memory research

Just over 50 years ago, Scoville and Milner (1957) first reported that bilateral resection of the medial temporal lobes resulted in dense anterograde amnesia in a patient called H.M., a condition that produces a persistent inability to acquire new memories whilst leaving intact early memories and overall intelligence. Two of the main implications of this report were that a bilateral lesion of medial temporal lobe structures placed recent memory functions at risk; and that the formation of new memories had a distinct neural substrate. While disturbing memory for new experiences, other cognitive functions and sensory capacities could be unimpaired (Corkin et al., 1997).

The amnesic syndrome has then been defined as ‘an abnormal mental state in which memory and learning are affected out of all proportion to other cognitive functions in an otherwise alert and responsive patient (Victor et al., 1971). In particular, anterograde amnesia has been typified by a failure to acquire or retain ‘episodic’ information (e.g. Tulving, 1983) that occurred after the onset of brain injury, a condition that can be separated from loss of memory for acquired facts (Nielsen, 1958) which we now call semantic amnesia (Kopelman, 2002).

Neuropathological studies have repeatedly highlighted that damage in the medial temporal lobes or/and the medial diencephalon can result in anterograde amnesia. As a matter of anatomy, the medial temporal lobe comprises the hippocampus, the amygdala and underlying perirhinal, entorhinal and the

parahippocampal cortices (Buckley and Gaffan, 2006) whereas the mammillary bodies and the medial thalamus are both medial components of the diencephalon, which is composed of the thalamus, hypothalamus, epithalamus, and subthalamus (Aggleton and Brown, 1999).

Some evidence suggests that amnesia is often associated with damage to either the hippocampus in the medial temporal lobe (Zola-Morgan et al., 1986) or the mammillary bodies of the medial diencephalon (e.g. Dusoir et al., 1990; Kopelman, 2002; Miller et al., 2003), and a massive tract of fibres known as the fornix provides a powerful connection between these two structures (Saunders and Aggleton, 2007). Since the fornix forms a vital bridge between medial temporal and medial diencephalic regions (Aggleton et al., 1986; Aggleton and Saunders, 1997; Aggleton and Brown, 1999), and that there is a single functional pathway subserving memory runs from the hippocampus through the fornix to the mammillary bodies, disruption of this pathway at any of its stages should result in amnesia (Delay and Brion, 1969; Gaffan and Gaffan, 1991).

1.2 Anatomy of the fornix

As the role of the fornix in memory is of significance in understanding the functional basis of amnesia, I would first describe the anatomical details of this structure in the primate. Axons from within the hippocampus proper and the subiculum project into and are joined in the fimbria, exiting the posterior part of the hippocampal complex (composing of the hippocampus proper, the dentate gyrus, the subiculum, and the entorhinal cortex). This pathway now moves rostrally and joins to the ventral surface of the corpus callosum, and at this point it becomes the main body

of the fornix. The fornix extends in the anterior direction, still attached to the ventral surface of the corpus callosum, and the fornix in the two hemispheres joins together for a period. This is convenient for the neurosurgeon interested in fornix function, as transection of the fornix at this level has bilaterally symmetrical effects. When the body is formed from the two crura, a small number of fibres cross over to the other side at the hippocampal commissure. Most fibres stay on their original side. The forward projections of the fornix form distinct sections. The projections to the mammillary bodies and to the thalamus largely originate from subicular neurons, whilst the projections to the septal nucleus and other regions of the basal forebrain arise mainly in the hippocampus proper (Amaral and Insausti, 1990) (See Figure 1 and Figure 2).

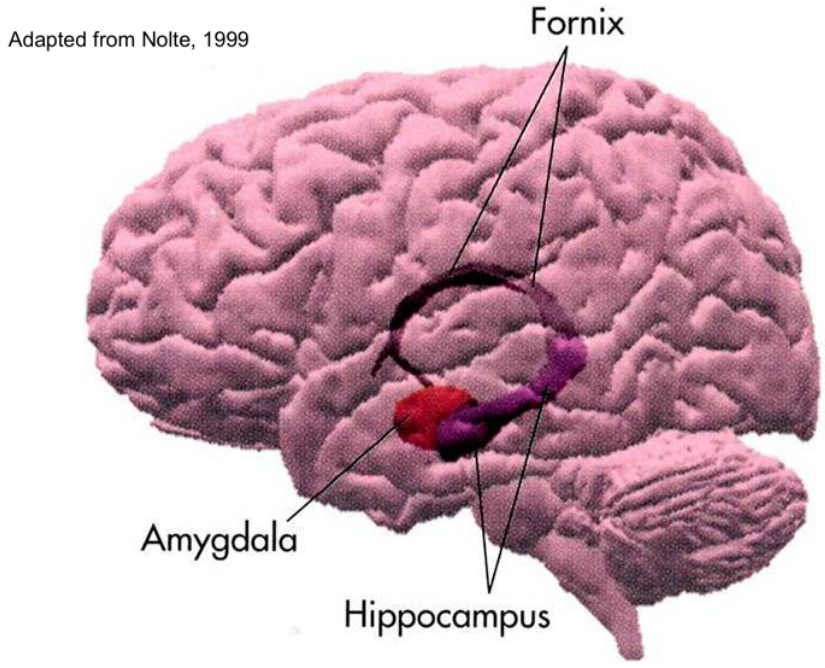


Figure 1. A simplified depiction of the location of the fornix and the hippocampus (and the Amygdala) within the medial temporal lobe in a primate brain (Nolte, 1999).

The fornix is a C-shaped bundle of fibres (axons) (Gray, 1918) and is

estimated to contain, in each hemisphere, some 2,700,000 fibres in the human brain and ~500,000 fibres in the monkey brain (Simpson, 1952; Daitz and Powell, 1954; Saunders and Aggleton, 2007). The fornix is not only the principal tract linking the hippocampal region with subcortical areas, but also links the hippocampal region with some cortical areas; for example, it contains projections to the prefrontal cortex (Vann et al., 2000; Saunders and Aggleton, 2007). The fornix also connects the hippocampus to some subcortical regions such as the diencephalon, striatum, basal forebrain (Amaral and Insausti, 1990), medial septum, and the supramammillary region in the caudal hypothalamus (Saunders and Aggleton, 2007). It projects to the thalamus and mammillary bodies reciprocally (Swanson and Cowan, 1975; Aggleton et al., 1986). Another group of subcortical fornical input comes from the medial and lateral supramammillary nuclei. The supramammillary region is of particular interest as it controls the frequency of theta activity within the hippocampus (Vertes and Kocsis, 1997). Theta rhythm is thought to set conditions for plasticity within the hippocampus, and so has been linked with a variety of mnemonic processes (Vertes, 2005). Beyond the hippocampal formation, the primate fornix also provides some afferent and efferent connections for the entorhinal and perirhinal cortices (Saunders et al., 2005). Since ascending fibres from the basal forebrain are known to travel through the fornix (Ridley et al., 1992; Gaffan et al., 2001), section of this structure would disrupt some of the routes of cholinergic projection to the entorhinal cortex, subiculum and the hippocampus (Browning et al., submitted). Fornix transection also severs the major cholinergic projection from the septum and the vertical limb of diagonal band of Broca to the hippocampus and cuts GABAergic and other hippocampal afferents, and efferents which are largely glutamatergic (Ridley et al., 1992).

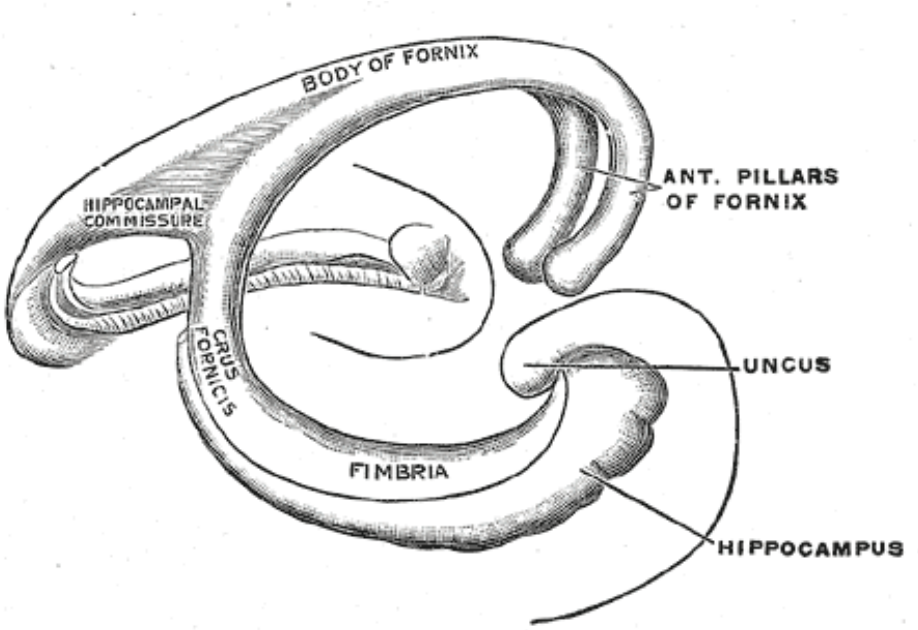


Figure 2. A diagram of the fornix (Latin, 'vault' or 'arch') (Gray, 1918).

The existing evidence converges to indicate that there are at least four sets of connections within the fornix that might have an important role for normal memory functions. The very dense projections to the mammillary bodies and anterior thalamic nuclei link the hippocampus with sites thought to be responsible for diencephalic amnesia and, hence, these connections appear to be necessary for episodic memory (Delay and Brion, 1969; Gaffan, 1992a). It is noteworthy that this pathway is not reciprocated via the fornix (Saunders and Aggleton, 2007). The second set of projections comprises the cholinergic inputs from the septum and basal forebrain to the hippocampus and rhinal cortex, some of which use the fornix. A few learning studies reported evidence for the importance of these cholinergic afferents in monkeys (Ridley et al., 1991; Gaffan et al., 2001; Easton et al., 2002; Browning et al., submitted). The fornix is also the route for at least some of the inputs from the supramammillary nucleus to the hippocampus. Given the importance of this nucleus for setting the frequency of theta (Vertes and Kocsis, 1997), it is possible that the loss

of this fornical input might alter hippocampal plasticity (Vertes, 2005). Fourthly, the fornix contains efferents from the entorhinal and perirhinal cortices, so the loss of these projections following fornix damage could add to the consequences of the concurrent hippocampal disconnection (Saunders and Aggleton, 2007). There is growing evidence that the parahippocampal cortical areas have some functional properties that are independent of the hippocampus (Aggleton and Brown, 1999; Malkova et al., 2001), so the disconnection of these regions could create additional deficits to those brought about by hippocampal dysfunction.

Further insights into the anatomy of the fornix can be drawn from the laboratory rat. Using immunohistochemical visualisation of Fos, the product of c-fos, multiple brain sites can be simultaneously compared to identify regions likely to contribute to learning processes (Vann et al., 2000). In the rat, Vann et al. (2000) confirm that widespread hippocampal changes are associated with fornix transection. All three hippocampal subfields (dentate gyrus, CA3 and CA1) and both the dorsal and ventral parts of the hippocampus, as well as the ventral subiculum, presubiculum, and parasubiculum are all affected with decreased Fos levels after fornix transection. The changes might reflect the loss of fornical projections from the medial septum, diagonal band of Broca, locus coeruleus and raphe nuclei (Vann et al., 2000). However, these disconnections are not complete because, in the rat, some 45% of the cholinergic input from the septal region, ~90% of the noradrenergic input from locus coeruleus and ~70% of the serotonergic input from the raphe nuclei to the hippocampal is via non-fornical routes (Amaral and Kurz, 1985; Burwell et al., 1995; Cassel et al., 1997), all of which might help explain why fornix lesions do not always mimic the full effects of hippocampectomy (Whishaw and Jarrard, 1995). Likewise, in the primate, it is also known that the hippocampus and parahippocampal region are

connected to some sites via multiple, parallel routes (Saunders et al., 2005; Saunders and Aggleton, 2007), and some of them are non-fornical. Moreover, some hippocampal afferents are without a fornical component, these include the direct projections from the amygdala, thalamic nucleus centralis medialis, the claustrum, central gray, and locus coeruleus (Amaral and Cowan, 1980; Saunders and Aggleton, 2007). A minority of cholinergic projections to the hippocampal region and entorhinal cortex is also known to be independent of the fornix (Saunders and Aggleton, 2007).

Although fornix transection has the advantages of being reproducible and producing some disconnections of the hippocampus from other sites, we now know that the disconnection of the hippocampus is by no means a complete one. It is also difficult to ascertain which of the four sets of connections mentioned above are implicated. I acknowledge that fornix transection should not be taken as a substitute for direct hippocampal lesions. On one hand, transection of the fornix cuts off substantial inputs and outputs of the hippocampus, and so it seems likely that the hippocampus could not process information fully normally after this lesion. But unlike direct hippocampal ablation, fornix transection does not cut off substantial fibres of the cortex surrounding the hippocampus and it inflicts negligible damage to extrahippocampal structures (Murray and Baxter, 2006). Basal forebrain projections via the temporal stem, amygdala, and entorhinal cortex to the hippocampus are also relatively unaffected by fornix transection (Gaffan et al., 2001). So, as Gaffan has argued (e.g., Rupniak and Gaffan, 1987), fornix transection may be the most selective way to disrupt the functions of the hippocampus. On the other hand, others view fornix transection as at the same time too extensive and too limited a substitute for a hippocampal lesion. Fornix transection can be viewed as too extensive because it eliminates connections of all hippocampal subdivisions, including those of the

subiculum, which is generally not considered as part of the hippocampal proper. Also, a fornix transection can be viewed as too limited because it does not interrupt connections between hippocampal subdivisions and the cortex, which are thought of by most as the main information-bearing conduits for hippocampal memory processing. Fornix transection also spares commissural connections of the hippocampus (Demeter et al., 1985). That is, even after a fornix transection, cortical information can reach the hippocampus, and the hippocampus can send the outcomes of its processing back to cortex. Hence, one might not expect a fornix transection to eliminate the main contributions of the hippocampus to cortical memory processing (Eichenbaum and Cohen, 2001).

Admittedly it is no longer common to make hippocampal ablations in monkeys, given problems with inadvertent damage to the underlying cortex. Workers in the field now would much prefer to administer neurotoxic hippocampal lesions rather than making hippocampal ablations. If the question is solely on what the hippocampus does, neurotoxic hippocampal lesions are undoubtedly more desirable than fornix transection. However, it has become increasingly important to examine the functions of the fibres coursing through the fornix in its own respect given some dissociable effects of these two related structures (e.g. McDonald et al., 1997). An investigation into the effects of the fornix transection procedure is thus justified and necessary. Moreover, neurotoxic lesions of the hippocampus do have some major drawbacks, and one of them is the technical difficulty of obtaining a complete coverage of the hippocampus by the excitotoxin. As reported by Murray and Mishkin (1998), the mean percentages of cell loss caused by excitotoxin in the hippocampi of their seven monkeys range from 55% to 98%, clearly indicating a strong inconsistency between animals and incompletion of the damage.

In short, as a complete transection of the fornix disrupts cholinergic and GABAergic function as well as electrical activity and induces morphological reorganisation in the hippocampus, fornix transection produces significant disruption of information processing and output of the hippocampus. However, a fornix transection does not disconnect the parahippocampal region from the neocortex. Accordingly, a fornix transection may not disrupt functions that can be carried out by the parahippocampal region independent of processing by the hippocampus and hence would not be expected to produce the full-blown amnesia seen following more complete hippocampal system damage (Eichenbaum and Cohen, 2001). For instance, fornical and hippocampal lesions are found to be dissociable in long-lasting DNMS memory impairments in macaques (Zola-Morgan et al., 1989), in acquisition of three configural discriminations (McDonald et al., 1997) and spatial conditional learning (Dumont et al., 2007) in rats, as well as on postoperative retention of a visuospatial conditional task learnt prior to surgery in marmosets (Ridley et al., 1992).

Therefore, given it is difficult to challenge the ample findings that fornix transection and hippocampal lesion often produce comparable impairments (for example, monkeys are similarly impaired on delayed matching to location (Murray et al., 1989; Hampton et al., 2004; Lavenex et al., 2006), disrupted on spatial discrimination and reversal learning (e.g. Mahut and Zola, 1973) and on visual-spatial conditional tasks in the Wisconsin General Test Apparatus (Gaffan et al., 1984c Exp. 5; Parkinson et al., 1988; Gaffan and Harrison, 1989b Exp. 1; Angeli et al., 1993), but also equally unimpaired on concurrent object discrimination (Gaffan, 1994b; Teng et al., 2000), even similarly facilitated on transverse patterning tasks (Saksida et al., 2007); and similar effects are also reported in human patients (e.g. Tucker et al., 1988)), I would rather be cautious not to proclaim definitely as to whether the findings

presented in the succeeding five experimental chapters, all of which were obtained in monkeys with bilateral fornix transection, are wholly transferable to results that would have been caused by neurotoxic hippocampal lesions. I would instead endeavour to build a case to illustrate various aspects of the mnemonic role of the fornix with data revealed by my recent empirical works in the macaque, hoping to offer a more global picture of how the fornix is implicated in our memory. After reviewing the anatomical evidence of the fornical interconnections with other parts of the brain, I would now turn to discuss some of its cognitive functions in the following sections.

1.3 Effects of fornical damage in humans

In view of the potential importance of this tract for normal medial temporal lobe function, the effects of the fornical damage on memory has received discussion in the human literature. In spite of the fact that the extent and nature of aetiologies reported in the following human studies vary immensely, with a similar neuropsychological amnesic pattern observed and that fornix damage was the only common factor across cases, it becomes compelling to attribute the resultant amnesic deficits to the effect of fornix damage.

Interest in the role of the fornix has intensified after reports linking cases of anterograde amnesia in humans with damage to this tract (Gaffan and Gaffan, 1991; Gaffan et al., 1991; McMackin et al., 1995; Aggleton et al., 2000; Papanicolaou et al., 2007; Vann et al., 2008). For example, Aggleton and colleagues (2000) tested a series of twelve patients who have received surgical removal of a colloid cyst, and concluded that fornix damage is sufficient to induce anterograde amnesia. Gaffan and colleagues (1991) also reported clear amnesic impairments in two patients with

fornical damage in some of the psychological (visual) memory tests. Integration of these findings with other single case studies of fornix damage associated with other aetiologies, such as stroke (Moudgil et al., 2000), tumours (Rudge and Warrington, 1991; Yasuno et al., 1999), tumour removal (Calabrese et al., 1995), traumatic injury (Papanicolaou et al., 2007), wounds (Grafman et al., 1985), vascular accidents (Brion et al., 1969), gunshot injury (D'Esposito et al., 1995), and carbon monoxide poisoning (Kesler et al., 2001) further suggests that fornix damage can cause wide-ranging memory disturbances (Gaffan et al., 1991) and even induce persistent and marked loss of episodic memory (Aggleton et al., 2000; Papanicolaou et al., 2007). Moreover, it has been reported after bilateral lesions to the fornix, a patient had a severe retrograde amnesia for autobiographical episodes that covered his entire lifetime and also a time-limited retrograde amnesia for semantic memory (Poreh et al., 2006). Interestingly, a recent study also found that bilateral deep brain stimulation of the hypothalamic/fornix region evoked detailed autobiographical memories and led to enhanced recollection but not familiarity-based recognition in a patient with morbid obesity (Hamani et al., 2008), a finding that supports a possible role for the fornix in autobiographical memories. Also relevant are findings that fornix damage in human does not necessarily cause dense amnesia as some subjects demonstrate relatively intact performance in tests of recognition memory (McMackin et al., 1995; Aggleton et al., 2000), which is largely in accordance with findings in monkeys with experimental transection, often bilaterally, of the fornix (e.g. Charles et al., 2004). The following sections are devoted to review the literature of various mnemonic effects of fornical damages in macaque monkeys.

1.4 Fornix damage and amnesia in macaques

1.4.1 Object recognition memory

Gaffan (1974) was the first to report that monkeys with fornix transection were impaired in performance on a delayed matching to sample (DMS) task with trial-unique stimuli, although the task was taught postoperatively and a substantial deficit only appeared if the delay was increased to above 1 min. A series of later DMS or delayed non-matching to sample (DNMS) studies found that fornix transection produced little or even no deficit in monkeys. While some studies showed that fornix lesions in monkeys can spare delayed object matching (Gaffan et al., 1984a; Gaffan et al., 1984b) and delayed non-matching tasks (DNMS) (Bachevalier et al., 1985b; Zola-Morgan et al., 1989) using single, discrete items, others found mild impairments (Bachevalier et al., 1985a) or no further decrement in DMS performance in addition to bilateral amygdalectomy (Parker and Gaffan, 1998). Some earlier studies in monkeys however found more substantial impairments in object recognition, in delayed matching to sample tests (Gaffan, 1974; Gaffan, 1976, 1977a, b) or in delayed non-matching to sample tests (Gaffan and Weiskrantz, 1980; Owen and Butler, 1981, 1984). In these cases where severe impairments were found, I recognise that the tests were not pure tests of recognition as monkeys might be confused in discriminating the relative familiarity of the stimuli when the same pool of stimuli were used repeatedly across testing sessions. For instance, Owen and Butler (1981; 1984) only found impairments in performance when their fornix transected animals were learning a set of familiar objects (Owen and Butler, 1984) or when the same two objects were presented repeatedly (Owen and Butler, 1981) across trials, but were unable to demonstrate any impairment in performance when the correct stimulus was always a completely novel one (Owen and Butler, 1984). These severe impairments were

probably caused by a greater interference from previously learnt material within sets on the performance of FNX monkeys than on controls, instead of a FNX-induced deficit in object recognition as previously suggested (Charles et al., 2004). Taken together, these findings suggest that fornix transection does not generally produce dense anterograde amnesia in recognition memory of objects in both humans and monkeys. Compared to the vast quantity of work done on object recognition, the amount of data on recognition memory of objects' spatial locations, which is also an integral element of episodic memory, in monkeys is relatively sparse. This hence provided the impetus for the experiment reported in Chapter 5 in which I consider in greater details of the effect of fornix lesions on a spatial recognition memory task in macaques.

1.4.2 Memory of spatiality

Whilst fornix lesions in monkeys can spare recognition of single, discrete stimuli (Mahut et al., 1982; Gaffan et al., 1984a; Gaffan et al., 1984b; Owen and Butler, 1984; Bachevalier et al., 1985b; Bachevalier et al., 1985a; Zola-Morgan et al., 1989; Charles et al., 2004), the same lesions can, however, impair concurrent discriminations when each pair of items to be discriminated is placed in a consistent position in a unique background or 'scene' (Gaffan, 1994b; Parker and Gaffan, 1997). Likewise, the recognition of complex scenes is disrupted (Gaffan, 1994a). This is presumably because the performance of such tasks demands other attributes, such as spatiality, of episodic memory; as the animal is aided by the memory of not just the stimulus but also by its placement in relation to its context or scenes (Gaffan, 1994b). An accumulation of evidence suggests a role of the fornix in spatial information processing.

Fornix transection is known to produce impairments in tasks that require particular types of spatial processing. These impairments are shown in tests of explicit spatial information processing, such as spatial delayed non-matching to sample in a T-maze (Murray et al., 1989 Exp. 1), spatial reversal learning (Mahut, 1972; Gaffan and Harrison, 1989b Exp. 3), and simple spatial discrimination learning (Gaffan and Harrison, 1989b Exp. 3; Gaffan and Harrison, 1989a Exp. 3; Gaffan, 1994a Exp. 2) as well as less explicit forms of spatial memory such as object-spatial conditional tasks (Gaffan and Harrison, 1989b Exp. 1). In a spatial-visual conditional task, the position (either left or right) of two stimuli in a modified Wisconsin General Test Apparatus (WGTA) determines which one of the two objects is rewarded; whereas in a visual-spatial conditional task, a specific set of two objects covering food wells in the WGTA informs the location (either left or right) where food is placed, regardless of which object may cover it (Gaffan et al., 1984c Exp. 5; Gaffan and Harrison, 1989b Exp. 1). Performance in each of these tests is impaired following fornix transection. Also relevant is the finding that fornix transection severely impairs monkeys' perfromance in a task that requires the animal to learn in what spatial direction, either approach or withdrawal, to move in relation to the visual stimuli on a touch screen (Rupniak and Gaffan, 1987). In another form of the task, the same type of stimuli instructed a non-spatial response, specifically whether to repeatedly tap or to make sustained contact with the stimuli. Fornix transection did not impair the non-spatial version of this task (Gaffan and Harrison, 1988). This impairment implies that the fornix transected monkeys fail to learn about the significance of the monkey's own spatial movements. After Gaffan and Harrison (1989a Exp. 1, 3 and 5) demonstrated in a series of experiments that the memory disrupted by fornix transection is like a snapshot memory, which stores not only the identity of visible stimulus items but also

their spatial arrangement within the witnessed scene, a few studies then extended these previous findings with either naturalistic scenes and objects (Gaffan, 1992b, 1993a) or artificially constructed scenes (Gaffan, 1994b) instead of three-dimensional objects. These studies show that fornix transection produces impairments in tasks in which the spatial layout of objects make up a scene, such as scene discrimination learning (Gaffan, 1992b, 1993a) and object-in-place scene memory (Gaffan, 1994b; Parker and Gaffan, 1997) suggesting the fornix performs a role in memory for the spatial arrangement of multiple objects. However, it is important to note that neither the naturalistic scene task nor the algorithmically generated scene task is overtly spatial as monkeys can aid their performance with information from the foreground objects or background detail of each.

Since scene learning is not a purely spatial task, and existing direct evidence reviewed above for a fornical role in spatial memory comes exclusively from tasks involving learning about food-reward locations, either in front of a test tray on a pedestal (Gaffan and Harrison, 1989a) or in a WGTA (Gaffan, 1994a Exp. 2) or a T-maze (Murray et al., 1989), Buckley (2004) identified potential discrepancies between these two kinds of apparatuses which might reflect two different kinds of learning strategies. Buckley and colleagues (2004) then tested this possibility by assessing the effects of fornix transection on purely spatial memory using a touchscreen apparatus. They devised some novel spatial discriminanda in the appearance of ‘tadpoles’, each of which is defined by three spatial variables: position on the screen, size of the tail, and orientation of the tail. Accurate performance across sets of problems required the ability to encode and remember multiple distinct configurations of spatial cues, making this task a complex spatial memory task. Fornix transected monkeys are impaired in relearning and new learning of this kind of

concurrent spatial discrimination problems, especially those with a greater degree of spatial overlap. The authors also found that new learning about configurations of spatial features is impaired after fornix transection. Given that the fornix is necessary to support new learning (Buckley et al., 2004) and that the hippocampal system might underlie rapid learning of new declarative information (McClelland et al., 1995; Brasted et al., 2003), I raise the speculation of whether the fornix is crucial throughout the whole new learning process, or whether its contribution might be more important during the early stages of acquisition, as previously suggested by others (Brasted et al., 2003). In Chapter 2, I elaborate on a distinction between 'fast' (initial) and overall new learning with findings from a conditional visuospatial discriminations task.

1.4.3 Retention of spatial memory

One notable finding of Buckley's et al. (2004 Exp. 1) study is that recall of preoperatively learnt concurrent spatial information is not impaired at all after fornix transection, whereas new postoperative spatial learning is markedly impaired. The absence of impairment of spatial recall is particularly striking given the high spatial memory demands of that task. More recently, Buckley and colleagues (2008) reported in another study that postoperative recall of 288 concurrent visuospatial discrimination problems acquired preoperatively was also unaffected after fornix transection in the macaque, whereas new postoperative learning of 72 problems was impaired. The selective impairment in new spatial learning but not recall, consistently observed in these studies, indicates it is possible that fornix transection does not result in a global and severe retrograde memory loss.

Prior to these two concurrent spatial discrimination studies, Gaffan (1993a; 1994b) also demonstrated with different paradigms that while new acquisition was

impeded, retention was sometimes preserved. For example, in Gaffan's (1994b Task 1) study, monkeys were trained to learn to discriminate 192 algorithmically generated complex scenes in which one-third of the scenes was of each of the three types (place, object-in-place and object discrimination scenes). Half of the monkeys then received fornix transection, and were tested subsequently together with the unoperated controls on three sessions of retention tests of the scenes learnt before operation. The fornix lesion had no effect on the retention of scenes. Similarly, Gaffan (1993a) trained unoperated control and fornix transected monkeys to learn to discriminate 320 complex naturalistic scenes and subsequently tested on their extent of forgetting of these scenes after reaching criterion. Despite a slower rate of acquisition in the FNX group, the number of scenes forgotten was found to be equal in both groups of monkeys on a retention test 49 days later. Although a couple of studies have reported that fornix transection did impair the retention memory of a large number of complex naturalistic scenes learnt preoperatively when comparisons between preoperative and postoperative performances were made (Gaffan, 1992b; Gaffan, 1993b), it should be noted that these complex naturalistic scenes, and even the algorithmically generated scenes, do not necessarily demand much spatial memory per se as memories for foreground objects or background detail can aid performance of each, so it is not possible to infer from these studies that retrieval of spatial memory is unaffected by fornix transection, nor is it possible to directly compare the magnitude of the retention deficit with a deficit in new learning. Furthermore, the retention tests in all of the studies reviewed in this section were administered within a relatively short period of time after problems were acquired (approximately 2 weeks on average, except in Gaffan's (1993b) study where the scenes were tested six months later). Findings in respect to the fornical role in memory retention are inconclusive. The experiment

reported in Chapter 4 was thus designed to address these issues.

1.4.4 Beyond the spatial domain

The evidence reviewed in the last two sections points to an established role of the fornix in spatial memory and this is consistent with considerable behavioural data in nonhuman primates that the hippocampal system functions primarily in spatial localisation or navigation (Mahut and Moss, 1986; Parkinson et al., 1988; Gaffan, 1998). Some neuropsychological evidence, however, indicates that the hippocampal system also functions in 'non-spatial' tasks, and hence may contribute to processes beyond spatial memory such as relational (Bunsey and Eichenbaum, 1995), declarative (Squire and Zola, 1997), and episodic (Gaffan, 1994b) memory.

The fornix in the monkey has been implicated in aspects of non-spatial memory (Brasted et al., 2002; Brasted et al., 2003; Charles et al., 2004; Brasted et al., 2005; Wilson et al., 2007). For example, several demonstrations of non-spatial impairments after fornical damage have implicated the fornix in memory for temporal context (Charles et al., 2004; Wilson et al., 2007). Monkeys with fornix transection are impaired at making judgements of relative recency, but not of absolute novelty (Charles et al., 2004) and are impaired in a concurrent object discrimination task in which the reward assignments and pairings of stimuli in the task have been randomly reassigned relative to a previous stage of training (Wilson et al., 2007). These studies suggest that even non-spatial memory tasks may be impaired after damage to the fornix in monkeys, provided they place some demand on processing of temporal context.

Other studies show that fornix transection also causes non-spatial deficits in conditional visuomotor tasks that are not confined to the spatial domain as monkeys

with fornix transection were slower to associate non-spatially differentiated visual cues with non-spatially differentiated responses (Brasted et al., 2003; Brasted et al., 2005). Brasted and colleagues (2003; 2005) thus proposed that associative learning impairment, including initial stage acquisition, should by no means be restricted to the spatial domain. In contrast, as reported in Chapter 2 in this thesis, monkeys with fornix transection manifested marked impairments in the initial phase of acquisition of visuospatial associative problems. It remains uncertain of whether fornix transection generally affects rapidly acquired associations of all kinds or is restricted within the spatial domain. In order to help reconcile these two conflicting opinions, I developed a conditional discrimination task that entailed only stimulus-stimulus associations. Chapter 3 is devoted to report data from this experiment and address some pertinent issues on this generality-versus-spatiality debate.

1.4.5 Exploration in open space

Readers may be reminded that all the studies reviewed so far sought to assess one (or maybe two) very specific aspect of memory functions, and mostly involved small-scale environments in which monkeys responded by reaching, rather than by traveling to, different locations in space. I recognize that a holistic picture of normal functioning of an organism might be better shown with testing conducted under more naturalistic, or at least larger and immersive, environments. In their seminal book *Hippocampus as a Cognitive Map* (1978), O'Keefe and Nadel postulated a theoretical link between hippocampal circuitry and exploratory behaviour, in which they stated exploration is fundamental in building and updating the internal representation of the spatial layout of an environment and that an intact hippocampus is pivotal to a functional exploratory repertoire. Other workers in the

field have also advocated similar theories that the hippocampal formation is essential for spatial relational learning and memory (Eichenbaum et al., 1994; Eichenbaum, 2000), which is the formation and use of allocentric representations of space (O'Keefe and Nadel, 1978; Nadel and Hardt, 2004; Lavenex et al., 2006).

Both exploration and spatial relational learning inevitably entails locomotion, which is a conspicuous feature of hippocampal-dependent spatial memory tests such as the Morris water maze (Morris, 1984) and the radial-arm maze (Olton and Papas, 1979) that typically conducted with rodents. Comparable studies that involved locomotion or exploration on the role of the nonhuman primate hippocampal system are, however, very few. Following the theoretical framework developed from rodent studies, a handful of ambulatory studies on nonhuman primates have been conducted and are described here.

One of the earliest ambulatory studies on nonhuman primates was carried out by Murray and colleagues (1989 Exp. 1) in which macaque monkeys were trained preoperatively on delayed non-matching to sample in a T-maze, and were then retrained postoperatively to the same criterion they had attained before surgery. The results demonstrate that monkeys with fornix transection are severely impaired on this spatial working memory task requiring locomotion. However, strictly speaking, this task could not be regarded as an exploratory task because behaviour at the choice point in the maze was simply a choice between two spatial directions of movement and so this task is at best qualified as a delayed non-matching to locations test that involves locomotor actions. Interestingly, it has been shown that normal marmoset monkeys can be trained to perform very well at this task too (Easton et al., 2003).

Until more recently, two other studies, both conducted in an open-field, set out to investigate the effects of selective bilateral hippocampal lesions on spatial

learning in ambulatory macaque monkeys. The first study was a series of open-field experiments (Hampton et al., 2004) that allowed tethered monkeys to walk about in a large-scale environment. Monkeys with bilateral hippocampal excitotoxic lesions were tested in match-to-location tasks. The results demonstrate that selective hippocampal lesions in monkeys impair memory for location in an open-field. Although the data indicate that the monkeys encoded some allocentric information about the goal locations, the study was not able to demonstrate a reliance on an allocentric representation of space in normal monkeys by ruling out the use of an egocentric strategy; and thus the authors were unable to determine whether their monkeys were using a true 'cognitive map'. In contrast, in Lavenex and colleagues' (2006) study, freely moving monkeys were trained to forage for food located in six goal locations among 18 locations distributed in an open-field arena. Multiple goals and four pseudorandomly chosen entrance points precluded the monkeys' ability to rely on an egocentric strategy to identify food locations. Trials were also divided into either under a local visual cue condition or a spatial relational condition involving distant environmental cues. Both hippocampal-lesioned and control monkeys discriminated the food locations in the presence of local cues. In the absence of local cues, control monkeys discriminated the food locations, whereas hippocampal-lesioned monkeys were unable to do so. The results demonstrate that the monkey hippocampal formation is critical for the establishment or use of allocentric spatial representations and that selective damage of the hippocampus prevents spatial relational learning in nonhuman primates.

There are also some unconventional mazes for nonhuman primates, such as a large virtual space in a virtual navigation task (Hori et al., 2005), a visual maze (Crowe et al., 2004) and a V-shaped maze (McDermott and Hauser, 2004). However,

these mazes can usually at most test spatial memory but not the type of cognitive function (i.e. learning of new spatial relations) lost in subjects with hippocampal damage. Furthermore, it has been very recently reported that some other more sophisticated monkeys maze models are being developed (Zhang et al., 2008), which will be valuable for future studies involving spatial learning and memory.

It is worth noting that all of these studies were designed more to assess spatial relational learning and less on exploratory behaviours per se; and mainly focused on selective hippocampal lesions. As I have established earlier on, fornix transection might produce different functional deficits from selective hippocampal lesions. In view of these considerations, I conducted an exploratory study with unrestrained monkeys in an enclosed chamber; in which they were allowed to explore the environment freely. Their exploration behaviours were examined and findings in respect to the effects of fornix transection on locomotion, exploration and learning are detailed in Chapter 6.

1.5 The fornix and visual memory

The discovery that the fornix is vital for normal memory highlights the need to further elucidate the contribution of the fornix to mnemonic processes in primates; and one of the ways to help achieve this is by testing animals with bilateral section of the fornical tract, on tasks carrying various mnemonic demands.

Fornical lesions can lead to deficits in various forms of spatial learning and the deficits are especially sensitive to new learning in monkeys; yet we are not certain whether the impairments in new learning are present throughout the learning process or are mainly confined to the initial ‘fast’ acquisition stage. In Chapter 2, I assess the

contribution of the fornix to both initial and overall acquisition stages in the learning of a large number of concurrent visuospatial conditional discriminations; with this task, I also assess whether set size affects the severity of impairment. I show that fornix transection impairs the initial acquisition, and particularly so in the smallest set of problems wherein 'fast learning' is more important.

The role of the fornix has been shown to extend beyond the spatial domain in learning; and evidence points to a 'fast learning' role of the fornix in a visuomotor learning task and a visuospatial discrimination learning task (Chapter 2). In Chapter 3, I seek to ascertain whether this deficit is spatially dependent or not with a visuovisual conditional task. I show that fornix transected monkeys are not impaired and in fact facilitated in this visuovisual conditional task.

Some evidence indicates that the fornix is not implicated in retention memory of spatiality. I recognise the lengths of retention after criterial learning of these studies are short, on average just a matter of weeks. Therefore in Chapter 4 I set out to assess the retention of a large number of visuospatial discriminations that were learnt to criterion postoperatively, at two independent retention tests, i.e. three months and 15 months after the criterial acquisition. I confirm that the fornix takes no role in retention of the visuospatial information.

I outlined above that fornix transection does not produce dense anterograde amnesia in recognition memory of objects in monkeys, and summarised evidence showing the crucial role the fornix in learning materials that carry spatial components. However, to my knowledge no work has been done thus far on explicit spatial recognition memory in fornix transected monkeys. In Chapter 5, I examine the effects of fornix transection on spatial recognition memory, under different delay and spatial separation conditions. I find that fornix transected monkeys are partially impaired in

spatial recognition memory in respect to manipulations of delay lengths and spatial distance between choices.

Some of the studies reviewed above sought to test freely moving monkeys in open space, and also the effects of hippocampal lesions on their spatial learning and cognition in those apparatuses. In Chapter 6, I report the first experiment ever to explore the exploratory behaviours of fornix transected monkeys in an enclosed arena and I show evidence that damage to the fornix distorts monkeys' exploratory patterns and impairs learning of the environments.

Finally, in Chapter 7, I present a general discussion of the results obtained in the experiments undertaken in this thesis and consider the implications they offer to understand the role of the fornix in visual learning and memory.

CHAPTER 2

FORNIX TRANSECTION SELECTIVELY IMPAIRS FAST LEARNING OF VISUOSPATIAL DISCRIMINATIONS

Sze Chai Kwok & Mark J. Buckley
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CHAPTER 2: FORNIX TRANSECTION SELECTIVELY IMPAIRS FAST LEARNING OF VISUOSPATIAL DISCRIMINATIONS

2.1 Abstract

As the fornix has previously been implicated in the rapid learning of associations, we hypothesised that fornix transection in macaques would selectively impair the acquisition of rapidly learnt conditional visuospatial discrimination problems. Macaque monkeys learnt, postoperatively, three sets of concurrent problems of increasing sizes containing 8, 32 and 64 problems respectively. Each problem consisted of four identical visual stimuli and animals had to learn which stimulus position was rewarded. The lesioned animals made significantly more errors-to-criterion on the smallest set of problems, consistent with the idea that the most rapidly acquired sets would be more vulnerable to fornical damage. Moreover, during the early stages of acquisition across all three sets, fornix transection selectively impeded monkeys' abilities to eliminate non-perseverative errors in correction trials, consistent with an inability to monitor or correct erroneous spatial responses made further back in time than the last trial. Both one-trial learning and an errorless learning (facilitation of performance) were observed in control and fornix lesioned animals but neither were fornix dependent and overcoming the deleterious effect upon subsequent learning of having made prior errors was also unaffected by fornix transection. The data indicate that the fornix is not important for all forms of new learning; rather it is selectively concerned with the relatively rapid acquisition of spatial and temporal relationships between stimuli and responses.

2.2 Introduction

A distinction can be made between fast and slow associative learning mechanisms. Fast learning can manifest in several forms, ranging from one-trial learning (a hallmark of episodic memory) to rapid acquisition of sets of associations across several trials within either a single or a very small number of sessions, depending on the set size. Fast learning is distinguished from slow learning that typically occurs more gradually across numerous testing sessions. Rescorla and Wagner's (1972) classical learning theory stipulates that an organism accrues a larger share of the associative strength when the unconditional stimulus is most surprising while the association is first encountered, and the associative strength available to be learnt slows progressively trial by trial as the unconditional stimulus gets less surprising, until an asymptote of that association is reached. This theory predicts that learning can occur in stages. It has been theorised, from the perspective of connectionist modelling, that the hippocampus might underlie the most rapid learning stages of new associative information (McClelland et al., 1995). Lesion studies in macaques have provided empirical support for this idea. Macaques trained on a conditional visuomotor leaning task with non-spatially differentiated cues have been shown to be impaired on one-trial learning after fornix transection (Brasted et al., 2005). In that study, removal of the hippocampus, subiculum, and subjacent parahippocampal cortex, added to fornix transection, did not exacerbate the impairment, indicating that at least in tasks like these, transecting the fornix, a major input and output pathway of the hippocampus, may be functionally equivalent to hippocampal system lesions. Other rapidly acquired visuomotor discriminations are similarly impaired after fornix transection (Rupniak and Gaffan, 1987; Brasted et al.,

2003). Rapid within-session acquisition of concurrent object-in-scene discriminations is likewise impaired after fornix transection (Gaffan, 1994) and animals with fornix transection fail to habituate rapidly to novel environments (Kwok and Buckley, 2006). However, in tasks where problems are acquired slowly, performance is usually not affected by fornix transection, including transverse patterning (Brasted et al., 2003), visual-visual associations (Murray et al., 1993) and a tap-hold version of visuomotor associations (Gaffan and Harrison, 1988).

The present study aimed to investigate the generality of fast learning deficits after fornix transection, by examining the effect of this intervention on a concurrent visuospatial conditional discrimination learning task. These kinds of conditional learning tasks have previously been shown to be impaired after fornix transection (Buckley et al., 2004; Buckley et al., 2008) but it is not known whether the contribution of the fornix might be particularly important during the early stages of acquisition of these tasks. Here, two groups of monkeys, one which had already received fornix transection and a second group of unoperated controls with identical behavioural experiences, learnt 104 visuospatial discrimination problems to criterion. The problems were acquired by the monkeys in three sets of increasing size, consisting of 8, 32 and 64 concurrently presented problems respectively. We reasoned that with different set sizes the greater separation between repeated presentations of the same problem in larger sets would allow us to distinguish fast from slow learning in the larger sets, and that we might therefore expect a greater overall impairment in the smaller sets wherein a greater proportion of the overall learning may be attained by fast learning.

It has been reported that fast learning deficits after fornix transection were not absolute, but reflected a slowing in the learning rate so that control monkeys

eliminated errors at a three-fold faster than fornix transected monkeys (Brasted et al., 2003). In the current study we therefore adapted the task used by Buckley et al. (2008) so that each trial now had the addition of multiple incorrect foils to enable us to elucidate the effects of fornix transection on the elimination of different kinds of errors that can be commissioned during correction trial procedures. We also sought to analyse the effects of fornix transection on two other types of fast learning that could occur in the context of this task, namely one-trial learning and errorless learning (such as McClelland, 2001; Kessels and De Haan, 2003; Brasted et al., 2005; Clare and Jones, 2008).

2.3 Materials and Methods

Subjects

Six male cynomolgus monkeys (*Macaca fascicularis*) took part in this experiment. Their mean weight at the start of behavioural testing was 5.8 kg (range 4.9 kg to 6.7 kg), and their mean age was 4 years and 6 months. All six monkeys had identical pre- and postoperative experience in concurrent discrimination learning tasks, in a series of experiments that were carried out before the present study began (Buckley et al., 2008). They were housed together in a group enclosure (except for one who was housed in a pair with another animal not involved in this experiment) in an enriched environment in which they were able to forage daily for small food items (seeds etc) and all had automatically regulated lighting and with water available *ad libitum*.

Surgery

Three monkeys had received bilateral fornix transection (group FNX) six months before the present study began and the other three had identical behavioural experience as the FNX group but remained unoperated controls (group CON). Prior to this study all six animals had identical postoperative experience in retention and learning of different kinds of concurrent visuospatial discriminations (reported in Buckley et al., 2008). All licensed procedures were carried out in compliance with the United Kingdom Animals (Scientific Procedures) Act of 1986. The operations were performed in sterile conditions with the aid of an operating microscope, and the monkeys were anesthetised throughout surgery with barbiturate (5% thiopentone sodium solution) administrated through an intravenous cannula. A D-shaped bone flap

was raised over the midline and the left hemisphere up to the midline. The dura mater was cut to expose the hemisphere up to the midline. Veins draining into the sagittal sinus were cauterized and cut. The left hemisphere was retracted from the falx with a brain spoon. A glass aspirator was used to make sagittal incision no more than 5 mm in length in the corpus callosum at the level of the interventricular foramen. The fornix was sectioned transversely by electrocautery and aspiration with a 20-gauge metal aspirator insulated to the tip. The dura mater was drawn back, the bone flap was replaced, and the wound was closed in layers. The operated monkeys rested for 11 – 14 days after surgery before beginning postoperative training. Unoperated CON monkeys rested for the same period of time between preoperative and postoperative training.

Histology

At the conclusion of this experiment and a series of following experiments (Kwok and Buckley, 2006; Wilson et al., 2007) the animals with fornix transection were deeply anaesthetised, then perfused through the heart with saline followed by formol-saline solution. The brains were blocked in the coronal stereotaxic plane posterior to the lunate sulcus, removed from the skull, and allowed to sink in sucrose-formalin solution. The brains were cut in 50 μm sections on a freezing microtome. Every fifth section was retained and stained with cresyl violet. Microscopic examination of the stained sections revealed in every case a complete section of the fornix (see Figure 1, panels B, C and D) with no damage outside the fornix except for the incision in the corpus callosum as described in the surgical procedures and at most, only slight damage to the most ventral part of the cingulate gyrus at the same anterior-posterior level in only one hemisphere of one animal

(Figure 1, panel B). A coronal section of a normal control monkey's brain with an intact fornix is also shown for comparison (Figure 1, panel A).

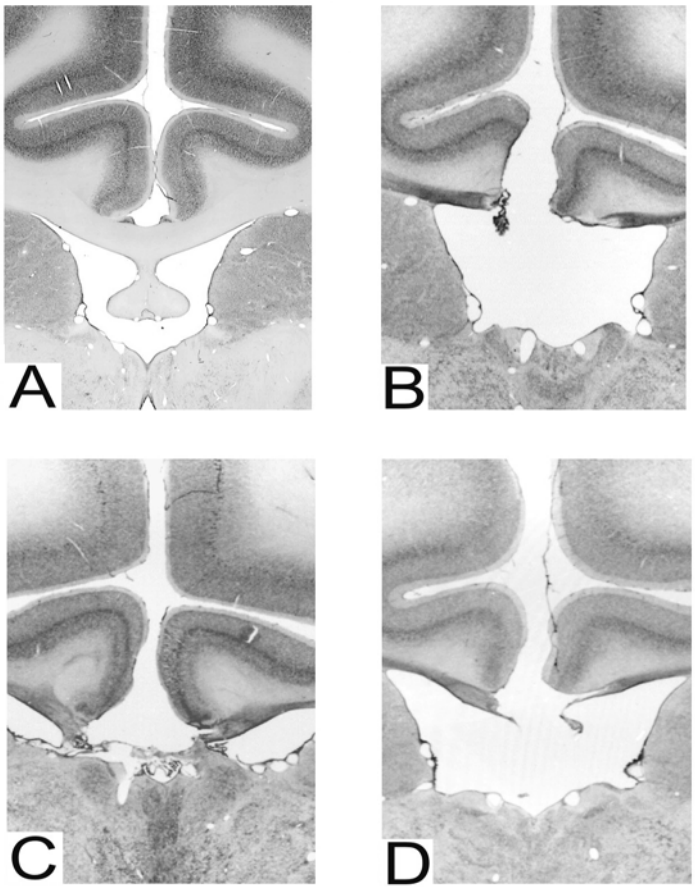


Figure 1. (A) Coronal section from the brain of a normal unoperated macaque just posterior to the level of the interventricular foramen; (B, C, D) coronal sections from the brains of three fornix transected monkeys showing that the fornix transection was complete (the anterior-posterior level of the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

Apparatus

The present tasks were performed in an automated test apparatus. The subject sat in a wheeled transport cage fixed in position in front of a touch-sensitive screen (380 mm x 280 mm) on which the stimuli could be displayed. The subject could reach out

between the horizontal bars (spaced approximately 50 mm apart) at the front of the transport cage to touch the touchscreen. An automated pellet delivery system, controlled by the computer, delivered reward pellets into a food well (approximately 80 mm in diameter) that was positioned in front of and to the right of the subject. Banana-flavoured reward pellets (190 mg; P. J. Noyes, Lancaster, NH) were delivered only in response to a correct choice made by the subject to the touch screen. Pellet delivery was accompanied by an audible click. An automated lunch box (length 200 mm, width 100 mm, height 100 mm) was positioned in front of and to the left of the subject. It was spring-loaded and opened immediately with a loud crack on completion of the task to deliver the animal's daily diet of wet monkey chow, pieces of fruits, raisins, and peanuts. A closed-circuit TV infrared camera positioned above the touch screen and in front of the monkey was used for observation from another room from which the stimulus display, food delivery, and experimental contingencies were computer-controlled. The entire apparatus was housed in an experimental cubicle that was dark apart from the background illumination from the touch screen.

Stimulus material

The visual stimuli presented on the touchscreen were taken from a large library of individual clipart images obtained from commercially available internet sources. The visual problems in the experiment consisted of a white background containing four identical stimuli. Each clipart bitmap image was 128 x 128 pixels in dimension and comprised a unique foreground multi-colored cartoon-like image on a white background. The background of the whole touchscreen was set so as to match this colour, with the effect that the visible borders of our stimuli matched the outlines of their actual shapes and not the rectangular border of each clipart image. A total of 120

images were used in the experiment (including the preliminary training stage). The particular stimuli assigned to each problem set were chosen at random (without replacement) from a library of over 6,000 clipart stimuli. The resolution of the visual display on the touchscreen was set at 800 x 600 pixels with the effect that each visual stimulus on the screen subtended a visual angle of approximately 11.5^0 from the typical viewpoint and perspective of a macaque in its transport cage. The four identical stimuli of each problem were presented on four fixed positions, symmetrically with two on top and two at the bottom on the touchscreen. Some examples of the stimuli used in this study are shown in Figure 2.

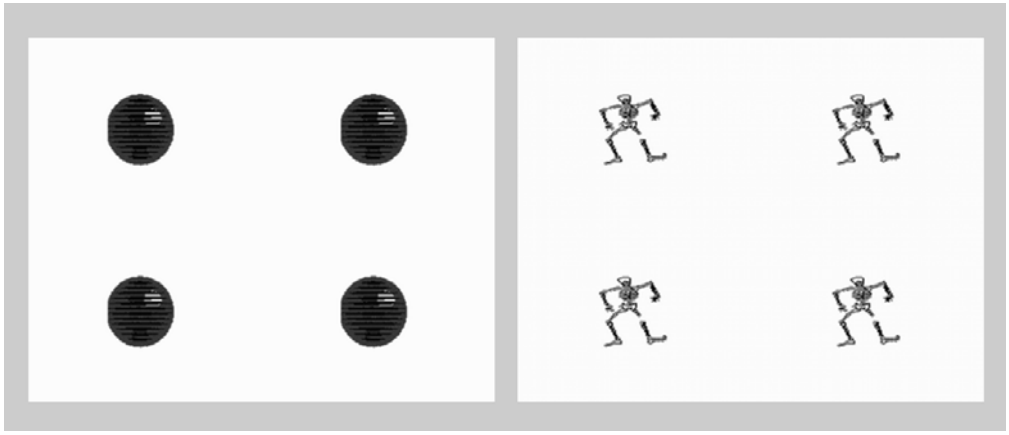


Figure 2. Two examples of problems from the conditional visuospatial discrimination task showing the four identical stimuli that appear in each trial. In each problem the animal has to learn by trial and error which of the four positions is correct for that particular problem. Large numbers of such problems were learnt concurrently with each of the four positions rewarded equally often across the entire set.

Behavioural testing

Preliminary training

To familiarise the animals with the demands of the task we first administered a preliminary practice stage which consisted of only 16 problems in total. The nature of

individual problems is described in detail below and none of the problems in this preliminary training stage appeared in any of the three experimental sets (A – C). The preliminary training sessions introduced these 16 problems gradually in 5 stages: (i) new learning of 4 problems (problems 1 – 4), (ii) new learning of 4 problems (problems 5 – 8), (iii) concurrent testing with all 8 problems (problems 1 – 8), (iv) new learning of 4 problems (problems 9 – 12), and (v) new learning of 4 problems (problems 13 – 16). Correction trials were employed in these practice sessions as an aid to task acquisition. Each new stage (i to v) commenced when performance reached 90% correct or better on the preceding stage. The mean length of the preliminary training period was 20 sessions. Thus before the animals commenced training on the first experimental set (Set A) they were already well practiced at acquiring these kinds of concurrent visuospatial discrimination problems.

Overview of behavioural testing and the experimental task (Sets A to C)

The experimental task used here was a modified version of the concurrent visuospatial discrimination learning task used by Buckley et al. (2008); the primary difference was that in the current task, four identical stimuli rather than two identical stimuli were presented in each trial. In each trial the monkeys were presented with four identical copies of a stimulus upon the touchscreen (see Figure 2). For each unique stimulus, one out of the four positions in which one of the copies appeared was pre-designated as the rewarded choice (S+) and the other three were designated as foils; the position-reward contingency for each particular problem remained constant for each problem throughout the entire experiment. The different problems that together constituted a set of problems were counterbalanced so that across each set as

a whole, there were equal numbers of problems occurring in each of the different locations rewarded.

At the start of each problem, all four stimuli appeared on the screen at the same time and all four remained on the screen until the computer registered that one of them had been touched. A touch to the S+ was followed immediately by delivery of a reward pellet and the immediate removal of the three S-; the S+ remained on the screen alone for a further second to provide visual feedback for a correct response. The screen would then be blanked for an intertrial interval of 10 s before the next trial began. Alternatively, a touch to an S- immediately blanked the screen and started a longer intertrial interval of 16 s after which the same trial was presented again as a correction trial. Correction trials were repeated in this manner until the monkeys eventually made the correct response and the number and types of errors were recorded. A touch to a location not occupied by a stimulus had no effect, excepting for the case where a touch was made to the screen during an intertrial interval which had the effect of restarting that intertrial interval. After successfully completing the final problem in the session, the monkey would be rewarded by the opening of the lunch box. The criterion for completing a session was either that the required numbers of full sets of problems were completed, or that the animal had accrued more than a designated number of errors.

Six monkeys were tested daily on sets of concurrent visuospatial discrimination problems of this kind. The first set (Set A) comprised 8 problems and animals would proceed to the next set once they had attained a level of at least 90% correct responses within a single daily session. The second set (Set B) comprised 32 problems and on the day after attaining performance level of 90% or better in a single daily session on Set B they progressed to Set C (which contained 64 problems). Set C was the final set.

The monkeys performed one session per day and were trained 6 – 7 days per week until all three sets (and therefore 104 problems in total) were acquired to criterion.

Due to the nature of the task, we could not determine in advance how many errors and then how many attempts at the correction trials each animal would make, and to avoid unduly length sessions, a session would be terminated if a monkey accumulated more than 100 errors in the smaller set (Set A) and 150 errors in the larger sets (Sets B and C), within a single session. The implications of this are that the precise number of times animals attempted each unique problem varied form animal to animal and from session to session. However, in a hypothetically perfect scenario where not a single error was made, a daily session would comprise 128 trials in total and each problem in a set would be presented once (in random order) before the whole set was reshuffled and presented again in a new random order. Furthermore, in this hypothetical case, problems in each set would be presented an equal number of times during each session with the actual number of times each problem was presented depending upon the size of that problem set. That is, each problem would be presented more frequently during a daily session with a small set (e.g. each problem would be presented 16 times per session in Set A assuming no errors were made) but less frequently in a large set (e.g. each problem would only be presented 2 times per session in Set C).

2.4 Results

1. Preliminary training stage

We analysed the total number of errors to criterion between groups in the five task acquisition stages on the logarithmically transformed data and found that there were no significant differences in acquisition of the task between groups in any of the task acquisition stages [largest t (4) = 1.27, all $p > 0.1$].

2. Main task

Errors-to-criterion in main task

The overall performance of the FNX group was compared with that of the CON group to assess whether there were any deficits in the overall learning of new visuospatial concurrent discriminations. We scored the total number of errors to criterion (including correction trials) for each problem set. The CON group accumulated a mean of 114 errors in learning Set A to criterion, 596 errors in learning Set B, and 930 errors in learning Set C. The corresponding means for the FNX group were 251, 771, and 973 errors respectively. Here, and elsewhere in the paper, all of our error data were logarithmically transformed prior to analysis following the recommendations of Kirk (1982). We conducted a repeated measures ANOVA with two levels of the between-subjects factor 'Group' (CON, FNX), and three levels of the within-subjects factor 'Set' (Sets A – C) on the logarithmically transformed number of errors to criterion. This analysis showed that although there was no main effect of Group [$F < 1$] there was a significant Group*Set interaction [F (2, 8) = 5.84, $p = 0.027$] and the linear trend component of this interaction was also significant [F (1, 4) = 7.85, $p = 0.049$] confirming what is illustrated in Figure 3 that the relative size

of the impairment in the FNX group is inversely correlated to set size. Inspection of the individual animals' scores indicates that whereas there is overlap in these scores in Sets B and C, there is no overlap in the scores between animals in the two groups in Set A.

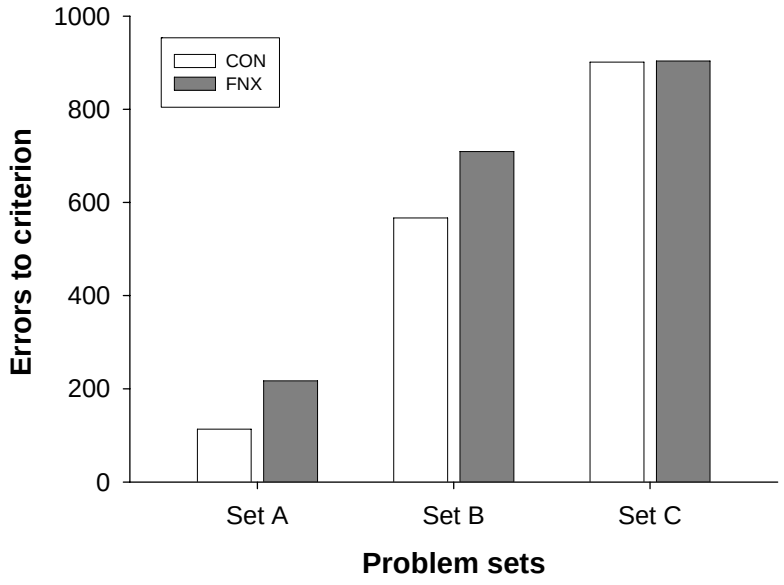


Figure 3. Geometric mean of the errors to criterion for controls (CON) and fornix transected monkeys (FNX) for each problem set.

Fast learning: error elimination in the early stages of learning

In order to analyse the rate at which the animals eliminated errors during an early stage of learning we had to decide upon a fixed number of trials that we could examine in each animal in each set. As individual animals learnt at different rates, to avoid picking an entirely arbitrary fixed number of trials to analyse in each set that might correspond to an early learning stage and which would be comparable between animals, we instead formalized a procedure by which we calculated the mean number of trials it took the animals to attain criterion on each set and designated 20% of that number as the 'early stage' for each set. By this measure, for each animal, the 'early

stage' in Set A consisted of the first 80 trials, the 'early stage' in Set B consisted of first 300 trials in Set B, and the 'early stage' in Set C consisted of first 400 trials. The remaining 80% of all trials of individual animals was designated as the 'later stage'.

The mean numbers of total errors/problem accrued by the CON group during this 'early stage' were 2.89, 2.42, and 2.28 for Sets A, B and C respectively, the corresponding numbers of the FNX group were 6.33, 4.38, and 3.00. We ran a 2-way repeated measures ANOVAs on the logarithmically transformed number of total errors/problem commissioned during the early stage of learning, containing two levels of the between-subjects factor 'Group' (CON, FNX) and three levels of the within-subjects factor 'Set' (Sets A – C), and found that a main effect of 'Group' [Group: F (1, 4) = 10.70, p = 0.031] and no Group*Set interaction [F (2, 8) = 1.01, p > 0.1], confirming that the FNX group made more errors than the CON group across the early stage of each set. A similar analysis of the later stage of learning found no main effect of Group [Group: F (1, 4) = 2.14, p > 0.1], and no Group*Set interaction [F (2, 8) = 1.55, p > 0.1].

In order to further investigate whether the FNX group might be particularly impeded with their 'fast learning' we examined the rate at which the monkeys could eliminate different kinds of errors during the early stages of acquisition of each of the three sets. To do this we divided up the total errors accumulated by each animal into three mutually exclusive sub-classes of errors, namely first-time errors, non-perseverative errors, and perseverative errors. First-time errors (1st time) refer to those errors made by a monkey to a problem on the very first occasion it encountered any problem. As there were three foils on each trial and a correction procedure was employed, if a monkey made a first-time error on a problem (touching *any* S- on the trial), then two different types of repetitive errors were possible on subsequent

presentations for that particular problem. The first kind of repetitive errors are the non-perseverative errors (non-P) and this refers to those errors made when an animal went on to pick a different S- from the preceding one in the ensuing correction trial. The second type of repetitive errors are perseverative errors (P) which refer to those errors made when an animal went on to choose exactly the same spatial position as chosen erroneously in the preceding trial (i.e. touching the *same* S- again). Due to the correction procedure, it was possible for the monkey to accrue several non-perseverative and perseverative errors before a correct response was made which completed that problem.

We ran a 3-way repeated measures ANOVAs on the logarithmically transformed number of errors/problem commissioned during the early phase of learning, containing two levels of the between-subjects factor 'Group' (CON, FNX), three levels of the within-subjects factor 'Set' (Sets A – C) and three levels of the within-subjects factor 'Error-type' (Error-types: 1st time, non-P and P). Although there were no interactions of Group*Set and Group*Set*Error-type [all $F < 1$], we did find a main effect of Group [Group: F (1, 4) = 9.25, p = 0.038] and a significant Group*Error-type interaction [F (2, 8) = 5.97, p = 0.026, Huynh-Feldt corrected], which prompted us to look at each of the three types of errors separately.

Whether or not the monkey made an error when it encountered any problem for the very first time depends entirely upon chance, and in this case a 75% 1st time error rate is expected. The error rate of the animals averaged across the early stage of acquisition was 67% for the CON group and the error rate was 75% for the FNX group. We ran a 2-way repeated measures ANOVAs on the logarithmically transformed number of 1st time errors/problem commissioned during the early stage of learning, containing two levels of the between-subjects factor 'Group' (CON, FNX)

and three levels of the within-subjects factor 'Set' (Sets A – C), and found that no main effect of 'Group' [Group: F (1, 4) = 1.72, $p > 0.1$] and no Group*Set interaction [F (2, 8) = 2.83, $p > 0.1$], confirming that the FNX group was not different from the CON group in making 1st time errors across the early stage of each set. As animals experienced the same problems again as training proceeded, and remembered the correct solutions, the 1st time error rate is expected to fall. The error rate of the animals averaged across the later stage of acquisition was 27% for the CON group and the error rate was 25% for the FNX group. A similar analysis of the later stage of learning also found no main effect of Group [Group: F (1, 4) < 1], and no Group*Set interaction [F (2, 8) = 3.48, $p = 0.082$].

In addition to 1st time errors as described above, animals could accrue a variable number of non-P and P errors in each problem prior to making a correct response. Analysing these types of within-problem errors allows us to probe the strategy used by the animals to eliminate errors. We therefore ran two additional 2-way repeated measures ANOVAs, one for each type of error (non-P and P errors), each with two levels of the between-subjects factor 'Group' (CON, FNX) and three levels of the within-subjects factor 'Set' (Sets A – C) on the logarithmically transformed number of errors/problem accrued in this stage. For non-P errors, the mean numbers of errors/problem accrued by the CON group in the early stage of acquisition were 1.32, 1.05, and 1.17 for Sets A, B and C respectively; the corresponding numbers of the FNX group were 2.91, 2.03, and 1.45. We found that there was a significant main effect of 'Group' [Group: F (1, 4) = 11.10, $p = 0.029$] and no Group*Set interaction [F (2, 8) < 1] confirming that the FNX group made more non-P errors than the CON group across the early stage of each set. For non-P errors, the mean numbers of errors/problem accrued by the CON group in the later stage of learning were 0.14,

0.21 and 0.23 for Sets A, B and C respectively; the corresponding numbers of the FNX group were 0.23, 0.23, and 0.25. A similar analysis of the later stage of learning found no main effect of Group [Group: F (1, 4) = 1.24, $p > 0.1$], and no Group*Set interaction [F (2, 8) < 1]. For P errors, the mean numbers of errors/problem accrued by the CON group in the early stage of acquisition were 0.95, 0.67, and 0.42 for Sets A, B and C respectively, the corresponding numbers of the FNX group were 2.60, 1.62, and 0.84. We found no main effect of 'Group' [Group: F (1, 4) = 7.46, p = 0.052] and no Group*Set interaction [F (2, 8) < 1], confirming that the FNX group was marginally not different from the CON group in making P errors across the early stage of each set. For P errors, the mean numbers of errors/problem accrued by the CON group in the later stage of learning were 0.1, 0.09, and 0.06 for Sets A, B and C respectively, the corresponding numbers of the FNX group were 0.17, 0.16, and 0.13. A similar analysis of the later stage of learning found no main effect of Group [Group: F (1, 4) = 5.54, $p > 0.05$], and no Group*Set interaction [F (2, 8) < 1].

Taken together these analyses confirm that fornix transection caused a selective impairment in eliminating non-perseverative errors in the initial stages of learning in each set. During initial acquisition, FNX monkeys appeared likely just as able as CON monkeys to monitor their most recent action, and rectify it if it was an error (i.e. no strong evidence of deficits in correcting perseverative errors). However, if an error was made further back in time (more than one preceding trial) then FNX monkeys showed deficits in their monitoring and rectification of such errors.

Fast learning: one-trial learning

To test whether fornix transection causes a deficit in one-trial learning, we measured performance on the first versus the second presentation of the 104 problems

(i.e. all of the stimuli from Sets A to C combined) irrespective of when the first and second presentation of each problem occurred in their respective sessions. In order to complete each trial, if the animals failed to get the correct response on the first attempt then the monkeys had to perform correction trials on that problem until they eventually responded correctly, thereby completing the trial. Thus, at the time of the second presentation of each problem, the monkeys had performed and experienced only one correct, reinforced response to that stimulus. At the time of the first presentation of each problem, the stimuli presented were novel and, accordingly, the mean percentage correct score for all animals on the first presentation of each problem was 25.6% (mean percent correct for CON and FNX monkeys are 26.6% and 24.7% respectively) which was indistinguishable from chance performance in a four choice test and the two groups did not differ from each other with respect to their performance on the first presentation of each problem [t (4) = -0.37, $p > 0.5$]. Upon the second presentation of each problem, the monkeys had experienced a single correct and reinforced response to that problem (from completing the first trial of that problem). With that one trial of experience, the mean score on the first attempt at the second presentation of each problem had now increased to 34.1% correct for all monkeys (mean percent correct: 32.7% and 35.5% for CON and FNX monkeys respectively) which was now significantly above chance [t (5) = 4.02, p = 0.01] thereby demonstrating one-trial learning; again there was also no significant difference in performance between groups [t (4) = 0.59, $p > 0.5$]. Furthermore, in order to assess the effect of set size upon one-trial learning account, we also conducted a 3-way repeated measures ANOVA with two levels of the between-subjects factor 'Group' (CON, FNX), three levels of the within-subjects factor 'Set' (Sets A, B and C), and two levels of the within-subjects factor 'Trial'

(Trials 1 and 2) on the percent correct score. We found no main effect of group [$F < 1$], and no interaction between Group*Set*Trial or Group*Set [largest $F = 2.40$, $p > 0.1$]. The 3-way repeated measures ANOVA helped us rule out any effect or interaction of set size on one-trial learning. Altogether, these analyses showed that both groups of monkeys were capable of some degree of one-trial learning of these associative problems across all three sets but we found no evidence that the one-trial learning expressed in this task was dependent upon the fornix.

Fast learning: errorless learning

Errorless learning refers to situations in which an animal has no experience with making an incorrect response prior to the first instances of making a correct one. The absence of prior error expedites learning (McClelland, 2001; Kessels and De Haan, 2003; Tailby and Haslam, 2003; Brasted et al., 2005; Clare and Jones, 2008). The number of errors prior to the first correct response on each problem corresponded to the number of correction trials, if any, on the first presentation of each problem. Here we assessed the effect of the number of prior errors on the performance for the second trial of a given problem, at which time the monkeys had always made only one correct response to that stimulus. A two-way repeated measures ANOVA, with two levels of the between-subjects factor 'Group' (CON and FNX) and four levels of the within-subjects factor 'Number of prior errors' (0, 1, 2 and >2) on the percent correct of the second trial of 104 problems from all three sets showed that the number of prior errors had a highly significant effect [Number of prior errors: F (3, 12) = 6.60, p = 0.007], which was the same in both groups [no Group effect: F (1, 4) < 1 and no Group*Number of prior errors interaction: F (3, 12) = 1.10, $p > 0.1$]. When the monkeys had made no errors prior to the first correct response, their performances on

the second trial were well above chance [t (5) = 7.05, $p < 0.001$], but not so when prior errors had been made [t (5) = 0.07, $p > 0.5$, for combined percent correct across all three conditions: 1, 2 and >2 errors] (See Figure 4).

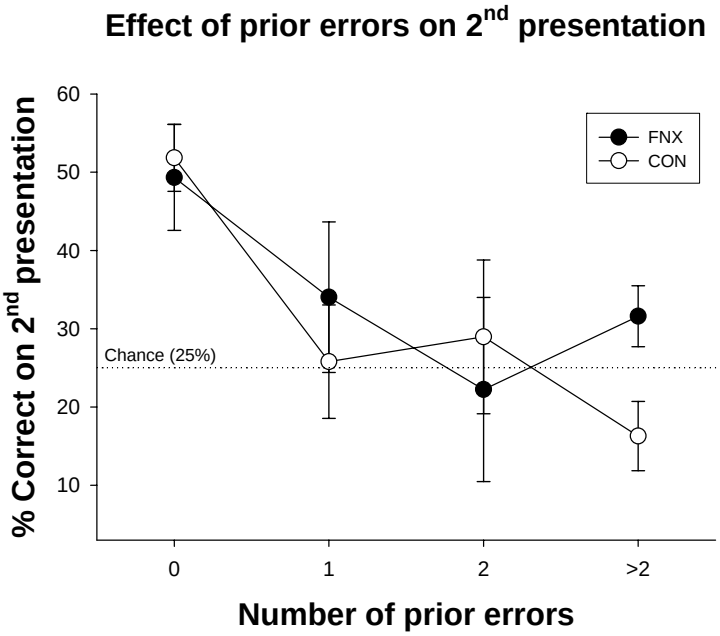
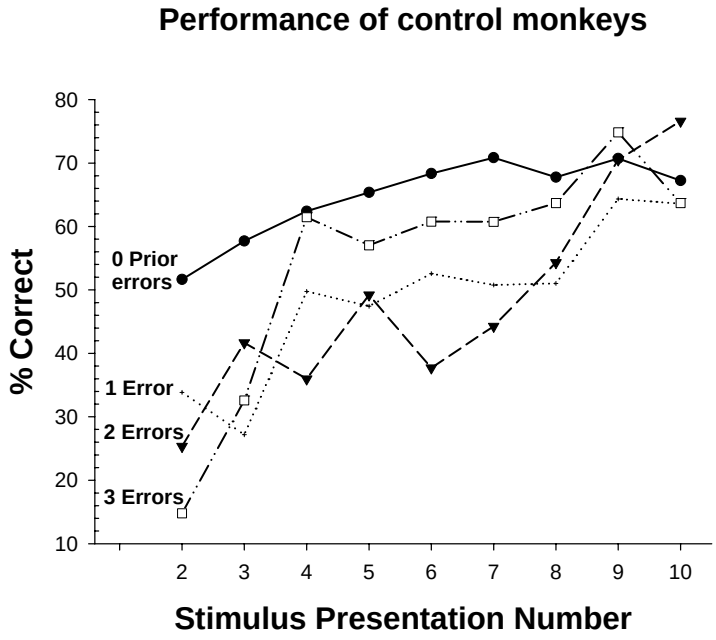


Figure 4. Effect of the number of prior errors (0, 1, 2 or >2 errors) on subsequent learning by depicting the mean (±SEM) percent correct performance on the second presentation of problems for controls (CON) and fornix transected monkeys (FNX) for all 104 problems.

Given that prior errors are detrimental to learning in both groups we went on to explore how many subsequent presentations of each problem it took to overcome the effects of errors made prior to the first correct response (Figure 5). A three-way repeated measures ANOVA, with two levels of the between-subjects factor 'Group' (CON and FNX), four levels of the within-subjects factor 'Number of prior errors' (0, 1, 2 and 3), and ten levels of the within-subjects factor 'Presentation number' on the percent correct of the largest set showed a significant effect of presentation number [F (8, 32) = 21.49, $p < 0.001$] and that the main effect of group and all interactions involving group were not significant [all $F < 1$]. These confirm that an intact fornix is

not critical in overcoming the detrimental effects caused by errors made prior to the first correct response. When the monkeys had made no prior errors to the first correct response, their performance approximated plateau levels after first few presentations of a stimulus. When the monkeys had made some number of errors prior to the first correct response, they reached their learning asymptote by the time of the ninth or tenth presentation of each stimulus. Both groups of monkeys approached their asymptotes in a comparable manner, indicating that the number of presentations required to overcome the effects of prior errors was independent of the fornix. In summary, these analyses showed that any errors made prior to the first correct response retarded one-trial learning; as this effect was present in both groups of monkeys, we conclude that the facilitatory effect of errorless learning was independent of the fornix.

Panel A



Panel B

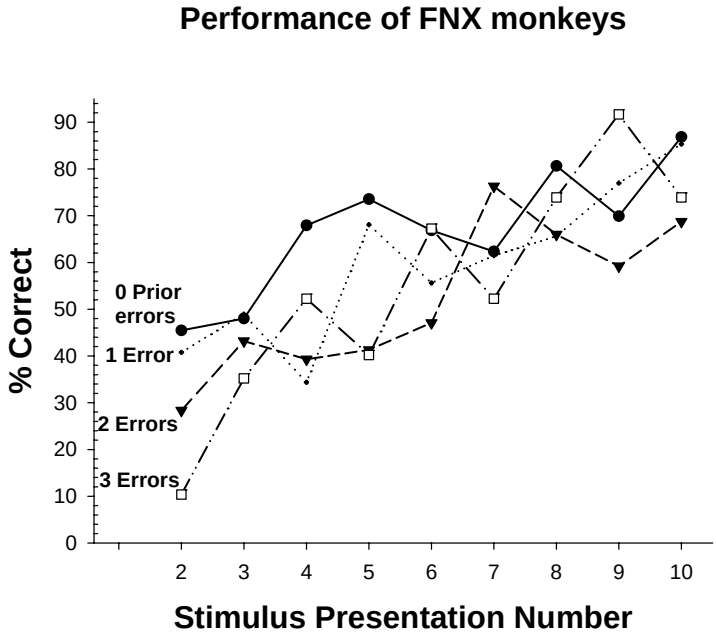


Figure 5. Mean percent correct performance (Panel A: Control monkeys, Panel B: Fornix transected monkeys) on the second through tenth presentations of each stimulus in the largest set according to number of errors made prior to the first correct response, if any.

2.5 Discussion

The main findings from this study were as follows. Firstly, bilateral fornix transection in macaques led to a greater impairment in learning smaller than larger sets of concurrent visuospatial discriminations to criterion. Secondly, an analysis of error types on correction trials (each problem had multiple foils) revealed that fornix transection impeded the initial learning stages of each set by slowing the rate at which non-perseverative errors were eliminated, while the perseverative error rate was largely unaffected. Thirdly, we found that monkeys could perform above chance on this task after a single trial of training but that this form of one-trial learning was not fornix dependent. Finally, we showed that any errors animals made prior to the first correct response slowed their subsequent learning (i.e. errorless learning facilitates acquisition) and we ascertained that errorless learning in this context was not fornix dependent, nor was overcoming the deleterious effect of having made prior errors. Each of these findings is discussed in more detail below.

Our hypothesis that fornix transection would impair fast learning of visuospatial information was confirmed. The FNX group was impaired in acquiring the smallest set of concurrent visuospatial discriminations (Figure 3). This is consistent with an impairment in fast learning of visuospatial problems because the interval between successive presentations of the same problem was much shorter in the smaller sets which are acquired more rapidly. In contrast, impairments were not observed in the largest set, wherein learning might be predominantly mediated by a 'slow learning' mechanism. Slow learning mechanism have previously been proposed to depend on structures other than the hippocampal system, either through the interaction of the neocortex with those parts of the basal ganglia that receive inputs from the neocortex (Fernandez-Ruiz et al., 2001) or through the neocortex acting

through sensorimotor, corticocortical connections (McClelland et al., 1995), or both mechanisms (Brasted et al., 2005).

Previous studies in macaques, that have used different tasks, may also be interpreted as supporting a distinction between fast and slow learning mechanisms, with the former but not the latter dependent upon the fornix. For example, in a study by Rupniak and Gaffan (1987), monkeys learnt postoperative visuomotor conditional problems (involving associating either approach or withdrawal with visual stimuli) very quickly (with the control group averaging ~8 errors/problem to attain a criterion of 90% correct responses) and fornix transection was observed to impair this task. Similarly, the control monkeys in Brasted's et al. (2003) study who learnt visuomotor conditional discriminations of a different kind (involving associating three temporally distinct motor responses with visual stimuli) rapidly, averaging ~15 errors/problem to learn three-choice problem sets to a 90% criterion, were similarly impaired. In contrast to these findings, Gaffan and Harrison (1988) observed that fornix transection was without effect in a visuomotor conditional discrimination task that was learnt much more slowly (control subjects averaging ~90 errors/problem to criterion for the first five postoperative problem sets and ~55 errors to criterion for the second five problem sets). Note that the distinction we make between fast and slow learning illustrated by these studies, and the different sets in the current study, concerns only rate of acquisition and is independent of the nature of the task. It is quite different from earlier distinctions drawn between memory and habit learning based upon the effects of lesions to the medial temporal lobe on recognition memory versus concurrent discrimination learning, particular comparison has since been shown to be unfounded once set size is taken into account (Gaffan, 1996; Buckley and Gaffan, 1997; Buckley, 2005).

When we probed the nature of the errors made by the FNX group in the early learning stages we found that FNX animals made significantly more non-perseverative errors than CON animals. During the early stage of learning of these sets of problems, FNX animals were as able as CON animals to monitor their most recent action (i.e. the preceding spatial response) and correct it if it was an error as evidenced by the FNX group not accruing more perseverative errors. However the FNX group was less able to remember incorrect responses from further back in time than the preceding trial, as evidenced by their greater likelihood than the CON group to return back to previously unrewarded places from responses made more than one trial ago, thereby generating more non-perseverative errors. Although FNX monkeys' abilities in monitoring the most recent action principally remained intact, their deficits in remembering multiple stimuli chosen over extended periods of time and in monitoring earlier errors is consistent with an impairment in the processing of temporal context (Wilson et al., 2007) or more generally, with an impairment in processing information about temporal order (Charles et al., 2004). It is unlikely that the impairments observed in the present study may be attributable solely to deficits in remembering information over longer periods of time because a previous study showed that fornix transected monkeys were as good as controls at recognising stimuli irrespective of when the item was presented (i.e. early or late) in an extended list of samples (Charles et al., 2004). Likewise, a purely spatial learning deficit would also be insufficient to explain the present deficits because we can infer from the lack of perseverative impairments in the FNX group that FNX monkeys performed just as well as CON monkeys in learning and rectifying their immediate spatial errors. This leads us to propose that the impairments in our study should be attributable to a deficit in encoding the spatio-temporal context in which associations were learnt. This is

consistent with other recent studies that have argued that the deficits after fornix transection in reversal learning paradigms may also be attributed to deficits in learning about temporal context (Wilson et al., 2007).

Apart from early stage error elimination, two other phenomena of 'fast learning' were examined in the current study, namely one-trial learning and errorless learning. The existing literature strongly suggests a contribution of the fornix to one-trial learning. For example, Gaffan et al. (1984) showed a significant effect of fornix transection on only one of two versions of a one-trial, object-reward associative learning task; monkeys with fornix transection were impaired in performing according to a win-shift, but not with a win-stay, rule. The selective impairment on the win-shift rule after fornix transection might reflect an inability to recall the information presented on the acquisition trials, which would represent a form of one-trial learning. Also, Gaffan (1994) showed in a scene learning task that monkeys improved their performance considerably after only a single trial of training when required to discriminate two objects on algorithmically generated complex scenes; and fornix transection greatly attenuated the one-trial learning improvement. More recently, Brasted et al. (2005) reported that monkeys achieve significant one-trial learning in a non-spatial version of a conditional motor learning task and fornix transection eliminated this capability. Nevertheless, we showed that, in the context of the present study, one-trial learning was not dependent on the fornix. This highlights that the previous findings of impaired one-trial learning after fornix transection do not necessarily generalise to all learning tasks.

Finally, like in a previous study (Brasted et al., 2005), we observed a facilitatory effect of errorless learning in control macaques, but whereas Brasted et al. (2005) found that fornix transected animals performed at chance on the second

presentation of problems when other problems intervened between the first correct response and the second presentation of a particular problem, we observed that both control and FNX animals showed a facilitatory effect of errorless learning despite the ubiquitous presence of intervening problems in our task. Furthermore, we found that this facilitatory effect was unaffected by fornical damage (Figure 5). The commission of prior errors on one-trial learning in our control monkeys provides further support to McClelland's (2001) idea that prior execution of erroneous responses to a given stimulus impairs associative learning because of a maladaptive Hebbian learning mechanism (see a detailed discussion in Brasted et al., 2005). But another aspect of McClelland's (2001) theory states that the hippocampal system is involved in overcoming the deleterious effect of prior errors on subsequent learning and indeed, there is some evidence that amnesic patients benefit from errorless learning (Wilson et al., 1994; Kixmiller, 2002; Grandmaison and Simard, 2003; Kessels and De Haan, 2003; Tailby and Haslam, 2003). Our results indicate that, at least in tasks such as these, impaired errorless learning is not a necessary effect of damaging the fibres that course through the fornix, and that the fornix does not generally contribute to animals' abilities to overcome the detrimental effects upon learning of having made prior errors either. The same may be true for amnesic patients with fornical damage given the previously noted similarities in the effects of fornix transection in the two species (Gaffan, 1994; Aggleton et al., 2000).

To conclude, fornix transection impairs some, but not all, aspects of fast learning in the context of a conditional visuospatial concurrent discrimination learning task. Learning was disproportionably impeded in the smallest set wherein information could be learnt most rapidly with a 'fast learning' mechanism that was presumably more susceptible therefore to fornix transection. We also found that, during the initial

stages of learning, fornix transected monkeys appeared unable to keep track of incorrect responses from further back in time, implying that cortical-subcortical connections via the fornix, while being important to support new learning (Buckley et al., 2004; Buckley et al., 2008), are not important for all forms of new learning; rather, they may be selectively concerned with relatively rapid acquisition of the spatial and temporal relationships between stimuli and responses.

2.6 References

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CHAPTER 3

FORNIX TRANSECTION SELECTIVELY FACILITATES INITIAL ACQUISITION OF CONDITIONAL VISUOVISUAL DISCRIMINATIONS

Sze Chai Kwok & Mark J. Buckley
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CHAPTER 3: FORNIX TRANSECTION SELECTIVELY FACILITATES INITIAL ACQUISITION OF CONDITIONAL VISUOVISUAL DISCRIMINATIONS

3.1 Abstract

Previous studies with macaque monkeys have found fast learning of associative information to be impaired in both spatial (visuospatial) and non-spatial (visuomotor) associative tasks after fornix transection. In order to test theories that posit a general role for the fornix in associative learning, we investigated whether monkeys with fornix transection would also be impaired in the rapid acquisition of visuovisual conditional associations. We trained monkeys, postoperatively, on three sets of conditional stimulus-stimulus concurrent associations. Fornix transection did not impair early learning of these associations; rather, animals with fornix transection accrued significantly fewer perseverative errors during the initial acquisition stages. These results challenge the idea that the hippocampal system plays a general role in the rapid acquisition of all kinds of associative knowledge. We suggest that the lesion induced facilitation of this non-spatial task may be secondary to an impairment in visuospatial processing, which might act to bias animals away from attempts to learn about spatial strategies for solving novel tasks. Both fornix and control monkeys were observed to adopt a change-shift response strategy on successive trials that were different; this strategy use was also fornix independent.

3.2 Introduction

While there is ample evidence showing that the hippocampus and fornix are implicated in spatial associative learning (Gaffan and Harrison, 1989; Murray et al., 1989; Gaffan, 1994a, b; Buckley et al., 2004; Buckley et al., 2008), it has also become apparent that there is a distinction between the effects of hippocampal system disruption on the early and late stages of learning associative problems (Brasted et al., 2003; Kwok and Buckley, submitted).

Brasted et al. (2003) trained monkeys on a series of conditional stimulus-response associations involving complex visual stimuli, each instructing one of three non-spatially differentiated visuomotor responses. They found that fornix transection impaired the initial learning of these associations and the impairments were mainly attributed to the fact that the unoperated control monkeys eliminated errors at about a three-fold faster rate than fornix transected monkeys. More recently, Kwok and Buckley (submitted) showed that fornix transection selectively impaired the early learning stages of acquisition of sets of concurrent visuospatial conditional discriminations. Our intention with the present study was to ascertain whether fornix transection affects rapidly acquired associations generally by investigating the effects of fornix transection on visuovisual associative learning.

Further impetus for the present study came from studies that have investigated the role of the hippocampal system in associative learning. Murray et al. (1993) showed that visuovisual associative learning was unaffected by complete bilateral removal of the hippocampus and concomitant damage to underlying structures in the medial temporal lobe, which implies that the hippocampal system as a whole may be uninvolved in associative learning when neither component of the

association has a spatial attribute. However, monkeys in that experiment required a large number of trials to learn the associative problems (approximately 2,000 and 2,800 trials on average were required to acquire five and ten new pairs of visuovisual paired associates, respectively) and there remains a possibility that, despite the equated overall errors to criterion in learning between groups, hippocampal and control monkeys might actually differ in the early stages of acquisition of visuovisual paired associates even without involvement of a spatial element.

In order to investigate whether the fornix supports the initial stages of acquisition of non-spatial stimulus-stimulus associations, we trained monkeys on a conditional visuovisual discrimination learning task. Given recent findings of deficits after fornix transection on the early stages of animals' abilities to learn arbitrary mappings between visual and temporally differentiated responses (Brasted et al., 2003) and between visually and spatially differentiated responses (Kwok and Buckley, submitted), we reasoned that deficits in the present visuovisual conditional task would amount to evidence in favour of the view that the fornix supports a general fast learning mechanism for acquiring conditional associations. Alternatively, if fornix transection did not impair initial acquisition of the current task then this would support the alternative hypothesis that the fornix plays a more restricted role in spatial and temporal learning, consistent with some views of the role of the hippocampal system in learning about context (e.g. Gaffan, 1994b; Charles et al., 2004; Wilson et al., 2007; Kwok and Buckley, submitted). Like the visuospatial task used by Kwok and Buckley (submitted) here we also incorporated multiple choice items in each trial as the presence of more than one incorrect stimulus in each trial allows the possibility for animals to make different kinds of errors which could be

analysed to better elucidate monkeys' learning strategies in the early acquisition stages.

3.3 Materials and Methods

Subjects

Six male cynomolgus monkeys (Macaca fascicularis) took part in this experiment. Their mean weight at the start of behavioural testing was 7.1 kg (range 6.2 kg to 8.1 kg), and their mean age was 6 years and 1 month. All six monkeys had identical pre- and postoperative experience in concurrent discrimination learning tasks, in a series of experiments that were carried out before the present study began (Kwok and Buckley, 2006; Buckley et al., 2008; Kwok and Buckley, submitted). They were housed together in a group enclosure (except for one who was housed in a pair with another animal not involved in this experiment) in an enriched environment in which they were able to forage daily for small food items (seeds etc) and all had automatically regulated lighting and with water available *ad libitum*.

Surgery

Three monkeys had received bilateral fornix transection (group FNX) 25 months before the present study began and the other three had identical behavioural experience as the FNX group but remained unoperated controls (group CON). Prior to this study all six animals had identical postoperative experience in retention and learning of different kinds of concurrent visuospatial discriminations (reported in Buckley et al., 2008). All licensed procedures were carried out in compliance with the United Kingdom Animals (Scientific Procedures) Act of 1986. The operations were performed in sterile conditions with the aid of an operating microscope, and the monkeys were anesthetised throughout surgery with barbiturate (5% thiopentone sodium solution) administrated through an intravenous cannula. A D-shaped bone

flap was raised over the midline and the left hemisphere up to the midline. The dura mater was cut to expose the hemisphere up to the midline. Veins draining into the sagittal sinus were cauterisd and cut. The left hemisphere was retracted from the falx with a brain spoon. A glass aspirator was used to make sagittal incision no more than 5 mm in length in the corpus callosum at the level of the interventricular foramen. The fornix was sectioned transversely by electrocautery and aspiration with a 20-gauge metal aspirator insulated to the tip. The dura mater was drawn back but not sewn, the bone flap was replaced, and the wound was closed in layers. The operated monkeys rested for 11 – 14 days after surgery before beginning postoperative training. Unoperated CON monkeys rested for the same period of time between preoperative and postoperative training.

Histology

At the conclusion of this experiment and a series of following experiments (Wilson et al., 2007) the animals with fornix transection were deeply anaesthetised, then perfused through the heart with saline followed by formol-saline solution. The brains were blocked in the coronal stereotaxic plane posterior to the lunate sulcus, removed from the skull, and allowed to sink in sucrose-formalin solution. The brains were cut in 50 μm sections on a freezing microtome. Every fifth section was retained and stained with cresyl violet. Microscopic examination of the stained sections revealed in every case a complete section of the fornix (see Figure 1, panels B, C and D) with no damage outside the fornix except for the incision in the corpus callosum as described in the surgical procedures and at most, only slight damage to the most ventral part of the cingulate gyrus at the same anterior-posterior level in only one hemisphere of one animal (see Figure 1, panel B). A coronal section of a normal

control monkey's brain with an intact fornix is also shown for comparison (Figure 1, panel A).

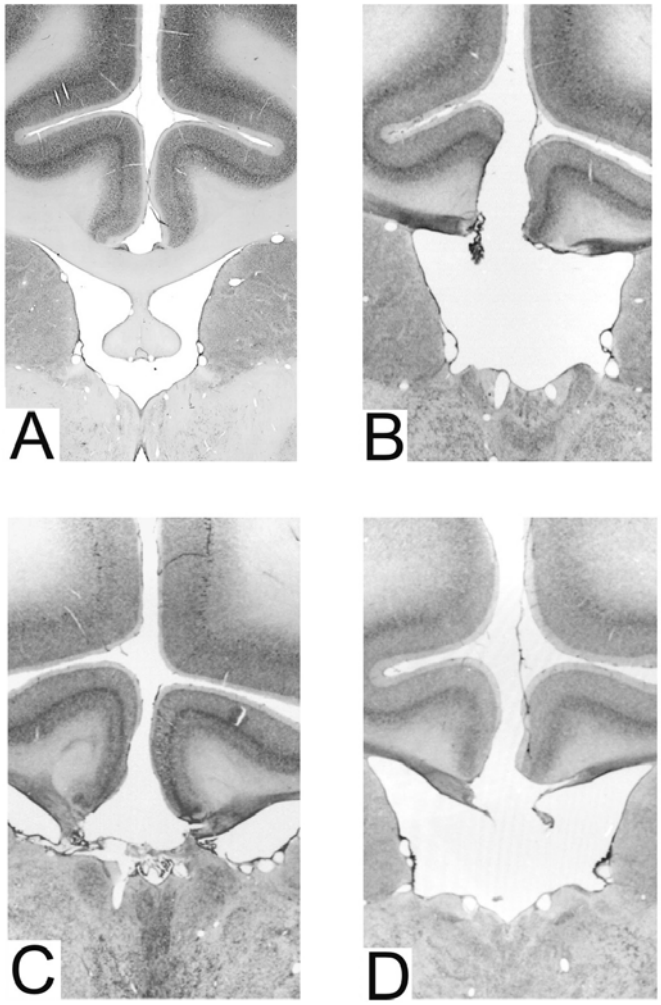


Figure 1. (A) Coronal section from the brain of a normal unoperated macaque just posterior to the level of the interventricular foramen; (B, C, D) coronal sections from the brains of three fornix transected monkeys showing that the fornix transection was complete in each case.

Apparatus

The present tasks were performed in an automated test apparatus. The subject sat in a wheeled transport cage fixed in position in front of a touch-sensitive screen (380

mm x 280 mm) on which the stimuli could be displayed. The subject could reach out between the horizontal bars (spaced approximately 50 mm apart) at the front of the transport cage to touch the touchscreen. An automated pellet delivery system, controlled by the computer, delivered reward pellets into a food well (approximately 80 mm in diameter) that was positioned in front of and to the right of the subject. Banana-flavoured reward pellets (190 mg; P. J. Noyes, Lancaster, NH) were delivered only in response to a correct choice made by the subject to the touchscreen. Pellet delivery was accompanied by an audible click. An automated lunch box (length 200 mm, width 100 mm, height 100 mm) was positioned in front of and to the left of the subject. It was spring-loaded and opened immediately with a loud crack on completion of the task to deliver the animal's daily diet of wet monkey chow, pieces of fruits, raisins, and peanuts. A closed-circuit TV infrared camera positioned above the touchscreen and in front of the monkey was used for observation from another room from which the stimulus display, food delivery, and experimental contingencies were computer-controlled. The entire apparatus was housed in an experimental cubicle that was dark apart from the background illumination from the touchscreen.

Stimulus material

The visual problems in the experiment consisted of a white background containing one sample stimulus in the middle and three choice stimuli positioned equidistantly around the sample stimulus in the periphery of the touchscreen. With the constraint of being equidistant to the sample stimulus, the positions of the three choice stimuli varied across trials. The sample visual stimuli were taken from a large library of individual clipart images obtained from commercially available internet sources. Each clipart bitmap image was 128 x 128 pixels in dimension and comprised

a unique foreground multi-coloured cartoon-like image on a white background. The background of the whole touchscreen was set so as to match this colour, with the effect that the visible borders of our stimuli matched the outlines of their actual shapes and not the rectangular border of each clipart image. A total of 12 images were used as sample stimuli in the experiment and these were chosen at random (without replacement) from a library of over 6,000 clipart stimuli. None of these stimuli had ever been seen by the animals prior to this study. The choice stimuli, namely a red circle, a blue square and a green triangle, all of comparable size to the sample stimulus, were drawn by the computer and they were repeatedly used across all problem sets. The resolution of the visual display on the touchscreen was set at 800 x 600 pixels with the effect that each visual stimulus on the screen subtended a visual angle of approximately 11.5^0 from the typical viewpoint and perspective of a macaque in its transport cage. Two examples of trials used in this study are shown in Figure 2.

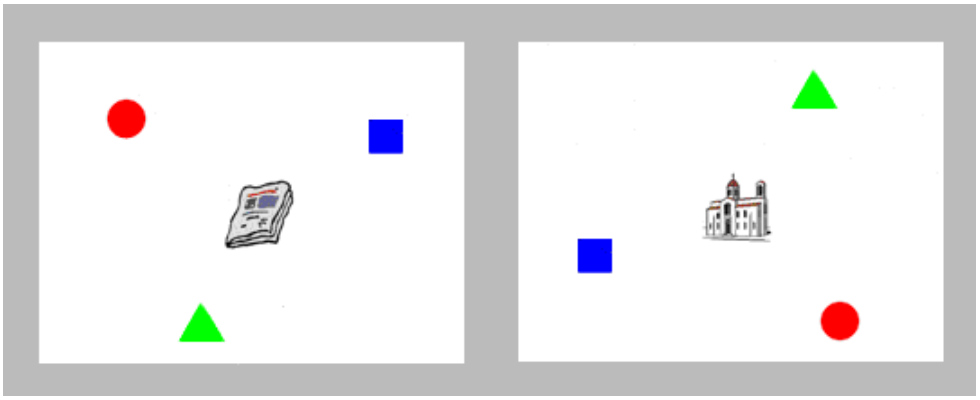


Figure 2. The two panels illustrate two examples of problems from the visuovisual conditional discrimination task showing a sample stimulus in the centre and three choice stimuli in the periphery.

Behavioural testing

Previous behavioural experience

Before commencing preliminary training on this task, the subjects had been acclimatized to the apparatus and had been taught in stages to touch visual patterns appearing on the touchscreen for food rewards; they then had on average 18 months of experience in a series of visuospatial memory tasks and a study of exploratory behaviour that did not form part of the present experiment and were reported separately (Kwok and Buckley, 2006; Buckley et al., 2008; Kwok and Buckley, submitted). To control for the effects of previous experience, we provided preliminary training for the task proper, as described below, until each monkey reached its own stable level of performance.

Preliminary training

The preliminary training consisted of three stages: (i) new learning of a 2-choice problem set (problems 1 – 2), (ii) new learning of a 2-choice problem set (problems 3 – 4), (iii) new learning of a 3-choice problem set (problems 5 – 7). Correction trials were employed. The criterion for progression beyond stages i and ii was a performance level of 80% correct or better in a single session whereas the criterion for stage iii was 500 correct responses being accrued by monkeys across sessions. The mean length of the first two stages i and ii was 40 sessions and that of stage iii was 7 sessions. Thus before the animals commenced training on the first experimental set (Set A) they were already well practiced at acquiring these kinds of concurrent visuovisual conditional problems.

The experimental task (Sets A to C)

Each session progressed as follows: in each trial the monkeys were presented with one sample stimulus in the centre of the touchscreen surrounded by three choice stimuli in the periphery of the touchscreen. Whereas the sample stimulus was always presented in the same position in each trial, the three choice stimuli were presented in different positions from trial to trial with the constraint that they were equidistant from the sample and equidistant from each other (the choice stimuli were positioned along an imaginary circle centred on the sample). This design feature was incorporated so that spatial position did not enter into the associations that had to be learnt in this task and to encourage animals to focus on identify which was the behaviourally relevant feature, and not location, which was not.

For each sample stimulus, one out of the three choice stimuli was arbitrarily pre-designated as the rewarded choice (S+) and the other two were designated as foils; the sample-choice-reward contingency for each particular problem remained constant throughout the experiment. Within each problem set, each choice item was designated as correct for one sample item. A trial began with a sample stimulus displayed in the centre of the touchscreen and the three choice stimuli would only be displayed after a touch by the monkey to the sample stimulus. All stimuli then remained on the screen until the computer registered a second touch to the touchscreen. A touch to the S+ was followed immediately by delivery of a reward pellet, the two S- were removed immediately upon the touch, and the S+ remained on the screen alone for a further second to provide visual feedback for a correct response. The screen would then be blanked for an intertrial interval of 5 s before the next trial presentation. Alternatively, a touch to an S- immediately blanked the whole screen and started a longer intertrial interval of 10 s before a correction trial commenced. In the correction trial procedure, separated by the intertrial interval, the

same sample stimulus as on the previously incorrect trial appeared again in the centre but the choice stimuli were now presented in different positions from that on the previous trial. Correction trials were repeated in this manner until the monkeys eventually made a correct response and the numbers and types of errors were recorded. A touch to the sample stimulus had no effect and a touch to a location not occupied by a stimulus also had no effect, excepting for the case where a touch was made to the screen during an intertrial interval which had the effect of restarting that intertrial interval. After successfully completing the final problem in the session, the monkey would be rewarded by the opening of the lunch box. The criterion for completing a session was when 100 correct responses were made within a single session.

Thus in this task, conditional upon the sample stimulus, the monkeys learnt, by trial and error, which one of the three possible choice stimuli was the target that a sample instructed. Six monkeys were tested daily on sets of concurrent visuovisual discrimination problems and would proceed to the next stage once they had accrued 300 correct responses in a problem set. In the task proper, the monkeys started with Set A, and progressed to Set B on the day after accruing 300 correct responses across sessions. On the day after attaining 300 correct responses on Set B they progressed to Set C. Set C was the final set. The monkeys performed one session per day and were trained 5 – 7 days per week until 300 rewards were made for each problem set. The number of trials in each daily session varied depending on how quickly a monkey made 100 correct responses. Within a session each problem in a set was presented once (in random order) before the whole set were reshuffled and presented again in a new random order until the monkey completed the session.

3.4 Results

Preliminary testing

We analysed the total number of errors to criterion between groups in stage i (problems 1 – 2), stage ii (problems 3 – 4) and stage iii (problems 5 – 7) from preliminary training. Independent samples t-tests on the logarithmically transformed data confirmed that there were no differences in learning performance between CON and FNX monkeys in stage i (t = 1.28, df = 4, p = 0.270, 2-tailed), in stage ii (t = 0.23, df = 4, p = 0.833, 2-tailed) and stage iii (t = 1.30, df = 4, p = 0.262, 2-tailed). Thus there was no indication of any differences in task acquisition between groups at this stage.

Total errors accrued in each set

The overall performance of the FNX group was compared with that of the CON group to assess their rates of learning new visuovisual conditional problems. We scored the total number of errors accrued towards attaining the 300 correct responses required for each problem set. The CON group accumulated a mean of 276 (SD = 87) errors in Set A, 253 (SD = 104) errors in Set B, and 305 (SD = 86) errors in Set C. The corresponding means for the FNX group were 192 (SD = 119), 185 (SD = 127), and 200 (SD = 74) errors respectively. Here, and elsewhere in the paper, all of our error data were logarithmically transformed prior to analysis following the recommendations of Kirk (1982). A repeated measures ANOVA, with two levels of the between-subjects factor 'Group' (CON, FNX) and with three levels of the within-subjects factor 'Set' (Sets A, B and C) on the logarithmically transformed data confirmed that that there was no significant main effect of 'Group' [Group: F (1, 4) =

1.17, $p = 0.34$] and no significant Group*Set interaction [Group*Set: F (2, 8) < 1]. Therefore, although FNX animals performed numerically better than CON animals in terms of total number of errors accrued while making 300 rewards across three problem sets, the difference did not reach statistical significance.

As there were two foils on each trial and a correction procedure was employed, if a monkey made an initial error on a problem (touching *any* S- on the trial), then two different types of errors were possible on subsequent trials for that particular problem that we call non-perseverative errors and perseverative errors. We divided up the total errors accumulated by each animal into three mutually exclusive sub-classes of errors, namely first-time errors, non-perseverative repetitive errors, and perseverative repetitive errors. First-time errors (1st time) refer to those errors made by a monkey to a problem on the very first occasion it encountered any problem. As there were three foils (Genovesio and Wise, 2008) on each trial and a correction procedure was employed, if a monkey made a first-time error on a problem (touching *any* S- on the trial), then two different types of repetitive errors were possible on subsequent trials for that particular problem. The first kind of repetitive errors are the non-perseverative errors (non-P) and this refers to those errors made when an animal went on to pick a different S- from the preceding one in the ensuing correction trial. The second type of repetitive errors are perseverative errors (P) which refer to those errors made when an animal went on to choose exactly the same spatial position as chosen erroneously in the preceding correction trial (i.e. touching the *same* S- again). Due to the correction procedure, it was possible for the monkey to accrue a single first-time error in addition to several non-perseverative and/or perseverative errors before a correct response was made which completed that problem.

Fast learning: within-session learning

In order to investigate whether the FNX group might be particularly impeded with their 'fast learning' we examined the rate at which the monkeys could eliminate these kinds of errors during the earlier stages of acquisition of each of the three sets. We ran a 3-way repeated measures ANOVAs on the logarithmically transformed number of errors/problem commissioned during the first 40 trials of learning, containing two levels of the between-subjects factor 'Group' (CON, FNX), three levels of the within-subjects factor 'Set' (Sets A – C) and three levels of the within-subjects factor 'Error-type' (Error-types: 1st time, non-P and P). Although there were no main effect of Group [Group: F (1, 4) = 3.85, $p > 0.1$] and no interactions of Group*Set*Error-type [$p > 0.1$], we found a significant Group*Error-type interaction [F (2, 8) = 6.38, $p = 0.022$, Huynh-Feldt corrected], which prompted us to look at each of the three types of errors independently.

We then ran four 2-way repeated measures ANOVAs, one for each of the four groups of errors (total, 1st time, non-P and P errors), each with two levels of the between-subjects factor 'Group' (CON, FNX) and three levels of the within-subjects factor 'Set' (Sets A – C) on the logarithmically transformed number of errors/problem accrued from the first 40, the first 100 and the first 200 trials during the initial learning stages (these numbers were chosen to represent different stages of acquisition within the first session, see also Brasted et al. (2003)).

The mean numbers of total errors/problem accrued during the first 40, 100, and 200 trials were 4.4 (SD = 1.14), 4.3 (SD = 1.38), and 3.9 (SD = 1.39) for the CON group, and the corresponding numbers were 3.1 (SD = 1.08), 2.7 (SD = 1.14), and 2.3 (SD = 1.10) for the FNX group. We found that the groups were not different in the total number of errors/problem commissioned during the first 40, 100 and 200 trials

[greatest $F = 2.21$, $p > 0.1$]. The mean numbers of 1st time errors/problem accrued during the first 40, 100, and 200 trials were 1.7 (SD = 0.27), 1.6 (SD = 0.36), and 1.5 (SD = 0.32) for the CON group, and the corresponding numbers were 1.3 (SD = 0.25), 1.2 (SD = 0.29), and 1.0 (SD = 0.31) for the FNX group; and that of non-P errors/problem were 1.6 (SD = 0.52), 1.6 (SD = 0.54), and 1.4 (SD = 0.61) for the CON group, and 1.3 (SD = 0.66), 1.2 (SD = 0.67), and 1.0 (SD = 0.59) for the FNX group. The groups were not different in either the numbers of 1st time errors or non-P errors [all $p > 0.1$]. However, the mean numbers of P errors/problem accrued during the first 40, 100, and 200 trials were 1.1 (SD = 0.37), 1.1 (SD = 0.48), and 1.0 (SD = 0.48) for the CON group, and the corresponding numbers were 0.5 (SD = 0.19), 0.4 (SD = 0.23), and 0.3 (SD = 0.23) for the FNX group. We found that FNX monkeys made significantly fewer perseverative errors than controls for the first 40 trials [F (1, 4) = 10.12, $p = 0.033$] and for the first 100 trials [F (1, 4) = 12.24, $p = 0.025$] but despite noticeable numerical differences (see Figure 3) the group difference failed to reach significance when the first 200 trials were analysed [F (1, 4) = 4.43, $p > 0.1$]. All Group*Set interactions for perseverative errors were insignificant [greatest $F < 1$]. Thus the FNX monkeys demonstrated enhanced abilities relative to controls in eliminating perseverative errors during initial stages.

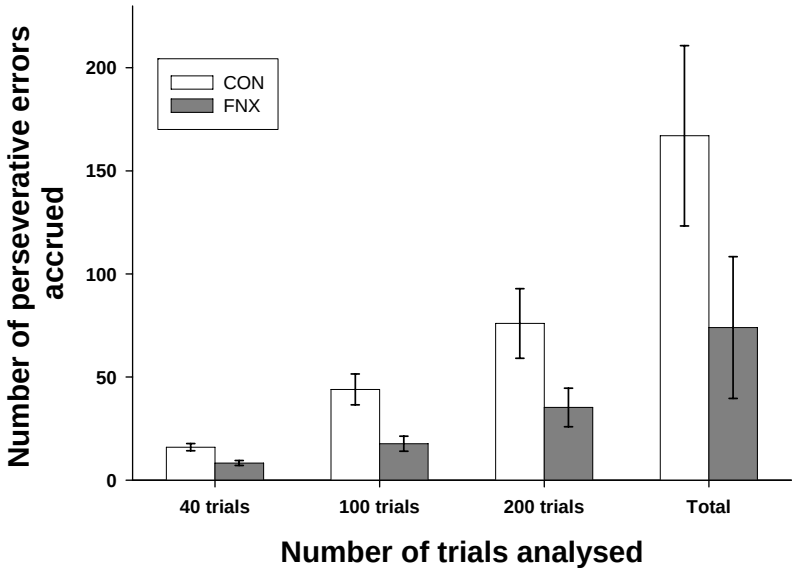


Figure 3. This figure depicts the number of perseverative errors accrued when different numbers of trials were analysed: the first 40, 100, 200 and total number of trials towards making of 300 rewards for the control and fornix transected monkeys.

Abstract response strategies

We recognised the possibility that the monkeys might have employed an abstract response strategy to reduce their error rate, one that they could apply to novel stimuli even before they learning about the specific stimulus-stimulus contingencies (Genovesio and Wise, 2008), thus we also examined whether either group might have adopted response strategies named 'Repeat-stay' and 'Change-shift' strategies on monkeys' performance. In our three-choice task, each trial began with the presentation of a problem selected randomly from the set and with a correction trial procedure, each problem always ended with a correct response. On any subsequent given trial, the monkeys would either be presented with a different problem from the one that appeared in the previous trial, or less frequently, with the same problem as it had been presented with in the previous trial. We called the former 'change trials' and

the latter 'repeat trials'. On repeat trials, if the monkeys' most recent response had yielded a reward, then they could simply remember the sample and its corresponding choice stimulus over the intertrial interval and then just repeat the same response that they had just made if the same sample reappeared in the next trial. This strategy is referred to as 'Repeat-stay'. On change trials, the monkeys could similarly remember the sample and its corresponding choice stimulus over the intertrial interval, and if a different sample appeared in the next trial, they could shift their choice to one of the two remaining possibilities. This strategy is referred to as 'Change-shift'. In a three-choice task, perfect use of the Change-shift strategy would yield a 50% correct return on change trials compared to 33% correct return if choice stimuli were chosen completely randomly. Furthermore, consistent application of the Repeat-stay strategy would lead to a 100% return in repeat trials. Thus, by employing these strategies perfectly, either alone or in combination, the monkeys would be able to demonstrate better than chance performance; and this could occur even in the absence of any learning about which sample mapped on to which choice stimulus.

We analysed the first daily session of each problem set (that is 99 problem trials because no strategy whatsoever could be used on the very first trial) to test whether the monkeys employed these strategies before learning of the problems occurred. The mean percent correct for CON and FNX groups were 45.2% (SD = 11.67) and 59.5% (SD = 10.52) for change trials, and 36.8% (SD = 7.10) and 26.4 % (SD = 18.95) for repeat trials, respectively. Independent samples t-tests confirmed that there were no differences in performance between CON and FNX monkeys for both change trials (t (4) = -1.580, $p > 0.1$, 2-tailed) and repeat trials (t (4) = 0.891, $p > 0.1$, 2-tailed) during the first session. The overall mean percent correct for all six monkeys was 50.0% (SD = 11.49) and an one sample t-test showed that all six monkeys as a whole group

performed better than the chance level of 33% in this three-choice task (t (5) = 3.615, p = 0.015, 2-tailed). Further t-tests show that the monkeys performed better than chance on change trials (t (5) = 3.741, p = 0.013, 2-tailed) but not so on repeat trials (t (5) = -0.241, $p > 0.5$, 2-tailed). This indicates the superior performance could be attributed to a Change-shift strategy on change trials but not to a Repeat-stay strategy on repeat trials as the monkeys benefited from employing the Change-shift strategy, alone, to exceed chance levels of performance.

Moreover, we ran a 2-way repeated measures ANOVA on the combined number of first-time correct responses (wherein response strategy could not be employed to aid learning) from the first 200 trials of each set, with two levels of the between-subjects factor 'Group' (CON, FNX) and ten levels of the within-subjects factor 'First-time correct response' (each level comprised 20 trials), and found neither a significant main effect of 'Group' [Group: F = 1] nor a significant 'Group*First-time correct response' interaction [Group*First-time correct response: $F < 1$]; the linear component of the 'Group*First-time correct response' interaction was also insignificant [F (1, 4) = 1.31, $p > 0.1$]. These suggest that the monkeys did not show any significant group differences in the learning of the problems in the first 200 trials. In fact, the chance level performance on repeat trials also confirms the absence of any learning of the problems in the first session of training. Therefore, we can attribute the monkeys' superior performance to a Change-shift response strategy, even before learning of the visuovisual associations occurred, and we can conclude that the fornix does not mediate the adoption of such a strategy.

3.5 Discussion

Unlike in previous studies which showed that fornix transection impaired the initial learning stages of learning visuospatial and temporally differentiated visuomotor conditional problems, the group of monkeys with bilateral fornix transection in the current study remained unimpaired relative to an unoperated control group in acquiring conditional stimulus-stimulus discriminations. These results therefore fail to support the hypothesis that the fornix supports rapid acquisition of all kinds of concurrent conditional discriminations. Rather, the data support the hypothesis that the fornix is selectively involved in learning arbitrary mappings in the spatial and temporal domains. In fact, we found evidence that the FNX group were actually facilitated in their elimination of perseverative errors during the early course of acquiring these visuovisual conditional discriminations.

In comparison to other fast learning impairments after fornix transection, such as those in learning associations of stimuli and responses that were non-spatially differentiated (Brasted et al., 2003), as well as those in learning conditional visuospatial discriminations (Kwok and Buckley, submitted), the lack of impairments in the present study clearly indicates fast learning deficits after fornix transection do not necessarily generalise to all conditional learning tasks. The present results also provide evidence that the learning deficits produced by lesions of the fornix in the foregoing studies are neither centred on conditional rules nor execution of motor control.

To consider the present findings on a wider anatomical perspective, reference can be made to a previous report which has shown that removal of the hippocampus plus subjacent cortex was without effect in a visual stimulus-stimulus

associative learning task (Murray et al., 1993). This failure of hippocampectomy to affect new learning of stimulus-stimulus associations correspondingly argues against the suggestion that the hippocampus is generally important for all kinds of associative learning. The present study thus extends this finding to the fornix which implies that the wider hippocampal system is not crucial for such tasks.

As mentioned above, we observed not merely an absence of a deficit in the FNX group but in fact we found that the FNX group was significantly facilitated in terms of their reduced perseverative error rate, particularly in the earlier stages of acquisition. One potential explanation for this finding is that whereas control monkeys might attempt to seek out 'spatial' solutions to this task, fornix transected animals, who are known to have deficits in visuospatial learning (e.g. Buckley et al., 2008), might be biased away from such strategies. The constant changing of the positions of choices around the sample in the present task might have primed the CON animals to attempt to find certain spatial, though ultimately non-existent, patterns in these problems, and the finding that FNX animals perseverated significantly less than CON animals in choosing the same foil across successive trials also suggests that FNX animals did not 'over-attend' to changes of stimuli's positions across trials to the same degree as the CON group. This finding, that fornix transection has facilitatory effects, is not an isolated observation as similar facilitatory effects have been observed on a number of other occasions in different tasks after both fornix transection or hippocampal lesions. For example, monkeys with fornix transection have been observed to be significantly facilitated in other non-spatial tasks including object reversal learning (Zola and Mahut, 1973; Mahut et al., 1981) and concurrent object discrimination task (Moss et al., 1981). Fornix transection has also been seen to facilitate acquisition of transverse patterning tasks, another

non-spatial learning task, in both monkeys (Brasted et al., 2003) and rats (Bussey et al., 1998). Moreover, the additional of a hippocampal and parahippocampal cortex ablation to an existing rhinal cortex lesion was observed to significantly reduce the recognition impairment produced by rhinal cortex lesions alone in a delayed non-matching to sample task (Meunier et al., 1996). The authors pointed to the possibility that if hippocampal lesioned monkeys were unable to use information concerning the location of the objects, then they may be more likely to associate rewards with the object identity alone, leading to an enhanced association of reward with the object identity by discounting the location of objects altogether. This idea is speculative but does illustrate another plausible possibility in which disruption of spatial mnemonic processing might yield an improvement in performance in recognition in monkeys with hippocampal damage. This amelioration of a recognition memory deficit after disruption of the hippocampal system can be linked to the facilitation in learning conditional visuovisual problems after fornix transection observed here.

Our detailed analysis of the different kinds of errors sought to probe the nature of the fornix dependent facilitation. We were able to specify that the FNX group benefited from an enhanced and selective ability to minimise their perseverative errors, right from the earliest stages of acquisition. Thus FNX monkeys are superior to CON monkeys in the early stages due to their improved ability to monitoring their most recent actions and correcting them if erroneous. This is in contrast to transverse patterning studies which have shown enhancements following hippocampal damage; in these cases facilitation was not manifested early in acquisition but only became significant later on when a third stimulus pair was introduced to two existing stimulus pairs (Saksida et al., 2007). Similarly, the

facilitatory effect of fornix lesions on acquisition of a transverse patterning problem set was also not observed in first two stages during which only one or two stimulus pairs were involved (Bussey et al., 1998). However, in an absence of systematic analyses of the error subtypes commissioned in Bussey's et al. (1998) and Saksida's et al. (2007) studies, no direct comparison can be drawn to confirm these apparent differences with regard to the period or periods in which a facilitatory effect emerges.

We note that fast learning deficits are not always present in all arbitrary mapping tasks after fornix transection. Irrespective of the nature of tasks, fast learning impairments are generally observed only in tasks that can be acquired quickly (Rupniak and Gaffan, 1987; Brasted et al., 2003; Kwok and Buckley, submitted) and not in tasks that are learnt much more slowly (Gaffan and Harrison, 1988), wherein learning as a whole might be predominantly mediated by a 'slow learning' mechanism.

Our study also sheds light on the role of the fornix in learning to apply abstract response strategies (Genovesio and Wise, 2008). Whereas Brasted et al. (2002; 2003) found no evidence for their control or lesioned monkeys using either 'repeat-stay' and 'change-shift' response strategies in their three-choice non-spatial visuomotor conditional task, in the present study we showed evidence of the adoption of a 'change-shift' strategy but not a 'repeat-stay' strategy in both controls and fornix lesioned animals. That is, after a correctly performed trial if the next sample stimulus changed, our monkeys were more likely to choose one of the two remaining choices and in effect, reduce their error rates by one-third. Indeed, while the monkeys were slow to learn the specific associations instructed by each sample stimulus, they quickly mastered the change-shift strategy and employed it, alone, to exceed chance levels of performance. Fornix transection did not affect the use of the change-shift

strategy. The absence of a repeat-stay strategy in the present study is not unexpected given that our monkeys had less experience of encountering 'repeat' trials in this task.

In summary, the lack of impairment in fornix transected monkeys reported here in a non-spatial conditional visuovisual discrimination learning task poses a challenge to hypotheses which posit a general role for the hippocampal system in the rapid acquisition of all kinds of associations. The finding of a selective reduction in perseverative error rate after fornix transection, lends further support to previous studies which have also observed facilitated learning after fornical or hippocampal damage. Our finding that fornix transection does not impair learning non-spatial visuovisual mapping alongside previous studies reporting that fornix transection does impair learning visuospatial and visuomotor mappings is consistent with the idea that one role of some of the fibres coursing through the fornix (Vann et al., 2000) is to selectively support rapid learning about the context of stimuli. This is consistent with previous studies that speak towards a fornical role in the learning about the spatial and temporal relationships between stimuli (Charles et al., 2004; Wilson et al., 2007).

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CHAPTER 4

FORGETTING OVER THE VERY LONG-TERM IS UNAFFECTED BY FORNIX TRANSECTION

Sze Chai Kwok & Mark J. Buckley
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CHAPTER 4: FORGETTING OVER THE VERY LONG-TERM IS UNAFFECTED BY FORNIX TRANSECTION

4.1 Abstract

As part of an earlier experiment, six macaque monkeys (three with fornix transection and three unoperated controls) were trained postoperatively to discriminate a total of 104 new concurrent visuospatial conditional problems to criterion. The present experiment measured and compared long-term retention of these problems with two 1-trial postoperative retention tests administered 3 and 15 months respectively after acquisition. All animals showed some forgetting of these problems but still remembered well above chance after 15 months. The amount forgotten by each group did not differ significantly at either time point. These results show that long-term retention of visuospatial information and the reliability in retention between tests are independent of the fornix. The present findings suggest the fornix transected monkeys learnt the task differently from the control monkeys, perhaps with less emphasis placed on contextual cues; and that forgetting could be attributable to either interference or memory decay, or both. A similarity in resistance to forgetting was drawn between fornix transected macaques and patients with anterograde amnesia and its implications on clinical rehabilitation are discussed.

4.2 Introduction

Whereas many reports have indicated that anterograde amnesia in humans may be linked to damage to the fornix (e.g. Gaffan and Gaffan, 1991; Gaffan et al., 1991; Hodges and Carpenter, 1991; McMackin et al., 1995; Aggleton et al., 2000; Papanicolaou et al., 2007; Vann et al., 2008), the evidence that fornix damage produces retrograde amnesia in humans is less strong (Hodges and Carpenter, 1991; Poreh et al., 2006). On the other hand, it is established in macaque monkeys, where highly selective and complete fornix transection may be surgically induced, that fornix transection affects new postoperative learning far more than it affects retrieval of preoperatively acquired memories. For example, earlier studies which looked at the effects of fornix transection upon retention of postoperatively learnt discriminations between complex naturalistic scenes (Gaffan, 1993), or within algorithmically generated complex scenes (Gaffan, 1994), found that while retention was spared in both studies, new learning was impaired. In Gaffan's (1993) study, unoperated control and fornix transected monkeys were trained postoperatively to learn to discriminate among 320 complex naturalistic scenes and were subsequently tested on their extent of forgetting of these scenes after reaching criterion. Despite a reduced rate of acquisition of the fornix transected monkeys, the number of forgotten scenes was equal in the two groups of monkeys on a retention test 49 days later. However, neither the complex naturalistic scene task nor the algorithmically generated complex scene task necessarily require spatial memory per se (as memories for foreground objects or background detail can aid performance of each), so it is not possible to infer from these studies that retrieval of spatial memory is unaffected by fornix transection.

For this reason, Buckley et al. (2004) assessed the effects of fornix transection on a more direct test of spatial memory using a concurrent spatial discrimination learning task. In that study, Buckley and colleagues (2004) found that immediate postoperative retention of 40 preoperatively learnt problems was unaffected, while subsequent new learning of similar problems was impaired. Albeit with a different kind of stimuli, Buckley et al. (2008) similarly showed that immediate postoperative recall of 288 preoperatively learnt visuospatial problems likewise remained completely intact after fornix transection, while new learning was again observed to be impaired. Both of these studies employed an 1-trial postoperative retention test of large numbers of preoperatively learnt problems to avoid confounding retention with re-learning. Nevertheless, the retention tests in these two studies (and also in Gaffan, 1994) were administered within a relatively short period of time after problems were acquired and after surgery was administered (approximately 2 weeks on average). Thus these studies, while providing evidence that fornix transection does not affect short-term retention, do not speak towards the effect of fornix transection on long-term retention.

Although Gaffan's (1993) study employed a longer period of 49 days before a subsequent retention test was given, as highlighted above, the paradigm used does not necessitate spatial memory per se and the delay before retention was not particularly long. Here, in order to facilitate a robust assessment of the effect of time on long-term spatial memory acquired after fornix transection, we carried out an experiment on fornix transected and control monkeys, employing two 1-trial postoperative retention tests, both administered after much greater periods of time had elapsed after acquisition, namely 3 and 15 months after postoperative learning occurred. The same animals were previously reported to be impaired in the initial

phases of acquisition of these problems (Kwok and Buckley, submitted).

4.3 Materials and Methods

Subjects and surgery

The subjects were six male cynomolgus monkeys (*Macaca fascicularis*). The monkeys had previously been acclimatized to an automated test apparatus (described below) and had been taught in stages to touch visual patterns appearing on the touchscreen for food rewards, and then had on average 10 months of experience in a visuospatial concurrent discrimination learning task that did not form part of the present experiment and which has been reported separately (Buckley et al., 2008). During the course of that study the six animals were divided into two performance matched groups, one group (CON group) remained unoperated controls and the other received bilateral transection of the fornix (FNX group). After that study was completed the six animals commenced further postoperative testing involving acquisition of a further 104 new postoperative visuospatial conditional problems of a different kind (i.e. the kind described in this study) and the FNX group was reported to be impaired in the early stages of acquisition of these problems (Kwok and Buckley, submitted). The current study, which concerns only the analyses of the long-term forgetting of these 104 problems, took place subsequently, and was completed 15 months later. In between the two retention tests of this study, the monkeys were also trained to discriminate randomly changing pairings in an object discrimination learning task (Wilson et al., 2007). The mean weights of the animals at the start of this study was 6.1 kg (range 5.2 kg to 7.0 kg), and their mean age was 4 years and 9 months.

Microscopic examination of the stained histology sections confirmed in every case a complete section of the fornix with no damage outside the fornix except for the

incision in the corpus callosum and at most, only slight damage to the most ventral part of the cingulate gyrus at the same anterior-posterior level in only one hemisphere of one animal. Detailed photomicrographs of the histology for the lesioned animals together with descriptions of the surgical method have already been presented elsewhere (Buckley et al., 2008). All licensed procedures were carried out in compliance with the United Kingdom Animals (Scientific Procedures) Act of 1986.

In common with most ablation experiments using macaque monkeys, this study did not have an operated control group. This means that we cannot completely rule out the possibility that the effects seen in the animals with fornix transection were because of the callosal incision, the anesthesia, or some other component of the surgical operation other than the fornix transection itself. However, in earlier experiments with fornix transection an operated control group was sometimes included, and a change from the control group's preoperative ability was never reported (Gaffan et al., 1984a; Gaffan and Saunders, 1985). Similarly in the human brain, patients with fornix transection in the course of colloid cyst removal showed memory impairments, but those patients in which a similar surgical operation was performed while leaving the fornix intact showed no memory impairments (Aggleton et al., 2000).

Apparatus

The present task was performed in an automated test apparatus. The subjects were sat in a wheeled transport cage that was secured about 60 cm opposite a touch-sensitive screen (380 mm wide and 280 mm high) on which the stimuli could be displayed. The subject could reach out between the horizontal bars (spaced approximately 50 mm apart) at the front of the transport cage to touch the

touchscreen. Banana-flavoured reward pellets (190 mg; P. J. Noyes, Lancaster, NH) were delivered by an automated pellet delivery system, accompanied by an audible click, into a hopper centrally placed beneath the touchscreen, in response to correct choices made by the subject. An automated lunch box was positioned in front of and to the left of the subject. It was spring-loaded and opened immediately with a loud crack on completion of the task to deliver the animal's daily diet of wet monkey chow, pieces of fruits, raisins, and peanuts.

Behavioural testing: acquisition of visuospatial problems and retention tests

Each of the problems in the experiment consisted of four identical multi-coloured cartoon-like stimuli (each subtending a visual angle of approximately 11.5^0) presented upon a white background (see Figure 1). The four identical stimuli in each problem were presented in four fixed positions, symmetrically with two on top and two at the bottom on the touchscreen. A total of 120 images were used in the experiment (16 for preliminary training and 104 for experimental testing as described below).

For each problem, one out of the four positions in which the four copies of the identical stimulus appeared was pre-designated as the rewarded position (S+) and the other three positions were unrewarded foils; the position-reward contingency for each particular problem remained constant throughout the experiment and there were equal numbers of problems in which each of the four positions were rewarded. Thus, conditional upon a problem's object identity, the monkeys learnt, by trial and error, which one of the four possible positions was the choice that each problem instructed.

In Kwok and Buckley's (submitted) study each monkey received preliminary training in stages on 16 initial problems (problems 1 – 16) after which they

commenced acquisition of three sets of concurrent visuospatial discrimination problems of different set sizes. Set A comprised 8 problems, Set B comprised 32 problems, and Set C comprised 64 problems; they commenced learning Set A and progressed to the next two sets in turn on the day after attaining a performance level of 90% or better in a single daily session on the previous set. For each trial, a correct response was followed immediately by delivery of a reward pellet whereas trials that ended with an incorrect response were followed by correction trials, that is, repeated presentation of the same problem. Correction trials were repeated an unlimited number of times until a correct response was achieved. Normally each daily session comprised 128 trials in total. The monkeys performed one session per day and were trained 6 – 7 days per week until all three sets (and therefore 104 problems in total) were acquired to a 90% performance level criterion.

In the current study the monkeys were then given two retention tests (RET1 and RET2), administered independently 3 months and 15 months after they had reached the criterion on the last problem set, to assess long-term retention. In the retention tests, each of the 104 problems which they had acquired earlier (in Sets A to C) was now presented just once in a single session to avoid confounding retention with re-learning. For that monkeys had one single trial for the recall of each problem, we called this paradigm an 1-trial postoperative retention test. On the day prior to each of the retention testing days, a short refamiliarization test was given to remind the monkeys about the testing conditions and to ensure that the monkeys had not altered their motivation as a consequence of the extended breaks from testing. The refamiliarization test comprised just 12 problems (taken from the preliminary training stage) and each was presented in three repetitions in this single session. The order of presentation of stimuli in both retention tests was randomised and these 12 problems

did not appear again in the retention tests proper. Only performance data from the retention tests proper were analysed.

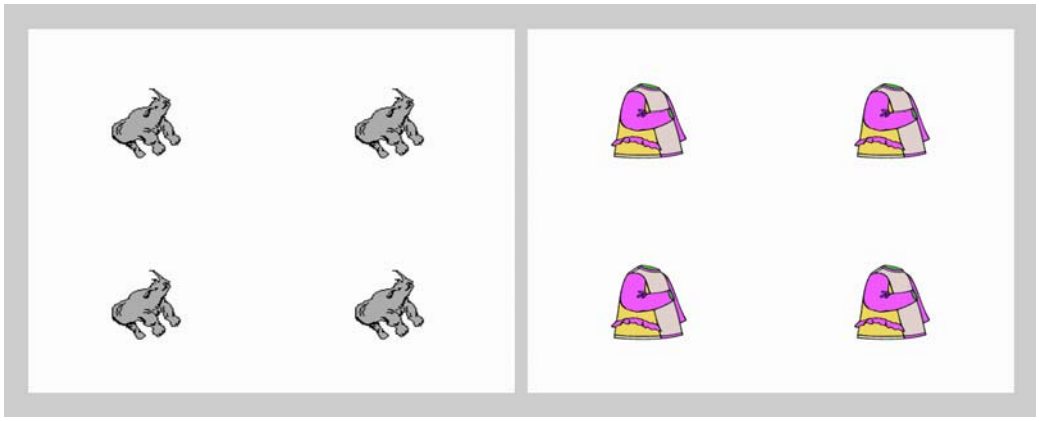


Figure 1. These two panels show two illustrative examples of problems from the visuospatial conditional discrimination.

4.4 Results

Acquisition of problems

Detailed analyses of the acquisition phases have been reported in Kwok and Buckley's study (submitted). In short, although there were no differences in the total number of errors to criterion between groups in learning the 104 problems to criterion, the FNX group were significantly impaired in making non-perseverative errors, relative to the CON group, at the early stages of acquisition. This deficit in the FNX group at this stage was primarily one of eliminating errors in the correction trials.

Retention tests

In the current study we report analyses of the retention of these 104 problems by the FNX and CON monkeys at two independent time points, 3 months and 15 months, after they had learnt the problems to criterion. The retention rates of these 104 problems for the CON group were 54.2% (SD = 6.75) and 45.5% (SD = 8.04) respectively at the end of 3 months and 15 months, the corresponding retention rates for the FNX group were 55.8% (SD = 6.93) and 49.4% (SD = 6.18). Based on the fact that all individual monkeys had attained 90% performance level before these retention tests were carried out (i.e. when the problems were learnt to criterion), paired samples t-tests showed significant forgetting in both groups after 3 months [CON: t (2) = 9.19, p = 0.012; FNX: t (2) = 8.55, p = 0.013] and after 15 months [CON: t (2) = 9.58, p = 0.011; FNX: t (2) = 11.39, p = 0.008]. In order to ascertain whether forgetting proceeded at the same rate in the two groups, we ran a 2-way repeated measures ANOVA with two levels of the between-subjects factor 'Group'

(CON, FNX) and two levels of the within-subjects factor 'Time' (retention rates at 3 months and 15 months) on the percentage of correct responses from the retention tests. We found neither a main effect of 'Group' [Group: F (1, 4) < 1] nor a main effect of 'Time' [Time: F (1, 4) = 2.94, p > 0.1]. The 'Group*Time interaction' was also insignificant [Group*Time: F (1, 4) < 1]. These suggest that FNX animals were not more impaired than CON animals in their retention of these 104 problems at either time point, thus the groups did not differ in their forgetting rates. We also found that despite the forgetting exhibited by both groups of monkeys over a period of 15 months, the animals still remembered above a chance level of 25% (as our task was a series of independent four-choice problems) after 3 months [CON: t (2) = 7.48, p = 0.017; FNX: t (2) = 7.69, p = 0.017] and after 15 months [CON: t (2) = 4.43, p = 0.047; FNX: t (2) = 6.83, p = 0.021].

In order to test whether animals retained the same specific problems as each other, we ran a series of pairwise comparisons between individual animals within groups to assess what degree of correspondence there might be between individuals with regard to which specific problems were remembered; this was done for both retention tests separately. All but two within-group correlations between individuals were insignificant. The mean correlation coefficients on RET1 and RET2 for the FNX group were 0.127 and 0.161; the corresponding coefficients for the CON group were 0.199 and 0.108. There were no group differences [all p > 0.5], suggesting that there was no correspondence between which particular problems individual monkeys remembered within, or between, groups. Moreover, we also ascertained the proportion of problems correctly remembered at the first retention test that were subsequently also retained in the second retention test. In RET2, the CON group remembered a mean of 58% of the problems that were retained correctly on RET1,

the corresponding mean for the FNX group was 60%. There were no group differences in this respect [$p > 0.5$].

Taken together, these analyses suggest that, while all monkeys demonstrated significant forgetting, the FNX group exhibited a forgetting rate indistinguishable from that of CON monkeys, as determined by our samples of how much and what was forgotten at 3 and 15 months after acquisition respectively. We conclude that long-term retention of these visuospatial problems is not fornix dependent.

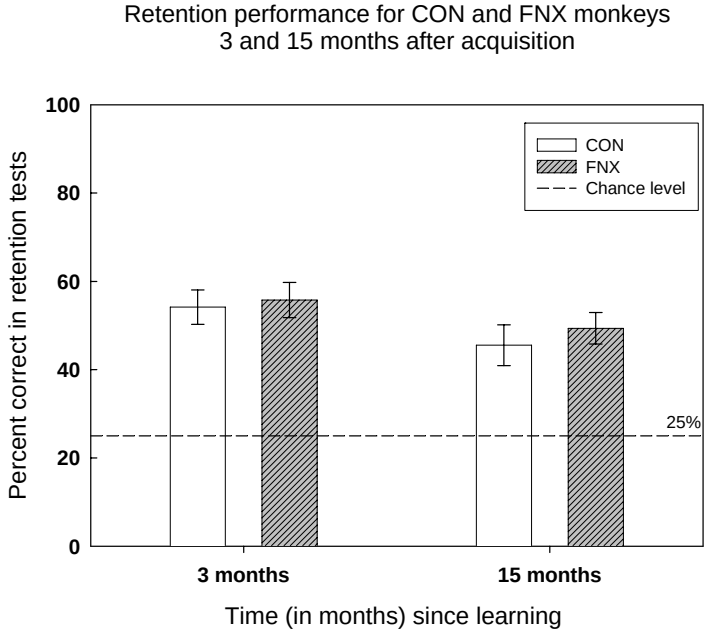


Figure 2. This figure depicts the level of retention of 104 visuospatial concurrent discrimination problems in two retention tests administered 3 and 15 months respectively after postoperative learning to a 90% performance criterion occurred.

4.5 Discussion

The present finding adds to existing evidence (Gaffan, 1993, 1994; Buckley et al., 2004; Buckley et al., 2008) that the slower learning rate following fornix transection was not caused by a susceptibility to forgetting as the extent of forgetting observed in this study was found to be equal in both control and fornix transected monkeys after 3 and 15 months. Thus the current study extends Gaffan's (1993) claim that impairment in discrimination memory does not result from an accelerated rate of forgetting over a period of 49 days, to a much longer period of time, namely up to 15 months.

In addition to the finding that specific problems remembered on RET1 were also more reliably retained on RET2 in both groups of monkeys, we showed that fornix transected monkeys, while clearly being unimpaired compared to control monkeys in long-term retention memory, still remembered the problems significantly above chance even after 15 months. The observation of a lesion-independent long-term retention of some problems in memory in fornix transected monkeys leads us to believe that once a memory trace is laid down, some components of the trace are preserved for at least 15 months (extrapolation of forgetting rates would suggest for much longer periods than this), and that the fornix is not implicated in the long-term storage of such memories.

Previous studies have shown that the fornix is important for discriminating between scenes that vary in the spatial arrangements of features (Gaffan and Harrison, 1989b) and for learning about the spatial organization of complex scenes (Gaffan, 1991), moreover the fornix is necessary for spatial configural learning (Buckley et al., 2004 Exp. 3) and spatial conditional learning paradigms (Gaffan and

Harrison, 1989a). Although the role of the fornix in spatial learning is well established, it is equally apparent that the fornix is not necessary for all forms of spatial learning. Discriminating among locations in space, and associating those locations with objects is independent upon the fornix (Murray et al., 1989), as is place discrimination if the scenes are sufficiently distinct (Gaffan and Harrison, 1989b). In the present task, the 'scenes' are unique to each problem, moreover, even spatial conditional discriminations are found to be unimpaired after fornix transection providing that they are successively and not concurrently learnt (Gaffan et al., 1984b Exp. 6), and so the use of a correction procedure in the current study might arguably promote some degree of successive learning. Thus it is likely that learning of the current task, in normal animals, may proceed by a combination of fornix dependent and fornix independent learning strategies. It would follow that recently observed impairments following fornix transection in concurrent spatial discrimination learning paradigms (such as Buckley et al. (2004) and Buckley et al. (2008)) might be attributable to deficient contextual or configural learning rather than a loss of simple spatial learning per se. We speculate that fornix transected monkeys can employ spatial learning strategies that are independent of the fornix particularly when they are faced with tasks of a familiar format that do not place high demands on contextual and configural learning. This hypothesis is consistent with Gaffan's (1993) suggestion that the fornix transected animals learnt differently from control animals, relying more on local object cues and less on whole-scene spatial cues than the normal animals. Nevertheless, our study shows that even if CON monkeys do have additional strategies for learning compared to FNX monkeys, both groups forget at the same rate and hence different routes to learning do not dictate how things are subsequently remembered. That short-term retention is unaffected by fornix

transection has previously been established (Gaffan, 1993; Buckley et al., 2004; Buckley et al., 2008).

It is established that longer retention intervals lead to more forgetting (King et al., 2002) and forgetting functions typically decrease with time, as is evident with the present findings too. At least three candidate factors have been proposed to explain forgetting in animals, most commonly proactive interference (PI), retroactive interference (RI) and memory trace deterioration (memory decay) (see Wixted, 2004 for a review). For example, it is known that prior learning can profoundly affect the forgetting of subsequently learnt material (Underwood, 1957), particularly when the prior learning trials were massed (Underwood and Ekstrand, 1966, 1967). Ample evidence also points to a theory of forgetting processes associated with the formation of new memories which retroactively interfere with previously formed memories that are still undergoing the process of consolidation (Villarreal et al., 2002; Wixted, 2004). With respect to the current study, the monkeys were trained on tasks such as concurrent spatial discrimination learning (Buckley et al., 2008) prior to commencement of the current study and were exposed to tasks including object discrimination learning with randomly changing pairings (Wilson et al., 2007) before the retention tests. According to the interference theories, these pre- and post-exposure to various kinds of learning problems and scenarios should lead to both proactive and retroactive interferences to the learnt material in the current task, and so the extent to which each of these interfering factors contributes is difficult to determine. One also cannot rule out that time-dependent ‘trace deterioration’ itself may also play a significant role in forgetting (Bailey and Chen, 1989).

The present results, together with previous findings in fornix transected monkeys (Gaffan, 1993; Buckley et al., 2004; Buckley et al., 2008) and in amnesic

patients (Huppert and Piercy, 1978; Squire, 1981; Kopelman, 1985), provide support to the notion that anterograde memory deficits ascribed to fornical disruption in amnesic patients and macaques are unlikely caused by increased susceptibility to forgetting. From a rehabilitation viewpoint, the literature has described a number of memory enhancement techniques, including visual imagery, the PQRST (Preview, Question, Read, State, and Test), rehearsal techniques and errorless learning (Tailby and Haslam, 2003; Clare and Jones, 2008), in the rehabilitation of amnesic patients (Miotto, 2007). Our data here imply that if appropriate techniques are utilized to teach amnesic patients to learn certain associations or re-acquire what was forgotten, patients may not only eventually succeed in acquisition, but importantly, may retain what has been learnt at levels similar to the retention abilities of normal healthy subjects.

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CHAPTER 5

FORNIX TRANSECTION IMPAIRS SPATIAL RECOGNITION MEMORY IN MACAQUES

Sze Chai Kwok & Mark J. Buckley
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CHAPTER 5: FORNIX TRANSECTION IMPAIRS SPATIAL RECOGNITION MEMORY IN MACAQUES

5.1 Abstract

Whereas the role of the fornix in associative learning is well established, its role in recognition memory is less clear. While several studies have examined the effects of fornix transection in macaques on object recognition memory, only one study has to our knowledge investigated the importance of the macaque fornix for spatial recognition memory, and in that it was done in the context of a t-maze. Here we tested the postoperative ability of macaque monkeys with and without fornix transection to perform an automated delayed matching to position task presented on a touchscreen. The fornix transected group (FNX) were unimpaired in acquiring the basic task but were significantly impaired when the delays between sample and choice phases were varied in Experiment 1; however the FNX group remained unimpaired when the spatial separation between choice stimuli was varied in Experiment 2. The unimpaired performance of the FNX group in the acquisition phase and in Experiment 2 appears to rule out explanations based simply in terms of motor, percept or motivational deficits. The absence of delay-dependent deficit in Experiment 1 suggests that the deficit is not necessarily in memory per se. Rather, we propose that fornix transection leads to an impairment in acquiring new task-sets in certain circumstances. Our data also suggest that the contributions of the fornix and hippocampus to recognition memory are dissociable.

5.2 Introduction

Recognition is the ability to judge whether stimuli, information, or events have been encountered before. It is argued that tasks that assess recognition, including standard recognition memory paradigms such as delayed matching or non-matching to sample (DMS and DNMS), may draw upon both knowledge that the information was learnt (i.e. familiarity) as well as remembrance of specific contextual details about the situation in which the information was learnt (i.e. recollection) (e.g. Mandler, 1980). Disagreement exists about the neural substrates of these two mental states. Whereas some authors argue that the hippocampus is necessary for both recollection and familiarity (for review see Squire et al., 2007), others suggest that the hippocampus mediates only recollection while other structures in the medial temporal lobe, such as the perirhinal cortex, mediate familiarity (for reviews see Brown and Aggleton, 2001; Eichenbaum et al., 2007).

The evidence from hippocampal lesions in macaque monkeys is equivocal on this debate; some studies have shown that neurotoxic hippocampal lesions produce substantial deficits on object recognition memory (Zola-Morgan and Squire, 1986; Beason-Held et al., 1999), whereas other studies show mild deficits (Zola et al., 2000), or no deficits at all (Murray and Mishkin, 1998; Nemanic et al., 2004). Possible accounts of these discrepancies have been considered elsewhere (Baxter and Murray, 2001).

The fornix is a major input/output pathway of the hippocampus and accordingly, transecting the fornix has often been considered a useful means of indirectly assessing the role of the hippocampal system. In this respect, fornix transection has some advantages in that, unlike neurotoxic lesions which are rarely

complete, complete section of the fornical tract can be assured in every animal, and there is very minimal damage to other structures. However, caution has to be exercised as although fornix transection often produces similar effects to neurotoxic hippocampal lesions (monkeys are shown to be equally impaired on place-in-scene learning (Gaffan, 1994; Murray et al., 1998), delayed matching to location (Murray et al., 1989; Hampton et al., 2004), but unimpaired on concurrent object discrimination (Mahut et al., 1982; Gaffan, 1994; Teng et al., 2000), as well as similarly facilitated on transverse patterning tasks (Bussey et al., 1998; Saksida et al., 2007)), this is not always the case (as evidence show that the effects of fornical and hippocampal lesions are dissociable in visuomotor learning (Brasted et al., 2005), place-discrimination and reversal (Mahut, 1972; Murray et al., 1998), object discrimination and reversal (Mahut, 1972; Murray et al., 1998), as well as in long-lasting DNMS memory (Zola-Morgan et al., 1989) in macaques. Similar dissociations are also seen in rodents (McDonald et al., 1997; Dumont et al., 2007)). Indeed, it is known that fornix transection disrupts functioning in areas beyond the hippocampus, and that the fornix carries some fibres that are non afferent or efferent to the hippocampus too (Vann et al., 2000; Saunders and Aggleton, 2007) so it is therefore important to assess the importance of the fornix in its own respect.

Although a number of studies have reported object recognition memory impairments following fornix transection in macaques (Gaffan, 1974; Gaffan, 1976, 1977a, b; Gaffan and Weiskrantz, 1980; Owen and Butler, 1981, 1984; Bachevalier et al., 1985b; Bachevalier et al., 1985a), this is not always the case, particularly when interference from using repeated stimuli within and across days is removed by using trial-unique stimuli (Mahut et al., 1982; Gaffan et al., 1984; Owen and Butler, 1984; Zola-Morgan et al., 1989; Charles et al., 2004). To the extent that fornix transection is

capable of informing about more general hippocampal system function, these studies also indicate that the hippocampal system may not always be necessary for recognition memory.

Many studies have indicated the existence of some extent of functional specializations within the medial temporal lobe according to stimulus domain. Whereas the hippocampus (Parkinson et al., 1988; Angeli et al., 1993; Murray et al., 1998) and the fornix (Gaffan and Harrison, 1989; Gaffan, 1994; Buckley et al., 2004; Buckley et al., 2008) have both been strongly implicated in spatial memory processes, the perirhinal cortex has been strongly implicated in object processing (Buckley and Gaffan, 1997, 1998b, 1998c; Bussey et al., 2002, 2003; Buckley, 2005; Bussey et al., 2005). Accordingly, a number of studies have turned to investigate the effects of hippocampal damage on spatial recognition memory paradigms. Some studies have shown that monkeys with neurotoxic hippocampal lesions were not impaired on tasks such as recognising spatial locations (Murray and Mishkin, 1998; Malkova and Mishkin, 2003; Bachevalier and Nemanic, 2008), whereas others show that monkeys with the same lesions were impaired on 10-min delayed non-matching to location (Alvarado and Bachevalier, 2005), on memory for locations in an open-field (Hampton et al., 2004), and on remembering long lists of locations (Beason-Held et al., 1999). However, despite these findings on hippocampal damage, it is not yet known whether fornix transection impairs spatial recognition memory.

This study therefore aimed to determine the effects of fornix transection on a delayed matching to position task in macaques. We tested the monkeys on five levels of delay intervals and four levels of spatial separations in the first and second experiments respectively to assess whether the effects of fornix transection on spatial recognition memory are delay dependent (Zola-Morgan et al., 1992; Zola et al., 2000;

Charles et al., 2004) or spatial separation dependent as shown in rodents with hippocampal lesions (Gilbert et al., 1998; Gilbert et al., 2001; Gilbert and Kesner, 2006). If fornix transection led to general impairment on this task then it would be consistent with the idea that the hippocampal system has a general role in recognition memory, and given the status that some authors accrue to recognition memory as a test of declarative memory, further support theories that consider the hippocampus to be important for both familiarity and recollection. If on the other hand, the task remained entirely unimpaired after fornix transection, then the idea that the hippocampal system is generally involved in recognition memory would be weakened, as would the idea that the hippocampus is generally involved in all kinds of spatial processing.

5.3 Materials and Methods

Subjects

Six male cynomolgus monkeys (*Macaca fascicularis*) took part in this experiment. Their mean age was 6 years and 6 months at the start of behavioural testing with a mean weight of 7.5 kg (range 7.0 kg to 8.25 kg). All six monkeys had identical pre- and postoperative experience in a series of experiments, with some of the results reported elsewhere. These studies include, in a chronological order: a two-choice concurrent visuospatial discrimination task (during which the three animals in the current FNX group received their fornix transection) (Buckley et al., 2008), a four-choice concurrent visuospatial discrimination task (Kwok and Buckley, 2006a), a study of exploration of a large arena (Kwok and Buckley, 2006b), a concurrent object-object discrimination task (Kwok and Buckley, submitted), and tasks that involved concurrent object discriminations (Wilson et al., 2007). They were all housed together in a group enclosure (except for one who was housed in a pair with another animal not involved in this experiment) in an enriched environment in which they were also able to forage daily for small food items (seeds etc) and all with water available *ad libitum*.

Surgery

Three monkeys received bilateral fornix transection (group FNX) during the course of a previous experiment (Buckley et al., 2008) and the other three animals remained unoperated controls (group CON). All licensed procedures were carried out in compliance with the United Kingdom Animals (Scientific Procedures) Act of 1986. The operations were performed in sterile conditions with the aid of an

operating microscope, and the monkeys were anesthetised throughout surgery with barbiturate (5% thiopentone sodium solution) administrated through an intravenous cannula. A D-shaped bone flap was raised over the midline and the left hemisphere up to the midline. The dura mater was cut to expose the hemisphere up to the midline. Veins draining into the sagittal sinus were cauterized and cut. The left hemisphere was retracted from the falx with a brain spoon. A glass aspirator was used to make sagittal incision no more than 5 mm in length in the corpus callosum at the level of the interventricular foramen. The fornix was sectioned transversely by electrocautery and aspiration with a 20-gauge metal aspirator insulated to the tip. The dura mater was drawn back, the bone flap was replaced, and the wound was closed in layers. The operated monkeys rested for 11 – 14 days after surgery before beginning postoperative training. Unoperated CON monkeys rested for the same period of time between preoperative and postoperative training.

Histology

At the conclusion of this experiment, the animals with fornix transection were deeply anaesthetised, then perfused through the heart with saline followed by formol-saline solution. The brains were blocked in the coronal stereotaxic plane posterior to the lunate sulcus, removed from the skull, and allowed to sink in sucrose-formalin solution. The brains were cut in 50 μm sections on a freezing microtome. Every fifth section was retained and stained with cresyl violet. Microscopic examination of the stained sections revealed in every case a complete section of the fornix (see Figure 1, panels B, C and D) with no damage outside the fornix except for the incision in the corpus callosum as described in the surgical procedures and at most, only slight damage to the most ventral part of the cingulate

gyrus at the same anterior-posterior level in only one hemisphere of one animal (Figure 1, panel B). A coronal section of a normal control monkey's brain with the fornix intact is also shown for comparison (Figure 1, panel A).

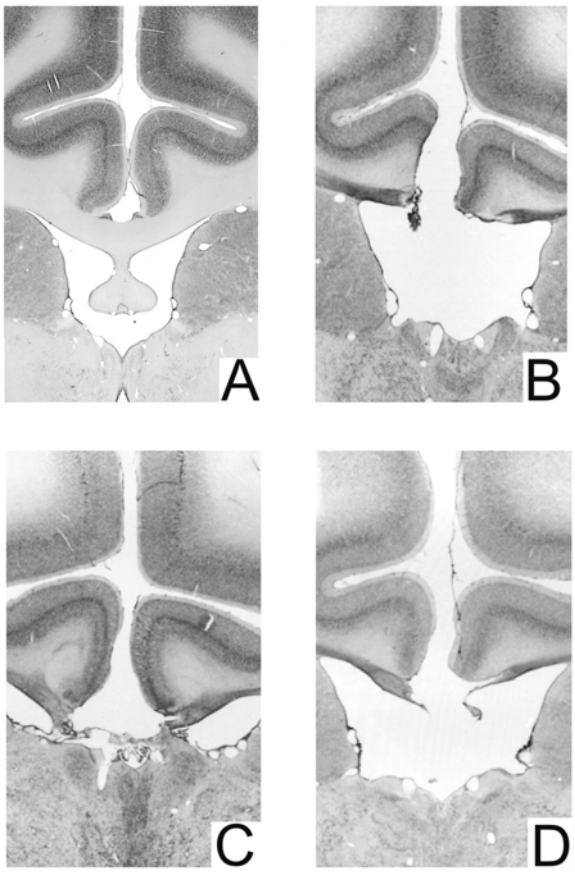


Figure 1. Panel (A): Coronal section from the brain of a normal unoperated macaque just posterior to the level of the interventricular foramen; Panels (B, C, D): Coronal sections from the brains of three fornix transected monkeys showing that the fornix transection was complete (the anterior-posterior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

Apparatus

The present tasks were performed in an automated test apparatus. The subject sat in a wheeled transport cage fixed in position about 60 cm in front of a touch-sensitive screen (380 mm x 280 mm) on which the stimuli could be displayed. The subject

could reach out between the horizontal bars (spaced approximately 50 mm apart) at the front of the transport cage to touch the touchscreen. An automated pellet delivery system, controlled by the computer, delivered reward pellets into a food well (80 mm in diameter) positioned in front of and to the right of the subject. Banana-flavoured reward pellets (190 mg; P. J. Noyes, Lancaster, NH) were delivered only in response to a correct choice made by the subject to the touchscreen. Pellet delivery was accompanied by an audible click. An automated lunch box (length 200 mm, width 100 mm, height 100 mm) was positioned in front of and to the left of the subject. It was spring-loaded and opened immediately with a loud crack on completion of the task to deliver the animal's daily diet of wet monkey chow, pieces of fruits, raisins, and peanuts. A closed-circuit TV infrared camera positioned above the touchscreen and in front of the monkey was used for observation from another room from which the stimulus display, food delivery, and experimental contingencies were computer-controlled. The entire apparatus was housed in an experimental cubicle that was dark apart from the background illumination from the touchscreen.

Behavioural Testing

Overview

Monkeys began pretraining and learning of the experimental tasks described in this paper postoperatively after a series of other tasks described above were completed (the CON group had identical experience, apart from that they rested for an equivalent period of time instead of having fornix transection). The spatial recognition memory test we employed required the animal, in each trial, to distinguish a familiar (i.e. previously presented as a 'sample') spatial position from a novel one (i.e. a position different from that of the 'sample') on the touchscreen. Each

trial consisted of two parts: a sample phase followed by a choice phase. In the sample phase the monkey was first presented with a sample (a red cross) in a pre-designated position on the touchscreen. After the monkey touched the sample a distracting cue (a blue square) appeared in the centre and remained on the screen until the animal subsequently touched this cue too. Immediately following a touch to the blue square, the choice phase commenced wherein two identical red cross stimuli appeared on the screen. One of these was the correct choice (S+) and this red cross appeared in the identical position to the red cross in the sample phase; the second red cross was the incorrect foil and this stimulus appeared in a position that was different to that of the preceding sample. A touch to the S+ was followed immediately by a delivery of a reward pellet, the S- was removed immediately upon the touch, and the S+ remained on the screen alone for a further second to provide visual feedback for a correct response. The screen would then be blanked for an interval of 6 s before the next trial presentation. Alternatively, a touch to an S- immediately blanked the screen and started a longer intertrial interval of 12 s before the next trial. There was no correction trial procedure in this experiment. A touch to a location not occupied by any stimulus had no effect at any stage, except for the case where a touch was made to the screen during an intertrial interval which had the effect of restarting that intertrial interval. Normally a session would be completed when the animal obtained 100 rewards in the task acquisition stage in Experiment 1, or 150 rewards in Experiment 2. Upon the last rewarded trial in a session the monkey would be rewarded by the opening of the lunch box containing its daily food ration.

The resolution of the visual display on the touchscreen was set at 800 x 600 pixels and the sample stimuli subtended visual angles of approximately 9^0 in preliminary training and in Experiment 1; and 6.8^0 in Experiment 2 from the typical

viewpoint and perspective of a macaque in its transport cage. The distractor stimulus (blue square) subtended a visual angle of 4.6^0 in all stages.

Task acquisition

The monkeys were preliminarily trained on the task with gradually increasing numbers of rewards across daily sessions until they reached a 90% performance level or better within a 100-reward session at which point we were confident that the monkeys were already well practiced at acquiring these kinds of delayed matching to position problems. All trials in this preliminary training phase had a delay interval of 1 s between cue and choices presentation, and a visual angle of 21.7^0 between the choice positions. The monkeys performed one session per day and were trained 6 – 7 days per week throughout this study.

Delayed Matching to Position (DMP) Task

In the task proper, the monkeys were tested in two separate experiments which allowed us to examine independently the effect of two factors, namely delay and spatial separation between choices, upon the monkeys' ability to perform the spatial recognition memory task.

Experiment 1: intermixed delays

This experiment consisted of three consecutive daily sessions of testing. The criterion for completing each daily session was when the animal obtained 100 rewards, so every animal worked for 300 rewards in total in this experiment (and so each animal therefore completed varying numbers of trials). Trials within a session were divided into five intervals of delay of 1, 2, 4, 8, and 16 seconds between the cue

and the presentation of choices. A set of five trials containing one of each of the delay conditions was first presented in a random order, after which the five delay lengths were presented in a new random order, and so on. The two choice stimuli in the choice phase were consistently separated by a visual angle of 21.7^0 throughout this experiment.

Experiment 2: intermixed spatial separations

Experiment 2 consisted of two levels. Level 1 and level 2 both consisted of one session of testing, the criterion for completing both sessions was when the animal obtained 150 rewards so the animals worked for 300 rewards in total in Experiment 2. Trials within a session were divided into four spatial separation distances subtending visual angles of 4.8^0, 8.6^0, 15.2^0 and 21.7^0 between choices on the screen. A set of four trials containing one of each of the delay conditions was first presented in a random order, after which the four separation distances were presented in a new random order, and this procedure repeated throughout the entire session. The two levels of this experiment differed only in the length of the delay between cue and choice presentation, being 1 s in level 1 and 8 s in level 2.

5.4 Results

Task acquisition

One CON monkey, who had previously been a reliable worker, failed to cooperate in acquiring the task by expressing reluctance to touch the screen; testing of this monkey was therefore discontinued and no data was obtained from it. Unfortunately the monkey could not be replaced by another animal at this stage as the remaining animals had all had extensive and identical experience on a battery of behavioural tasks that a replacement animal would not have had. Therefore we proceeded with the experiment with 5 animals. Their mean numbers of errors made before reaching criterion during preliminary training were 472 and 439 for CON and FNX groups respectively. The scores by different individuals in different groups overlapped and there were no significant differences between the groups in acquisition.

Experiment 1 performance: effects of delay length

Figure 2 clearly shows that the percentage of errors numerically increases with the interval of delay for both groups of monkeys and that the FNX group makes more errors at all delay lengths tested. Levene's tests showed that none of the five delay conditions suffered from any inhomogeneity of variance [the largest $F = 5.10$, all $p > 0.1$], so we proceeded to run a two-way repeated measures ANOVA with two levels of the between-subjects factor 'Group' (CON, FNX) and five levels of the within-subjects factor 'Delay' (delays in seconds: 1, 2, 4, 8 and 16) on the percentage error scores. We found that there was a significant main effect of 'Group' [$F(1, 3) = 18.74$, $p = 0.023$] but no 'Group*Delay interaction' [$F(4, 12) = 1.27$, $p > 0.1$]. There

was also a significant main effect of 'Delay' [F (4, 12) = 14.27, $p < 0.001$]. This confirmed that the FNX group was significantly impaired relative to the CON group in remembering the sample locations in the DMP task across all delay intervals tested. While it is clear that both groups of monkeys committed more errors as the delay increased, confirming that increasing the delay in this DMP task taxes the memory of the monkeys to a greater extent, the absence of a Group*Delay interaction in this task shows that the effects of fornix transection on performance are not delay-dependent.

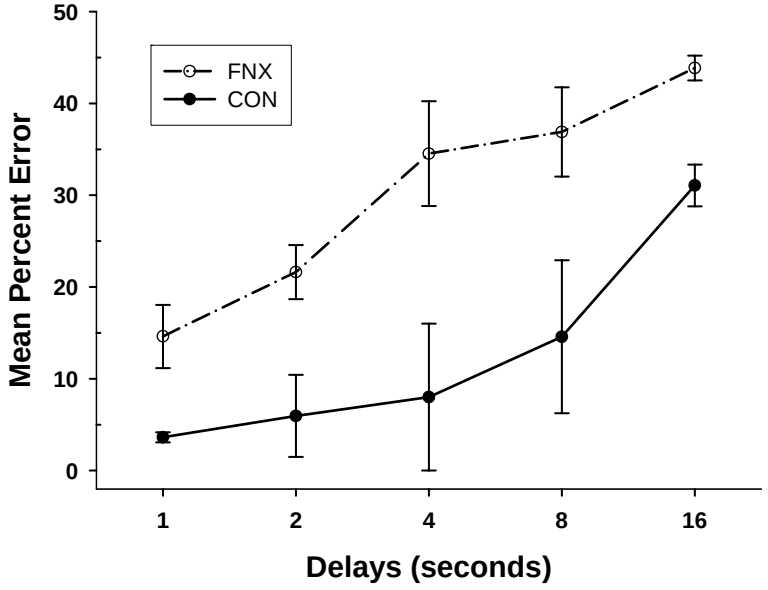


Figure 2. Performance expressed as mean percent error across five levels of delay interval (in seconds) of the fornix transected group (FNX) and the control group (CON) in the Experiment 1 of DMP task with variable delay lengths. Error bars depict the standard error of the means.

Experiment 2 performance: effects of spatial separation

Figure 3 shows that the percentage of errors increased in both groups as the two choice items became closer in spatial separation. In order to assess the effects of spatial separation, we ran a three-way repeated measures ANOVA with two levels of the between-subjects factor 'Group' (CON, FNX), four levels of the within-subjects

factor 'Separation' (spatial separation visual angle: 4.8^0, 8.6^0, 15.2^0 and 21.7^0) and two levels of the within-subjects factor 'Delay' (1 s and 8 s) on the percentage of errors. We found a significant main effect of 'Delay' [$F(1, 3) = 571.80$, $p < 0.001$] and a significant main effect of 'Separation' [$F(3, 9) = 27.60$, $p < 0.001$] but no main effect of 'Group' [$F(1, 3) = 1.12$, $p > 0.1$]. There was also no significant Group*Separation interaction [$F < 1$] and no significant Group*Separation*Delay interaction [$F < 1$]. One-sample t-tests show that for the hardest spatial separation condition (closest distance between sample and foil), all monkeys were better than chance (50% for a two-choice test) in the 1 s delay condition [$t(4) = -4.484$, $p = 0.011$], but were not significantly different from chance in the 8 s delay condition [$t(4) = -2.262$, $p > 0.05$]. Although the FNX group performed numerically worse than the CON group in terms of percentage of errors across the different spatial separations, these differences did not attain statistical significance. We did however confirm that the difficulty of the task was increased as monkeys had to wait longer before they could make their choices and as the separation distance between choices got shorter.

Finally, we ran a two-way repeated measures ANOVA with two levels of the between-subjects factor 'Group' (CON, FNX) and two levels of the within-subjects factor 'Experiment' (Experiment 1 and 2) on the percentage correct in the condition of 8 s delay and a separation of 21.7^0 between choices (a combination which appeared in both experiments). There was no main effect of Group [$p > 0.1$] but we found a significant Group*Experiment interaction [$F(1, 3) = 12.79$, $p = 0.037$], suggesting that lack of impairment in FNX animals in Experiment 2 may be due to the extensive experience gained on this particular delay/separation combination during the course of Experiment 1.

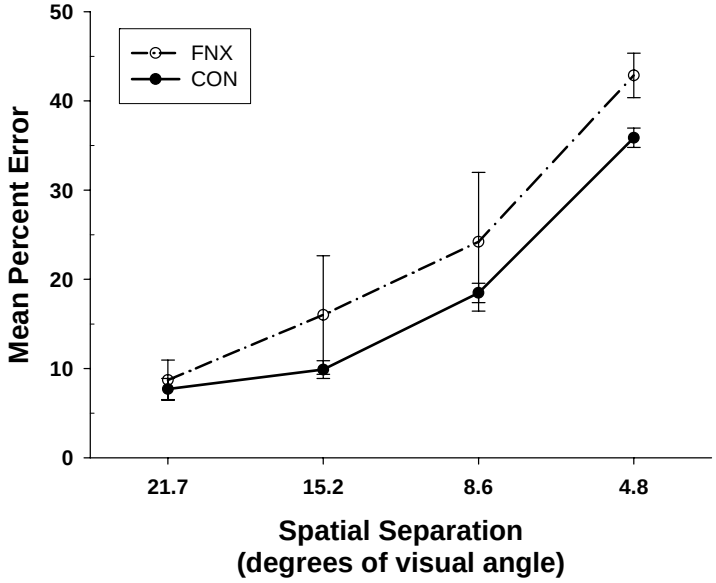


Figure 3. Performance expressed as mean percent error across four levels of spatial separation on the screen (visual angle in degree) of the fornix transected group (FNX) and the control group (CON) in Experiment 2 of the DMP task with variable spatial separations (with 1 s delay). Error bars depict the standard error of the means.

5.5 Discussion

The results of this study demonstrate, for the first time, that transection of the fornix in the macaque has a deleterious effect upon a spatial recognition memory task (DMP), performed on a touchscreen. However, the FNX group was not generally impaired on all aspects of the DMP task. The FNX group showed no impairment in acquiring the basic rules of the task (initial acquisition phase unimpaired). In the first performance test (Experiment 1), the FNX group was impaired relative to the CON group when we imposed various longer delays between sample and choice phases; however they were impaired across all delays and did not show a delay-dependent deficit. In contrast to this impairment, the FNX group was not impaired in the second performance test (Experiment 2) when we manipulated spatial separation between choices as a factor. The presence of impairment in Experiment 1 but not Experiment 2 cannot be explained as a transient effect of the lesion as the tests reported here were conducted over two years postoperatively. Thus the FNX group was neither generally impaired on DMP nor entirely unimpaired; rather there is some selectivity in the deficit, the nature of which is discussed below.

Firstly, the lack of any significant difference between the groups in the initial task acquisition phase preceding Experiment 1, together with the absence of any impairments in the FNX group in Experiment 2, where we varied the spatial separation between choice items, allows us to conclude that the fornix is not critical for a number of abilities demanded by these tasks including execution of motor movements, perception of the stimuli on the screen, short-term retention of stimuli, and motivation to perform the task.

In Experiment 1 we made the task more difficult than in the acquisition phase by introducing longer delays and we also introduced intermixed delays of

varying lengths between sample and choice phases in successive trials. Introducing these increased and more varied task demands produced robust impairments in the FNX group across all delays. However the impairments were not delay-dependent, in that they did not increase with longer delays as would be expected if the impairment in the FNX group was a deficit in memory loss per se.

As assessed by object DMS or DNMS tasks, fornix transected monkeys show abnormal rapid forgetting of object recognition over delays, scoring well on trials with very short retention intervals of a few seconds but being quite severely impaired on those with longer intervals of a few minutes (Gaffan, 1974; Gaffan and Weiskrantz, 1980; Owen and Butler, 1981, 1984), which clearly indicates delay-dependent deficits. Similarly, monkeys with hippocampal lesions also show impairments after long delays of many minutes in DNMS tasks (Zola-Morgan and Squire, 1986; Beason-Held et al., 1999; Zola et al., 2000). Nevertheless, these studies invariably tested performance at the different delays in successive order of incremental lengths, with monkeys only allowed to proceed to longer delays after having attained a criterion on some shorter delay conditions. This kind of design suffers from the problem of having trained the monkeys on easier levels first prior to testing on harder conditions later, which is thereby prone to confusing task acquisition deficits with delay-dependent deficits which these previous studies all claimed. We recognise that only studies that intermix delays in testing once the task is learnt (like the present task design) would be able to offer conclusive evidence in favour of true delay-dependent deficits and such evidence has not yet been provided (Murray et al., 1989). Indeed, despite the established role of the fornix in temporal processing (Charles et al., 2004; Wilson et al., 2007), there is, as is the case in the non-spatial domain, no incontrovertible evidence reporting delay-dependent effects

on spatial recognition in fornix transected monkeys either. The absence of any evidence for delay-dependent deficits in the current study, in spite of properly intermixed delays at test, leads us to argue against the notion that the present impairments are due to a deficit in stimulus memory per se.

Instead of a memory deficit, we suggest that the impairment which emerged as the animals moved from our relatively easy acquisition phase to our demanding Experiment 1 might be accounted for by monkeys' impeded ability in task acquisition, given the increased cognitive demands placed on the animals at this point. With respect to spatial recognition memory, Murray et al. (1989 Exp. 1) assessed monkeys' spatial recognition memory in the T-maze task having trained the animals preoperatively, but this meant that this particular study was unable to test the effects of fornix transection on task acquisition. In contrast, in the present study, monkeys were introduced to the task postoperatively enabling us to assess the effects of fornix transection on task acquisition. We found that FNX monkeys were not impaired in our initial task acquisition phase which animals became quickly familiar with having a short 1 s delay in every trial, confirming that fornix transection does not impair recognition of spatial positions after short delays. However, when the demands of the task were increased by the incorporation, and intermixing of, five kinds of delays of variable lengths within the sessions, FNX monkeys showed marked impairment. In the absence of delay-dependent deficits we favour an interpretation in terms of a deficit in acquiring the complex task. This substantial deficit was however short-lived, as the FNX animals were unimpaired in Experiment 2 in which a single delay condition was used continuously throughout the sessions.

In Experiment 2 we increased the burden on spatial discrimination by introducing trials in which the spatial separation between choice items was varied

parametrically. We found that FNX monkeys were not impaired compared to CON monkeys. Although there is currently no study reporting the effects of spatial separation on spatial recognition in monkeys with hippocampal disruption (either after neurotoxic hippocampal lesions or after fornix transection), evidence from rodent studies have shown that hippocampal lesions cause deficits in discriminating between spatial locations, and the deficits are exacerbated as a function of an increased spatial similarity, or decreased spatial separation, between the correct and foil locations on the choice test (1998; 2001; 2006). In the present study, a notable finding is the very significant detriment in performance in both groups of monkeys as we reduced the separation distance between choices (from a visual angle of 21.7^0 to 4.8^0). Yet, contrary to Gilbert's et al. (e.g. Beason-Held et al., 1999; Hampton et al., 2004; Alvarado and Bachevalier, 2005; Lavenex et al., 2006) prediction, we did not obtain an impairment after fornix lesion despite the manipulation of a rather close, and presumably very difficult, spatial separation (i.e. a visual angle of 4.8^0). A floor effect emerged with the most difficult spatial separation we used (monkeys were already at chance after an 8 s delay with this separation) and so we cannot expect to have found any evidence for a proportionally greater deficit in the FNX than CON group even if we had included still finer and even more demanding separations. However, it is plausible that in the context of spatial tasks presented upon a touchscreen, the degree of spatial separation between choice items might have less of an impact upon spatial recognition in monkeys than in rodents because of the lack of changes in visible background details that vary with spatial separation in the rodent studies. If varying spatial separation in our task was not equivalent to varying spatial separation in rodent studies then this might account for the lack of deficits in Experiment 2. Nonetheless, the lack of any deficit on even the closest separations in

Experiment 2 does suggest that fornix transected animals do not have profound deficits in spatial perception and discrimination.

The combined results from both experiments suggest that fornix transection neither leads to a general impairment in spatial recognition memory nor completely spares spatial recognition in monkeys, and as such does not provide full support to either of the hypotheses outlined in the introduction. Nevertheless, our findings help to indicate that the hippocampus and the fornix contribute differently to spatial memory and an explanation is given as follows. Excitotoxic hippocampal lesions in monkeys impair some delayed recognition of locations tasks in which delays lasted several minutes to several hours (Bachevalier and Nemanic, 2008). Nonetheless, when delays were restricted to 2 min, monkeys with the same kind of lesions were not impaired, for example, intact performance is found after 10 s delay in visual paired comparison of locations (Murray and Mishkin, 1998), with 30 s to 2min delays in delayed non-matching to location (Malkova and Mishkin, 2003) and 6 s delay in one-trial spatial memory (Bachevalier and Nemanic, 2008). It has thus been put forward that memory for spatial location can be maintained, by the medial temporal cortical areas, such as TH/TF and/or the lateral prefrontal areas, at least for short delays when the hippocampus is disrupted. This idea is supported by findings that damage to parahippocampal areas TH and TF alone produces deficits in spatial location recognition (Ploner et al., 1999), and that memory for spatial locations is impaired after non-selective (Bohbot et al., 1998; Bohbot et al., 2000) or selective (Sommers et al., 2005; Buffalo et al., 2006) damage to areas TH/TF in human patients. Studies also show that areas TH/TF are activated in normal subjects during Spatial Location memory (Kessels et al., 2001) and encoding of new locations during navigation (Saunders and Aggleton, 2007) in neuroimaging studies. It is known that

fornical damage leads to a loss of efferents from the entorhinal and perirhinal cortices (Aggleton and Brown, 1999; Malkova et al., 2001), and could disconnect the parahippocampal cortical areas from other cortical areas (Buckley et al., 2004; Buckley et al., 2008). Therefore, we suggest that the impaired spatial recognition after fornix transection with very short delays (less than 16 s) here may be explained by the disconnection of the parahippocampal cortical areas. If this is the case then similar deficits would be unlikely to follow neurotoxic hippocampal lesions, which would be further consistent with the idea outlined in the introduction that deficits after fornix transection are in certain cases dissociable from those brought about by neurotoxic hippocampal lesions.

In summary, we demonstrated that fornix transection does not impair spatial recognition memory under all conditions. FNX monkeys were impaired when we imposed various delays between sample and choice phases but not so when we manipulated spatial separation between choices as a factor. The discrepancies between the two experiments may be best explained by the different demands placed upon task acquisition and taken as support for the idea that the fornix supports new learning ; with the present results, we extend the role of the fornix in new learning to cover acquisition of task-sets as well. Interpreting these results in the light of known anatomical connections coursing through the fornix, suggests that the contribution made by the fornix to spatial recognition memory may well differ from that of the hippocampus.

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CHAPTER 6

FORNIX TRANSECTION IMPAIRS EXPLORATION BUT NOT LOCOMOTION IN AMBULATORY MACAQUE MONKEYS

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CHAPTER 6: FORNIX TRANSECTION IMPAIRS EXPLORATION BUT NOT LOCOMOTION IN AMBULATORY MACAQUE MONKEYS

6.1 Abstract

Prompted by the theoretical prediction that damage to the hippocampal system should abolish exploratory behavior, the present study examined exploratory movements in control monkeys (CON) and monkeys with transection of the fornix (FNX), a major input/output pathway of the hippocampus. CON and FNX monkeys were introduced to a novel octagonal chamber for six daily sessions each lasting 20 minutes. Both groups visited, punctuated by stops, the majority of the floor-space of the environment in each of the sessions. The exploratory movements of CON and FNX groups were not significantly different on most of the measures taken over 6 consecutive days. These measures included cumulative distance traveled, number and duration of stops, travelling patterns, and proportion of time spent in each of 12 designated zones of floor-space. The high degree of similarity in behaviour between CON and FNX groups suggests that an intact hippocampal system is not necessary for the display of normal exploratory movement per se. On the other hand, the CON and FNX groups did behave differently on two measures. First, the CON group exhibited a decrement in distance traversed over consecutive epochs within the first test session whereas FNX animals did not. Second, on those days in which the chamber was made visually asymmetrical, the CON animals tended to show a predilection for spending proportionally more time within one particular quadrant of the chamber. These observations are consistent with the idea that interrupting normal hippocampal system

function by means of fornix transection is detrimental to learning about the spatial layout of environments. We therefore suggest that while monkeys with fornix transection still display intact locomotor and exploratory behavior patterns, their new learning of visuospatial context is impaired.

6.2 Introduction

The hippocampal circuitry has long been linked to animals' exploratory behavior. In O'Keefe and Nadel's seminal discussion of environmental exploration in *Hippocampus as a Cognitive Map* (1978), they put forward a spatial mapping theory which states that exploration is fundamental in building and updating the internal representation of the spatial layout of an environment and that an intact hippocampus is pivotal to a functional exploratory repertoire. O'Keefe and Nadel's argument that animals with hippocampal lesions would cease to display exploratory behavior was based upon the hypothesis that the hippocampus serves as a cognitive map requiring environmental information derived from exploration (O'Keefe and Nadel, 1978; Clark et al., 2005). Although the hypothesis that the hippocampus serves as a cognitive map has received extensive examination (Redish, 1999), there has not been similar focus of research into the predication that the hippocampal system is required for exploration (Clark et al., 2005).

Studies have confirmed that there are alterations in exploratory behaviors displayed by hippocampal lesioned rats in open field tests (Whishaw et al., 1994), but other studies have reported that hippocampectomized rats still investigate novel objects and are sensitive to changes in their location (Harley and Martin, 1999). However, until recently there have been no detailed analyses of animals' ongoing movements and movement patterns after hippocampal system damage. For this reason, Clark and colleagues (2005) examined exploratory movements in control rats and rats with hippocampal lesions [produced with the neurotoxin N-methyl D-aspartate (NMDA)] attempting to test the hypothesis that damage to the hippocampus would abolish exploratory behavior. On four successive days, control and hippocampal rats

were placed onto a circular table near to which a large salient visual cue was positioned and their exploratory movements were measured. It was found that there was no difference in these measures between control rats and rats with hippocampal lesions on the first test day. However, by the time of the fourth day of testing control animals were found to be less active on most of the measures of exploration, whereas the behavior of hippocampal rats remained unchanged from that observed on the first day. Thus Clark and colleagues (2005) suggested that the hippocampus may not be necessary for the display of normal exploratory movements by making inference from the high degree of similarity in behavior between hippocampal lesioned and control rats on Day 1, and the persistence of this behavior in hippocampal rats on Day 4. This finding also suggests that hippocampectomized rats' behavior might be related to a spatial memory impairment impeding acquisition of familiarity with the environment that the animal finds itself in (Clark et al., 2005).

Despite a number of studies conducted with rodents, to date there have not been any similar systematic investigations conducted on macaque monkeys to test the predictions arising from O'Keefe and Nadel's theory (1978) since its conception almost three decades ago. In light of the finding that exploration behavior is not devastatingly affected by hippocampal lesions in rats (Clark et al., 2005), the present study aimed to examine whether, in macaque monkeys, transection of the fornix (a major input and output pathway of the hippocampus) might alter their patterns of exploratory behavior. To facilitate this we designed and built a novel octagonal test chamber within which unrestrained monkeys were able to freely ambulate around (see figure 1, panel 1). At the start of this experiment the chamber was a completely novel environment to all of the experimental animals and for six consecutive days we observed and recorded the exploratory behavior of a control group and a group of

fornix transected macaques by means of an automated movement tracking system. We hypothesized that if the role of the hippocampus in exploration and spatial learning is conserved between rodents and primates, then we would observe that macaques with fornix transection would continue to explore the chamber at a similar rate across days, whereas the control macaques might show reduced exploratory behavior as the chamber becomes an increasingly familiar environment. Furthermore, we also manipulated the degree of visual symmetry of the chamber between days to assess whether any such effect would only be seen on days where the directionality within the chamber was made explicit by an addition of asymmetrical visual cues.

6.3 Materials and Methods

Subjects

Six male cynomolgus monkeys (*Macaca fascicularis*) took part in this experiment. Their mean weight at the start of behavioural testing was 6.4 kg, and their mean age was 5 years and 3 months. All six monkeys had identical pre- and post-operative experience in concurrent discrimination learning tasks in series of experiments that were carried out before the present study began (Buckley et al., 2005). They were housed together in a group enclosure, excepting two, whom were housed together as a pair; all had automatically regulated lighting and with water available ad libitum.

Surgery

Three monkeys had received bilateral fornix transection (group FNX) and the remaining 3 were unoperated controls (group CON). All procedures were carried out in compliance with the United Kingdom Animals (Scientific Procedures) Act of 1986. The operations were performed in sterile conditions with the aid of an operating microscope, and the monkeys were anesthetized throughout surgery with barbiturate (5% thiopentone sodium solution) administered through an intravenous cannula. A D-shaped bone flap was raised over the midline and the left hemisphere. The dura mater was cut to expose the hemisphere up to the midline. Veins draining into the sagittal sinus were cauterized and cut. The left hemisphere was retracted from the falx with a brain spoon. A glass aspirator was used to make a sagittal incision no more than 5 mm in length in the corpus callosum at the level of the interventricular foramen. The fornix was sectioned transversely by electrocautery and aspiration with a 20

gauge metal aspirator insulated to the tip. The dura mater was drawn back but not sewn, the bone flap was replaced, and the wound was closed in layers. The operated monkeys rested for 11 – 14 days after surgery before beginning postoperative training. Unoperated control monkeys rested for the same period of time between preoperative and postoperative training. Although histological sections are not yet available (as the animals are partaking in further investigations), visual inspection at the time of surgery by two expert neurosurgeons confirmed that the fornix was completely sectioned bilaterally in each animal and that there was no obvious inadvertent damage elsewhere. Histology for other animals who have undergone the identical procedure by the same neurosurgeons in the same laboratory may be consulted in recent publications (Buckley et al., 2004; Charles et al., 2004).

Ambulatory apparatus

The ambulatory apparatus was a symmetrical eight sided chamber with opposing sides of either 128 cm or 120 cm long (see Figure 1, panel 1 for a diagrammatic plan). The length across the chamber was 3.2 m and the total floor area was approximately 7.4 m^2. Four touch screens (each with a visible screen area of 20 inches diagonally) were embedded in four of the walls for the purpose of displaying stimuli and registering the monkeys' responses to such stimuli (Figure 1, panel 1). An infra-red camera equipped with a wide angle lens was mounted centrally on the ceiling of the chamber (2.1 m above floor level) and was used with motion analysis software to continually record the movements of monkeys during the experiment. The walls and the floor of the chamber were coated with a durable waterproof material colored white and light yellow respectively upon which the dark coat of the monkey always stood out (due to differences in contrast) in the video images, thereby facilitating software

analysis of the animal's motion in the captured video. In addition, four separate infra-red CCTV cameras equipped with infra-red light sources were also mounted high upon the ceiling in a symmetrical manner which allowed each one to capture a direct view of one of the four touch screens, enabling the experimenter to observe the monkeys' screen touching behaviors from outside of the closed arena. Four extraction fans were installed, located on the top part of four of the wall panels to provide adequate ventilation within the enclosed arena during the testing session and the noise which these four fans generated while operated also acted as a mask of extra-chamber noise. One wall contained a large hinged door through which the experimenter could enter or leave the chamber between sessions to clean the chamber (three other walls contained a 'false' door to maintain visible symmetry). This large door (when closed) also contained within it a smaller door that could be raised or lowered to allow the monkey to enter or leave the chamber via a transport cage which could be securely abutted to the outside of the chamber at this point. When closed, the rectangular outline of this smaller door could be discerned from the inside and so the outlines of three 'false' small doors were drawn on three other walls to maintain visible symmetry. Altogether, a convincing visually-symmetrical environment was achieved within which human subjects quickly became disorientated after walking around for a few moments. However, on certain days, a non-symmetrical environment was created with the addition of visual aids to provide salient cues to directionality. A dramatic landscape poster (91 x 60 cm) was positioned above one touchscreen, a picture of a bird (30 x 27 cm) was positioned above another, and a 60W lamp shone through the extraction fan hole above a third screen (the effect of this lamp was to allow light to flood into the chamber from one point only in the room, akin to an artificial 'sun'). The asymmetrical environment was set up exactly the same way on each of these

asymmetrical days. A mechanism to deliver food rewards to the monkey inside the chamber was incorporated by means of four white food pellet bowls positioned to the lower left hand side of each touchscreen; food rewards could be delivered from dispensers installed outside the chamber via a tube system connected to these bowls. However, these pellet dispensers (and the touchscreens themselves) were not used in this study.

Procedure

For each session, a monkey was brought to the testing room in a wheeled transport cage, which abutted a sliding door of the apparatus, through which the monkey was introduced into the chamber. Each exploratory session lasted 20 minutes in which the animal was free to ambulate and explore the chamber at will. At the end of the session, the monkey left the chamber to be fed in its transport cage before being returned to its home cage. This procedure was repeated on 6 successive days, of which days 1, 3 and 5 were carried out in the symmetrical environment whereas days 2, 4 and 6 were carried out in the non-symmetrical environment. During the course of this study monkeys were given their daily food ration at the end of the daily session.

Movement tracking and arena design

Each session was captured digitally by an overhead camera attached to a computer. TopScan software 1.0 (Clever Sys. Inc. Virginia, VA, 20190, USA) was used to determine the monkey's position and locomotion at a capturing speed of 30 frames/second. The video file was then compressed and analyzed within TopScan to generate the trace paths, cumulative traveled distance, number and duration of stops, traveling patterns and proportion of time spent in each of 12 designated zones. Figure

1 (panel 2) depicts each of the 12 zones used for analysis: the floor of the chamber was divided into 4 quarters (with a touchscreen centered in each) and each of these was subsequently split up into three smaller areas. This particular zoned design allowed us to assess which screen the monkey was closest to at any point in time and to observe whether or not a monkey had any predilection to spend time in particular areas within the chamber.

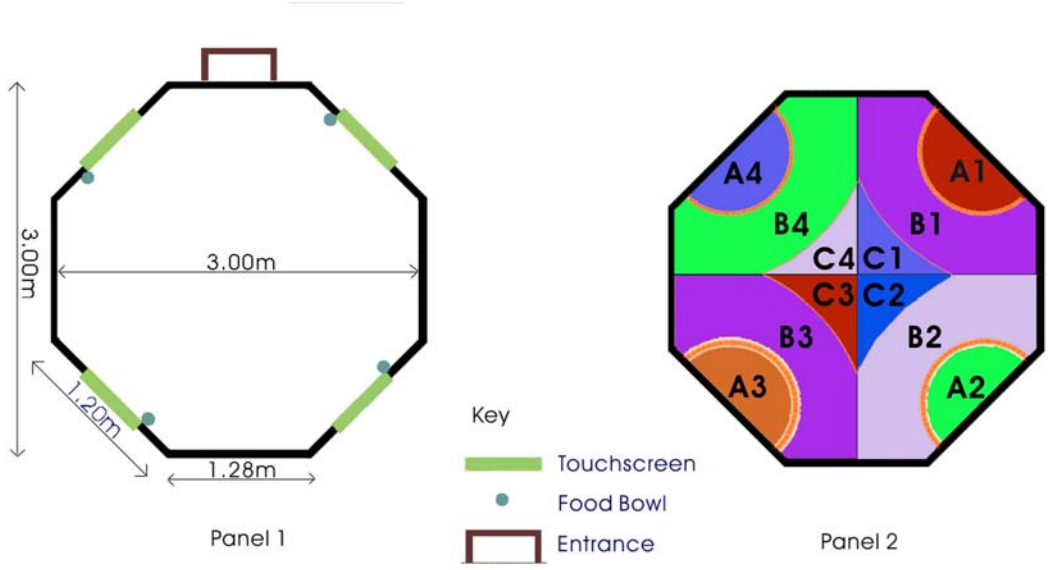


Figure 1. Panel 1. A plan of the ambulatory apparatus showing its dimensions and the positions of internal features, such as four touchscreens embedded in four of the walls, the four white food pellet bowls positioned next to the touchscreens, and the door through which a monkey enters into the chamber. Panel 2. A topographic representation of the apparatus arena (consisting of 12 areas).

Behavioural measures

We recorded a number of behavioral measures in both the CON and FNX groups, across six consecutive days of testing. However on days 1, 3, and 5 we presented no visual cues to symmetry whereas on days 2, 4, and 6 we included the same set of cues to make the room visually asymmetrical. The following measures were used to

examine the monkeys' exploratory movements on each daily session which lasted a total of 20 minutes:

(1) *Cumulative distance*. The exploratory paths taken by each monkey on each day were reconstructed and measured in length. The cumulative distances traveled by each monkey on the first day were also examined and analyzed within five consecutive four-minute epochs.

(2) *Number and duration of stops*. A stop was defined by a ratio of displacements of an animal's body between two consecutive time frames according to TopScan's motion measure function. An animal was classified as static (stopped) when the motion measure was below a value of 0.5. The duration of total stops was also measured.

(3) *Designated zones and screen quadrants*. The floor was divided into four main quadrants, each of them representing the area in front of a screen (i.e. screen quadrants 1, 2, 3 and 4) and each of these screen quadrants were further divided into three smaller zones (i.e. an 'outer' Zone A, an 'intermediate' zone B and an 'inner' zone C). Figure 1 (panel 2) shows the layout of each of the twelve zones. The proportion of time spent in each screen quadrant and zones were analyzed.

(4) *Trace paths and thoroughness of exploration*. A plan of chamber floor was divided into 244 individual squares by means of a regular grid superimposed upon the plan. An average monkey body occupied roughly 10 squares of the grid. Trace paths of monkeys' movements were generated in accordance to the center of a monkey's

body mass (calculated automatically by TopScan software) and each trace of exploration was then analyzed with reference to this grid to determine the number of squares on the grid that were crossed either i) never, ii) once, or iii) more than once, this was done independently for every session of every monkey to examine the thoroughness of each daily exploration with respect to the proportion of floor-space visited.

6.4 Results

Behavioural results

Both the control and the fornix groups were active in that they locomoted extensively throughout the chamber during six 20-minute test sessions. Behavior consisted of mainly locomotor progressions punctuated with periodic stops. Locomotion was mainly directed to the periphery of the chamber, but also included occasional trips across the center of the chamber. Comparisons between the control group and the fornix group across a wide range of behavioral measures showed that the two groups did not differ greatly in their behavioral profile across the six exploratory sessions.

Cumulative distance

a) Across six daily sessions

The mean distances traveled per 20-minute session averaged across the six sessions were 502 m for the CON group (range 359 m to 587 m) and 340 m for the FNX group (range 129 m to 621 m). An ANOVA of the average distance traversed per day showed that there were no significant differences between groups [$F(1, 4) < 1$]. A repeated measures ANOVA of the distance traveled on each of the six days of testing showed that there were also no significant changes in distance traveled by the groups across successive days [Session: $F(5, 20) = 2.10$, $p > 0.1$; Group*Session: $F(5, 20) = 1.379$, $p > 0.1$, and the linear trend component of this interaction was also insignificant] (see Figure 2 for examples of trace paths on the first and last days). An ANOVA considering the within-subjects differences between the mean daily distance

traveled on symmetrical versus non-symmetrical days also showed no significant differences between groups [F (1, 4 < 1].

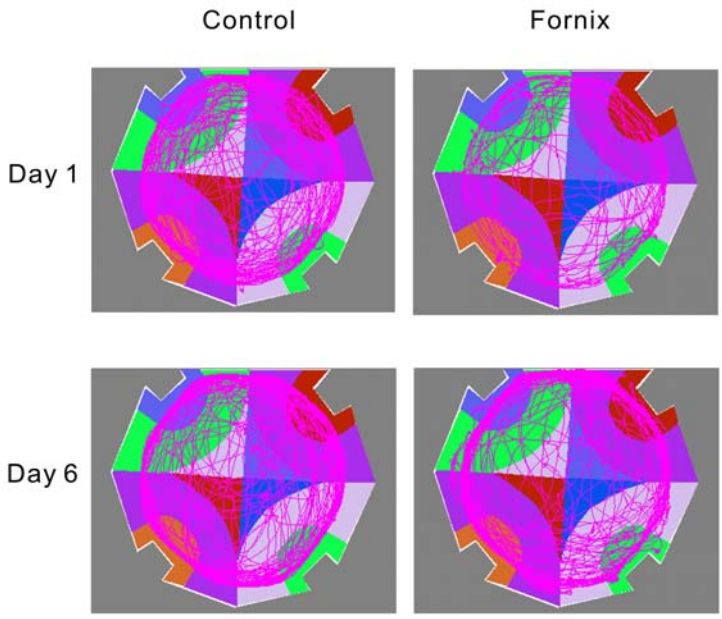


Figure 2. Traces of the exploratory paths followed by a representative control monkey (left panel) and a fornix lesioned monkey (right panel) on the first (day 1) and last day (day 6) of testing.

b) Across epochs within the first test session (Day 1)

As there was considerable individual variation in the mean distances traveled in the first epoch of the first testing session (Day 1) we conducted a repeated measures ANOVA on the logarithmic transformed distance traveled in five consecutive epochs within Day 1. This analysis showed that there was a significant difference between the groups in the within-subjects changes in distance traveled across these epochs [Group*Epoch: F (4, 16) = 3.601, p = 0.028] (see Figure 3 for example trace paths of animals from each group in the first and last half of session 1). Further, the linear trend component of this interaction was also significant [F (1, 4) = 10.468, p = 0.032] reflecting the fact that the difference between groups was apparent in the early epochs

but not the latter epochs; indeed, despite marked individual differences in distance traveled in the first epoch, the CON group animals showed a consistent tendency to travel less in later epochs while the reverse was true for FNX animals (see Figure 4 for regression lines of the mean changes in distance traveled by each group across the five epochs of the first session).

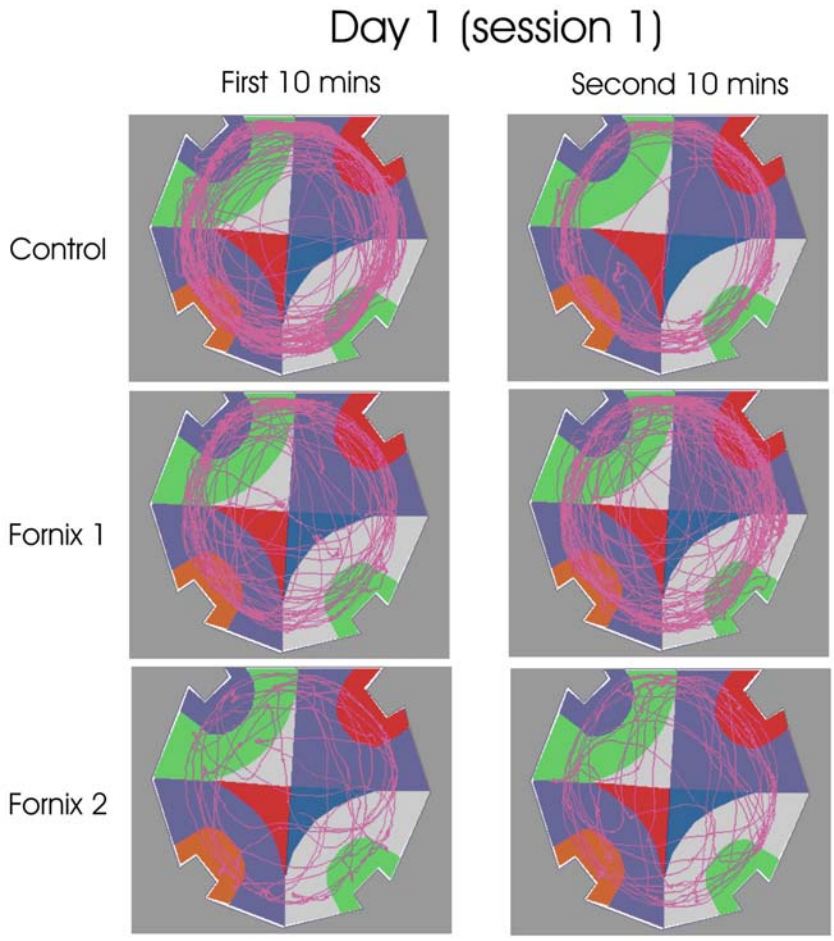


Figure 3. Traces of the exploratory paths followed by a control monkey (top panel) and two fornix lesioned monkeys (middle and bottom panels) in the first and second halves (10 minutes each) on the first day of testing.

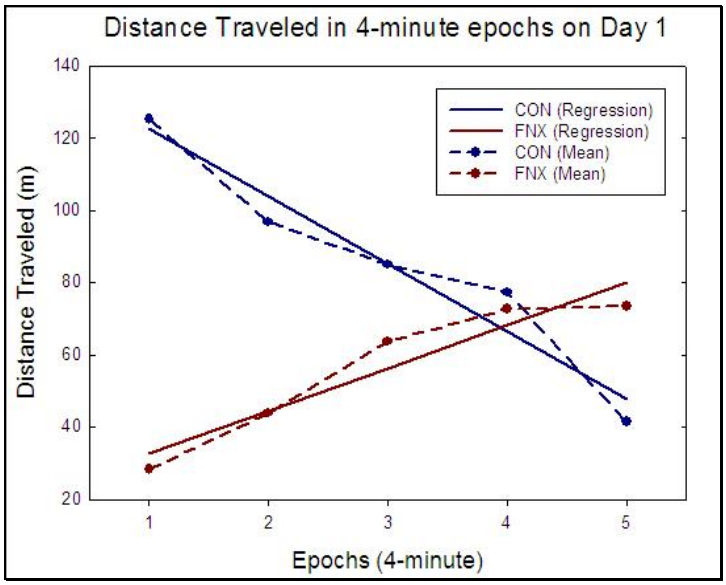


Figure 4. A graph showing the regression lines of the distances traveled in five 4-minute epochs by the CON and FNX groups on Day 1.

Stops and duration of stops

The mean numbers of stops averaged across the six sessions were 193 stops for the CON group (range 128 to 270) and 361 stops for the FNX group (range 234 to 536). An ANOVA of the average number of stop per day showed that there was no significant differences between groups [$F(1, 4) = 2.888$, $p > 0.1$]. A repeated measures ANOVA of the number of stops on each of the six days of testing showed that there were also no significant changes in the number of stops by the groups across successive days [Session: $F(5, 20) < 1$; Group*Session: $F(5, 20) < 1$, and the linear trend component of this interaction was also insignificant]. An ANOVA considering the within-subjects differences in the mean daily number of stops on symmetrical versus non-symmetrical days also showed no significant differences between groups [$F(1, 4) < 1$].

The mean duration of stops averaged across the six sessions were 271 seconds for the CON group (range 195 s to 391 s) and 429 seconds for the FNX group (range

184 s to 575 s). An ANOVA of the average duration of stops per day showed that there was no significant differences between groups [F (1, 4) = 1.319, $p > 0.1$]. A repeated measures ANOVA of the duration on each of the six days of testing showed that there were significant changes in the duration of stops across successive days [Session: F (5, 20) = 9.709, $p < 0.0001$] but there was no significant effect between groups [Group*Session: F (5, 20) = 1.232, $p > 0.1$, and the linear trend component of this interaction was also insignificant]. An ANOVA considering the within-subjects differences in the mean daily duration of stops depending upon whether the day was symmetrical or non-symmetrical also showed no significant differences between groups (F (1, 4) = 3.039, $p > 0.1$).

Zone preference

The mean percentage of time spent inside the designated zones (see Figure 1, panel 2 for the division of zones) averaged across all of the six sessions were: zones A $^{1-4}$ (33.1% for CON and 33.0% for FNX); zones B $^{1-4}$ (63.6% for CON and 60.6% for FNX) and zones C $^{1-4}$ (3.3% for CON and 6.4% for FNX) respectively. ANOVAs showed that there were no significant differences between groups in the proportion of time which they spent in each of the different zones averaged across all days: zones A [F (1, 4) < 1], zones B [F (1, 4) < 1], and zones C [F (1, 4) = 4.836, $p = 0.09$] respectively. A repeated measures ANOVA indicated that there were some significant changes in the proportion of time spent by the animals in different zones across successive days of testing. The proportion of time spent by the monkeys in zones B varied significantly between days [Session: F (5, 20) = 3.550, $p = 0.018$] but there was no indication that the patterns of exploration exhibited by the two groups differed in this regard [Group*Session: F (5, 20) < 1]. As for other zones (zones A and zones C),

there were no significant changes in zone preference across successive days and no significant changes between groups differences. ANOVAs considering the within-subjects mean differences in the percentage of time spent inside each of the zones depending upon whether the day was symmetrical or non-symmetrical also showed no significant differences between groups [Zone A: $F(1, 4) = 4.192$, $p > 0.1$; Zone B: $F(1, 4) < 1$; and Zone C: $F(1, 4) < 1$].

Screen quadrant preference and effects of symmetry

The mean percentage of time that the CON and FNX monkeys spent inside each of the screen quadrants (see Figure 1, panel 2) were as follows: for quadrant 1 (30.5% for CON and 24.7% for FNX); for quadrant 2 (19.9% for CON and 23.0% for FNX); for quadrant 3 (21.1% for CON and 25.8% for FNX); and for quadrant 4 (28.5% for CON and 26.4% for FNX) respectively. A repeated measures ANOVA of the mean percentage of time spent in each of the four quadrants (with 4 levels of the within-subjects factor quadrant, 2 levels of the within-subjects factor symmetry, and one between-subjects factor group) indicated that quadratic component of the group x quadrant interaction approached significance [$F(1, 4) = 5.646$ $p = 0.076$]. This, together with our observations that there were marked individual differences in preferences to quadrants and the feature of our design which ensured differential availability of different kinds of cues to each quadrant in asymmetrical days prompted us to examine the data in closer detail. Thus, even despite individual differences in preferences we discerned that the CON group appeared on average to prefer to spend proportionally more time in the two quadrants on either side of, and therefore equally closest to, the exit (see Figure 1, panel 2). A t-test confirmed that averaged over the 6 days the CON group's summed preference scores for these two 'exit' quadrants as

opposed to the other two 'non-exit' quadrants was indeed significantly greater than that of the FNX group ($t = 2.379$, $df = 4$, $p = 0.038$, 1-tailed). Furthermore, only one of the exit quadrants (quadrant 1) was illuminated by the artificial sun while the other (quadrant 4) remained in relative darkness. Although individual subjects exhibited varying preferences to quadrants with a single 'attractive' feature (exit proximity or lightness) examination of the data showed that a clear consensus in preference emerge in the CON group towards spending proportionally more time in the zone with both attractive features present (see Figure 5). Indeed, an ANOVA considering the within-subjects differences in the percentage of time spent inside each of the four quadrants depending upon whether the day was symmetrical or non-symmetrical showed that the CON group tended to increase the amount of time spent within a particular quadrant (quadrant 1) when the chamber was made asymmetrical but that the FNX group showed no such bias on asymmetrical days [$F (1, 4) = 14.266$, $p = 0.019$]. No such effect was found with the other three quadrants (all F's $(1, 4) < 1$).

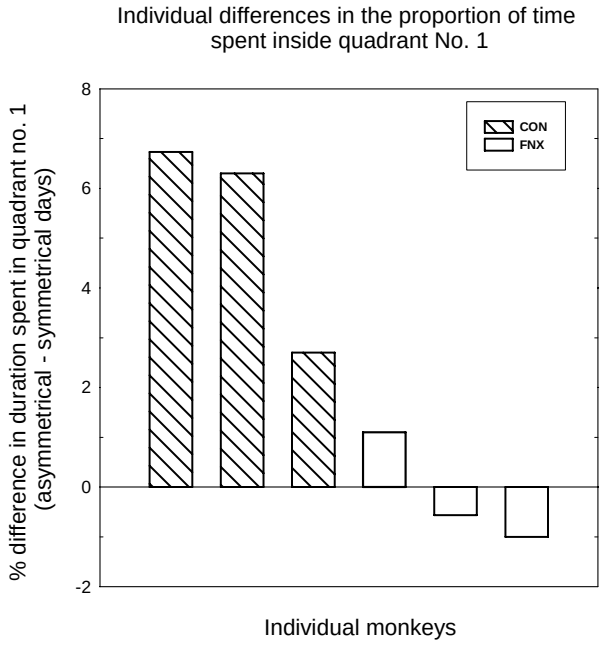


Figure 5. A graph showing the differences in the mean percentage of time (asymmetrical days – symmetrical days) spent within quadrant no. 1 for six individual monkeys.

Proportion of arena visited

The average proportions of the arena visited by the monkeys averaged across the six sessions were 92.5% for the CON group (range 84.6% to 98.4%) and 94.2% for the FNX group (range 87.9% to 98.7%). An ANOVA of the percentage of squares visited showed that there were no significant differences between groups in all three measures: i) squares never visited, $F(1, 4) = 0.106$, $p > 0.1$; ii) squares visited only once, $F(1, 4) = 0.053$, $p > 0.1$; and iii) squares visited more than once, $F(1, 4) < 1$. A repeated measures ANOVA of the proportion of the arena visited on each of the six days of testing showed that there were no significant changes in the proportion of the arena visited by the groups across successive days [Session: $F(5, 20) = 0.839$, $p > 0.1$; Group*Session: $F(5, 20) < 1$, and the linear trend component of this interaction was also insignificant]. ANOVAs of the within-subjects differences in the mean proportion of the arena visited on symmetrical versus non-symmetrical days also

showed no significant differences between groups in all three measures [squares never visited: $F(1, 4) < 1$; squares visited only once: $F(1, 4) = 1.561$, $p > 0.1$; and squares visited more than once: $F(1, 4) < 1$].

Individual differences

Despite the overall pattern of the main effects of the behavioral measures, there were some striking individual differences in the behavior pattern of FNX monkeys. Similar to hippocampal rats [(O'Keefe and Nadel, 1978), Table A14], the effect of fornix lesion on hyperresponsivity in our FNX monkeys was also not invariant. For instance, the most and the least active monkeys of all were both from the FNX group. In the measure of distance traveled, the least active monkey (FNX3) traveled merely 129 meters on average across six sessions, whereas the most active one (FNX1) traveled 621 meters (compared to 421 meters overall mean of all six monkeys). Evidence of this bipolar activity level within FNX group also manifested itself in other measures such as the number of stops and the total duration of static state. In the aspect of traversing patterns, one lesioned monkey (FNX2) traversed distinctly differently from others (e.g. FNX1) in a manner that it did not explore in a circling pattern around the periphery of the chamber but making many crosses and shortcuts across the chamber instead (see Figure 3, contrasting middle and bottom panels). In order to minimize the variances due to individual differences, many of our analyses above have taken individual differences into account by considering within-subjects measures such as change in individual activity levels over epochs, and over days, and by using comparisons of within-subjects activities on symmetrical versus asymmetrical days.

6.5 Discussion

Following Clark's et al. (2005) findings of intact exploratory behavior in hippocampal lesioned rats on an open circular table, the present study tested the prediction that transection of the fornix in monkeys would not abolish their exploratory behavioral patterns in a novel environment. On 6 successive days, control and fornix transected monkeys were introduced into an octagonal chamber in which their locomotion and movements of exploration were assessed according to a number of measures. Both CON and FNX monkeys visited, punctuated by stops, the majority of the floor-space of the chamber in each of the 20-minute sessions. CON and FNX monkeys were surprisingly similar on most measures of exploratory movements: including the cumulative distance traveled, the proportion of the floor-space of the chamber traversed, the number of stops and the amount of time spent still. We observed that FNX monkeys were as active as controls, but showed no signs of hyperactivity relative to controls, in locomoting within the chamber across all 6 days. The similarity of activity level between groups is taken as evidence that FNX monkeys are not un-motivated relative to the CON monkeys. The present results confirm that locomotor ability and motivation for exploring in a novel environment are well preserved in macaque monkeys with fornix lesions. This strengthens arguments (Gaffan, 1998) against the claim made by Whishaw and Jarrard (1996) that the hippocampal system, rather than contributing to learning per se, might instead mediate integrative processes providing movements that lead to learning. Our findings indicate that disrupting the normal function of the hippocampal system in non-human primates, by means of fornix transection, has no effect upon these proposed motor processes. Thus, in so far as our behavioral measures assess the extent of exploratory

behavior, our results do not support the hypothesis put forward by O'Keefe and Nadel (1978) that, if subject to hippocampal system disruption, animals would cease to display exploratory behaviors.

Clark et al. (2005) showed that whilst unoperated control rats exhibited 'habituation' (in terms of sustaining reactivity to salient aspects of a testing situation) over successive days of testing in a novel environment; rats with hippocampal lesions in contrast did not. In our experiment in which macaques were similarly introduced into a novel environment, neither the CON nor FNX group showed signs of habituation over successive days as both groups manifested similar exploratory movements across the six daily test sessions. However, we did find evidence for habituation with regard to exploratory behavior *within* the first test session as our CON group exhibited a consistent decrement in traveled distance within consecutive epochs of the first test session whereas our FNX group did not. The failure of our FNX group to display 'habituation' within the first session is consistent with previous evidence in rat studies that animals with hippocampal system disruption behave differently, with regard to sustaining reactivity to salient aspects of a testing situation (Clark et al., 2005).

With regard to Clark's et al. (2005) study, one could estimate a ratio of the body mass of rats (250 – 300 g on average) to the area of the circular table (5.1 m^2) that could be explored by the rats in that study. In order to allow our macaques to explore an environment with the same body mass to area ratio we would have had to build an arena measuring approximately 16 times larger than the 7.4 m^2 of floor space available in our current arena. Not only was our novel environment relatively smaller in scale than the novel environment given to rats by Clark and colleagues (2005), it may also be considered to be relatively simplistic in terms of the extent of

distinguishing visual features present a real naturalistic environment for macaques, say in a forest. Therefore, it is at least possible to speculate that the relatively small size and low complexity of our environment may have been a contributing factor towards our CON macaques' abilities to habituate within a very short period of time (within the first 20 minutes) so that no further decrements in measurements could be observed across subsequent days. However, it is also interesting to note that preliminary studies have indicated that when rats are allowed to explore 'infinite' virtual environments will freely locomote (or 'explore') vast environmental ranges covering up to 220 meters in a single 10-minute session (Hölscher et al., 2005) suggesting that the environmental ranges given to rats in experimental contexts are similarly restrictive in allowing the expression of natural exploratory tendencies. Thus, while our data is at least suggestive that non-human primates may be able to habituate to visual environmental cues at a faster rate than rodents, in the absence of any means to evaluate the relative efficacy of perceptual cues to directionality employed by these two different studies, with different species, and with different environmental ranges, this interpretation must remain speculative at present. Furthermore, other potentially important aspects of species differences, such as the relative importance of visual exploration in monkeys compared to olfactory and tactile exploration in rats, would also need to be taken into account before making any strong claims about general species differences in habituation rate.

We found one further measure that distinguished the behavior of the unoperated control macaques from that of those with fornix transection in our novel experimental arena. Unlike fornix transected animals, control animals showed stronger preferences for spending time in certain regions of the environment. In particular we found that control animals exhibited a mild but statistically significant

preference for spending relatively more time in one particular quadrant of the arena (quadrant number 1) on those days in which asymmetrical visual cues gave a stronger sense of directionality to the environment whereas fornix transected animals did not show such a preference on either symmetrical or asymmetrical days. We speculate that the preference for quadrant number 1 might have resulted from a combination of factors. Firstly, with the aid of visual landmarks on asymmetrical days, the CON group may have had a greater capacity to remember from which direction they were introduced into the arena and therefore might have been better able to keep track of the location of the entrance/exit to the arena. Quadrants 1 and 4 happened to be the two quadrants located right next to the exit and all things being equal monkeys might have preferred to locate themselves in and around these quadrants with a view to expediting their exiting from the arena when the opportunity arose (overall, our analyses confirmed that control monkeys indeed expressed a preference for locating themselves within these two quadrants, but that this was not the case in fornix transected animals). However, the chamber was also set up with an 'artificial sun' shining light into the arena from above quadrant 4 and which primarily illuminating the other three quadrants. All things being equal, monkeys may have preferred to spend more time in quadrants 1 – 3 either because these positions afforded a view of the source of the light form outside the arena, or perhaps because the relative brightness of these zones afforded a greater sense of security while in an unfamiliar environment. The combination of these two factors may explain why quadrant 1 was much preferred over other quadrants for normal monkeys on asymmetrical days as it was the only quadrant which presented both potential benefits.

There are a number of potential reasons for why fornix lesioned macaques may have failed to habituate and to develop regional preferences in response to salient

visual cues to directionality available on asymmetrical days. One potential explanation is that our lesioned animals might simply have been impaired at recognizing non-spatial or spatial visual features. However we judge this as unlikely as monkeys with fornix transection have been shown to be completely unimpaired at discriminating objects in the context of trial-unique object-recognition memory tasks (Charles et al., 2004), and fornix lesioned macaques have also been shown to remain unimpaired at discriminating large numbers of spatial problems exposed to preoperatively (Buckley et al., 2004; Buckley et al., 2005). Indeed, object and spatial recognition memory has been shown, under some circumstances, to even survive neurotoxic lesions directed towards the hippocampus and amygdala in macaques (Murray and Mishkin, 1998). Moreover, differences in forgetting rate between CON and FNX groups are also deemed to be unlikely to account for the group differences because fornix transection in macaques was shown not to affect the rate at which over 100 visuospatial discrimination problems were forgotten even after an interval as long as 90 days (Buckley et al., 2005) (see also Chapter 4).

On the other hand, the lack of selective habituation in the FNX group may reflect the necessity of an intact hippocampal system to build and update the internal representation of the spatial layout of an environment during exploration (O'Keefe and Nadel, 1978; Clark et al., 2005). Indeed, some spatial learning theories stipulate that the hippocampal system contributes to exploratory behavior indirectly by facilitating animals' acquisition of spatial information regarding their environment (Hines and Whishaw, 2005). While fornix transection has previously been shown to impair macaques' abilities to learn about scenes (Gaffan, 1994a) such scene learning tasks do not explicitly demand spatial learning. Nevertheless, other tasks that do explicitly tax spatial learning have been shown to be impaired after fornix transection

(Gaffan and Harrison, 1989; Murray et al., 1989; Gaffan, 1994b; Buckley et al., 2004; Buckley, 2005; Buckley et al., 2005; Bussey et al., 2005). Furthermore, as recent experiments by Charles and colleagues (2004) and Brasted and colleagues (2003) have indicated that an intact fornix is also required to support various kinds of temporal information processing tasks, thus one can infer that fornix lesions impairs learning about spatial-temporal context. We propose that a general impairment in learning about context could have impeded the ability of FNX animals to develop location preferences as well as their ability to habituate to the novel environment at the same rate as controls.

Nonetheless, we still cannot completely rule out that the behavioral differences between our FNX and CON monkeys might be attributable to underlying deficits of a perceptual nature. According to the MTL (Medial Temporal Lobe) Memory System theory, the MTL contributes exclusively to mnemonic processing (Squire and Zola-Morgan, 1991). However, more recently some authors have challenged this theory on account of evidence that some MTL structures, particularly the perirhinal cortex, contribute to perception [for recent reviews see: (Murray and Bussey, 1999; Buckley, 2005; Bussey and Saksida, 2005; Bussey et al., 2005; Lee et al., 2005a; Buckley and Gaffan, 2006)]. By this account, MTL structures are critical to higher order perceptual as well as mnemonic processes, and therefore the hippocampus might be involved in perception, of scenes and spatial information in particular. This would be consistent with recent new evidence demonstrating deficits in a variety of spatial processing tasks which place little or no demands on memory following hippocampal damage (Hartley et al., 2005; Lee et al., 2005a; Lee et al., 2005b). Although in the context of the current study, we are unable to conclude whether the failure of the FNX group to habituate and develop location preference

was caused either by mnemonic deficits, perceptual deficits or both, we advocate the view that a role for the hippocampus in spatial perception is not necessarily contradictory to a relational memory view of the hippocampus (Eichenbaum et al., 1994). The hippocampal system could be involved in the binding of perceptually distinct items both for explicit long-term memory as well as for more immediate processing of the relationships among perceptually distinct elements of scenes or events that give rise to perceived spatio-temporal context.

In conclusion, in this study FNX monkeys displayed a behavioral pattern that was very similar to that of control animals overall excepting for the fact that they failed to habituate to a novel environment in the same time frame as controls and that they failed to develop a predilection of a particular quadrant. We are able to conclude that motivation and locomotor ability remain intact after FNX transection and to reject the hypothesis that fornix lesions abolish exploratory behavior per se. Based on the fact that FNX monkeys failed to habituate and to develop location preferences, we suggest that an intact fornix is necessary for habituation to a novel environment, as well as to build and update the internal representation of the spatial layout of an environment during exploration. Whether the underlying nature of this deficit is perceptual or mnemonic remains to be established, and this is therefore a topic of considerable interest that future work will aim to address.

6.6 References

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CHAPTER 7

GENERAL DISCUSSION

CHAPTER 7: GENERAL DISCUSSION

On the supposition that fornix transection would be expected to have different functional consequences as damage to the hippocampus itself, the research undertaken in this thesis primarily aims to extend the current understanding of the fornical role in various forms of visual learning and memory in the macaque monkey. In this general discussion, I consider the contributions of each of the experimental studies reported in Chapters 2 – 6, in light of the evidence reviewed in Chapter 1, so as to offer a more global view of the mnemonic roles performed by the fornix in the primate.

It is important to begin by establishing that the absence of impairment noted in some of the tasks in this thesis, such as the larger sets in Chapter 2, the acquisition stage and Experiment 2 in Chapter 5, as well as most of the measures in Chapter 6, support the idea that the fornix is not critical for a range of abilities which include perception of the stimuli on the touchscreen, attention to and short-term retention of stimuli, execution of motor movements and motivations to learn the tasks. By eliminating those abilities which are not affected by a fornix transection, other cognitive attributes which might be mediated by the fornix could then be identified.

7.1 Fornical role in contextual learning

One common theme throughout this thesis is the support it provides for the existing literature that speaks towards a fornical role in the learning of spatial and temporal contexts of stimuli. In Chapter 6, under a large and more naturalistic setting,

fornix transected monkeys are impaired in their exploration of the environment within the chamber (FNX monkeys did not habituate as quickly as controls and they failed to show any predilection for any regions inside the chamber) in comparison to control monkeys. I also found in Chapter 2 that fornix transected monkeys are retarded on holding visuospatial memory of prior errors across time in a task which demands visuospatial memory (FNX monkeys made more non-perseverative errors than controls, but not on count of perseverative errors, suggesting the fornix is involved in monitoring or correcting temporally distant errors). Fornix transection is already known to impair performance in tasks that involve learning about associations of stimuli with temporally differentiated responses (Brasted et al., 2002; Brasted et al., 2003), temporal processing (Charles et al., 2004; Wilson et al., 2007), as well as spatial memory (Murray et al., 1989; Buckley et al., 2004; Buckley et al., 2008). However, the effects of fornix transection upon object recognition memory are inconsistent (Gaffan, 1974; Gaffan, 1976, 1977a, b; Gaffan and Weiskrantz, 1980; Owen and Butler, 1981; Mahut et al., 1982; Gaffan et al., 1984a; Owen and Butler, 1984; Bachevalier et al., 1985a; Bachevalier et al., 1985b; Zola-Morgan et al., 1989; Charles et al., 2004), and fornix transection has been shown not to impair associative learning about objects (Moss et al., 1981) and object-reward associations (Gaffan et al., 1984b). Together with the previous evidence, our findings in Chapters 2 and 6 therefore lend support to the idea that cortical-subcortical connections via the fornix are not important for all forms of new learning but are selectively concerned with new learning of contexts comprising spatio-temporal information.

7.2 Fornical role in conditional learning

On the basis of findings gathered in Chapter 3, I intend to put forward two arguments regarding the learning of conditional rules. Firstly, I suggest that learning of conditional rules generally does not depend crucially upon an intact hippocampal system. Fornix transection has been shown to impair performance of conditional tasks that have spatial or non-spatial elements. Monkeys with fornix transection are impaired on visuospatial conditional discriminations (Chapter 2), visual-spatial or spatial-visual conditional learning (Gaffan et al., 1984b Exp. 5; Gaffan and Harrison, 1989b Exp. 1), visuomotor conditional learning that directs spatial movements (Rupniak and Gaffan, 1987), as well as a non-spatial visuomotor conditional learning task (Gaffan and Harrison, 1988; Brasted et al., 2002; Brasted et al., 2003; Brasted et al., 2005). However, the performance in fornix transected monkeys was facilitated in the experiment in a visuovisual conditional learning task (Chapter 3) and this provides a strong indication that the fornix is not crucial for acquiring the conditional rules per se. Previously fornix lesions have also been found to be without detrimental effects in monkeys trained on different conditional tasks. These include foodstuff-object conditional learning (Gaffan et al., 1984b Exp. 3), auditory-visual conditional learning (Gaffan et al., 1984b Exp. 7), three spatial-visual conditional tasks (Murray et al., 1989), visuomotor conditional learning with a non-spatial instrumental response (Gaffan and Harrison, 1988), and a non-spatial conditional task in marmosets (Ridley and Baker, 1997). Coupled with other findings that monkeys have a preserved ability to learn certain conditional tasks after hippocampal lesions (Murray et al., 1993; Gaffan, 1998) and that fornix transection in rats also does not disrupt the acquisition of conditional learning (Dumont et al., 2007), it is thus possible that the general performance and acquisition of conditional rules does not seem to depend critically on an intact hippocampal system. In addition, a study that has recorded neuronal

responses in macaques found that neurons responded much less commonly in the hippocampus than in the rhinal cortices and area TE during performance of a visual conditional discrimination task (Xiang and Brown, 1999), which taken together with evidence from lesion studies, offers no strong evidence for a major contribution of the hippocampus to visual conditional discrimination.

Instead, involvement of other regions in addition to the rhinal and TE areas is possible (Xiang and Brown, 1999), and one such region at the seat of conditional tasks is the prefrontal cortex. Selective lesions of the prefrontal cortex impair performance of conditional tasks (Halsband and Passingham, 1982; Petrides, 1982) and task-related neuronal activity is found in the premotor cortex (Mitz et al., 1991). Moreover, cutting the uncinate fasciculus (a direct cortico-cortical projection between the temporal and prefrontal cortical regions) impairs conditional visual discrimination tasks (Eacott and Gaffan, 1992; Gutnikov et al., 1997; Parker and Gaffan, 1998), indicating that one important role of the prefrontal cortex is in solving conditional tasks. Note that the fornix carries some projections to the prefrontal cortex, but seemingly the prefrontal-temporal lobe interaction via the fornix is not needed to support all conditional tasks.

Secondly, the findings in Chapter 3 (facilitation in a visuovisual conditional task) suggest that the learning of conditional rules is independent of the way of how stimuli are presented. Eichenbaum and Bunsey (1993; 1995) reported that rats with aspirations of the hippocampus were superior to control animals in distinguishing ‘paired-associates’ from ‘mispairs’. The kinds of discrimination problems used in Bunsey and Eichenbaum’s (1993) task and in transverse patterning tasks (Bussey et al., 1998; Brasted et al., 2003; Saksida et al., 2007) are similar to those employed in Chapter 3 in that the problems must be solved using stimulus-stimulus associations or

conditional rules of the type. However, I note that conditional problems might be solved in different ways depending on the way a task is administered: two stimuli can be presented simultaneously and configurally on a given trial (Bussey et al., 1998; Brasted et al., 2003; Saksida et al., 2007) or they can be presented without a well defined configural arrangement like the case in Chapter 3. The former simultaneous arrangement promotes a configural representation whereas the latter discontiguous arrangement should encourage the two stimuli to be represented individually (Holland, 1991). Because the design of our task in Chapter 3 constantly varies the configuration of sample and choices across trials and can obviate any rote learning strategy that might be assisted by 'cue compression' or 'stimulus fusion' (Eichenbaum and Bunsey, 1995) in which often repeated stimulus arrangements may become encoded as a single percept, our task readily represents a case of discontiguous arrangement. Considering that facilitations are obtained in all of these foregoing studies irrespective of the way of presentation, I therefore suggest that the facilitations in performance, and by extension the general effects as well, after fornix transection on learning conditional problems are not affected by how stimuli are presented and represented.

7.3 Fornical role in fast learning

Another key finding of this thesis is that I demonstrated that the early phases of new learning of visuospatial information are particularly susceptible to fornix transection. This prediction holds valid if learning in organisms can be assumed to operate in stages, for instance the early (fast learning) and late acquisition phases respectively, albeit these stages may be overlapped to some extent. With respect to classical conditioning, Rescorla and Wagner's (1972) seminal theory stipulates that an

organism accrues a larger share of the associative strength when the unconditional stimulus is most surprising while the association is first encountered, and the associative strength available to be learnt slows progressively trial by trial as the unconditional stimulus gets less surprising, until an asymptote of that association is reached. According to this theory, the learning curve of associations is steeper at the outset and levelling off subsequently towards the asymptote. It predicts that associative learning does occur in phases; an organism learns more quickly at the beginning and then the learning slows down as the process progresses.

Fornical damage is known to impair performance in tasks that have some spatial attributes, as previously reported in instances such as spatial recognition in T-maze alternation (Murray et al., 1989), visuospatial conditional learning (Gaffan and Harrison, 1989a; Buckley et al., 2008), spatial discrimination learning (Buckley et al., 2004), and objects discrimination within complex scenes (Gaffan, 1994), but none of these has examined the modulation of phases in initial acquisition. Chapters 2 and 6 advance our understanding of the role of the fornix in this respect, by demonstrating that impairments in new learning can be modulated by the phases of acquisition.

In Chapter 6, although monkeys with fornix transection habituated to their new environment in a chamber at a slower rate than their normal counterparts during the first 20-min session, their habituation caught up after the first testing session and matched the controls' level eventually. Since exploratory learning of an environment involves learning of a wide range of information, I cannot ascertain in that experiment whether the slower learning of the environment was centred on deficits in learning the visuospatial or just the spatial elements of the context, or in perceptual processing of the context, or other non-mnemonic factors such as differential responses to 'stress-inducing' features of the testing situation (Gray, 1982). The finding however

suggests a possibility that the learning deficits caused by fornix transection are not always of an all-or-none manner. It might be a slowing in the initial phases wherein associative information can be learnt rapidly with an intact hippocampal system (McClelland et al., 1995; Brasted et al., 2003) as it has been theorised, from the perspective of connectionist modelling, that the hippocampus might underlie the most rapid learning phases of new associative information (McClelland et al., 1995). The experiment in Chapter 2 attempted to test this 'fast learning' idea more rigorously by training animals with or without fornix transection to acquire conditional visuospatial problems and found that monkeys with fornix transection are impeded in acquiring this kind of problem. Added on to the fast learning impairments already known in visuomotor conditional problems (Brasted et al., 2003) and exploratory tasks (Kwok and Buckley, 2006), the study in Chapter 2 provides new evidence to extend the scope of fast learning impairments to a new kind of problem, namely conditional visuospatial. Data from neurophysiological studies showing that neuronal activity changes relatively early in the hippocampus during the learning of location-scene associations (Wirth et al., 2003) also indicate a wider hippocampal role in subserving the process of fast learning of spatial associations.

Nonetheless, in Chapter 3, I showed that fornix transection does not cause fast learning impairments in a conditional stimulus-stimulus learning task. Monkeys with fornix transection are actually facilitated in that task. The results indicate that fast learning deficits after fornix transection do not necessarily generalise to all learning tasks. Furthermore, it is reported that fornix transection eliminates one-trial learning (which is an important part of fast learning) in a non-spatial visuomotor task (Brasted et al., 2003), but no strong evidence for such a fornical role in this capability, nor for a facilitatory effect of errorless learning, was obtained in Chapter 2. So again,

the impaired one-trial learning after fornix transection does not necessarily generalise to all learning tasks.

In fact, the relevant evidence on fast learning deficits after fornix transection in monkeys has been equivocal. Impairments are noted in spatial (e.g. visuospatial learning in Chapters 2 and 6) and non-spatial tasks (e.g. conditional visuomotor tasks in Rupniak and Gaffan, 1987; Brasted et al., 2003). However, it is not always the case, as associative learning could be unimpaired in non-spatial tasks (e.g. the absence of impairments in visuovisual associative learning in Chapter 3). Thus, it is difficult to determine a specific role for the fornix in associative learning if modality of tasks is the only factor taken into account.

On the other hand, in accord with the 'fast learning' idea, an emphasis has been placed on associations that are learnt rapidly. Indeed, impairments after fornix transection were severest in the smallest problem set of a visuospatial associative task (Chapter 2) or the first 20 min of habituation to a new environment (Chapter 6) wherein 'fast learning' is more importantly involved than the 'slow learning' mechanism. Even though evidence covered here is based on a limited range of tasks, I note that fast learning deficits are not always present in all arbitrary mapping tasks after fornix transection. Irrespective of the nature of tasks, fast learning impairments are generally observed only in tasks that can be acquired quickly (e.g. Rupniak and Gaffan, 1987; Brasted et al., 2003; Kwok and Buckley, submitted) and not in tasks that are learnt much more slowly (Gaffan and Harrison, 1988) wherein learning as a whole might be predominantly mediated by a 'slow learning' mechanism. Therefore, I propose that the discrepancy in these 'fast learning' studies may be differentiated by how quickly associations can be learnt, rather than by the modality of the associations.

7.4 Fornix transection versus neurotoxic hippocampal lesions

Due to the intimate anatomical relationship of the fornix and hippocampus, bilateral fornix transection in primates often produces similar effects to neurotoxic hippocampal lesions (monkeys are shown to be equally impaired on place-in-scene learning (Gaffan, 1994; Murray et al., 1998), delayed matching to location (Murray et al., 1989; Hampton et al., 2004), but unimpaired on concurrent object discrimination (Mahut et al., 1982; Gaffan, 1994; Teng et al., 2000), as well as similarly facilitated on transverse patterning tasks (Saksida et al., 2007)). However, mounting evidence begins to suggest that the effects of the fornix and the hippocampus can be functionally dissociated (as evidence show that the effects of fornical and hippocampal lesions are dissociable in visuomotor learning (Brasted et al., 2005), place-discrimination and reversal (Mahut, 1972; Murray et al., 1998), object discrimination and reversal (Mahut, 1972; Murray et al., 1998), as well as in long-lasting DNMS memory (Zola-Morgan et al., 1989) in macaques. Similar dissociations are also seen in rodents (e.g. McDonald et al., 1997; Cassel et al., 1998; Sziklas and Petrides, 2002; Dumont et al., 2007)).

Monkeys with excitotoxic hippocampal lesions were found to be unimpaired in recognising spatial locations in visual paired comparison (VPC) (Bachevalier and Nemanic, 2008), delayed non-matching to location (DNML) (Murray and Mishkin, 1998) and one-trial spatial memory (Malkova and Mishkin, 2003) after short delays. The lack of impairment in these three previous studies may be attributed to the relatively short delays used in all (i.e. 10 s in VPC, between 30 s to 2 min in DNML and 6 s in the one-trial spatial memory task). In the absence of a functional hippocampus, some authors proposed that memory for spatial location could be

maintained, at least for short delays, by the medial temporal cortical areas, such as TH/TF and/or the lateral prefrontal areas (Bachevalier and Nemanic, 2008). Some workers also showed that regions that process spatial information project heavily to the parahippocampal cortex in the monkey (Burwell et al., 1995).

Conversely, in Chapter 5, damage to the fornix is shown to affect spatial recognition memory even after some very short delays (the longest is 16 s). This contrasts with the lack of impairment in neurotoxic hippocampal monkeys in comparable locations recognition tasks (Murray and Mishkin, 1998; Malkova and Mishkin, 2003; Bachevalier and Nemanic, 2008) (but also see (Hampton et al., 2004)), a comparison leading to the conclusion that the fornix and hippocampus might mediate spatial recognition memory differently. In fact, this behavioural dissociation of effects of fornical and hippocampal damage needs not be surprising in view of the predominantly subicular origin of fornix fibres for the following reasons. As the fornix contains efferents from the entorhinal and perirhinal cortices, the loss of these projections following fornix damage could then add to the consequences of the concurrent hippocampal disconnection (Saunders and Aggleton, 2007). Moreover, the parahippocampal cortical areas might have some functional properties that are independent of the hippocampus (Eichenbaum and Bunsey, 1995; Aggleton and Brown, 1999; Malkova et al., 2001), therefore the disconnection of these regions could also create additional deficits to those brought about by hippocampal dysfunction.

I suggest the impairments observed in Chapter 5 can be linked to the disconnection of the parahippocampal cortical areas following fornix transection and some evidence is as follows. A study has shown that damage to parahippocampal areas TH and TF alone can result in deficits in spatial location recognition

(Bachevalier and Nemanic, 2008). Non-selective (Ploner et al., 1999) and selective (Bohbot et al., 1998; Bohbot et al., 2000) damage to areas TH/TF are also found to impair memory for spatial location in human patients. Recent neuroimaging studies also show that areas TH/TF are activated in normal subjects during Spatial Location memory (Sommers et al., 2005; Buffalo et al., 2006) and encoding of new locations during navigation (Kessels et al., 2001). Therefore, in line with the results in Chapter 5, I argue for the view that the effects of a fornix lesion could be dissociated from that of a hippocampal lesion on some memory processes, such as spatial recognition memory, in the macaque.

Furthermore, although I proposed in Chapter 5 that the deficits caused by the fornix in that spatial recognition task lies in task acquisition, I cannot ascertain in that study which fibres in the fornix are supporting the task. One explanation for the pattern of impairments is the hypothesis that fibres in the fornix carry a cholinergically mediated reinforcement signal that is required for new learning (e.g. task-set acquisition) but not for retrieval (a non-fornical contribution to retention is discussed in Chapter 4). In support of this hypothesis, Gaffan and colleagues (2001) produced dense amnesia in monkeys as they compromised all of the projections into the temporal lobe cortex that flow from the basal forebrain. According to this account, fornix transection alone would not produce dense amnesia, because cholinergic fibres connecting the basal forebrain and temporal lobe cortex, through the amygdala and anterior temporal stem, remain intact and therefore can support some degree of learning (Buckley et al., 2004). Indeed, the extent to which learning processes is fornix dependent is elucidated by noting that not all recognition memory tasks require the fornix, as recognition of objects (DNMS) does not, especially when interference from using repeated stimuli within and across days is removed by using trial-unique

stimuli (e.g. Gaffan and Harrison, 1989a; Murray et al., 1989). Thus, fornix transection would only be expected to impair learning processes that critically depend on that specific route, and I suggest task-set acquisition might be one of them.

7.5 Implications for human amnesia

Whilst much of this thesis is devoted to exploring the role of the fornix in macaque monkeys, the importance of its implications on human studies also warrants a discussion. One implication I can draw from macaque studies is that, as shown in Chapter 4, the very long-term retention of visuospatial information is independent of the fornix and can be kept as long as up to 15 months after criterial acquisition by the monkeys. As discussed in Chapter 4, it is possible that learning of the visuospatial problems in that experiment, in normal animals, may proceed by a combination of fornix dependent and fornix independent learning strategies given that the fornix is not necessary for all forms of spatial learning (Gaffan and Harrison, 1989a; Murray et al., 1989 Exp. 2). If this speculation holds true, and given that both groups of monkeys forgot at the same rate, I argue for the view that different routes to learning by monkeys do not dictate how things are subsequently remembered (Gaffan, 1992a, 1993). Similarly, on a conditional visuomotor associative task, fornix transection also does not affect the retention of postoperatively learnt problems (Brasted et al., 2003). These findings coincide with human literature that reports normal recognition after fornical damage (Huppert and Piercy, 1978; Squire, 1981; Kopelman, 1985; Gaffan, 1991, 1992b; Aggleton et al., 2000) and help draw parallels in the effects on retention of previously learnt material between fornical damage in clinical cases and those observed following more selective surgery in nonhuman primates. The observed

pattern of results from these human studies, together with the present findings in fornix transected monkeys, rules out the notion that the difference between normal and amnesic memory is to be explained by increased susceptibility to forgetting, implying that the anterograde amnesic deficit in amnesic patients and macaques is more likely an acquisition or learning deficit instead. In addition to visuospatial information, this acquisition deficits account might apply to cover acquisition of task-sets too, as detailed in Chapter 5.

From a rehabilitation viewpoint, the literature has described a number of memory enhancement techniques to target people with various degrees of memory impairments (Miotto, 2007) and one of the more successful techniques used is 'errorless learning' (Tailby and Haslam, 2003; Clare and Jones, 2008). The errorless learning technique involves learning or encoding new information without error. To achieve this, individuals are given the correct information during each learning episode. In a typical experiment, this involves the examiner providing the same new information to the patient over multiple learning trials, with the patient repeating or writing down the information (Tailby and Haslam, 2003). This technique was first applied to the treatment of memory disorders by Wilson et al. (1994) and subsequently shown to enhance learning and reduce forgetting rates in amnesic patients (Baddeley and Wilson, 1994), assist learning of extended word lists (Hunkin et al., 1998) and word pairs (Squires et al., 1997), help restore premorbid knowledge (Parkin et al., 1998) as well as aid develop procedural skills in a work setting (Andrewes and Gielewski, 1999). However, the benefits are not evident for all cases, particularly not so when acquiring more complex information (Tailby and Haslam, 2003; Clare and Jones, 2008). In Chapter 2, in line with these clinical observations, I presented experimental evidence that extends the beneficial effect of errorless learning

to a different kind of information, namely visuospatial associations, which accordingly strengthens the notion that errorless learning does have a useful role to play in memory rehabilitation in fornix lesioned subjects.

7.6 On episodic memory and future directions

Impairments on animal tasks that are designed to model aspects of human episodic memory (e.g. one-trial learning and learning within a context) after fornix transection or hippocampal lesions are consistent with the repeated findings that fornix damage in humans, like hippocampal pathology, is sufficient to produce anterograde amnesia (Gaffan et al., 1991; McMackin et al., 1995; Aggleton et al., 2000; Poreh et al., 2006). Although domains of spatial and temporal and object processing have been considered separately in the experiments on fornix transected animals, it is interesting to speculate whether these domains of information, when bound together, will be impaired by fornix transection. On the supposition that the neuronal architecture of brain areas (likely including the hippocampal formation given its role in learning about context) thought to be involved in episodic memory are substantially similar in mammals and, arguably, all vertebrates (neuroanatomical data reveal possible homologues of hippocampus in mammals, birds, and reptiles (e.g. Northcutt, 1981; Butler and Hodos, 1996; Rodríguez et al., 2002)), it has been proposed that certain animals possess an episodic-like memory system (Morris, 2001). Clayton and colleagues (1998; 2001; 2003) developed a paradigm based upon criteria for episodic memory defined by Tulving (1972), in which scrub jays showed that they were sensitive to the spatio-temporal relations of events. This type of task then, binds together the ‘what’, ‘where’ and ‘when’ information that captures the behavioural

criteria for episodic memory. Future research in the ambulatory apparatus described in Chapter 6 aims to clarify the role of the fornix in a primate analogue of this task.

Finally, as subcortical damage leading to amnesia in humans is usually not restricted to a single structure, dense amnesia may be more readily explained if the contributions of various cortical and subcortical structures and/or pathways are taken into account together. For example, a recent study shows that an addition of fornix transection to monkeys who had received a disconnection of frontal and inferotemporal cortex significantly increases impairment on a scene learning task. The additive effect of fornix transection suggests that the functions of the fornix in scene learning – and, by extension, episodic memory – are distinct from that of frontal-temporal interaction (Wilson et al., 2008). The findings of Wilson et al. (2008) argue against the traditional hypothesis that the fornix forms a unitary system with the mammillary bodies as part of a proposed cortico-cortical association pathway for episodic memory (Parker and Gaffan, 1997). Also of particular relevance is Mitchell's et al. (Mitchell et al., 2008) study which shows that monkeys with combined magnocellular mediodorsal thalamus (MDmc) and fornix lesions are impaired in retention and relearning of preoperatively acquired object-in-place scene problems, as well as new learning of the same kind of problems. The impairment in new scene learning of combined lesions was much greater than the effect of MDmc lesions alone. With the addition of fornix transection, monkeys made three times as many as errors as monkeys with only selective MDmc lesions in a previously reported study on the same task (Mitchell and Gaffan, 2008). These findings again show that when fornical damage is combined with other brain lesions, the severity of impairment could be additive. These two reports not only challenge the emphasis placed on identifying one critical region responsible for dense amnesia and other cognitive processes associated

with the medial diencephalon, but also reinforce the view that episodic memory is supported by a widespread cortical and subcortical network. In light of these recent findings, I suggest interactions between the fornix and other subcortical (e.g. magnocellular mediodorsal thalamus in Mitchell et al., 2008) and cortical structures (e.g. disconnecting frontal and inferotemporal cortex in Wilson et al., 2008) in various aspects of episodic memory shall form the base of future investigations.

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LIST OF ABBREVIATIONS

CON	Normal unoperated controls
DMS	Delayed matching to sample
DNMS	Delayed non-matching to sample
DNML	Delayed non-matching to location
FNX	Fornix transection
MDmc	Magnocellular mediodorsal thalamus
MTL	Medial temporal lobe
VPC	Visual paired comparison task
WGTA	Wisconsin General Test Apparatus