Helen Browncross
University College

THE ROLE OF THE PRIMATE FRONTOPOLAR CORTEX IN MNEMONIC AND CHOICE BEHAVIOUR

Thesis submitted for the degree of Doctor of Philosophy

Trinity Term 2014

Table of Contents

ABSTRACT **2**

DETAILED ABSTRACT **3**

ACKNOWLEDGEMENTS **9**

INTRODUCTION **12**

CHAPTER 1: Lesions to frontopolar cortex impair recency memory in macaque monkeys **65**

CHAPTER 2: The role of the macaque frontopolar cortex in recollection **96**

CHAPTER 3: Monkeys with lesions to anterior cingulate cortex differ from controls and from monkeys with lesions to frontopolar cortex in the influence that the outcomes of previous trials exert on current behavioural choices **117**

CHAPTER 4: The role of the macaque frontopolar cortex in social valuation **173**

CHAPTER 5: Lasting reductions in resting state functional connectivity after frontopolar lesions in monkeys **191**

CHAPTER 6: Recovery of function after principal sulcus lesions assessed by longitudinal behavioural testing **218**

DISCUSSION **247**

REFERENCES **264**

APPENDIX **282**

Helen Browncross
University College

DPhil thesis
Trinity Term 2014

The role of the primate frontopolar cortex in mnemonic and choice behaviour

ABSTRACT

The role of the primate frontopolar cortex (FPC) has been investigated using human neuroimaging, lesion and disruption techniques. The results of these investigations have led to a variety of theories regarding the function of this region. It has been linked to the formation of task sets, the performance of multiple tasks, reasoning, context-specific memory (including episodic memory, prospective memory and source memory), attention to internally or externally generated information, mentalising and decision-making. It has not previously been possible to study this area using animal lesion techniques. Here, behavioural experiments conducted using non-human primates (rhesus macaque monkeys) who have received lesions to the frontal pole investigate the contribution of this region to context-specific memory, decision-making and social cognition. Functional magnetic resonance imaging (fMRI) is used to investigate changes in functional network connectivity which occur after lesions to this region. A long-lasting impairment is observed in contextual memory judgements (specifically, how recently a stimulus was encountered) after lesions to the frontal pole. An analysis of the influence of the outcomes of previous choices on behaviour on an analogue to the Wisconsin Card Sorting Test (WCST) indicate that monkeys with lesions to area 10 may be less influenced by the outcomes of an extended history of rewards than control animals. Long-lasting widespread disruption to functional networks after lesions to this region indicate that indirect anatomical connections from this region to posterior areas play a crucial role in the normal functioning of posterior networks.

DETAILED ABSTRACT

Evidence collected in humans has implicated frontopolar cortex (FPC) in retrieval of context-specific information, although the contribution of this region to this process is unclear. One possibility is that FPC is involved in the recall of temporal context information. In Chapter 1, I investigated the contribution of the macaque frontal pole to the recall of short-term temporal context information (specifically, which of two stimuli was presented more recently). I trained three rhesus macaque monkeys who had received lesions to the frontal pole three years previously on a task designed to test their ability to distinguish how recently a stimulus had been presented. Five visually distinct cartoon-style stimuli were presented sequentially on a touchscreen. After monkeys responded to each of these stimuli in turn (by touching the picture on the touchscreen), two stimuli from this sequence were presented simultaneously on the touchscreen. Monkeys were rewarded for touching the stimulus which had been presented later in the sequence. Performance data from these animals was compared to previously collected control data (Charles et al., 2004). FPC-lesioned monkeys were significantly impaired compared to control monkeys on this task.

In Chapter 1, I also tested monkeys on a task designed as a control for the recency task. I tested four animals on this task three years after lesions to FPC, and three animals both pre-operatively and two weeks after lesions to FPC. In this task, macaques were again shown five visually distinct cartoon-style images, presented sequentially on a touchscreen. After they had responded to these stimuli (by touching the picture on the screen), two stimuli were presented on the screen. One of these stimuli was derived from the sequence of stimuli already presented, and the other was novel. Macaques were rewarded for touching the already-presented stimulus. This experiment tested macaques' ability to retain and retrieve multiple stimuli over a short period of time, but did not require them to retain any contextual information. Analysis of performance data on this task indicated that macaques that had received lesions thirty-five months previously were not impaired on this task relative to controls; however, macaques that had received lesions two weeks previously

were impaired relative to their pre-operative performance. Taken together, the results presented in this chapter indicate that macaques with lesions to FPC receive an enduring selective deficit to recency memory; however, they also receive a short-term impairment in recognition memory. These results indicate that the macaque FPC may be functionally homologous to human Brodmann's area 10 (BA10), as both regions are implicated in memory for contextual information. Furthermore, these results indicate that the time of testing relative to lesion may constitute a critical variable in animal lesion studies.

In Chapter 2, I extended the research conducted in Chapter 1 by conducting an examination of the role of the macaque FPC in recollection. Three animals with lesions to FPC (sustained an average of 26.5 months previously) and two unoperated control animals were trained on a task which utilised the Receiver-Operating-Characteristics (ROC) technique; the two unoperated animals were retested on this task shortly after receiving frontopolar lesions. This technique has previously been used in experiments with rats (Eichenbaum et al., 2010) and monkeys (Guderian et al., 2011) to assess the differential contributions of an episodic-like recollection process and a context-independent familiarity process to recognition memory. In this task, monkeys were presented with three visually distinct cartoon-like images displayed sequentially on a touchscreen. After they responded to these images, they were presented with six recognition memory trials, in which a stimulus and a red square were displayed on the screen, in the top half of the area. Monkeys were rewarded for touching the stimulus if it had been previously displayed, or for touching the red square if the stimulus had not been previously displayed. Additionally, they were biased towards touching either the stimulus or the red square by the presentation, in the bottom half of the screen, of a number of smaller items, which were either replications of the cartoon-like stimulus or the red square. Monkeys were required to touch all of the items of one kind on the screen in order to move onto the next trial and before receiving any reward. By varying the number of items presented in the bottom half of the screen (from zero to eight), it was possible to assess monkeys' recognition memory performance at varying levels of bias towards making an "old" or "new" response. By

plotting the likelihood of a correct "old" response (a "hit") versus the likelihood of an incorrect "old" response (a "false alarm") at varying bias levels, it is possible to draw a curve from which estimates of the contribution of recollection (which is a high-confidence, threshold process) and familiarity (which is a signal detection process) to recognition memory in these animals may be extracted. By assessing the estimated contribution of recollection to recognition memory in FPC-lesioned and unoperated animals, we hoped to assess whether lesions to FPC impair recollection in macaques. However, as we were not able to reliably bias animals towards one response or another in this task, it was not possible to adequately address the hypotheses of this experiment.

In Chapter 3, I examined the contribution of the FPC and other prefrontal regions to decision-making in macaques. Rhesus macaque monkeys were tested on an analogue of the Wisconsin Card Sorting Test (WCST) developed by Buckley et al. (2009) before and after lesions to FPC, superior dorsolateral prefrontal cortex (sdlPFC), principal sulcus (PS), orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC); control monkeys were tested before and after a rest period. Multiple analytic approaches were taken in this chapter. As in previous papers (Buckley et al., 2009), monkeys with lesions to PS, OFC and ACC were significantly impaired in overall performance on this task post-operatively compared with control monkeys and monkeys with lesions to sdlPFC or FPC. The influence of the outcome of previous trials on monkeys' choices on future trials was assessed using multiple logistic regression. The influence of rewarded outcomes to the two possible sorting rules ("match shape" and "match colour") on future choices varied depending on both how recently the outcome occurred, and the lesion experienced by the monkey. A rewarded outcome in the most recent trial had a large positive influence on the likelihood of the monkey choosing the same rule in the next trial; however, this influence was reduced in ACC- and OFC-lesioned animals. A rewarded outcome in the third most recent trial had a small positive influence on the likelihood of preoperative and control animals choosing the same rule in the next trial; however, this effect was reversed in FPC-lesioned monkeys. The influence of a non-rewarded outcome in previous trials on rule choice also varied significantly based on the lesion-status of the monkey and the recency of the

outcome, although it was not possible to isolate the source of this interaction. This was not significantly influenced by the level of uncertainty that monkeys experienced regarding the correct rule choice. The influence of the concrete properties of the stimulus (location on screen, shape, colour) chosen by the monkey on the most recent trial on choices in future trials was also considered. Monkeys with lesions to OFC were more likely to repeat choices of location after a non-rewarded choice to that location. These results indicated that FPC may contribute to decision-making in macaques, as in humans, although further research is required in order to firmly establish the nature of this contribution.

In Chapter 4, I investigated the role of the FPC in social cognition. Neuroimaging research in humans and research with brain-damaged patients indicates that medial FPC is functionally involved in cognition about one's own and others' mental states. In this chapter, macaques were tested prior to and after lesions to FPC, or prior to and after a rest period on a task designed to measure animals' attention to social, neutral and fear-inducing stimuli. In this task, monkeys were given the opportunity to obtain a valuable food reward. This was presented on top of a clear Perspex box. On each trial, either an object was placed in the box (a neutral object or a rubber snake) or a video clip was played on a screen behind the box (a neutral screensaver animation, a human displaying threatening behaviour, or a monkey engaged in one of a variety of behaviours). The time that it took monkeys to reach out and retrieve the food reward was monitored. A longer reaching latency was associated with a greater level of attention towards the distracting fear-inducing, threatening, neutral or social stimuli. Monkeys with lesions to FPC showed shorter reaching latencies to social stimuli post-operatively when compared with pre-operative reaching latencies, indicating a lower level of attention to these stimuli post-operatively; however, no significant difference could be observed when this reduction in reaching latencies was compared with the difference between pre- and post-rest period reaching latencies recorded in control animals. It is possible that this pre- and post-operative change in performance was due to habituation to the social stimuli used in this task.

In Chapter 5, I analysed functional connectivity in anaesthetised monkeys. Compared with unoperated controls, monkeys with lesions to frontal pole (scanned an average of forty months post-lesion) showed a significant decrease in functional connectivity, as measured by correlation of the blood-oxygenation-level-dependent (BOLD) signal between regions in functional magnetic resonance imaging (fMRI) scans. This decrease was noted in widespread networks, and was not restricted to networks which are anatomically linked to FPC. Functional connectivity measured in monkeys scanned eight weeks after lesions to FPC did not significantly differ relative to either control animals or monkeys scanned forty months post-lesion. This result indicated that indirect anatomical connections between FPC and posterior brain regions are important for normal network activity between those regions. No improvement in functional connectivity was noted between animals scanned eight weeks post-lesion and animals scanned forty months post-lesion (there was a decrease in functional connectivity between these time-points), indicating that it was not possible to parallel the improvement in behavioural performance observed in Chapter 1 between similar time-points with recovery in functional networks in the brain.

In Chapter 6, I built upon the research conducted in Chapters 1 and 5 into recovery after experimentally-inflicted lesions. I tested two rhesus macaque monkeys on two experimental tasks (delayed match-to-sample and delayed match-to-position) pre-operatively, after unilateral lesions to left principal sulcus, and after symmetrical lesions were added to right principal sulcus (thus making the lesions bilateral after the second surgery). These lesions were expected to impair performance on the delayed match-to-position task but not on the delayed match-to-sample task. These tasks were tested periodically: monkeys received three continuous days of testing of each task at 21 day intervals. This meant that it was possible to periodically record monkeys' performance on this task without an extensive period of retraining after the lesion was performed. The results of this study indicated that at least one monkey showed a significant effect of recovery on the delayed match-to-position task after the unilateral lesion was performed, although it was not possible to determine whether this improvement in performance was due to retraining on the task or spontaneous

recovery from the lesion. Forthcoming research will indicate whether this behavioural recovery can be paralleled with recovery in functional connectivity (as measured by periodic fMRI scans).

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Dr. Mark Buckley, for his guidance on all aspects of the work presented in this thesis, and for his feedback on draft chapters of the thesis. I would also like to thank Dr. Buckley for his work in performing all the surgeries in our laboratory. I would like to thank Biomedical Services and veterinary staff for their work in providing excellent daily care for the animals tested in these experiments, and Dr. Erica Boschin for her guidance in how to conduct experimental training with monkeys. I would like to thank Stuart Mason for perfusing the animals and Greg Daubney for preparing histological sections of the monkey brains. The research was funded by contributions from a Medical Research Council (MRC) project grant (Dr. Buckley), a MRC programme grant (Prof. Matthew Rushworth and Dr. Buckley and others) and a MRC programme grant (Dr. David Gaffan) and the Department of Experimental Psychology. I also thank MRC for their generous financial support through a Doctoral Training Award and an Early Career Centenary Award which enabled me to undertake this programme of study.

I performed all of the behavioural training and testing described in Chapter 1, with the exception of testing control animals in the recency task. The recency task was originally designed and implemented by Dr. David Charles, Dr. David Gaffan and Dr. Mark Buckley and the control data for this experiment was gathered by these researchers. I designed and implemented the recognition memory task (adapted from a program originally written by Dr. Buckley). I performed all data analysis and interpretation described in this chapter.

I designed and implemented the task described in Chapter 2, and performed all the behavioural training and testing in this chapter. The DPSDSSE spreadsheet provided by the Yonelinas laboratory was essential for the analysis of these results. I conducted this analysis and interpreted the results.

In Chapter 3, I extensively recoded and reanalysed data previously collected by Dr. Buckley's laboratory in Oxford and Dr. Keiji Tanaka's Laboratory for Cognitive Brain Mapping at RIKEN Brain Science Institute, Wako, Japan. I designed the data analysis, wrote the program which extracted the data, implemented the data analysis and interpreted the results.

In Chapter 4, I scored and analysed data previously collected by Dr. Jerome Sallet and Dr. Carinne Piekema. The experimental paradigm was originally designed by Dr. Peter Rudebeck, Dr. Mark Buckley, Dr. Mark Walton and Prof. Matthew Rushworth. Dr. Sallet, Dr. Piekema, Dr. MaryAnn Noonan and I scored the data. I conducted all data analysis and interpretation. Dr. Sallet kindly provided comments on a draft of this chapter.

Dr. Andrew Bell and Dr. Anna Mitchell operated the MRI scanner to collect the data analysed in Chapter 5. Anaesthesia for the scans was provided by Dr. Anna Mitchell and the Biomedical Services and veterinary staff. I provided post-anaesthesia supervision for these animals. Dr. Daniel Mitchell and Dr. Andrew Bell provided invaluable support and advice, including analysis scripts, to help me to conduct fMRI data processing. I designed and conducted the data analysis on extracted BOLD correlation coefficient scores and interpreted the results. Kathy Mitchell and Dr. Jerome Sallet provided advice regarding the interpretation of anaesthetic effects. Dr. Anna Mitchell helped me to prepare figures showing the location of ROI seeds.

Chapter 6 constitutes one part of a wider study conducted as a collaboration between my laboratory, and the laboratories supervised by Dr. Anna Mitchell, Dr. Andrew Bell and Prof. John Duncan. In collaboration with these researchers, I designed and implemented the behavioural tasks (adapted from programs originally written by Dr. Buckley). I performed all behavioural training and testing. These animals were tested on the project licence held by Dr. Anna Mitchell. I conducted data analysis with advice from Prof. John Duncan. I interpreted the results.

Personal acknowledgements

I would like to thank my partner, Jonathan Piper, my family and my friends (especially Zara Ahmed and Mark Mills) for their support over the last few years. My office mates (especially Erica Boschin and Julia Badger) have provided a good deal of moral support throughout the period of this DPhil, especially the last few months. Jonathan Piper, Jonathan Brooks, Mark Petersen, Edward Lacey and Erica Boschin all deserve a great deal of thanks for proofreading drafts of individual chapters.

INTRODUCTION

The frontopolar cortex (FPC), the most anterior region of the prefrontal cortex, has attracted increasing attention in research in recent years. It constitutes a larger proportion of the frontal lobe cortex in humans than in other apes (Semendeferi et al., 2001) and its location within the frontal lobe cortex may place it at the top of a proposed rostral-caudal prefrontal hierarchy (Petrides, 2005), in which more anterior regions process information and goals at successive levels of abstraction; this speculation is supported by the fact that cells in the human FPC are relatively more interconnected than other regions of the brain (Jacobs et al., 2001). However, research in this region poses several challenges: it has proved very difficult to target this region using transcranial magnetic stimulation, animal neurosurgery or neuronal recording, and very few patients have been found with selective damage to this area. Furthermore, despite a large number of functional neuroimaging studies which have found activation in this region in response to a wide range of different tasks and scenarios (a selection of these studies is reviewed below), patients with lesions to this area show a surprising lack of cognitive impairment; they typically have no impairment in verbal or non-verbal IQ, no anterograde or retrograde memory impairment, and no difficulty in performing inhibition tasks such as the Stroop or set-shifting tasks, such as the WCST: this sparing of cognitive ability can be seen both in patients with extensive damage including almost the entirety of bilateral FPC but also extending to other areas, such as patient AP/NM (the same man) in Shallice and Burgess (1991) and Wood and Rutterford (2004) and in patients with damage restricted to the FPC, such as the patient described by Hoffmann and Bar-On (2012). However, studies suggest that patients with lesions to this region may struggle in unstructured scenarios in which they have to weigh the competing demands of multiple tasks, and in considering their own and others' emotional states (Burgess et al., 2000; Roca et al., 2011; Hoffmann and Bar-On, 2012; these studies are discussed in greater detail below). Additionally, there is some evidence which may suggest that individuals with autism

spectrum disorders, attention deficit hyperactive disorder and schizophrenia may have anatomical and functional abnormalities in FPC (see Dumotheil et al., 2008, for a review).

There are a number of competing theories of the function of FPC, relating to cognitive functions as wide-ranging as prospective and episodic memory, mentalising, counterfactual evaluation, integrating the results of multiple cognitive operations and the direction of attention towards internally or externally-generated information. However, while these theories have each accounted for a selection of the evidence relating to this region, few have been able to encompass the diversity of scenarios in which FPC is activated while explaining the relative lack of cognitive deficit in FPC patients. In this introduction, the current state of research regarding the function of the FPC in human and non-human primates is described. I describe the anatomy of this region, and proceed to examine the evidence for the major competing theories of FPC function.

ANATOMY OF THE HUMAN FRONTOPOLAR CORTEX

The human FPC is roughly equivalent with the cortical region identified by Brodmann as area 10 (Brodmann, 1909). Its limits are defined in terms of cytoarchitectonic structure, which makes it difficult to define any structural landmarks which could be detected by neuroimaging. It is surrounded by Brodmann's area 9 (dorsally), Brodmann's areas 32, 45, 46 and 47 (caudally) and Brodmann's areas 11 and 12 (ventrally). However, there have been disagreements regarding the precise extent of the region: for example, Öngür et al. (2003) identify three separate subregions of area 10, of which areas 10r and 10m extend caudally on the ventral cingulate gyrus, an area which was considered a part of area 32 in Brodmann's map. There is also disagreement regarding the size of BA10. Some researchers have claimed that it is the largest region of the frontal lobes (e.g. Burgess et al., 2006 – "…in volumetric terms probably the largest single architectonic region of the frontal lobes…", Dumontheil et al., 2008 – "Rostral prefrontal cortex (PFC), which corresponds approximately to Brodmann area 10 (BA10), is the largest single architectonic region of the frontal lobes of the human brain"). However, this claim is based on a table given by Christoff et al. (2001) of

volumetric sizes of prefrontal anatomical regions (the regions of interest in their study), in which the combined volume of all BA10 sub-regions is listed as 33.6 cm^3. However, the basis for this claim is dubious: not only is BA10 not the largest region listed by Christoff et al. (adding half of the volume of the medial BA8/9 subregion to the calculation of volume of either BA8 or BA9 leads to a larger estimation of volume for those regions), but this estimation is not in line with other estimations of the volume of human BA10: for example, Semendeferi et al. (2001) list the volume of right BA10 as 14.2 cm^3. However, there is no doubt that BA10 is a large area of the frontal lobes; using Semendeferi's estimate, it constitutes 2.4% of the total brain volume of humans. Such a large area is likely to play an important role in human cognition.

Cytoarchitectonically, it is distinguished by a clear boundary between layers III, IV and V, a gradual increase in the size of pyramidal cells from upper to lower layer III, a peak cell density in layer IV, and clear subdivisions between layers Va and Vb, with a lower cell-density in layer Vb (Bludau et al., 2014; Semendeferi et al., 2001). Bludau et al. note that layer IV is particularly broad; this does not replicate the findings of Semendeferi et al., who note that it is thin relative to other cortical layers. Overall, cell density is lower in BA10 than in other regions of the human cortex: Semendeferi et al. (2011) found that BA10 had a mean horizontal spacing distance (HSD) between cells of 59.52 μm (standard deviation (S.D.) 12.77 μm); this was the largest in any of the human regions studied (BA3, BA4, BA10 and BA17; the second-largest was BA4: mean HSD 49.23 μm, S.D. 7.72 μm). It is possible that this cell density means that there is more space available for the spread of dendrites; this conclusion in supported by evidence that BA10 has a higher number and density of dendritic spines per cell and a greater dendritic length than other regions of the human brain. Jacobs et al. (2001) compared BA10 with BA3-1-2, BA4, BA22, BA44, BA6β, BA39 and BA11. Dendritic length in BA10 was 31.4% higher than in the least complex region examined (BA3-1-2); number of dendritic spines per cell was 19.9% higher in BA10 than BA3-1-2; dendritic spine density was 68.9% higher in BA10 than BA3-1-2. BA10 had a significantly higher number of dendritic spines and dendritic density per cell than any other region studied. These statistics suggest that BA10 may have an advantage in

processing information which requires integration of input from multiple sources; the fact that average dendritic length is longer in BA10 than in other regions may suggest that neurons in this region receive inputs from a widely distributed network, rather than forming reciprocal connections with neighbouring cells.

COMPARISONS BETWEEN HUMAN AND NON-HUMAN PRIMATE FPC

Much research in neuroscience relies on the use of animal models. This technique is important, as it allows us to make use of methods which would be unacceptable in human subjects: in particular, the infliction of targeted neurosurgical lesions. However, while behavioural research on lesioned animals allows us to learn about the localisation of cognitive function in those species, it is not possible to generalise these results to humans unless we have reason to believe that these regions are homologous between these species.

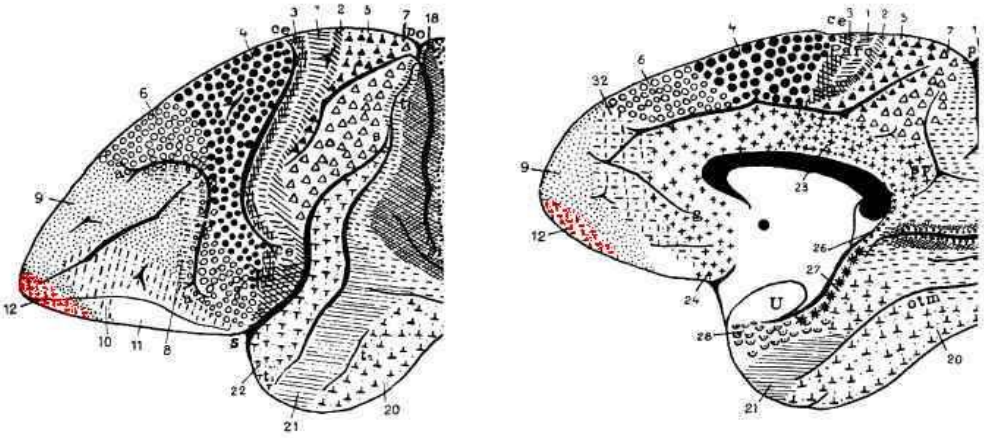
There are clear differences in the anatomy of FPC between primate species: most obviously, area 10 constitutes a larger brain area in humans (2.4% of total brain volume) than in other species of primate (0.92% - 1.48% in chimpanzees, orangutan, gorillas, bonobo and gibbons) (Semendeferi et al., 2001; although Holloway, 2002, disputes that this is a substantial increase). The low cell-body density which characterises this region in humans is not replicated in other primate species: Semendeferi et al. (2001) found that cell bodies constituted 15.17% (S.D. 1.89) of the total space in the region in humans (grey-level index; GLI); the closest value to this found in a primate species was in chimpanzees (GLI: 17.52 S.D.: 1.58), while macaques had a GLI of 20.34 (S.D. 1.86). In addition, human BA10 shows a greater level of horizontal spacing between cells than the equivalent region in great apes: the largest non-human area 10 HSD reported by Semendeferi et al. (2011) was 41.76 µm (S.D. 3.58 µm) (gorilla), compared to human figure reported above of 59.52 µm. This difference between humans and other great apes was not found for other regions of the brain (BA3, 4 and 17): in the primary motor cortex, mean HSD was larger in chimpanzees and gorillas than in humans (although this finding did not reach significance). In all other species, HSD was greater in BA4 than in

the frontal pole. Similarly, Spocter et al. (2012) compared neuropil distribution in the cerebral cortex of humans and chimpanzees. While neuropil space was significantly larger in the human BA10 than in other cortical areas examined (areas 45, FI, 4, 41/42 and 22), this pattern was not replicated in chimpanzees. Furthermore, the cytoarchitecture which defines BA10 in humans is not consistently found in the FPC of other primate species. According to Semendeferi et al. (2001), the distribution of cortical layers in BA10 is relatively homogenous in humans, with a much lower cell density in supragranular layers and a maximum cell density in level IV. While all other primates studied had a low cell density in the supragranular layer, bonobos and gibbons had much more pronounced peaks in cell density in layer IV, and gorillas and macaques had much more notable variation in the density of cortical layers. In particular, there is reason to believe that area 10 in the gorilla may not be homologous to the frontopolar region in other primate species. Layer II and Va are particularly prominent in this region in the gorilla; these distinctive cytoarchitectonic features are not found in other great apes.

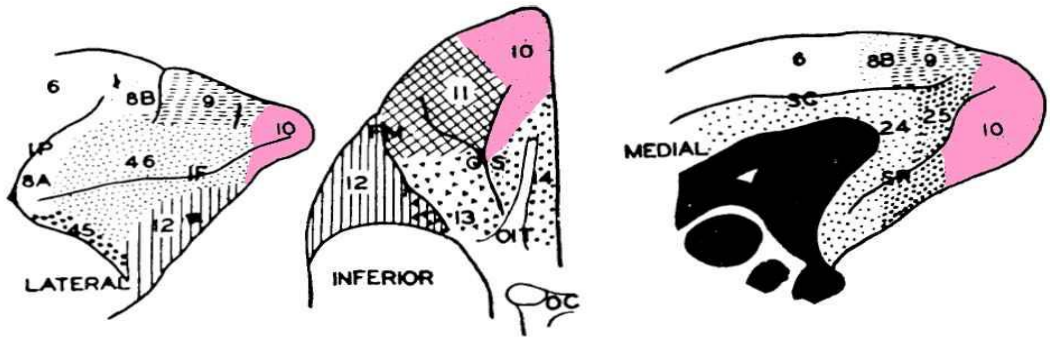
Despite these differences, it is clear that many of the features which mark BA10 in the human brain define a similar area in other primate species. Semendeferi et al. (2001) identify a region in the frontal pole of macaques, chimpanzees, bonobos, gorillas, gibbons and orangutan which has several characteristic cytoarchitectonic features shared with the human BA10: a layer I which is medium in width, a thin layer II, a broad layer III in which pyramidal cells increase in size as the layer is descended, a clearly distinguishable layer IV, a layer V which can be subdivided into two layers, of which layer Va is denser and populated by larger cells than layer Vb, and layer VI, which has a medium thickness and a clear boundary with white matter. Peak cell density was in level IV for all species. Similarly, Petrides and Pandya (1999) note the increasing size of pyramidal cells as layer III is descended, a “well-developed” layer IV and the population of layer Va with larger cells than layer Vb in both human and macaque area 10. This shared cytoarchitecture supports the view that this area is homologous in these species, and that it is valid to use macaque area 10 function as a model for the equivalent human brain region.

ANATOMICAL SUBDIVISIONS OF FPC

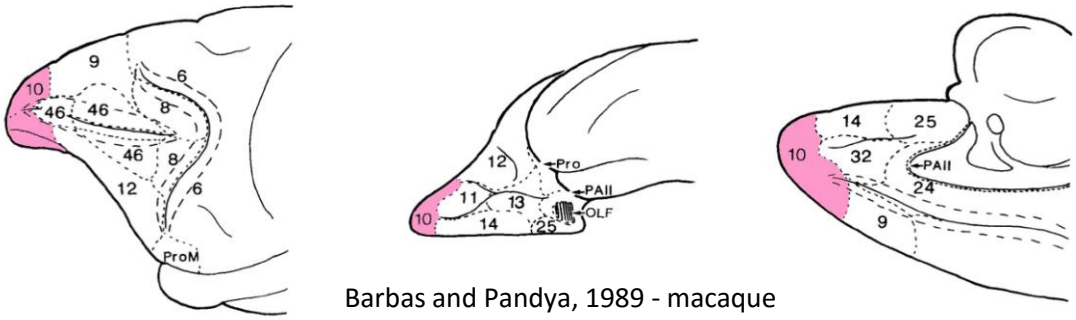
So far, the frontopolar cortex has been addressed as a whole. However, it seems to be the case that both anatomical and functional subdivisions may exist within this region in both macaques and humans. Brodmann (1905) provided a parcellation of the cerebral cortex of the guenon monkey, and much research has been conducted in order to extend these classifications to macaques; however, there has been disagreement regarding the extent of area 10 in the macaque monkey (while Brodmann labelled the guenon frontopolar region as area 12, this labelling has not been followed by other researchers), and regarding whether this area may be subdivided into distinct anatomical regions. While Walker (1940), Barbas and Pandya (1989) and Preuss and Goldman-Rakic (1991) described a cytoarchitecturally homogenous area, Carmichael and Price (1994) identify two distinct anatomical regions within macaque area 10: 10o (orbital) and 10m (medial) (they omit the dorsal part of area 10 from consideration in this paper). The medial area features horizontal organisation of granular cells within layer IV, a feature which is absent from the orbital area. In addition, vertical neurite bundles were observed in the medial area, but not in the orbital area. Semendeferi et al. (2001) arrive at a different subdivision: they note that in the dorsal part of the macaque frontal pole, layer Va is particularly pronounced, while in the orbital region of this area, layer V is less pronounced and more homogenous in nature: the subdivision between layers Va and Vb is less clear. These studies differ in their descriptions of the dorsal and medial extent of area 10 (see figure 1). These differences in definition may have important consequences in attempting to draw conclusions about the function of this region: fibres identified by Petrides and Pandya (2007) which project through the cingulate fasciculus originate in the dorsal area of the macaque frontal pole, but not the ventral orbital area.



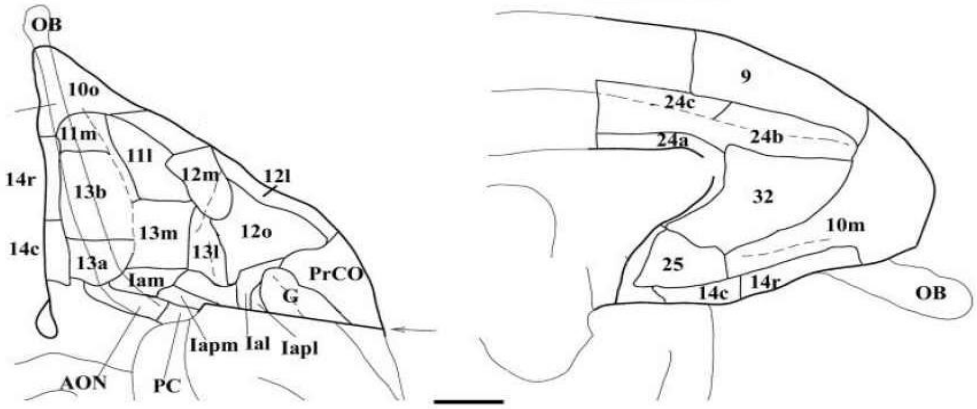
Brodmann, 1905 – guenon (note that the frontopolar region is labelled as area 12)



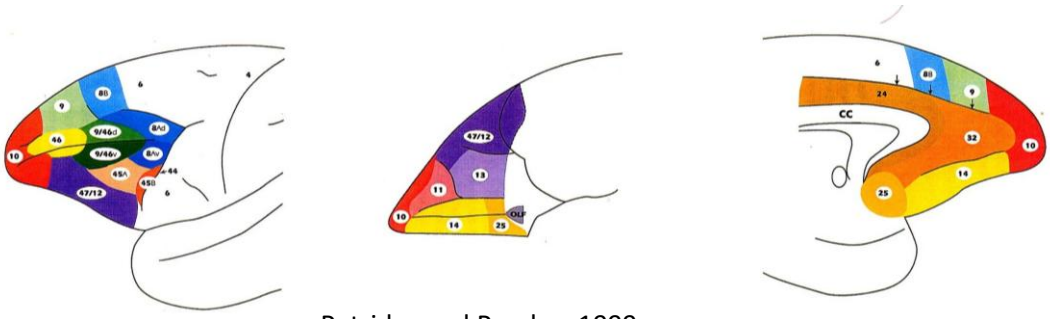
Walker, 1940 – macaque



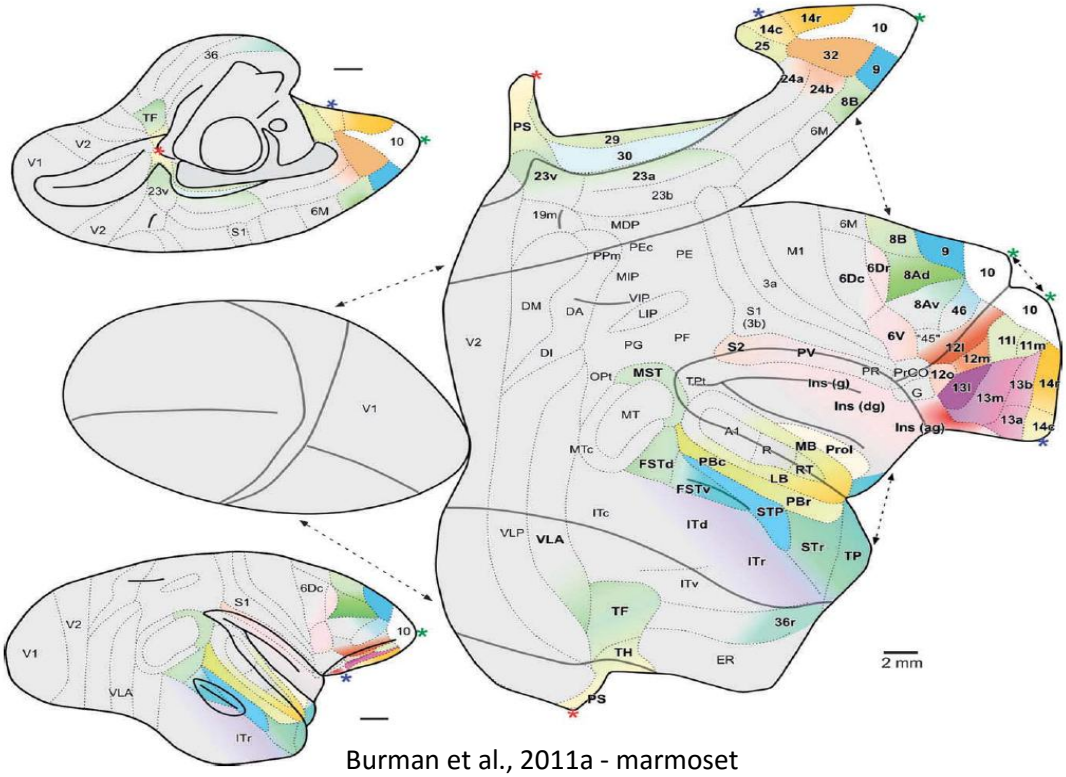
Barbas and Pandya, 1989 - macaque



Carmichael and Price, 1999 - macaque



Petrides and Pandya, 1999 - macaque



Burman et al., 2011a - marmoset

Figure 1 – Differing views on the dorsal and medial extent of area 10 in guenon, macaque and marmoset monkeys. From top: Brodmann (1905), Walker (1940), Barbas and Pandya (1989), Carmichael and Price (1999), Petrides and Pandya (1999), Burman et al. (2011a).

Sallet et al. (2013) used a different approach to study the organisation of the macaque and human frontal cortex. Using diffusion-weighted-tractography (DWT) (this technique is discussed in more detail below, p.21), these researchers parcellated the human dorsal frontal cortex into distinct regions; following this parcellation, they used functional connectivity measures (analysis of the

correlation of the time-course of the blood oxygenation level dependent (BOLD) signal between regions) in order to compare the "fingerprint" of connectivity between regions in humans and macaques. In the human parcellation, they identified an anterior region on the medial surface which they identified as area 10; while they also identified an adjacent lateral region, they argued that, based on its connectivity profile, it most closely resembled macaque area 46 (this conclusion supports the view of Petrides, 2005, who noted cytoarchitectonic similarities in layers III and IV between macaque area 10 and area 46). The area that was identified as area 10 could not be reliably subdivided. In a subsequent study with a similar methodology, Neubert et al. (2014) examined the human and macaque ventral frontal cortex. These researchers found two regions within the frontopolar area in humans, which they identified as FPm (frontopolar medial) and FPl (frontopolar lateral). The connectivity profile of area FPl, as noted by Sallet et al., resembled that of area 46, in both humans and macaques, but was treated as a distinct region in this study. A "cluster" of voxels was identified in the macaque frontal pole which shared a connectivity profile with the human FPm region. This study argued that the macaque FPC cannot be subdivided into two distinct regions, as there was no equivalent to human area FPl in terms of the connectivity of the macaque frontal cortex. However, it is important to note that connectivity was examined under different conditions in humans and macaques: humans were scanned while awake, while monkeys were scanned when anaesthetised; Hutchison and Everling (2012) review evidence of resting state connectivity in different anaesthesia conditions, and note that quantifiable differences exist between these states. Furthermore, the human FPl region connectivity profile resembled that of area 46 in both humans and macaques.

In humans, Öngür et al. (2003) examined frontopolar cytoarchitecture and replicated the subdivision observed by Carmichael and Price between the orbital and medial frontal pole in monkeys. They further subdivided the medial frontal pole into areas 10m (medial) and 10r (rostral) and renamed area 10o as area 10p (polar). These areas shared some cytoarchitectonic features, in particular a "crisscross" appearance which occurred as a result of apparent organisation of cells in

rows and columns. However, the regions could be distinguished in a number of ways: area 10p showed a higher level of granularity than areas 10m and 10r; layer III was more developed in area 10p than area 10r, and more developed in area 10r than area 10m; layer IV was thinner in area 10r than in area 10p or area 10m. Bludau et al. (2014), however, proposed a different subdivision of BA10 in humans based on cytoarchitectonic distinctions. These researchers described two regions in FPC as Fp1 (lateral) and Fp2 (medial); they did not subdivide the medial region. The main differences between these regions were a higher cell density in layer II and lower layer III, and a broader layer IV in Fp1 compared to Fp2. However, there were considerable similarities between Fp1 and Fp2 compared to adjacent prefrontal regions (BA9, BA11, BA46 and BA32), implying that these areas should be considered subdivisions within a larger frontopolar region. The volume of Fp1 was larger than the volume of Fp2 in both hemispheres of every brain examined by these researchers; the mean volume of Fp1 was more than double the mean volume of Fp2. This paper has the advantage of using an observer-independent method of distinguishing cytoarchitectonic variation (developed by Schleicher et al., 2009), and so this subdivision of the human frontal pole should be considered more reliable than others described here.

There is little agreement regarding the extent of the cytoarchitectonic region which occupies the FPC, and there is little agreement concerning whether and how it should be subdivided in humans or macaques. A detailed anatomical reanalysis of this region is beyond the scope of this thesis, which will focus on the functional role of the area; in lieu of agreement, this thesis will focus attention on the polar area which all studies agree constitutes area 10 in humans and macaques.

STRUCTURAL CONNECTIVITY OF FPC

It is possible to examine the connectivity of the frontopolar cortex in macaques through the injection of tracers into the area of interest, followed, after the passage of a number of days in order to allow diffusion of the tracer, by euthanasia and post-mortem examination of the brain; this method is the gold standard tract tracing method. This can be used to examine both the efferent

and afferent pathways of a region; in the former case, anterograde tracing is used to examine neural pathways from their source to their point of termination, while in the latter case, retrograde tracing is used to examine neural pathways from their point of termination to their source. This technique, clearly, is not available for the study of the human brain. It is necessary to use diffusion tractography to compare the similarity or otherwise of the white matter pathways emanating from and going to the frontal pole of the human and macaque brain. In a white matter pathway, water molecules will diffuse in the direction of the axon, rather than randomly; diffusion tractography uses neuroimaging to examine the primary direction of this diffusion across voxels. If multiple voxels show an aligned direction, it is possible to conclude that a white matter pathway has been detected.

Petrides and Pandya (2007) examined the efferent pathways of area 10 in the macaque brain using anatomical tracing. They found that the frontal pole was extensively interconnected with adjacent areas of the prefrontal cortex: a large number of fibres were observed which terminated in all areas of the prefrontal cortex. In addition, they observed three main pathways which projected from the frontopolar cortex to other regions of the cortex: a dorsal pathway via the cingulate fasciculus which contained fibres terminating in the anterior and posterior cingulate cortex and the retrosplenial area; a ventral pathway via the uncinate fasciculus which terminates in the amygdala, the rostral superior temporal gyrus and the temporal pole of the proisocortex; and a pathway via the extreme capsule and superior temporal gyrus, joining the middle longitudinal fasciculus and terminating in the superior temporal gyrus, the superior temporal sulcus, the insula and the circular sulcus. No fibres were observed which would connect area 10 with the parietal cortex, the occipital cortex or the inferior temporal cortex.

The study discussed above used anterograde tracing to examine efferent pathways from macaque area 10. Burman et al. (2011a, 2011b) examined both the cortical and subcortical afferent connections to area 10 in the marmoset monkey using retrograde tracing. They identified a similar range of connected regions to those discussed by Petrides and Pandya. On average, 57.7% of cortical

inputs to FPC identified in this study originated from other frontal regions, while 35.6% came from temporal regions. Of these, consistent projections to frontal pole were identified in the parabelt region, rostral superior and inferior temporal cortex, temporal pole and parahippocampal and perirhinal regions. Small but consistent projections were found from retrosplenial cortex. In contrast to Petrides and Pandya, projections from anterior and posterior cingulate cortex were considered "sparse" and "variable". When subcortical projections were studied, the strongest input to the frontal pole came from the claustrum (this constituted up to 50% of subcortical projections to rostral area 10), the mediodorsal thalamic nucleus and the ventral anterior thalamic nucleus. A "sparse" projection to area 10 was identified from the amygdala.

Thiebaut de Schotten et al. (2012) used an extension of diffusion tractography called spherical deconvolution tractography to evaluate the connectivity of the human frontal cortex. They found that both the pathway via the uncinate fasciculus and the pathway via the extreme capsule (which Thiebaut de Schotten et al. label as the inferior fronto-occipital fasciculus, although Schmahmann and Pandya (2006) reject that such a long-distance tract exists) can be observed in humans and connect to BA10. They did not observe a contribution of BA10 to the cingulate fasciculus. They additionally identify a frontal orbito-polar tract which connects BA10 to the posterior orbital frontal cortex, corroborating the evidence from monkey studies that BA10 is extensively interconnected with other areas of the prefrontal cortex. Sallet et al. (2013) and Neubert et al. (2014) compared the connectivity of the human and macaque frontal cortex using a combination of diffusion tractography (humans) and examination of correlations in the BOLD signal fluctuation during resting state fMRI (macaques). Sallet et al. replicated Petrides and Pandya's findings of connectivity between area 10 and anterior and posterior cingulate in both humans and macaques, of connectivity between area 10 and other areas of the prefrontal cortex (specifically, the ventral medial and ventral caudal aspects) and of connectivity between area 10 and the anterior temporal sulcus and temporal pole. Neubert et al. identify a lateral and medial subregion of the frontal pole in humans, and a medial region in monkeys: the lateral region largely showed connectity

with other prefrontal areas (areas 8, 9, 32, medial PFC and precentral gyrus) while the medial region, in both humans and macaques, showed connectivity with the temporal cortex (including temporal pole, fusiform face area/face superior temporal sulcus, parahippocampal place area/place inferior temporal gyrus) and the prefrontal cortex (including area 32, vmPFC, and areas 8 and 9). This is broadly consistent with the information reported above: the FPC in both humans and macaques shows extensive interconnectivity with other regions of the frontal cortex, some connectivity with regions in the temporal cortex, and little connectivity with regions in the parietal or occipital cortices.

Markedly different results were observed by Markov et al. (2014). Using retrograde tracing, these researchers identified inputs to macaque FPC from a wide range of brain structures; while major inputs were identified from frontal and temporal areas, weak projections were also noted from a range of posterior areas, including parietal and occipital regions (see figure 2).

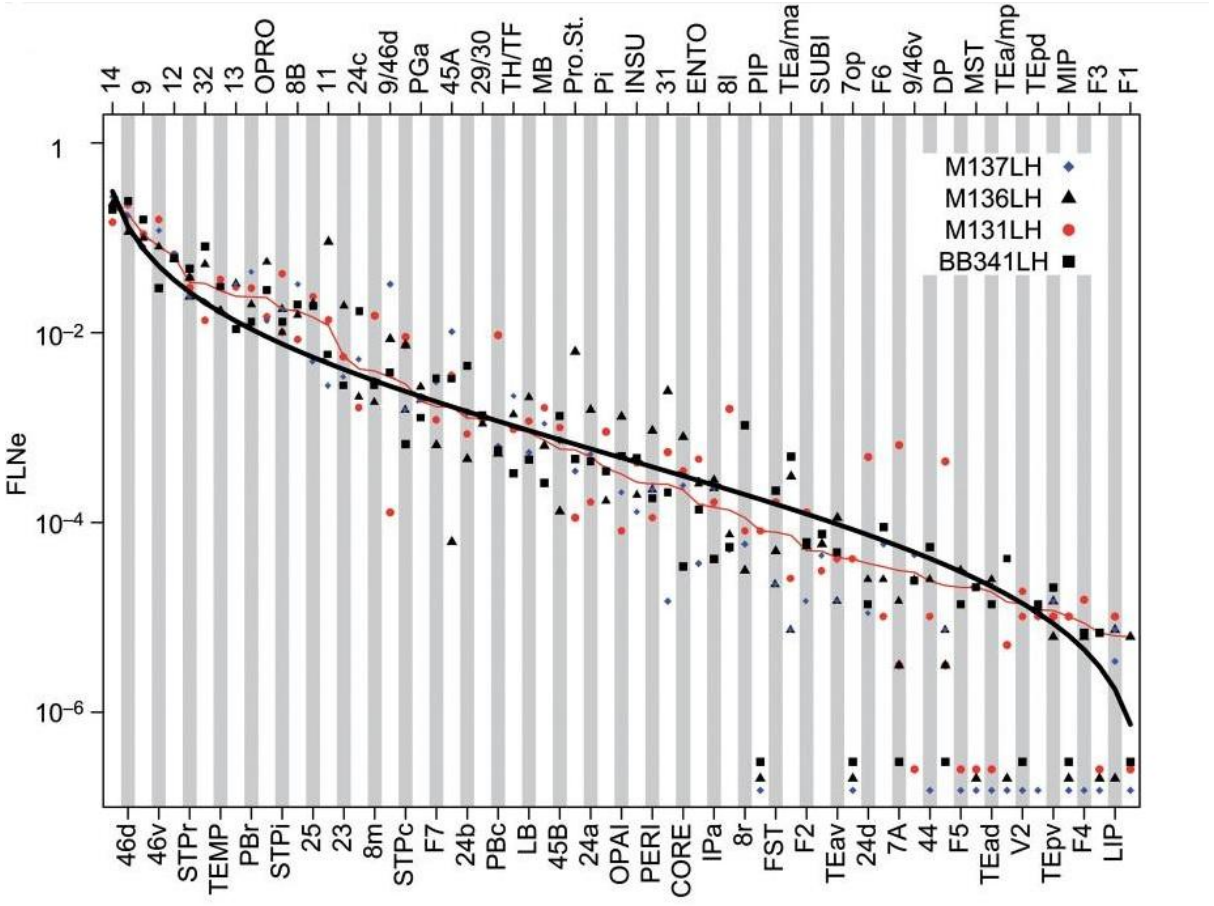


Figure 2 – Anatomical connections to macaque FPC from a wide range of brain regions, including parietal and occipital areas. For each region, the fraction of total labelled neurons (extrinsic to area 10) which were observed in that area is plotted. Data from four animals is included in the graph. From Markov et al., 2014.

THE FUNCTION OF THE FRONTOPOLAR CORTEX

The anatomy of the frontopolar cortex in both humans and macaques suggests some ideas regarding the function of this region. The low cell density and increased volume of dendritic spines in the human area 10 supports the view that this region may be involved in computations which integrate a relatively higher number of inputs than computations in other areas. FPC only receives very weak inputs from primary sensory areas (Markov et al., 2014), suggesting that the computations which occur in this area relate to stimulus-independent information or stimulus-dependent information at a higher level of abstraction. In the next section, theories regarding the

functional significance of this area in primates, and the evidence that supports them, are examined. Seven categories of theory will be examined:

1. Task set theories
2. Multiple task theories
3. Reasoning theories
4. Context-specific memory theories
5. Attention to internally/externally generated information
6. Mentalising
7. Decision-making theories

In order to propose a satisfactory account of the information-processing which is carried out within the FPC, theories must meet certain conditions. Below, I have discussed theories of FPC function and evaluated them against the following criteria:

- Firstly, it is important to have some kind of evidence that disruption of FPC leads to disruption of this processing (via neuropsychology, human non-invasive disruption studies or non-human lesion studies); human neuroimaging results are not adequate in themselves, as it is not the case that all neural activity that takes place while humans are performing some sort of task is task-relevant (especially considering evidence that BA10 is active in mind-wandering scenarios when participants are not attending to the task at hand e.g. Mason et al., 2007).
- Secondly, activity in BA10 should be observed for all tasks that obviously require cognitive processing of the type described in the theory. It is not possible to rule out a theory if one study fails to detect BA10 activity in a task in which it is predicted, as genuine activations may not pass the threshold for statistical significance in every instance; however, if a majority of studies do not find activation in FPC in a scenario in which it is predicted, the theory should be discarded.

- Less crucially, but still important, a theory cannot satisfactorily explain BA10 activation unless it contains some account of the cognitive process which underpins the observable behaviour; since behaviour is the result of the cumulative activity of the whole brain, it is rarely possible to attribute any form of complex behaviour to the specific activity of a particular brain region. Rather, some form of cognitive processing takes place in that brain region which enables some aspect of behaviour to occur. Noting behaviours which are reliant on the intact functioning of BA10 can only be a helpful first step towards understanding the processing which occurs in that region; it is necessary to supplement these observations with evidence from methodologies which analyse the activity of intact brains in order to understand the nature of the neuronal computations which take place in this region.
- Lastly, and least importantly, in order for a theory to be complete, it must attempt to explain a large proportion of the evidence with regard to when BA10 is activated and the deficits suffered when BA10 is disrupted. However, there may be a degree of functional specialisation within this region: in particular, dissociations have been observed between lateral and medial and left and right FPC. It may be that these sub-regions should be addressed separately in terms of theorising regarding their function.

1. Task set theories

Some researchers have suggested that the FPC is necessary for the preparation of cognitive sets; that is, the representation of a certain goal, and of all the processes which are necessary to achieve that goal, in preparation for executing those processes. There is substantial evidence which suggests that activity in FPC varies as a function of the task demands that participants are primed to expect, rather than as a function of the actual requirements of each trial, and which suggests that task-related activity can be observed in FPC before participants are given the necessary information to begin processing on an individual trial: for example, Burgess et al. (2001) used positron emission

tomography (PET) to observe activations while participants performed four prospective memory tasks. The details of these tasks differed, but all required a simple evaluation of an object or objects on the screen, with the instruction to press one of two keys depending on the outcome of the evaluation; if another condition on the screen was met, subjects were to press a third key. They observed an increase in activity in the lateral frontal pole bilaterally in all four tasks relative to activity when the same tasks were performed without a prospective memory component, regardless of whether or not the target that subjects were primed to expect actually occurred. Similar results were found by Simons et al. (2006), who noted bilateral lateral BA10 activation and medial BA10 deactivation when subjects performed a task with a prospective memory component; Haynes et al. (2007), who noted left lateral BA10 activation in the delay between making a decision to perform an action and implementing that decision; and Sakai and Passingham (2002), who observed right lateral BA10 activation in the delay between instructing participants on the actions in an upcoming trial, and implementation of those actions.

Sakai and Passingham (2002) found that interactions between right FPC and posterior frontal regions during the delay between instruction and task were modulated by the domain and type of task to be executed, although FPC activity itself was not modulated by either of these considerations: correlation between activation in the right FPC and the left inferior frontal gyrus was significantly higher in the condition in which participants had to verify whether verbal (rather than spatial) stimuli had been presented in a memory probe in the reverse order to the original presentation, rather than verifying that they had been presented in the original order, and an increase in activation in BA10 was associated with a greater increase in activation in the superior frontal sulcus in the spatial-backward condition than in other conditions. There was a significant main effect of the type of memory task on accuracy, which indicated that the backward task was more difficult than the forwards task. Sakai and Passingham (2006) developed this work subsequently and found that activity in left FPC was modulated by task domain: subjects were asked to classify a word in terms of its phonological (number of syllables), semantic (concrete or abstract)

or visual (upper- or lower-case) properties. In this task, phonological and semantic classifications were associated with greater lateral FPC activation in the delay between presentation of task instructions and target stimuli than visual classifications. Greater preparatory FPC activity in these trials was correlated with a lower RT. Furthermore, associations were found between task-domain and correlations between activity in left FPC and other regions: correlations were significantly higher between area 10 and area 6 in the phonological condition than either of the other conditions, and correlations were significantly higher between area 10 and area 47 in the semantic condition than in the other conditions. This evidence suggested that activity in FPC modulates activity in more posterior regions in order to prime these regions for completing the cognitive task once the stimulus is presented on the screen.

In addition to the evidence which implicates FPC in the creation of preparatory task sets, a number of studies have found that differences can be observed in FPC activations between blocks with differing task demands, even when these differences cannot be observed at the level of individual trials with varying task demands within those blocks. These studies include Koechlin et al. (1999), Braver et al. (2003), Wagner et al. (1998) and Rugg et al. (1999). However, many studies have also found modulation of FPC activity in relation to the task demands of individual trials (e.g. Boorman et al., 2009, Christoff et al., 2001, Kahn et al., 2004, Geake and Hansen, 2005; each of these studies is discussed in more detail below).

Konishi et al. (2005) suggested that the role of BA10 in relation to task sets may be in suppressing task-sets that have recently become irrelevant. Using the WCST paradigm, they found that activation in left lateral BA10 was significantly lower on trials in which the correct stimulus matched the target not only on the current dimension, but also on the dimension which had recently been switched away from (so that application of either rule would lead to a correct response), compared to trials in which a distractor stimulus matched the target in two dimensions that were not currently relevant. They suggested that deactivation in BA10 during these trials is due to the

temporary removal of the requirement to inhibit of previous task sets, which continue to be active for a short period of time after they become irrelevant. While this theory is somewhat plausible, evidence regarding WCST performance in FPC patients is mixed. Nagahama et al. (2005) found that reduced cerebral blood flow to area 10 was associated with a larger number of perseverative errors after a set shift in Alzheimer's patients. However, in case studies of patients with lesions to FPC (Shallice and Burgess, 1991, Hoffmann and Bar-On, 2012, Uretzky and Gilboa, 2010) and in studies of patients with frontal lesions, including BA10 (Mountain and Snow, 1993), no deficits in the WCST related to BA10 injury were observed. Macaques with FPC lesions are not impaired on the WCST (Mansouri et al., submitted; see also Chapter 3 of this thesis).

The evidence is fairly convincing that the lateral frontopolar cortex conducts preparatory activity based on expected task demands, and that part of this preparation involves the modulation of the activity of posterior regions. The majority of studies cited here localise this processing to the left hemisphere; however, Burgess et al. (2001) found bilateral activation and Sakai and Passingham (2002) found right-lateralised activation. However, "task-sets" as a theory of lateral BA10 function fail on multiple counts. If it were the case that FPC controlled the operation of task sets for every aspect of human behaviour, lateral BA10 would be activated by any situation in which participants were required to perform a cognitive task. This is clearly not the case: Christoff et al. (2004) compared activation when participants performed a low-demand task (pressing buttons which corresponded with the direction of arrows on a screen) with activation during a period of rest, when participants were engaged in spontaneous thought. The lateral FPC was more active in the rest condition than during the low-demand task, although this difference only reached significance when corrections for multiple comparisons were not applied. Furthermore, if the lateral FPC was necessary for normal performance in any situation in which it is necessary to apply a task set, we would expect frontopolar patients to display deficits on a much broader range of tasks than the limited range of difficulties that it has been reported that they experience.

The FPC is activated when task sets are shifted (multiple task theories of BA10 function are discussed below), but is also activated in situations in which task-sets are constant, and is activated at individual trial level in other contexts; defining the cognitive properties of situations which engage FPC, at task-set or individual trial level, is as necessary to understanding the function of this region as defining the level (task-set or trial-level) at which lateral BA10 is engaged in these scenarios. Furthermore, stating that BA10 is in some way involved in preparing to complete a task is not an adequate explanation of the role of BA10 in that preparation.

2. Multiple task theories

Many researchers have suggested that the frontopolar cortex is implicated in complex situations in which subjects have to balance the requirements of several tasks; I have used the term "multiple task" to refer to this kind of scenario, in contrast to the term "multi-tasking", in which participants are required to perform two tasks at once, or switch between two tasks rapidly and at short notice. Evidence which supports this theory is drawn from both neuropsychological observations that patients with damage which includes area 10 are impaired in situations in which they are required to balance multiple goals or switch between different tasks, and neuroimaging of healthy participants performing complex tasks with shifting rules and goals.

One large study providing support for this view was conducted by Burgess et al. (2000), who investigated the performance of 60 patients who suffered from lesions to various areas of the cerebral cortex on a complex multiple task scenario, in which participants were required to sample, but not complete, three tasks, and attempted to maximise their score by prioritising certain trials within those tasks. Patients with lesions to BA8, 9 and 10 were particularly poor at maximising their score: they broke the rules of the task more often than patients with lesions to other brain areas, and were impaired at task-switching. They were unimpaired when asked to recite the rules of the task (this was seen as evidence that patients had unimpaired retrospective memory) or formulate a plan for maximising the score within the task (this was taken as evidence of intact prospective

memory). However, as patients with lesions to BA8, 9 and 10 were grouped together, this study cannot provide evidence regarding the individual contribution of these regions.

Roca et al. (2011) were able to provide a more detailed view of the function of BA10. They compared patients who had received frontal lesions including BA10 with patients who had received frontal lesions excluding this area and with healthy controls. They found that the seven BA10 patients were significantly impaired on two of the tasks examined when compared with healthy controls: the Faux Pas task (which is discussed below), and the Hotel Task, in which subjects must divide their time between five tasks within a fifteen minute period in order to achieve a higher level goal (estimating how much time each of the five tasks would take to complete); performance on this task was measured by deviation from the optimal equal division of time between tasks and by the number of tasks attempted. Additionally, the BA10 patients were impaired compared to non-BA10 (n=8) patients on the Hotel Task. The extent of damage to right lateral BA10 was correlated with impairment on the Hotel Task.

While there is strong evidence that frontopolar patients find some aspect of dealing with complex environments and multiple task-goals difficult, it is necessary to examine further research to discover the cognitive deficits which underpin these behavioural difficulties. The Hotel task requires participants to create a plan, bear in mind sub-goals and higher-level goals simultaneously, recognise an appropriate cue for shifting tasks, and switch tasks multiple times within a short time-frame; impairment on any one of these cognitive requirements would cause BA10 patients to perform poorly on this task.

Rowe et al. (2007) examined four patients with lesions of the left rostral PFC which overlapped in areas 10 and 46. Participants were shown a sequence of four letters and four spatial locations and then, after a short delay, asked to answer a question about either the letter sequence or the spatial sequence while receiving an MRI scan. Participants were warned before the sequences were shown which one was relevant on that trial. Compared to controls, patients were less accurate

on trials in which the modality of the relevant sequence had shifted. As shifts were dictated by the task rather than controlled by the participants with regard to higher-level goals, this implied that participants were impaired at task-switching per se, although it is not impossible that other aspects of formulating and executing a plan with respect to goals and sub-goals are also impaired; these were not addressed by this study. In addition, Rowe et al. found that the activity in five other regions (pre-SMA, frontal eye fields, fusiform cortex, parietal cortex, area 8 and area 44) was less correlated in patients than in controls subjects, particularly on non-domain switch trials; these results imply that the computational deficit experienced by these patients related to coordination of activity in posterior brain regions, although the mechanism by which this deficit would impair task-switching while sparing many other complex tasks is unclear. However, the low number of patients included in this study means that the results must be treated with caution.

One theory which attempts to explain these deficits states that area 10 is responsible for cognitive branching (e.g. Koechlin et al., 1999, Charron and Koechlin, 2010, Koechlin and Hyafil, 2007, Koechlin and Summerfield, 2007). According to this theory, the frontopolar cortex enables human multitasking by providing a reservoir in which information relating to a pending task (or a pending task set) can be maintained in working memory in order to optimise retrieval of this task when it is reactivated. A typical branching study was conducted by Koechlin et al. (1999). In this task, participants were asked to perform letter-sorting tasks: in the delay condition, participants were asked to ignore lower-case letters and perform a task in relation to upper-case letters; in the dual-task condition, participants were required to perform an ongoing letter-sorting task and perform an additional task whenever there was a change between upper- and lower-case; in the branching condition, the case-change task only applied immediately after a upper- to lower-case shift and participants were asked to maintain the most recent upper-case letter in memory to resume the ongoing task after a run of lower-case letters. Lateral dorsal FPC activation was observed bilaterally in the branching condition, but not the dual-task or delay conditions. In a subsequent neuropsychological study by this laboratory, using the same behavioural paradigm, error rates in the

branching condition, and not in the dual task condition, were correlated with lesion size in left BA10 (Dreher et al., 2008). Similar findings were reported by Braver and Bongiolatti (2002), although in this case increased activity was observed only in the right lateral FPC. This activity was attributed to maintenance of information relevant to a pending task while another task was occupying limited resources in the posterior and medial prefrontal cortex (Dux et al., 2006, present evidence that the posterior lateral PFC cannot process information relating to multiple decisions simultaneously). This activation could not be attributed to simply maintaining task-relevant information over a delay or to task-switching; however, both reaction time and error rates were higher in the branching condition than any other condition (although these differences did not reach significance compared to the dual-task condition), implying that there was a difference in task difficulty between these conditions.

However, there is no consensus that BA10 is not activated in task-switching scenarios. Braver et al. (2003) found an increased level of activity in right anterior prefrontal cortex (lateral and medial), including BA10, during blocks of trials which included task-switching compared to single-task blocks, although, as in Koechlin et al. (1999) there was no trial-level effect. No information needed to be maintained in working memory over task switches, and participants were instructed to switch tasks, rather than doing so as a means to achieve an overarching goal, so this scenario did not require cognitive branching. It is possible, if switching frequently between two tasks, that maintaining the task set related to the currently non-performed task may optimise performance when it is resumed; however, it is also possible that the lack of trial-level effects may imply that FPC is recruited in dealing with complex, demanding and variable environments rather than operating at the level of controlling shifts between tasks on individual trials.

Furthermore, the branching theory does not immediately explain the scope of deficits which patients suffer: Rowe et al.'s patients were impaired after switching tasks, despite no requirement to maintain information in working memory. Most significantly, in the complex scenarios which Burgess et al.'s and Roca et al.'s patients were required to navigate, the optimal solution was to plan to

spend a certain amount of time on each task, and carry out the plan sequentially, not interrupt one task with another. This evidence, however, does not entirely rule out the branching theory: it is possible that the non-BA10 control patients with which the BA10 patients were compared do not have the optimal strategy when approaching a complex task such as the Hotel Task, and will interrupt one task with another, in which case an intact BA10 may assist in the task through the maintenance of a pending task set: evidence from Koechlin et al. (2000) suggests that, in healthy participants, BA10 is engaged to a greater extent when participants were not able to plan task shifts in advance.

The Tanaka laboratory conducted a study in which macaques that had received lesions to the FPC were tested on an analogue of the WCST (Mansouri et al., submitted). When the animals were working at a high level of accuracy (85% correct responses), they were sometimes interrupted with a single instance of a face/object discrimination task between WCST trials. After the monkeys responded to the discrimination task, they resumed the WCST, applying the same rule (shape/colour matching) as was in force before the interruption. Under the branching hypothesis, FPC-lesioned monkeys should be impaired on resuming the task, as they would not be able to store the WCST parameters (specifically, which rule was currently in force) when that task was interrupted by another one. However, these monkeys were not impaired at retaining the task rule across the interruption, and in fact performed better than controls when the WCST task resumed. While it is true that the face/object discrimination task was very easy, the combination of this evidence and the evidence cited in the previous two paragraphs makes it seem unlikely that the branching hypothesis provides a good account of the FPC function.

Another theory, suggested by Boorman et al. (2009), suggests that the FPC is involved in evaluating unchosen possibilities. This study is described in greater detail below; however, it is relevant to consider here, as it may account for the impairment of FPC patients in multiple-task scenarios and for the activation of FPC in task-switching experiments. In this study, participants were

asked to choose between different alternatives in the same task (the left or right side of the screen), rather than alternate between different tasks; however, as in scenarios in which participants are required to switch between different tasks, participants were asked to assess the value of different alternatives (following a different rule or different set of rules) and adjust their behaviour accordingly. Activity in lateral FPC was found to correlate with the estimated probability of the option that the participant did not choose relative to the estimated probability of the chosen option.

Generalising this account of the evaluation of counterfactual rewards to multiple-task situations does explain the behavioural deficits noted in some frontopolar patients: it may be difficult to switch between tasks at appropriate points in the Hotel Task if patients are only accurately able to estimate the reward associated with completing the current subtask, and not the reward available for sampling all of the subtasks. An alternative account of the findings of Boorman et al. is possible, however: these researchers found that FPC activation during the ITI predicted the likelihood of a behavioural shift on the next trial; increased activation in the FPC may have been associated with an increased estimation of the relative reward of the unfavoured option, or it may have encoded the likelihood of participants choosing to switch behaviours in upcoming trials; as reviewed above, FPC is implicated in task-switching scenarios when participants are instructed to change tasks, and not only when participants choose to do so in order to maximise reward.

Ramnani and Owen (2004), in a review of neuroimaging and neuropsychological findings relating to BA10, present a slightly different theory of FPC function. They suggest that BA10 is activated when the results of more than one cognitive process must be integrated in order to achieve an overarching goal. This theory would certainly explain the involvement of FPC in multiple task situations, and would account for Rowe et al.'s observations regarding the synchronisation of activity in posterior regions. This theory is inadequate, however, as it is possible to describe almost any task in these terms, including some tasks which do not result in BA10 activation. For example, in the reasoning experiment conducted by Kroger et al. (2002) (described below), success was only

possible by a) selecting the relevant information and b) reasoning about the relationship between the relevant pieces of information; however, this task did not recruit FPC unless the relations between the stimuli varied along a number of dimensions. Similarly, Boschin et al. (submitted) found that monkeys with lesions to FPC were not impaired when they were required to integrate the results of two rules (in this case "identical to" and "smaller than") in order to generate a correct response to a problem.

There is strong evidence that some feature of multiple-task scenarios recruits lateral FPC (in the studies described above, only Braver et al. (2003) detected medial FPC activation), and that patients with damage to this region are impaired in such situations. Processing related to multiple-task situations is probably not lateralised by hemisphere in this region. However, the research so far is inadequate to state the precise nature of lateral BA10 activity in such scenarios.

3. Reasoning theories

One theory of area 10 function suggests that the function of this area can be specified as "relational integration"; comparing and integrating higher-order relationships (i.e. relationships between relationships). This is linked to evidence regarding the function of neighbouring regions: for example, according to the rostral-caudal hierarchy of prefrontal function posited by Badre and D'Esposito (2007), the frontal pole, as the most rostral region, should process relations between stimuli at the highest possible level of abstraction from their perceptual features.

Christoff et al. (2001) presented participants with problems from Raven's Progressive Matrices which required them to consider relations between stimuli that varied along either zero, one or two dimensions, and integrate the results of these changes in order to arrive at the correct answer. These researchers observed a peak of activation in left lateral BA10 in trials which required participants to integrate changes over two dimensions compared with zero- and one-dimension trials. However, participants made significantly more errors and had slower reaction-times for two-

dimension trials, which suggests that these findings could be explained by a variation in the difficulty of the problems. Christoff et al. attempted to compensate for this problem by examining a zero-, one- and two-dimension trial for each participant in which reaction time was very similar: they found that the same pattern of activations could be observed even on RT-matched trials. However, the assumption that equal RT can be equated with equal difficulty when comparing individual trials for each subject is questionable, especially as the general error rates for the problems answered on the selected trials was not examined.

Kroger et al. (2002) went further in dissociating BA10 activity and difficulty. These researchers performed a similar study which included a set of control problems, all at zero-dimensions, which varied in difficulty level (distractors of varying complexity were added to the control matrices). Both error rates and solution times increased as the level of distractors or the complexity level increased, although solution times were significantly shorter for the highest level of distractor than for the highest level of relational complexity. These researchers found activation in anterior PFC (including left lateral BA10), among other areas, when subtracting the two levels with the greatest number of distractors from the two levels with the greatest relational complexity. However, it is possible that this result could be explained by dlPFC, not FPC, involvement in executive control, including suppression of distractors (Suzuki and Gottlieb, 2013); once this suppression has been completed, it is possible that zero-dimension control problems with a high distractor level were no more difficult than the zero-dimension experimental problems.

Further research has been found to support the relational integration theory in reference to analogical reasoning: for example, Bunge et al. (2005) found that left lateral FPC was activated when participants were asked to evaluate whether the relationships between the words in two word-pairs were analogous. Wendelken et al. (2008) observed bilateral lateral BA10 activation in the same task, and compared this task with one in which participants were asked to complete an analogy; lateral BA10 activation was greater in the “comparison” than the “completion” condition, suggesting that

this area is selectively activated by evaluation of relations rather than by reasoning tasks of the same level of difficulty (left medial BA10 was activated to a greater extent by the analogy task than the relational comparison task in this study).

However, not all studies agree that the role of FPC in reasoning can be explained by relational integration: for example, Geake and Hansen (2005) asked participants to solve analogical problems. They were presented with four different possible answers, all of which could be correct, but which required different numbers of analogical steps to move from one answer to the other. They found that activity on the lateral borders of BA10 (bilaterally) correlated with the number of analogical steps required by the answer that participants chose. While these reasoning problems required holding several relations in mind and combining them to form an answer, it is not obvious that increasing the number of steps needed to solve these problems required an increasing level of integration of these relations in the way specified by the relational integration theory. Furthermore, evidence that FPC activity is modulated by whether targets for reasoning are used to draw logically valid or probabilistic conclusions (Osherson et al., 1998; left lateral FPC) or drawn from the same or different semantic domain (Green et al., 2010; dorso-medial left FPC) cannot be explained simply through relational integration.

The most significant problem for the relational integration theory, however, is that there is no evidence to suggest that patients with lesions to FPC have deficits in this area. In the lesion study carried out by Burgess et al. (2000), patients had very similar Raven's Progressive Matrices scores when compared with controls, although they were only deliberately matched by age and reading performance. It does seem clear that left BA10 is recruited in some way by situations in which participants are required to carry out reasoning tasks, but is not modulated by the difficulty of these tasks; the majority of (although not all) research implicates lateral FPC in these scenarios. One common feature seems to be that BA10 is recruited to a greater extent when this reasoning is more

abstracted from the concrete semantic or perceptual relationship between the targets for reasoning. However, the nature of BA10 involvement in these tasks is unclear.

4. Context-specific memory

Area 10 has been consistently linked with episodic retrieval, source memory and prospective memory. There is considerable overlap between these processes: all depend on making and retrieving associations between pieces of information from different domains (e.g. what-where or what-when). However, there are crucial differences: one-trial learning is essential to both episodic retrieval and source memory, but not prospective memory; successful episodic and source memory are both passive, while successful prospective memory includes the performance of an action at some future time; episodic retrieval (and some forms of prospective memory tasks) require a subjective experience of mental time travel, while source memory (and other prospective memory tasks) do not require participants to report on their internal experience with relation to the past or the future.

Episodic retrieval

Right FPC activity has been consistently found in studies of episodic retrieval (e.g. Düzel et al., 1999) and has been linked with the establishment of an episodic memory "retrieval mode": a cognitive state associated with retrospective mental time-travel, but independent of the information actually retrieved (Lepage et al., 2000); it may be possible to identify "retrieval mode" as the task set associated with episodic memory. This activity is not diminished when comparatively few "old" targets were presented to subjects during a recognition task, and activation of retrieval mode was accompanied by little actual retrieval of information from memory (Kapur et al., 1995, although these results were contradicted by Rugg et al., 1998). In addition, Rugg et al., 1998 found that right lateral and left BA10 was activated by a cued-recall task, even when "old" targets were absent during the task (although the pattern of activation across BA10 in response to different task

conditions in this study was complex: right lateral BA10 was selectively activated when there were a high level of "old" targets compared to zero "old" targets and other regions of left and right BA10 were selectively activated by the opposite contrast), implying a task-set function for BA10 associated with retrieval in cued-recall conditions. While these experiments made little attempt to distinguish between recollection (in which successful recognition is based on episodic recall of the initial exposure to the target) and familiarity (in which the target is recognised as old, independently of recall of the context in which the target was encountered), the fact that patients with focal BA10 lesions are in no way amnesic (e.g. patients with lesions restricted to anterior medial frontal cortex are unimpaired on standard tests of verbal recall and recognition; Alexander et al., 2003) implies that the role of BA10 in retrieval may be based in information about the context of the original encounter with the stimulus or in a subjective sense of "remembering" rather than in stimulus retrieval per se.

However, it is clear that BA10 is not activated in all tasks involving the retrieval of episodic memory, but that its involvement is modulated by the conditions of the experiment. Wagner et al. (1998), for example, found that activation in right frontal pole (the Talairach coordinates of the centre of activation were (20, 63, -9); "at or near BA10 and 11") was higher when participants were primed to expect many "old" targets than when primed to expect many "new" targets. Similarly, Rugg et al. (1999) found that activity in left lateral BA10 was higher in a "high-density" item recognition task (in which familiar items constituted 80% of the stimuli presented) than in a "low-density" item recognition task (familiar items constituted 20% of the stimuli presented) or in a source memory task, despite identical instructions in the two recognition conditions.

In both studies reported above, increased activity in BA10 was observed in conjunction with lower response accuracy. Additionally, in the source memory task conducted by Rugg et al. (1999), which recruited left BA10 (discussed below), response times were slower than in the recognition memory conditions, which did not recruit left BA10. While there is no evidence to suggest that FPC is

recruited by difficult cognitive tasks generally (see above), it may be the case that, when retrieving information in the context of a memory task, FPC is selectively activated when the task demands are greater.

There is also evidence to suggest that activity in FPC during recognition tasks is modulated by the level of processing carried out on stimuli when they were first presented: for example, Fossati et al. (2004) found that left BA10 was more active when recognising words that were encoded deeply (participants were asked to assess whether a word described themselves or reflected a positive personality trait) rather than shallowly (participants rated whether a word contained a target letter), and that right BA10 was more active when retrieving a positive word that participants had rated in relation to themselves than when retrieving a negative word. Similarly, Reynolds et al. (2006) compared activation when participants performed a low integration task (participants determined whether words in a word pair referred to abstract or concrete nouns) and a high integration task (participants determined whether the words in the pair matched in whether they referred to abstract/concrete nouns), and when they retrieved word pairs which had been encoded in the high or low integration task. They found frontopolar regions which were more active in response to the high integration task (in left and right lateral BA10), and which were more active in response to retrieval than to the integration task (in left lateral BA10 and right BA10), and one region in right lateral BA10 which was most active when participants retrieved a word pair on which they had performed the high integration task.

While BA10 is activated by many tasks involving recognition memory, the evidence discussed thus far is insufficient to determine the common features of mnemonic tasks which recruit this region, or the nature of FPC involvement in these processes.

Source memory

Multiple researchers have found that BA10 is activated when participants are required to report on the context in which they first encountered a particular target. This activation occurs whether participants are asked to report on the task that they performed on a stimulus (Simons et al., 2005a and 2005b, Dobbins et al., 2002) or the perceptual details of the original image (e.g. whether it was presented as a smaller or larger image – Ranganath et al., 2000, or on the left or right of a screen – Rugg et al., 1999, Simons et al., 2005a). In contrast to right BA10 activation in general memory retrieval situations, source memory tasks tend to recruit left BA10 (e.g. Dobbins et al., 2002; Ranganath et al., 2000) or bilateral BA10 (e.g. Simons et al., 2005a and 2005b; Rugg et al., 1999 – although in this case activation was greater in left BA10 in the source task compared to the recognition memory task). Kahn et al. (2004) reported that left lateral BA10 was selectively activated when participants correctly recognised a word, and that this activation was increased when they remembered the source of a word that they had previously constructed a mental image of, in comparison to words that they had read aloud backwards; however, this study also found that right FPC was selectively activated in response to correctly identified novel items.

A dissociation was observed between lateral and medial BA10 by Simons et al. (2005a): activity in right and left lateral BA10 was increased in a perceptual source memory task (e.g. “was the stimulus originally displayed on the left or right-hand side of the screen?”), while left lateral and medial BA10 activity was increased in a task-based source memory task (e.g. “did you rate the stimulus as pleasant/unpleasant or politics/entertainment?”). Simons et al. (2005b) followed up these results, and found bilateral lateral activation in BA10 when responses to a task-memory and temporal-memory (subjects were asked to report on which of two temporally-distinct lists stimuli had been presented in) task were averaged together; when these results were examined independently, they found that left lateral and medial BA10 were selectively activated in the task-memory condition. They also found that lateral BA10 activity occurred earlier in the recollection process than medial BA10 activity; in fact, left lateral (but not medial) BA10 activity was observed on trials in which a cue was presented informing participants that they should perform a task-based

judgment on an upcoming target, but in which this cue was not followed by a target for retrieval, implying that lateral BA10 is activated by preparation of a "retrieval mode" while medial BA10 is activated a later stage of the process of retrieval (potentially memory search or evaluation).

This dissociation was further explored by Turner et al. (2008), who found that, while lateral BA10 was activated bilaterally when participants were asked to perform one of two possible source memory tasks (which of two lists a word was originally presented in; whether the word was perceived or imagined in response to being given its definition and initial letter), right medial BA10 was activated when participants were asked to recall the source of a word which had been originally imagined, and activation was found in both left lateral and left medial BA10 when activity related to items perceived at study was subtracted from activity related to items imagined at study.

Recency memory

There is conflicting evidence regarding whether FPC is activated when participants are required to report on how recently a stimulus was encountered. As discussed above, Simons et al. (2005b) found activity bilaterally in lateral BA10 in response to temporal-source and task-source memory when these conditions were examined together, and some bilateral lateral activation of anterior prefrontal cortex in response to temporal-source memory compared to baseline tasks, but did not find any areas in BA10 which were selectively activated by temporal-source memory in comparison with task-source memory. Dobbins et al. (2002) found left lateral BA10 activation when participants performed a task which required them to recall the previous semantic classification that they had made with regard to particular stimuli, but did not find activation in BA10 when participants were asked to make recency judgements about stimuli, although the recency task was more difficult than the source identification task (RTs and error rates were higher in the recency task than in the source task). However, the same group of researchers found opposing results the following year: right medial BA10 activation was observed in when participants made recency judgements, but no BA10 activity was observed when they made task-based source memory

judgements about stimuli (Dobbins et al., 2003). Dudukovic and Wagner (2007) observed left-lateralised frontopolar activation when participants made recency judgements compared to when they made novelty judgements.

Prospective memory

There is considerable agreement between multiple sources of evidence that the FPC is implicated in prospective memory. To some degree, this is unsurprising: while prospective memory (carrying out delayed intentions) is not identical to multi-tasking, there is considerable overlap between the ways these two processes are studied in laboratory contexts. In particular, both require the ability to switch between tasks, either in response to an explicit prompt, an implicit prompt (a predetermined cue which participants have been instructed in advance to respond to in some way), or due to some overarching goal demands (in which case participants may have planned privately to view an event in the task as a cue that it is time to switch tasks). However, as prospective memory encompasses much more than multi-tasking (planning, maintenance of a delayed intention, cue recognition, retrieval of a delayed intention, and finally enactment of the delayed intention, possibly including task-switching), and as deficits in retrospective memory have been linked with deficits in prospective memory, it is appropriate to examine separately the evidence which links prospective memory to BA10.

Neuroimaging

The neuroimaging evidence collected by Burgess et al. (2001) has been described above. Other studies, such as that conducted by Ramnani and Miall (2003), have found BA10 activation related to future expectations and plans: in this study, left FPC was activated by expectation of a reward on an upcoming trial, and right lateral FPC was activated when subjects were able to prepare a specific action in order to achieve a reward. Simons et al. (2006) attempted to dissociate the cue identification and intention retrieval components of prospective memory by interposing two

prospective memory tasks on the ongoing task. In the first prospective memory task, cue identification was difficult (either detecting whether two words were from the same semantic category or detecting whether two shapes were separated by a chess knight's move) and intention retrieval was easy (press a key); in the second, cue identification was easy (words were presented both in upper- or lower-case, or shapes were the same colour) and intention-retrieval was difficult (press a key indicating the number of syllables in both words or the number of sides on a complex shape). Both conditions were associated with bilateral lateral BA10 activation and medial deactivation, but the intention retrieval condition was associated with greater activity bilaterally in an area of lateral BA10 which was more medial than the area which was associated with both conditions.

These results were not replicated by Okuda et al. (2007), who in their first experiment found significant bilateral medial BA10 deactivation when the cue for performing a delayed intention was event-based (an appearance of the number seven in the ongoing task in experiment one, the appearance of the word "guitar" or a perfect square in experiment two), but no lateral BA10 response to either event-based or the more difficult time-monitoring-based (e.g. respond every minute) prospective memory. They did find that there was significantly more activity on the border between left BA9 and 10 when comparing the time-based prospective memory condition to the event-based condition. Cue identification in the time-based condition was more difficult (participants had to monitor their internal sense of time) and so participants may have viewed this as an ongoing task (e.g. counting seconds) rather than as a prospective memory task. In Okuda and al.'s second experiment, in which participants were provided with a clock in the bottom of their screen, they found activation in medial BA10 bilaterally in the time-based condition compared to the event-based condition, and left lateral BA10 activation in the event-based condition compared to the time-based condition.

Neuropsychology

Some studies have reported that patients with lesions to BA10 are impaired in prospective memory tasks. For example, Uretzky and Gilboa (2010) described a case-study of patient Z.P., who suffered from a lesion to a medial area of right frontal pole (BA9 and BA10). 23 years post-injury, he continued to suffer from a prospective memory deficit: he was severely impaired in remembering a set of actions he was supposed to perform after performing a distractor task (although he could perfectly recall the order of actions within each set of actions) and in responding to target words that are displayed as part of another, different, word-sorting task. He was also, anecdotally, impaired in multi-tasking, planning and prioritising tasks, although these deficits were not examined in this study.

Volle et al. (2011) tested 45 patients and 107 controls on word- and picture-based prospective memory paradigms. They found that, compared with other patients, patients with lesions to right area 10 were impaired on prospective memory tasks in which they were supposed to perform a target action (press a red button) at a particular time (every 30 seconds; participants were allowed to inspect the time passed since the last button press at any point). No significant difference in error rate existed between BA10 patients and other patients when the target action was tied to a specific event (e.g. press a button when you see an animal). This effect was seen whether the target stimuli were words or pictures.

However, not all BA10 patients are obviously damaged in prospective memory tasks: as described above, Burgess et al. (2000) found that patients with lesions to BA8, 9 and 10 were unimpaired when asked to formulate or follow a plan in order to maximise their score in a test in which they were required to balance the demands of several open-ended tasks. Furthermore, the participants tested by Volle et al. were impaired at time-estimation generally, and not only at performing a target action after a certain amount of time had elapsed; therefore it is difficult to conclude from neuropsychological evidence that the FPC is necessary for either time- or event-based prospective memory.

Human disruption studies

Relatively few results have been published which examine disruption in function after healthy human subjects have received transcranial magnetic stimulation (TMS) to BA10. However, in two studies, one laboratory has used TMS to connect BA10 disruption to impairments in prospective memory: Costa et al. (2011) applied continuous theta-burst stimulation (cTBS) TMS over left and right lateral BA10 and over the vertex (as a control site). These researchers found that cTBS over left BA10 inhibited participants' ability to respond to one of four target words while performing an ongoing task of remembering and reciting sets of words, while cTBS over right BA10 had no effect. This was interpreted as a deficit in verbal prospective memory. Costa et al. (2013) extended this research by replacing the verbal stimuli with the spatial positions of black squares. In this study, the researchers found that cTBS applied over right lateral BA10 impaired performance on the prospective memory task, while performance on the ongoing task was unimpaired; cTBS over left lateral BA10 had no effect.

Evidence from neuroimaging, neuropsychology and human disruption studies all indicate that BA10 is involved in prospective memory. However, the particular subregions which are implicated in prospective memory tasks vary with the details of the task: the task domain, whether the cue is time- or event-based, and whether identifying the cue or retrieving the intention to be enacted is the more difficult part of the study. This range of dissociations within prospective memory tasks strongly indicate that treating "prospective memory" as a unitary behaviour that can elucidate certain aspects of information processing in the brain is problematic.

In conclusion, while it seems certain that BA10 is recruited by some memory tasks, in particular source memory, it is equally clear that BA10 is not solely recruited by these tasks: Nyberg et al. (2003), for example, found activation in the same area of left BA10 in semantic, episodic and working memory tasks. Furthermore, as different areas of BA10 are activated depending on the

particular task demands of these memory tasks, it is necessary to identify the precise components of those tasks which recruit particular subregions of BA10.

5. Attention to internally/externally generated information

Several researchers emphasise the source of information processed by the FPC in theories of BA10 function. Some researchers have suggested that BA10 only processes "internally-generated information", while others suggest that the FPC is activated when the direction of attention is shifted between internally-generated information and attention to external stimuli. A rough behaviourist definition of "internally-generated information" might be "information which is not contained within the external stimuli of the task if they were considered individually", while "externally available information" would relate to information which is contained within the individual stimuli of the task.

Christoff et al. (2003) examined activations in response to self-generated or externally-generated information. In the self-generated condition, participants were shown two objects which differed from each other in a certain dimension (texture or shape). After a delay, participants had to determine whether two new objects varied along the same dimension or a different one to the original objects. In the externally-generated condition, participants had to determine whether a target object matched one of the two original objects in a specified dimension (texture or shape). Increased activity was found in lateral BA10 bilaterally at the evaluation phase of internally-generated trials, and was not affected by whether the original stimuli were displayed when participants made their judgement. They concluded that BA10 is primarily activated in the activation of internally-generated information. In this case, "internally-generated information" overlaps considerably with "relational integration"; however, this theory has been supported and developed by other researchers in ways which do not relate to relational integration. This theory supports other findings relating to FPC: as discussed above, left lateral and bilateral medial BA10 had greater activation when participants recognised an item that they had imagined themselves at study than when they recognised an item they had perceived at study (Turner, 2008). There is also evidence

that BA10 is activated when participants are not attending to external stimuli, or to the task at hand. BA10 is part of the "default mode" network of the brain (Buckner et al., 2008), which is activated when participants are not required to complete a behavioural task; this network is associated with high levels of "stimulus-independent thought" (SIT) (Mason et al., 2007). Mason et al. (2007) tested this association between the default mode and SIT, and concluded that activation in default mode regions, including medial BA10, was higher during task conditions in which participants reported a high level of SIT. However, the most significant problem for this account of BA10 function is that there are some tasks highly dependent on the analysis of internally-generated information which do not activate BA10: for example, Logie et al. (2011) reported no FPC activation in a mental rotation task.

Tsujimoto et al. (2010) carried out single-cell recordings from neurons within the right hemisphere of the macaque frontal pole while monkeys performed a task in which they responded to one of two targets on the basis of a cue which instructed them to respond either to the previously-chosen target or to the previously-unchosen target. These researchers found that cells in the FPC did not respond at decision, but were active before and after the monkey received delayed feedback. This activity was only seen when the correct response had to be inferred from the combination of the cue and the monkey's behaviour on the previous trial; no activity was observed when the correct response could be inferred directly from the cue. Tsujimoto et al. concluded that the FPC is likely to be responsible for the evaluation of self-generated actions in monkeys. Significantly, this study moved beyond human neuroimaging as a basis for theorising regarding the FPC, and provided evidence for the type of processing carried out by neurons in this region as well as simply the type of scenarios in which large numbers of these neurons are activated. Although the location of recordings taken differed in terms of medio-lateral distribution between the two monkeys, the results were similar in both subjects.

Burgess et al. (2006) proposed the "gateway hypothesis", in which FPC controls the balance between stimulus-oriented and internally-oriented attention while performing a task. These researchers suggest that the function of medial BA10 may be in biasing attention towards externally-available information, while lateral BA10 may be responsible for biasing attention towards internally-generated information. In a study by Gilbert et al. (2005), participants were asked to perform three tasks in which they either responded to a visual stimulus or imagined a continuation of a visual stimulus once the stimulus was no longer displayed (e.g. picturing a hand continuing to move around a clock face) and responded to that imagined stimulus in the same way as they had to the displayed stimulus. Higher activity was observed in right lateral rostral PFC during transitions between the two conditions of the task, whereas right medial rostral PFC was more active during phases of the experiment in which participants attended to externally available information; it was argued that this condition required both stimulus-independent and stimulus-oriented thought. They argued that these results provide evidence that BA10 is essential for situations in which participants must select between externally-available and internally-generated information, and that the roles of medial and lateral FPC in these situations could be dissociated. This evidence was combined with evidence that medial BA10 is activated by very basic stimulus-response tasks, and that greater activity in medial BA10 is associated with faster reaction times in these tasks (Gilbert et al., 2007), and evidence that lateral BA10 is activated when participants are asked to respond based on self-generated information (e.g. comparing the sum of two numbers with the sum of two numbers on a previous trial, contrasted with comparing whether one number in the current trial is greater or less than one number on the previous trial; Burgess et al., described in Burgess et al., 2006) to associate medial BA10 with externally-available information and lateral BA10 with internally-generated information.

Some support for this theory was provided by McCaig et al. (2011), who were able to train participants to increase or decrease activity in lateral BA10 by encouraging them to focus, respectively, on their own thoughts or on the external environment. A difference in activation

between the two conditions was seen immediately; furthermore, if participants received real-time feedback about the level of activation in lateral BA10 during the experiment, they were able to modulate activity in right lateral BA10 significantly more than if they received no feedback or sham feedback. While participants were encouraged to focus on internally-generated stimuli in order to increase activation in lateral BA10, they were able to maximise this activation when balancing attention to external stimuli (real-time feedback about activation in BA10) with internally-generated information (their subjective experience of their own mental state). Likewise, Ramnani and Passingham (2001) found that activity in right lateral BA10 was increased when participants were asked to tap their finger in time with a complex repeated rhythm (i.e. when they learned to synchronise their internally-predicted rhythm with the externally played rhythm) compared to a condition when the visual cues prompting finger taps were played randomly.

However, although it provides a theory which accounts for activation in both the lateral and medial subregions of BA10, the "gateway hypothesis" account of FPC function fails to account for all situations in which BA10 is activated. For example, Pollmann et al. (2000) found that activity in left BA10 was increased in a visual search task when the odd-one-out target was defined by a different dimension (colour or movement) than the odd-one-out on a previous trial. This task did require participants to shift the direction of their attention, but all of the information participants needed to complete the task was externally generated. Furthermore, it seems likely that it is possible to describe any task that contains any degree of complexity in terms of coordinating attention between external stimuli and internal processing; it is not surprising that tasks which recruit BA10 may be described in this way. In order to evaluate this theory, we need to consider whether there are tasks which obviously require this sort of coordination, but which do not recruit BA10.

In a study performed by Gilbert et al. (2006a), increased activity was found in left medial rostral PFC in a task which required monitoring of external stimuli (pressing a button when a stimulus passed a mark on a screen, and responding to repeated stimuli) compared to a task which

required attention to internally-generated information (generating letter- or number-sequences, and pressing a button when thinking of a new item in the sequence). Medial bilateral activation and left medial BA10 activation (although more lateral than the stimulus-oriented activation) was also found in a baseline condition (pressing a button when the whole screen is illuminated) compared to the stimulus-independent condition, the stimulus-oriented condition, and another condition which mixed stimulus-independent and stimulus-oriented behavioural requirements (validating visually-presented letters or numbers against an internally-generated sequence). In the baseline condition, increased activity in medial rostral PFC was associated with a lower reaction time. There was no increase in activity in BA10 in the condition which combined stimulus-oriented and stimulus-independent activity, which would have been expected under the "gateway hypothesis".

6. Mentalising

Other researchers have suggested that activation in BA10 is specifically recruited when attending to oneself or extending that awareness via empathy to imagining the mental state of others (mentalising). These theories might account for some of the results described above: for example, under some definitions of episodic memory, an autonoetic awareness of one's own subjective experience of time is necessary to experience true episodic recollection (e.g. Tulving, 2002), and attending to one's own state of being is certainly an example of attention to self-generated information (as evidenced by a high degree of overlap between those brain networks activated by social cognition and those identified as part of the default mode network previously discussed in relation to stimulus-independent thought; Mars et al., 2012, provide a meta-analysis of this data).

There is extensive neuroimaging evidence that BA10 is activated by mentalising tasks. For example, Ochsner et al. (2004) found increased activation in BA10 when participants were asked to rate their own emotional response to a photograph or rate the emotion displayed by the person in the photograph compared with when asked to rate whether it was taken inside or outside; this

activation was left-lateralised for self-judgements and too medial to determine a hemisphere for other-judgements. Similarly, Gusnard et al. (2001) found increased activation in medial BA10 when participants were asked to rate whether they found a photograph pleasant or unpleasant compared to making an inside/outside judgement, and Simons et al. (2005b) found activity in left medial BA10 when participants were asked to judge how pleasant or unpleasant they found a stimulus (in comparison to fixation on the stimulus). Amodio and Frith (2006) conducted a meta-analysis of literature relating to social cognition and the medial frontal cortex and concluded that medial BA10 was activated by tasks they defined as "self-knowledge" (e.g. tasks in which participants were asked whether traits applied to themselves, or where they were asked to report on their affective responses), "person perception" (e.g. tasks in which participants were asked to report on whether behaviours or adjectives could be true of people, or tasks in which participants were asked to view faces) and "mentalising" (tasks in which participants were required to represent the psychological perspective of another person).

There is also neuropsychological evidence relating FPC damage to impairment in Theory of Mind (the ability to distinguish between mental states, as they apply to oneself and other people, and external reality). Roca et al. (2011) found that patients with BA10 were impaired in comparison to healthy controls (although not in comparison to non-BA10 patients) in a task which required them to rate whether a short story contained an example of someone saying a hurtful thing to another person (the "Faux Pas" task). In a case study, Hoffmann and Bar-On (2012) investigated a woman who experienced a small haemorrhage isolated bilaterally in the medial frontopolar cortex (BA10). She scored in the normal range of almost all tests administered, including the Tower of London test, the Comprehensive Trail Making Test, the WCST, the Rey Complex Figure Test and almost all aspects of the Frontal Systems Behavioral Scale. The only tests in which she scored outside of the normal range were the Go-No-Go test (administered 24 hours after admission), in which she scored 3/10 (the normal score is 10) and the emotional self-awareness and assertiveness scales on the Emotional Quotient Inventory (she scored 86 and 89 respectively; the average score is 90-109; the test was

carried out five days post-admission). In addition, upon admission she seemed to experience a mild euphoria and showed a lack of interest in her condition. Hoffmann and Bar-On argue that this evidence supports the view that the medial frontopolar cortex is involved in self-referential processing, including processing of and awareness of internal states; however, as no evidence exists regarding the patient's pre-illness state and the impairment was quite mild, this study cannot provide strong positive evidence for this position. However, not all patients with lesions to the medial FPC are impaired in Theory of Mind tasks: Bird et al. (2004) described a patient with extensive damage to the medial frontal lobe (including medial area 10) who scored within the normal range on almost every test of Theory of Mind (with the exception of a higher-than-normal threshold for rating situations as "embarrassing"), administered three and six months after her stroke.

Christoff et al. (2009a) examined meta-awareness, which clearly depends on an attention to one's own mental state, in association with "mind-wandering". They combined a self-report measure of mind-wandering with an independent measure (performance on a low-demand, low response-frequency cognitive task). During the task, participants were periodically asked to report on whether, immediately prior to the probe, they had been focused on the task or not. They were then asked to report how aware they had been of their mental state prior to the probe. The accuracy of this self-reporting probe was supported by the fact that significantly more task errors were made prior to a self-report of mind-wandering. They found that left BA10 was most active in mind-wandering conditions when the participants had reported being unaware of their mental state, which the authors interpreted as mind-wandering without meta-awareness. However, it is highly likely that the periodic mental state probes promoted a general state of meta-awareness throughout the study, and participants must have had some degree of at least retrospective meta-awareness in order to report accurately on their previous mental state. It would be possible to clarify these findings by analysing the time-course of activity in BA10: in particular, whether it increased after

participants were required to report on their mental state. However, it is likely that it is not possible to achieve this degree of temporal resolution using fMRI.

The combination of neuroimaging and neuropsychological evidence linking medial BA10 to mentalising and Theory of Mind makes it likely that this region does play a role in this kind of cognition. However, evidence from human patient studies is mixed, and it is clear that it is neither true that medial BA10 activation is found in all conditions which require attention to mental states nor that medial BA10 is only activated by such conditions.

7. Decision-making

The FPC has repeatedly been implicated in studies in which participants had to choose between options associated with different probabilities of reward. Some researchers have associated this activity with the evaluation of the value of options which have not been chosen (counterfactual possibilities); however, it is clear that this account cannot explain all FPC activity in complicated decision-making scenarios.

Evaluation of counterfactuals

The study conducted by Boorman et al. (2009) has been mentioned above. In this experiment, participants were asked to choose between two options (presented to the left- and right-hand of the screen). The magnitude of the reward (in points) available for each option was shown to the participant in each trial, but the probability that the participant would receive the stated reward if they chose that option varied between the two options. Participants were not informed of this probability, and were forced to infer the likelihood of receiving a reward for choosing each option from the outcome of recent choices. The probability of a particular choice receiving a reward varied slowly throughout the experiment. A Bayesian model was used to track the optimal choice on each trial, based on the estimated probability that each option would be rewarded and the magnitude of the reward associated with the option. Activity in the lateral FPC

(bilaterally) was found to correlate with the estimated probability of the unchosen option relative to the estimated probability of the chosen option throughout the trial, but with peaks occurring at decision and in the inter-trial interval; it was not sensitive to the value of the foregone reward. This was true even for trials in which participants switched their choice from one option to the other; the FPC encoded value for the rejected option prior to the button-press which signalled the shift in behaviour. In this study, it was unclear whether this activity was related to counterfactual evaluation or the likelihood of a shift in behaviour on an upcoming trial; these overlap in most situations (as a high value associated with a counterfactual option is a sign that behaviour should change), but they are not identical.

Boorman et al. (2011) extended this research to three-choice situations. These researchers tested participants in a task in which three options were associated with different magnitudes of reward, which varied randomly throughout the experiment and participants were informed of at the start of each trial, and different probabilities of reward, which changed slowly throughout the experiment, and which participants had to discern through experience. These researchers found that activity in left lateral FPC correlated with the probability that the most optimal unchosen option was associated with a reward, and correlated negatively with the reward probabilities associated with the chosen option and the less optimal unchosen option. After information about the outcomes of both the chosen and unchosen options was presented, activity in lateral FPC correlated with the prediction error associated with the unchosen option (i.e. activity in this region after feedback correlated negatively with pre-feedback reward probability and positively with the outcome associated with the unchosen option). The researchers observed that participants who encoded this counterfactual reward probability more strongly were more likely to choose the option associated with this probability of reward when given an opportunity to choose between the two options that they had previously rejected; it was not reported whether activity in lateral FPC predicted a shift in behaviour on future trials.

Other decision-making studies

Rogers et al. (1999) found that right lateral BA10 was activated when participants had to choose between two mutually exclusive options, one of which was associated with a low probability of a high-value reward, and one of which was associated with a high probability of a low-value reward; left medial BA10, however, was significantly more active in a low-demand control condition (touch a stimulus when it is surrounded by a white border) than in the experimental condition. Both the relative probabilities of gaining a reward and the relative values of the reward varied throughout experimental trials; however, the lower-value reward was always associated with the higher probability. In the control condition, participants performed a simple stimulus-response task; the lack of a decision-making control condition means that it is difficult to assess the contribution of FPC to this task.

In an fMRI study in which participants were asked to choose between four choices, the pay-offs of which varied from trial to trial, Daw et al. (2006) found that bilateral FPC was significantly more active when participants made choices classed as exploratory compared with when they made choices classed as exploitative. They used a multiple regression analysis in order to exclude the possibility that this activation was due to task-switching. However, in a further study by this laboratory, these results were not replicated (Wittmann et al., 2008). Kovach et al. (2012) proposed an alternative account. These researchers used a similar paradigm to that employed by Daw et al. (2006) in order to study the impairment of patients with frontopolar lesions in this task. In this study, FPC damage was predominantly located in the right hemisphere although all but one patient (N=8) had some bilateral damage. Compared to healthy and brain-damaged controls, FPC patients did not take into account the outcome of recent trials in making their next choice; this effect was especially marked when recent trials had been rewarded (implying that the right choice would be to maintain the current behaviour), rather than when recent trials had not received a reward (implying that switching to other options might generate higher rewards). These researchers suggested that these

results could provide a reinterpretation of the results found by Daw et al. (2006), as exploratory trials tend to occur when the short-term trend of results indicates that the reward associated with a previously-favoured option has changed. This theory would also account for the difficulties suffered by FPC patients in adapting their behaviour to complex and varying environments. However, it is important to note that in this study, lesions in the FPC group contained a great deal of overlap posterior to FPC, that lesion volume was larger in the FPC group than in brain-damaged controls, and that 7/8 patients in the FPC group had bilateral damage while all patients in the non-FPC brain-damaged control group had unilateral lesions; when lesion volume was included as a covariate, there was no longer a significant difference between the FPC group and control subjects with damage to other regions.

While there is consensus which implicates the FPC in certain types of decision-making scenario, there is little agreement as to the type of information-processing which is conducted by this region in these cases. The striking common feature between these scenarios is that most involve shifting probabilities of reward; however, it is not clear whether BA10 is necessary for monitoring these shifts in probabilities or for planning behavioural responses to these probabilities.

FUNCTIONAL SPECIALISATION WITHIN FPC

Dissociations between activations in lateral and medial BA10 and between the left and right hemispheres of this region have been indicated above.

Lateral/medial

While there are some exceptions, evidence relating BA10 function to task sets, multiple task situations and reasoning almost exclusively implicate lateral BA10. Evidence relating BA10 to context-specific memory shows a complex pattern of dissociations between lateral and medial BA10. Evidence relating BA10 to internally-generated information and decision-making is mixed. Studies of mentalising almost exclusively implicate medial FPC.

Gilbert et al. (2006c) performed a meta-analysis of neuroimaging studies which identified activations in BA10. They found that reaction times relating to experimental trials which activated lateral BA10 were much longer than reaction times relating to trials which activated medial BA10; reaction times on trials which activated medial BA10 were, in general, as fast as or faster than reaction times for control conditions in those experiments. These researchers interpret this result as support for the subdivision suggested by the "gateway hypothesis", in which medial BA10 mediates attention towards external stimuli and lateral BA10 mediates attention towards internally-generated information. However, it is also possible that this dissociation reflects the fact that BA10 is associated with both task-relevant processing in challenging and complex tasks, and stimulus-independent default mode thought; lateral BA10 tends to be activated by very challenging tasks (e.g. task-switching, relational integration) and medial BA10 may be associated with mind-wandering, which is only possible during very simple tasks.

Rostral/caudal

Gilbert et al. (2006b), in a meta-analysis of neuroimaging results regarding BA10 activation identified a rostro-caudal axis in the typical areas of activation. They noted that studies which examined mentalising tended to note a relatively caudal activation in BA10, while studies which examined the coordination of multiple tasks tended to identify a relatively rostral activation.

Left/right

Some dissociations between activations in left and right BA10 can be noted in the studies described above. Those associated with reasoning tend to activate left BA10. Those associated with internally-generated information tend to activate right BA10. The TMS studies carried out by Costa et al. in 2012 and 2013 noted a domain-level dissociation between left and right BA10: TMS to the left FPC impaired verbal prospective memory and TMS to the right BA10 impaired spatial prospective memory. These results imply that dissociations between left and right BA10 noted above

may have been due to stimulus domain, rather than the computational demands of the task. There may be further dissociations between left and right medial BA10, but, as these activations may be very close to the midline, it is difficult to be certain that activation is confined to only one hemisphere.

THE RESEARCH IN THIS THESIS

Although FPC has attracted a substantial amount of research in the past years, and a number of theories regarding its function have been proposed, none of these theories have succeeded in accounting both for the wide range of tasks which activate this region and the smaller number of tasks which are impaired by damage to this region in a satisfactory way. One of the constraints on this field of research has been the difficulty in performing animal neurosurgery which targets the FPC. Decades of circumscribed lesion studies in monkeys have undoubtedly played a major part in shaping our understanding of primate prefrontal functional neuroanatomy by comparing the effects of lesion to different regions of PFC; it has not, before now, been possible to apply this technique to constrain theorising about FPC function compared to the function of other prefrontal regions. The research in this thesis moves beyond the current state of knowledge regarding FPC function by addressing the question of what can be learned from macaque monkeys with targeted neurosurgical lesions to this region.

One of the limitations of animal lesion research, particularly in macaque monkeys, is the small sample size that may be used in experiments: this limitation is imposed both for reasons of animal welfare (reduction of the numbers of animals used in research is one of guiding principles of the ethical use of animals in experimentation; Russell and Burch, 1959) and practicality (since the lesion must be individually imposed for every animal tested). This limitation is present in this thesis: the largest experimental group examined in any chapter has a sample size of four (Chapters 1, 3, 4 and 5); the largest control group has a sample size of 9 (Chapter 3). While this thesis follows the approach of applying parametric statistical tests in order to compare the results of tests applied to

experimental and control groups, as is standard in research in this field, the small sample sizes used in this research means that statistically significant results will only be achieved if the experimental group differs substantially from the control group (i.e. the effect size is large); if the effect size is small, a null result will be returned. Furthermore, the accuracy with which it is possible to test the assumptions inherent in parametric statistics (e.g. normality of distributions) is reduced in cases of small sample sizes; p-values estimated in these studies may not, therefore, be reliable. This may mean that even positive findings of statistical significance should be treated with caution. While the research presented in this thesis expands the range of techniques which have been applied in order to study FPC function in primates, further research using techniques in which a larger sample size is available is necessary in order to confirm the findings discussed throughout this thesis.

In Chapter 1, I examined evidence that frontopolar cortex is critically necessary for estimates of temporal context. Previous research has suggested that human patients may be impaired at estimated the passage of time (Volle et al., 2011) and that making recency judgements activates BA10 (Dobbins et al., 2003); this account may help to explain the recruitment of FPC by other cognitive processes, such as episodic memory (Lepage et al., 2000), and learning from the outcome of recent events (Kovach et al., 2012). I conducted an experiment which investigated FPC-lesioned monkeys' ability to learn about temporal context. I also examined performance on a control task which tested monkeys' recognition memory before and at two time-points after lesions to FPC.

In Chapter 2, I expanded on previous research conducted by my laboratory which shows that FPC-lesioned monkeys are impaired in one-trial learning, a component of episodic memory, and on the research in Chapter 1 which indicated that FPC-lesioned monkeys may be impaired at temporal-context judgements. Using an adapted version of the Receiver-Operating-Characteristics (ROC) technique, which has previously been used to examine recollection-like learning in monkeys (Guderian et al., 2011) and rodents (Fortin et al., 2004), I examined evidence for an episodic-like deficit in monkeys with FPC-lesions.

In Chapter 3, I expanded upon the research described in Chapter 1 which suggested that monkeys with lesions to FPC are impaired at estimating which of two stimuli were presented more recently. This finding may illuminate other research which suggests that FPC-lesioned patients are impaired at learning from the outcome of the most recent trial (Kovach et al., 2012), since it is possible that lesions to FPC impair memory for which outcomes to prioritise as the most recent. In order to investigate this possibility, I extensively reanalysed data previously collected by my laboratory relating to performance on an analogue to the WCST by animals with lesions to various regions of the prefrontal cortex, including FPC, to determine how they are influenced by the outcomes of recent trials in decision-making.

In Chapter 4, I examined the contribution of FPC to social valuation. Previous research indicates that lesions to FPC in humans impair sensitivity to social situations (Roca et al., 2011) and that medial FPC is activated by social cognition in humans (Amodio and Frith, 2006). I studied the behaviour of macaques prior to and after lesions to FPC in order to assess whether their responses to social stimuli were affected by lesions to this region.

In Chapter 5, I examined changes in functional connectivity that occur after lesions to FPC. I investigated these changes (as measured by correlation in the BOLD signal) in anaesthetised macaques in order to assess whether lesions to FPC affect connectivity in posterior brain networks (as suggested by Rowe et al., in relation to awake behaving human patients, albeit with non-circumscribed and non-complete rostral prefrontal lesions). The functional connectivity of the FPC has previously been investigated in anaesthetised macaques (Sallet et al., 2013; Neubert et al., 2014), but the effects of lesions to this region have not been examined. Furthermore, building on my work in Chapter 1, I assessed functional connectivity at two time-points after lesion in order to determine whether it is possible to detect changes in the effect of FPC lesions over time.

In Chapter 6, I built upon the research that I conducted in Chapter 1, which suggested that it is possible to observe behavioural recovery after lesion. I trained and then periodically tested

macaques on a behavioural task prior to and after the infliction of a targeted lesion to principal sulcus (this region was chosen due to its predictable behavioural effect) in order to measure recovery of function in these animals longitudinally. This behavioural study supplemented an fMRI project (which is not discussed in this thesis) which examined recovery in functional networks over the same period of time.

CHAPTER ONE

Lesions to frontopolar cortex impair recency memory in macaque monkeys

Previous research has established that human BA10 is activated by episodic and prospective memory tasks, although the contribution of the region to these tasks is unclear. One possibility is that BA10 contributes to the learning of spatial and temporal context information; previous studies have found activation in human BA10 during source memory tasks and Volle et al. (2011) have found that patients with lesions to BA10 are impaired at estimating the passage of time. While spatial context learning has been examined in macaques with frontopolar lesions (Boschin et al., submitted), temporal context learning has not been investigated in macaques with lesions to this region. I tested seven macaques, four of whom had received lesions to FPC and three of whom remained unoperated controls, on a task designed to test memory for recency, which may relate to short-term temporal context. This task relied on the ability to retain several stimuli over a delay. Since some researchers have suggested that macaque lateral FPC may be homologous with human dlPFC (Neubert et al., 2014), which subserves performance on delayed response tasks, I also tested these macaques on a control task designed to test this ability. Monkeys with FPC lesions, when tested 32-38 months post-lesion, were not impaired compared to unoperated controls on the control task, and were impaired compared to unoperated controls on the recency task, thereby showing the deficit in the task was selective to recency memory and not explicable by impaired item recognition judgements.

Introduction

In humans, BA10 is consistently implicated in tasks which require patients to remember contexts: activation in this region has been observed in tests of episodic memory (Duncan et al., 2000), prospective memory (Ramnani and Miall, 2003) and source memory (Simons et al., 2005a and 2005b); prospective memory deficits have been observed in patients with lesions to FPC (Uretzky and Gilboa, 2010; Volle et al., 2011), and prospective memory is impaired by continuous theta-burst

stimulation to area 10 (Costa et al., 2011 and 2013). While multiple explanations have been proposed regarding the nature of FPC functional contributions to episodic memory (this is discussed in more detail in Chapter 2), one possibility is that FPC is required in order to assess temporal context: Volle et al. (2011) found that patients with BA10 lesions were unimpaired when required to make an action after the occurrence of a particular cue (event-based prospective memory), but were impaired when required to make an action at a particular time (time-based prospective memory); they were also impaired in their ability to estimate the passage of time. Evidence from human neuroimaging studies is mixed regarding the activation of BA10 in tasks which require participants to judge the temporal context in which an item was presented (contrast Simons et al., 2005b; Dobbins et al., 2002; Dobbins et al., 2003; Dudukovic and Wagner, 2007), and it is possible that the patients tested by Volle et al. suffered from a strategy impairment (e.g. failing to count the seconds which passed between prospective actions). However, an impairment in judging the relative recency of events and outcomes may provide an explanation for other deficits observed in patients with damage to FPC: for example, Kovach et al. (2012) argue that patients with FPC damage failed to take into account the outcomes of recent trials in making subsequent choices. In order to integrate recent outcomes into a behavioural strategy, it is first essential to remember which outcomes occurred most recently, and thus have the greatest priority in influencing behaviours.

There are at least two distinct processes by which humans may assess temporal context. Firstly, they may access episodic information about the initial event, including information about any preceding or following occurrences or any particular temporal cues; secondly, they may assess the amount of interference or decay which a particular memory trace has undergone and thereby estimate how recently the memory trace was formed in comparison to another memory trace (this is only likely to be possible with regard to retrieval from working memory) (e.g. Portrat et al, 2008). These two processes may provide an explanation for the mixed results detected by human neuroimaging studies in relation to FPC activation when subjects make temporal context and recency judgements: in Simons et al. (2005b), for example, participants indicated which of two

temporally distinct lists a study item was drawn from, while in Dobbins et al. (2002 and 2003) and in Dudukovic and Wagner (2007), participants were required to judge which of two stimuli presented in the same study phase was presented more recently. In the study conducted by Simons et al., participants were asked to make one of two different types of semantic judgements about stimuli; these stimuli were presented in two temporally distinct lists. Researchers observed reduced frontopolar activation when participants made judgements based on the list in which a stimulus had been presented (i.e. in which temporal context the stimulus had been experienced), compared to when they reported what form of semantic judgement they had originally made regarding a stimulus; there was, however, some temporal context related activation in bilateral lateral anterior PFC compared to baseline (the coordinates of this activation were not reported). In this study, participants may have made temporal judgements based on episodic recollection of temporal cues associated with one list rather than the other. There is little evidence to suggest that this kind of episodic memory for temporal context cues is impaired in FPC patients: in the study conducted by Volle et al., each trial episode in the experiment was very similar to the others and the number of checks that subjects made to a stopwatch which would provide them with a temporal cue correlated positively with performance in both patients and healthy controls; it is more likely that FPC patients were impaired at judging how much time had passed (e.g. how much the memory trace had decayed) since the time that they last carried out the prospective action. However, there is no consensus regarding whether BA10 is activated when participants are required to judge which of two items in the same list were presented more recently: such activation was observed by Dobbins et al. (2003) and Dudukovic and Wagner (2007), but not by Dobbins et al. (2002). Furthermore, there is little consensus regarding which area of FPC may be activated by recency memory: Simons et al. (2005b) reported bilateral lateral activation of anterior PFC, while Dobbins et al. (2003) and Dudukovic and Wagner (2007) reported isolated activations in, respectively, the right and left hemispheres of BA10; these activations were not clearly lateral or medial.

No study has yet been conducted which assesses whether macaques with neurosurgical lesions to FPC are impaired in judgements of temporal context. Since such a deficit, if it exists, may explain the contribution of area 10 to multiple other cognitive processes (e.g. episodic memory, decision-making based on recent outcomes), it was essential to determine whether macaques were impaired in this area before assessing the effect of lesions on performance in other cognitive tasks (see Chapter 2 and Chapter 3). I therefore assessed FPC-lesioned macaques on a recency learning paradigm established by Charles et al. (2004) (Experiment 1) in order to assess whether these animals were impaired in judging the temporal context in which a stimulus occurred. In this task, macaques were shown five stimuli sequentially, and then given a choice of two stimuli that had occurred in the list; they were rewarded for choosing the stimulus which occurred more recently.

This task required animals to recall information from working memory. Neubert et al. (2014) noted that macaque lateral area 10 has a similar pattern of functional connectivity to both macaque area 46 and human BA46. In humans, BA46 is associated with the maintenance and manipulation of information in working memory, and lesions or neurological illness which impair function in this area result in deficits in these tasks (Keedy et al., 2006, Barbey et al., 2013). Research suggests that working memory is organised similarly in humans and macaques (Reinhart et al., 2012), and that macaques with dlPFC lesions are impaired on working memory tasks (Petrides, 1991, 1995). Double dissociations have been found between the effects of lesions to area 10 and area 46 in macaques, indicating that they are functionally independent:

- Lesions to area 46 cause an impairment to working memory for rule in the WCST analogue (Buckley et al., 2009) while lesions to area 10 do not (Mansouri et al., submitted).
- Lesions to area 10 cause impairments to one-trial object-in-scene learning (Boschin et al., submitted), whereas dorsolateral PFC lesions which include area 46 do not (Baxter et al., 2008).

- Lesions to PS (principal sulcus) impair conflict-induced behavioural adaptation in a modified WCST analogue (Mansouri et al., 2009), while lesions to FPC lead to enhancements in these scenarios (Mansouri et al., submitted).

However, monkeys with lesions to area 10 have not yet been clearly shown to be unimpaired at maintaining multiple items in working memory: Boschin et al. (submitted) found a one-trial learning deficit after frontopolar lesions in concurrent discrimination tasks which tested macaques' ability to remember which of a pair of items was rewarded, for ten pairs; while a delayed match-to-sample task was unimpaired, macaques were only required to recognise one item in that test. It was therefore essential to test this capacity before results from the recency memory task could be interpreted. I therefore conducted a task which tested monkeys' ability to recognise targets after presenting progressively longer sequences of stimuli (Experiment 2).

The hypothesis of this experiment was that lesions to the frontopolar cortex in macaque monkeys would selectively impair performance in recency memory tasks.

Method

Subjects

Seven experimentally experienced female rhesus macaque monkeys (*Macaca mulatta*) were used in these experiments. Their mean weight at the start of behavioural testing was 6.8 kg (range 4.98–9.01 kg), and their mean age was 6 years and 4 months. They were housed in small socially compatible groups. There was one group of three, one pair and two monkeys who were housed individually at the start of the experiments (due to a previous fight as part of a larger group) and moved together after recovering from injuries from that fight. They were housed in an enriched environment in which they could forage for small food items once a day. All had automatically regulated lighting and water available ad libitum. All monkeys had been pre-trained on a delayed match-to-sample task in the same apparatus that was used in these experiments.

At the start of behavioural testing, three animals were control animals, and four animals had received FPC lesions 32-38 months prior. After data had been collected in the tasks described below, the three control animals received FPC lesions and were retested on Experiment 2. No control animals from this group successfully passed the pre-training stage of Experiment 1 (described below), so data collected from the FPC-lesion group was compared with data collected from control animals by Charles et al. (2004). Details of those control monkeys are given in that paper. Those monkeys were housed in identical conditions to those already described.

Surgery

Four animals had received bilateral ablation lesions to FPC prior to the start of the experiment; the other three were tested as unoperated controls and again 7-12 months later after receiving identical lesions. All operations were performed under aseptic conditions with the aid of an operating microscope. The night before surgery, monkeys received steroidal anti-inflammatory (methylprednisolone, 20 mg/kg, intra-muscular [i.m.]) and antibiotic (amoxicillin, 8.75 mg/kg, i.m.) treatment to reduce the risk of intra-operative oedema and post-operative inflammation or infection. Additional doses of steroids were administered at 4-6 h intervals on the day of surgery. On the morning of surgery, monkeys were sedated with ketamine (10 mg/kg, i.m.), xylazine (0.5 mg/kg, i.m.) and/or midazolam (0.25 mg/kg, i.m.). After sedation, monkeys were given injections of atropine (0.05 mg/kg) in order to reduce secretions, an opioid (buprenorphine, 0.01 mg/kg, i.v.) and a non-steroidal anti-inflammatory (meloxicam, 0.2 mg/kg, i.v.) in order to provide analgesia, and a H_2 receptor antagonist (ranitidine, 1 mg/kg, i.v.) in order to protect against gastric ulceration which may have occurred as a result of combining steroidal and nonsteroidal anti-inflammatory treatments.

Monkeys were then moved to the operating theatre, where they were intubated and placed on isoflurane anaesthesia (1-2.75%, to effect, in 100% oxygen) and mechanically ventilated. An intravenous cannula was placed in order to allow intra-operative delivery of fluids (warmed sterile saline drip, 5 ml/h/kg). The head was shaved, placed in a head holder and cleaned with an

antimicrobial scrub and alcohol. A normal body temperature was maintained throughout surgery using adjustable heating blankets and vital signs (heart rate, oxygen saturation of haemoglobin, mean arterial blood pressure, end tidal CO_2, body temperature, respiration rate) were monitored continuously. A midline incision was made. The skin, underlying galea, and temporal muscles were retracted in anatomical layers to expose the skull surface and a bilateral bone flap was removed. The dura was cut to expose the cortex and the lesion was made by aspiration with a fine-gauge sucker at the intended lesion site. All brain tissue anterior to an imaginary line drawn 3mm posterior to the anterior tip of the principal sulcus was removed. After the lesion was complete, the wound was closed in anatomical layers. Anaesthesia was discontinued and the head holder was removed. Extubation occurred when a swallowing reflex could be observed and the monkey was then returned to the home cage. The animal was monitored continuously until it was able to sit normally, and checked regularly for a further 48 hours. After surgery, monkeys were treated with nonsteroidal anti-inflammatory analgesic and antibiotic medication as advised by veterinary staff. At least two weeks were allowed for recovery before testing was resumed.

All licensed procedures were carried out in compliance with the United Kingdom Animals (Scientific Procedures) Act of 1986. Histological analysis of the brains of the four animals that received lesions prior to the start of the experiment and the three animals that received lesions during the course of the experiment confirmed that the lesions were performed as intended, albeit with slight unilateral asymmetry in one animal, reflecting slight sparing in the most anterior ventral aspect of the right hemisphere (see figures 1 and 2).

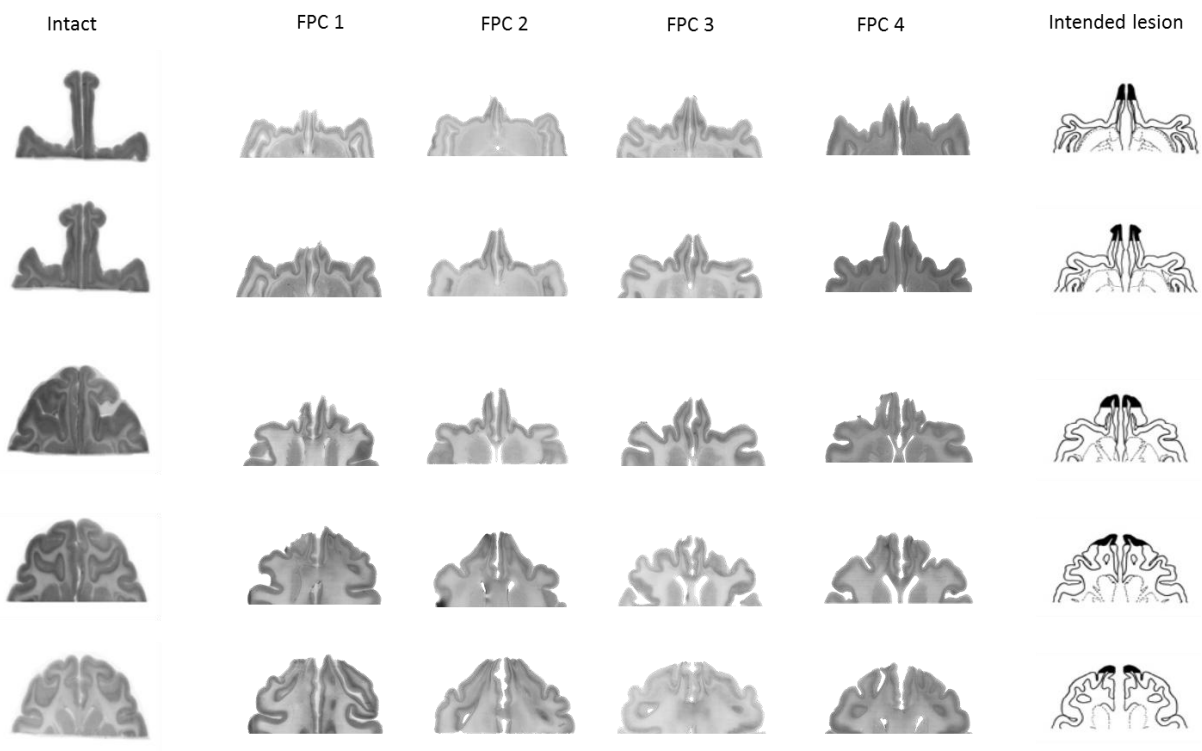


Figure 1 – Photomicrographs of stained coronal sections through the area of the intended lesion in the four animals with FPC lesions at the start of the experiment (FPC1 to FPC4) alongside drawings of the intended extent of the lesions on representative coronal sections (right column) and photomicrographs of stained coronal sections through the area of the intended lesion in an intact animal (left column). Adapted from Boschin et al. (submitted).

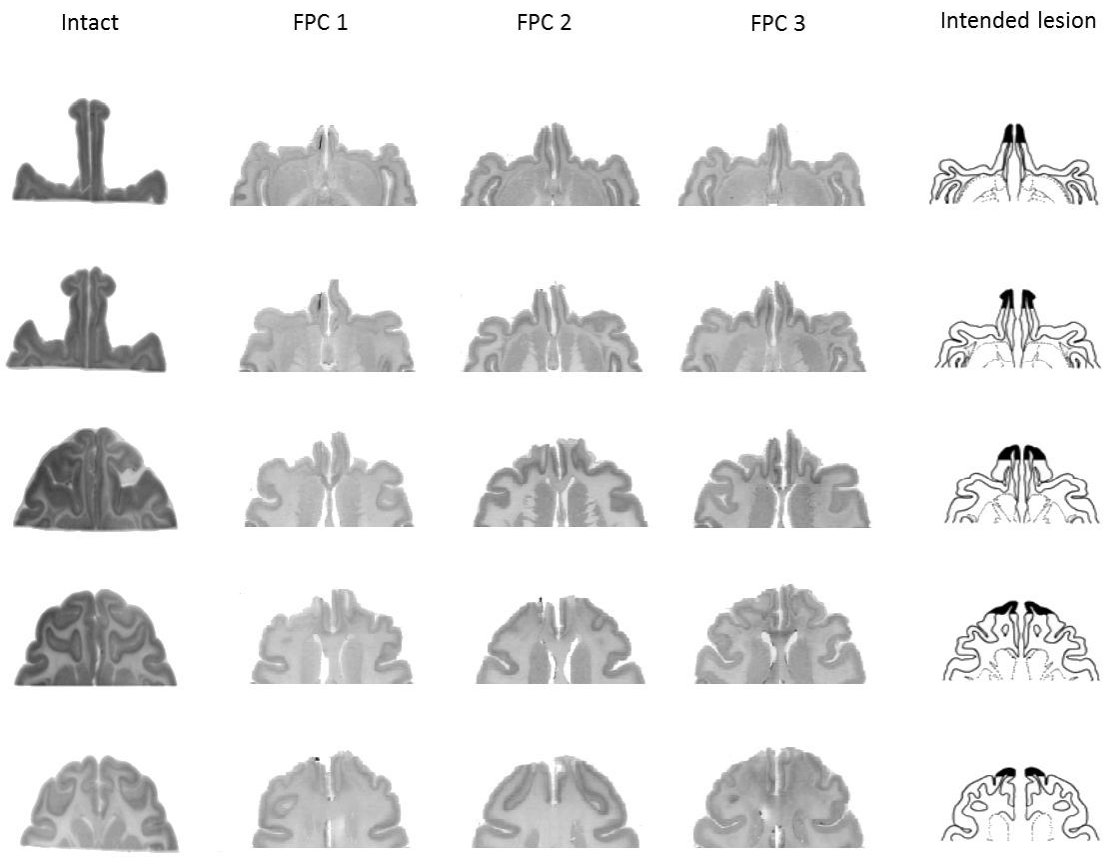


Figure 2 – Photomicrographs of stained coronal sections through the area of the intended lesions in the three animals that received frontopolar lesions during the course of the experiment (FPC 1 – FPC 3) alongside drawings of the intended extent of the lesions on representative coronal sections (right column) and photomicrographs of stained coronal sections through the area of the intended lesion in an intact animal (left column).

Apparatus

The task was performed in an automated test apparatus. The subject sat in a wheeled transport cage fixed in front of a touch-sensitive screen (380mm × 280mm; screen resolution was set at 800x600 pixels). Animals were unrestrained within this cage and could reach between the horizontal bars (spaced approx. 50mm apart) of the otherwise open frontage of the transport cage to touch the screen. A pellet delivery system, controlled by the computer, delivered reward pellets (190 mg, supplied by Noyes Company Inc.), peanuts or chocolate sweets into a food-well (approx. 80 mm in diameter) positioned beneath the screen. Reward delivery was accompanied by an audible click. An automated lunchbox (approximate dimensions: length, 200mm, width, 100mm, height,

100mm) was positioned beneath and to one side of the subject. The lunchbox was operated by a spring-loaded solenoid. It opened immediately with a loud crack on completion of the first correct trial after the monkeys completed an assigned number of trials. The lunchbox contained the subject's daily diet of wet monkey chow, slices of fruit and a few treats (peanuts, dates or prunes). An infrared camera was positioned to look down into the transport cage from above to allow the macaque to be observed from a separate room during the session. The apparatus was housed in a cubicle that was dark apart from background touch-screen illumination. The presentation of visual stimuli on the screen was controlled by a computer that also recorded the touches that subjects made to the screen and controlled the delivery of rewards and the opening of the lunchbox.

Experiment 1 – Recency

As this paradigm had previously been used by Charles et al. (2004), in order to avoid ambiguity, the method has been reported here in very similar terms to those used in that paper.

Subjects

Two FPC-lesioned animals and two control animals failed to pass the pre-training stages of this task. Of these, one of the FPC-lesioned animals was tested on the full task after achieving a score 2% lower than criterion in the final pre-training stage; the other three animals that failed to pass the pre-training stages were not tested on the full task. A third control animal passed all pre-training stages, but scored much lower than chance during the testing phase and was excluded from the analysis. As a result of failure to collect control data from this group of subjects, data collected from the three animals tested on the full task in the FPC-lesioned group was compared with control data collected from three subjects by Charles et al. (2004). FPC-lesioned animals were tested an average of 36 months post-lesion (range 34-38 months). No animal was tested pre- and post-operatively.

Stimuli

The visual stimuli presented on the touchscreen were individual clipart images obtained from commercially available internet sources. Each clipart image was 128×128 pixels in size, subtending a visual angle of 12 – 15 degrees to the typical viewpoint of a macaque. It comprised a distinct cartoon-like image, imposed on a white-coloured screen-congruent background. All stimuli were presented against a white background that occupied the whole touchscreen, and a touch-sensitive area extending 50 pixels beyond each side of each image was used to ensure the detection of stimulus-directed responses. A single pool of 600 images was used for all stages of testing in this experiment. Stimuli were used from the pool in a random order without replacement within each daily test session and were then reused in a new random order in the next session.

Pre-training stage 1: Charles et al. (2004) included an initial pre-training stage, in which monkeys were trained on a delayed match-to-sample task. As these monkeys had already been trained on this task, this stage was not included in this study.

Pre-training stage 2: "1 versus 5." Each trial consisted of a display stage (in which five stimuli were displayed sequentially) and a choice stage, separated by a delay. In the display stage, monkeys were required to touch a stimulus positioned in the centre of the screen. When the animal touched the picture, the stimulus disappeared and was replaced by a blank screen. After a one second inter-stimulus interval, the next sample in the sequence appeared and subjects were required to touch it, and so on, until the final sample had been touched. After the final stimulus was presented, there was a one second delay before the presentation of the choice trial. In this stage, the first and fifth samples in the sequence were displayed on the screen. They were displayed on the horizontal midline of the screen positioned equidistant on opposite sides of the central point; whether the first stimulus was displayed to the left or the right (and, correspondingly, whether the fifth stimulus was displayed to the right or the left) was determined randomly. In this stage, if the monkey touched the fifth sample, both stimuli disappeared from the screen and a reward pellet was immediately delivered. Touching the first sample caused both stimuli to disappear, but no reward was delivered.

Following a correct trial, the intertrial interval was five seconds; following an incorrect trial, the intertrial interval was ten seconds. Touching the screen during the intertrial interval reset this interval. The number of unique trials was varied according to the animals' temperament, with a minimum of 20 and a maximum of 50. Each session terminated on completion of the final trial. If the monkey completed the final trial incorrectly, the same sequence of samples was presented again, but followed by a choice screen in which both stimuli were the 5th stimulus in the sequence; this meant that the monkey was never rewarded for an incorrect trial with the presentation of their daily food. Training sessions ceased when animals attained a criterion of 90% correct trials in one session.

Pre-training stage 2.5: Several animals failed to progress through pre-training stage 2. For one monkey in the FPC-lesioned group, two adjustments were made: firstly, as she touched exclusively to the left side of the screen, the orientation of the choice stage was adjusted so that stimuli were presented along the vertical midline of the screen, equidistant on opposite sides of the central point for this animal. This orientation was preserved for this animal throughout the rest of the pre-training and test stages of the experiment. The second adjustment was to train this monkey for one day in which the same stimulus was presented five times (5/5/5/5/5), then one day in which one stimulus was presented, and then another one four times (1/5/5/5/5), then one day in which two stimuli were presented, and then another one three times (1/2/5/5/5), and then five days in which three stimuli were presented, and then the final stimulus twice (1/2/3/5/5). During this process, the first and last stimuli presented were always presented in the choice trial (1 v. 5), except on the first day, in which the choice was between a novel stimulus and the last stimulus presented. Over the course of this process, the monkey scored between 74% and 84%, so after completion of this process the animal was moved back onto the standard pre-training stage 2 and subsequently achieved the criterion score of 90%. Three animals (two control and one FPC-lesioned) failed to meet the 90% criterion on pre-training stage 2 and were not tested on this task despite adjustments similar to those described above.

Pre-training stage 3: "1 versus 4." This stage was the same in all respects to stage 2, with the exception that, in choice trials, subjects were required to choose between the first and fourth samples in the sequence. Choosing the fourth sample produced a food reward. Training sessions ceased when animals attained a criterion of 80% correct trials in one session. One monkey was tested on the main task after attaining a score of 78% correct trials in one session due to time constraints imposed by the licence for this project.

Test stage: After presentation of a sequence of five sample stimuli, monkeys received a trial in which they were required to choose between two samples presented in the sequence. They were rewarded for choosing the sample that occurred later in the sequence. There were ten possible different trial types: comparisons between the first and second, third, fourth or fifth stimuli (1 v. 2, 1 v. 3, 1 v. 4, 1 v. 5), between the second and the third, fourth or fifth (2 v. 3, 2 v. 4, 2 v. 5), between the third and the fourth or fifth stimuli (3 v. 4, 3 v. 5) and between the fourth and fifth stimuli (4 v.5). 1500 trials were collected on this task; the number of trials collected each day depended on the temperament of the animal and varied between 10 and 100. Figure 3 shows the display and choice stages of this task.

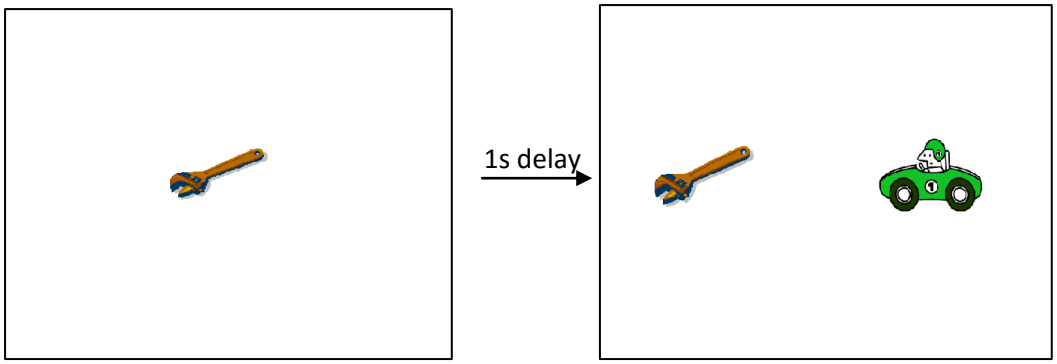


Figure 3 – An illustration of the display (left) and choice (right) stages for the recency task. Monkeys were shown five stimuli sequentially before a choice stage in which they were required to select the most recently displayed stimulus. Images are derived from free online resources, including Microsoft Clipart.

Experiment 2 – Recognition memory

Subjects

All subjects successfully learned this task. Three control animals and four FPC-lesioned animals (32 – 38 months post-surgery; average 35 months post-surgery) were tested. After receiving lesions to FPC, the three animals previously tested as controls were retested on this task; these animals were tested two weeks after surgery was conducted. Monkeys were given a few sessions of this task immediately prior to surgery in order to eliminate forgetting of the task over the delay between the first and second periods of testing; data from these sessions were not considered.

Stimuli

The visual stimuli presented on the touchscreen were taken from a library of clipart images obtained from commercially available sources. Each image was 128×128 pixels in dimension and comprised a distinct cartoon-like image. The stimuli assigned to each set of trials were chosen at random (without replacement) from a library of over 14,000 stimuli. The resolution of the visual display on the touch-screen was set at 800×600 pixels with the effect that each visual stimulus on the screen subtended approximately 12 to 15 degrees of visual angle from the typical viewpoint of the macaque.

Task

The monkeys tested in this study were already experienced at a delayed match-to-sample paradigm, so it was unnecessary to pre-train animals on this task. Each trial consisted of a display stage and a choice stage separated by a delay. In the display stage, a stimulus was displayed in the centre of the screen against a grey background. Monkeys were required to touch this stimulus, at which point it was removed from the screen. If the sequence length was greater than one, it was immediately replaced by a second stimulus; this procedure was repeated until all stimuli in the sequence had been presented. After all stimuli had been presented, there was a delay of one second before the choice stage of the trial. During this delay, a blank grey screen was displayed. In the

choice stage, one of the previously presented stimuli (the “old” stimulus) and a stimulus which had not been previously presented (the “new” stimulus) were displayed on the screen; at sequence lengths greater than one, the “old” stimulus was chosen randomly from the pool of potential stimuli. These stimuli were presented on the horizontal midline of the screen, on opposite sides equidistant from the central point. Whether the “old” or “new” stimulus was displayed to the left or right was determined randomly. The monkey was required to touch one of these stimuli. If the monkey chose the “old” stimulus, she was rewarded with a banana-flavoured pellet, and the inter-trial interval was eight seconds; if the monkey chose the “new” stimulus, she did not receive a food reward and the inter-trial interval was sixteen seconds.

There were five levels of this task, corresponding to sequence lengths of 1, 2, 3, 4 and 5 stimuli. Monkeys were tested on these levels sequentially, so that all trials of shorter sequence lengths were collected before testing on longer sequences of stimuli. 150 trials of sequence length 1 were collected; 150 of sequence length 2; 225 of sequence length 3; 300 of sequence length 4 and 375 of sequence length 5. This was calculated so that approximately 75 trials were collected for each possible position of the “old” stimulus in the sequence for each task level. Figure 4 shows the display and choice stages of this task.

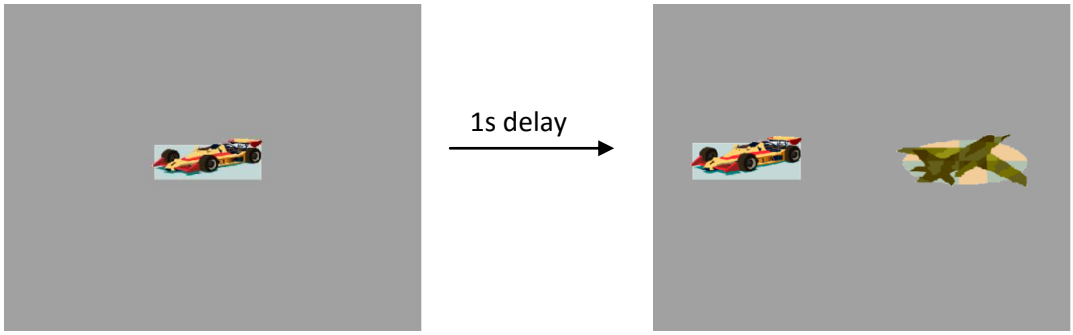


Figure 4 – An illustration of the display (left) and choice (right) stages in the sequence length task. Monkeys were shown between one and five stimuli, followed by a grey screen for 1 second, followed by a choice stage in which one previously displayed stimulus and one new stimulus was presented. Images are derived from free online resources, including Microsoft Clipart.

Results

Experiment 1 – Recency

One FPC-lesioned animal was moved onto the final stage of the task without achieving the 80% criterion level at the second pre-training stage. However, as this animal (monkey A) performed better than one of the other FPC-lesioned animals (monkey B) that were tested on this task (percent error, monkey A: 45.1%; percent error, monkey B: 46.2%), it cannot be concluded that FPC-lesioned animals performed worse than controls on this task as a result of inadequately learned rules in the pre-training stage. Overall, FPC-lesioned animals had a mean percentage error of 41.3% in this task; control animals had a mean percentage error of 28%. Figure 5 shows the mean percentage error on this task of FPC-lesioned and control animals.

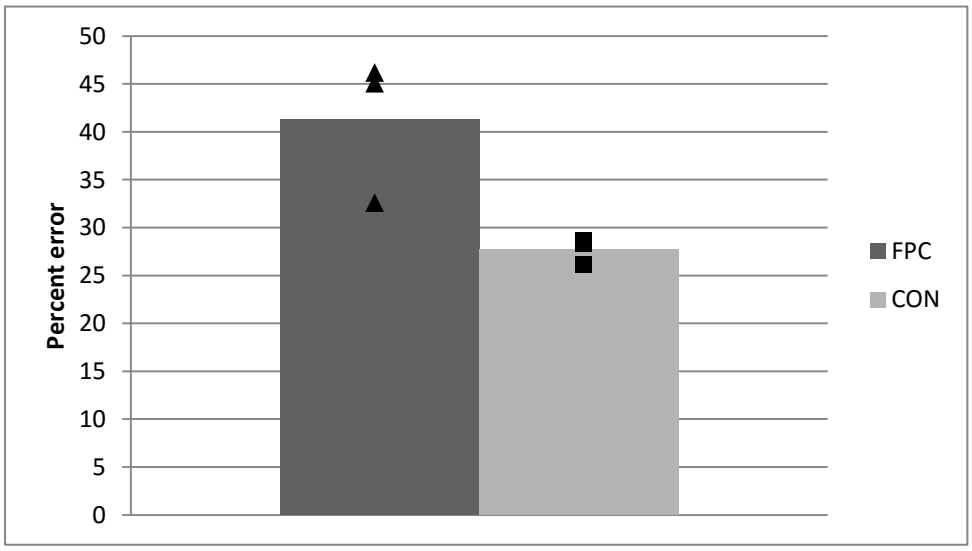


Figure 5 – Mean percent error of FPC-lesioned and control animals, for all possible recency comparisons

An independent-samples t-test was performed on the arcsine-transformed percentage error scores. Levene's test for equality of variances was significant (p=0.037), so equal variance was not assumed. A non-significant trend indicated that FPC-lesioned animals may have been more likely to record larger percentage error scores (an impairment): $t(2.159)$=3.133, p=0.080. Figure 6 shows the performance of FPC-lesioned animals across trial types; Figure 7 shows this data for control animals.

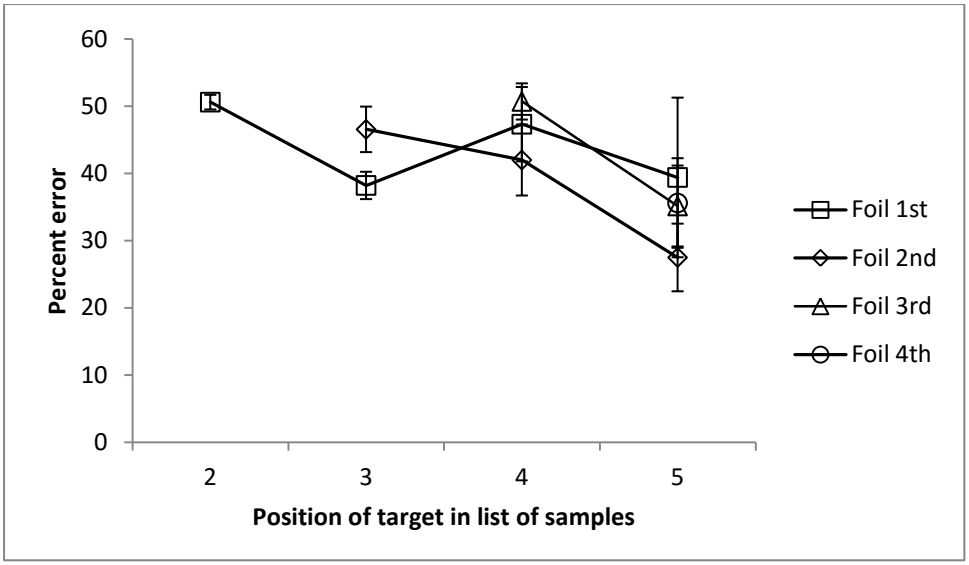


Figure 6 – Percent error of FPC-lesioned animals across all trial types

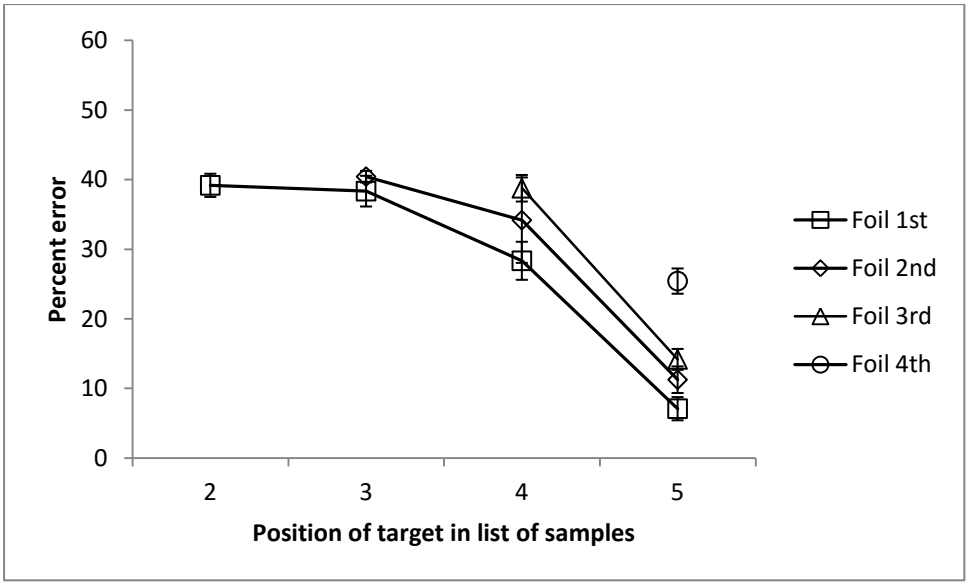


Figure 7 – Percent error of control animals across all trial types. Error bars show the +/- standard error of the mean.

In order to assess the effect of difficulty level on performance, a repeated measures ANOVA was conducted on the arcsine-transformed percentage error scores for each target position in the sequence of stimuli (one between-subjects factor of group: two levels – FPC-lesioned and control; one within-subjects factor of target position in sequence: four levels). This returned significant main effects of target position ($F(3,12)$=22.156, p<0.001) and group ($F(1,4)$=13.438, p=0.021) and a significant interaction effect between target position and group ($F(3,12)$=3.686, p=0.043). The main

effect of group detected by this ANOVA indicated that FPC-lesioned animals were significantly impaired on this task. Since it is possible that this data was affected by floor and/or ceiling effects, linear and quadratic components were examined in order to clarify whether these may explain the basis of the interaction. There was no significant linear or quadratic component to this interaction, although a quadratic trend could be observed ($F(1,4)$=5.499, p=0.079). In order to investigate the source of this interaction effect, I conducted independent samples t-tests on the arcsine-transformed percentage error scores for each target position. After the Holm-Bonferroni method (Holm, 1979) was applied to correct for multiple comparisons, FPC-lesioned animals differed from controls only at the highest difficulty level (target second in list of samples) ($t(4)$=5.711, p=0.005). However, clear trends towards impairment existed at target position 4 ($t(4)$=2.669, p=0.056) and target position 5 ($t(4)$=2.815, p=0.048).

Experiment 2 – Recognition memory

Three sets of data were collected for this task: one group of animals were only tested on this task 32-38 months post-lesion (FPC-late), one group of animals were tested as unoperated controls (CON), and this group of animals were also tested for a second time two weeks post-lesion (FPC-early). Three sets of analyses were thus carried out on this data: a between-subjects CON vs. FPC-late analysis, a within-subjects CON vs. FPC-early analysis, and a between-subjects FPC-early vs. FPC-late analysis (this analysis was conducted to determine whether any effect could be detected of time of lesion in relation to time of testing).

Two measures of recognition memory performance were used to assess monkeys' performance on this task: arcsine-transformed percentage error and d' values. The d' statistic has been used in preference to arcsine-transformed percentage scores in tests of recognition memory, as these may exaggerate differences close to 0% and 100% (Ringo, 1988). In this study, this technique was used to check the results observed when the arcsine-transformed percentage error

scores were analysed. The d' score for each animal was calculated according to the technique described by Baxter and Murray (2001).

CON vs. FPC-late (between subjects)

Percentage error for each animal was calculated as the mean of the percentage error scores for each sequence length. Mean percentage error was calculated for each sequence length by adding the total number of correct and incorrect trials at each sequence length and calculating the percentage of incorrect trials. Figure 8 shows the performance of FPC-late and control animals on the different sequence lengths of the task. An independent samples t-test was carried out on the arcsine-transformed percentage error scores; no effect of lesion was found ($t(5)$=0.341, p=0.747). Additionally, an independent samples t-test was used to compare the overall d' scores recorded in the CON and FPC-late groups; no effect of lesion was found ($t(5)$=-0.340, p=0.747).

A two-way repeated measures ANOVA was conducted on arcsine-transformed percentage error scores [one between-subjects factor of group (two levels: FPC-late and control) and one within-subjects factor of sequence length (five levels)]. In this analysis, a significant main effect of sequence length was detected ($F(4,20)$=13.708, p<0.001), in addition to a significant interaction effect between sequence length and group ($F(4,20)$=3.077, p=0.040). When this ANOVA was conducted on d' scores at different sequence lengths, the same results were detected: a significant main effect of sequence length ($F(4,20)$=11.999, p<0.001) and a significant interaction effect between sequence length and group ($F(4,20)$=3.419, p=0.028) were found. In order to examine this interaction effect, independent-samples t-tests were carried out on the arcsine-transformed percentage error scores to examine the effect of group at each sequence length. No significant differences were detected at any sequence length; the greatest between-group difference was detected at sequence length 1, in which FPC-late animals performed better on average (12.639% errors) than control animals (19.228% errors). The same result was detected when the d' scores

were analysed in a series of independent samples t-tests rather than the arcsine-transformed percentage error scores.

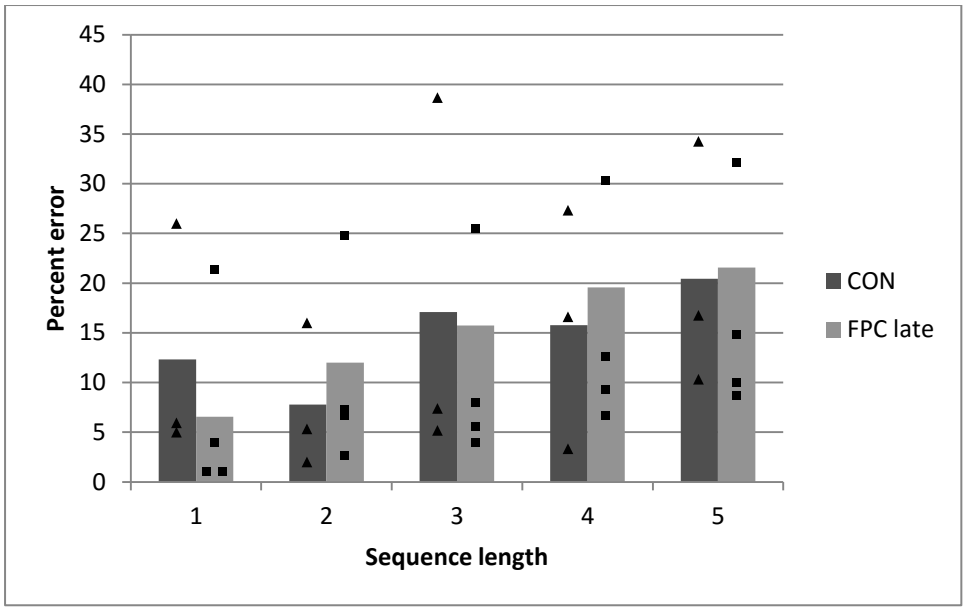


Figure 8 – Percent error at each sequence length of three control animals and four FPC-late animals

As the difficulty of the task was not modulated only by sequence length, but also by the number of stimuli presented between target and choice, I analysed data relating to performance at each possible target position (ranging from zero stimuli presented between target and choice to four stimuli presented between target and choice). Only data from the maximum sequence length (five stimuli) was included in this analysis in order to be consistent with the similar control task analysed by Charles et al. (2004). Figure 9 shows the performance of FPC-late and control animals at each possible target position. Percent error was calculated using the total number of correct and incorrect trials for each target position, regardless of sequence length.

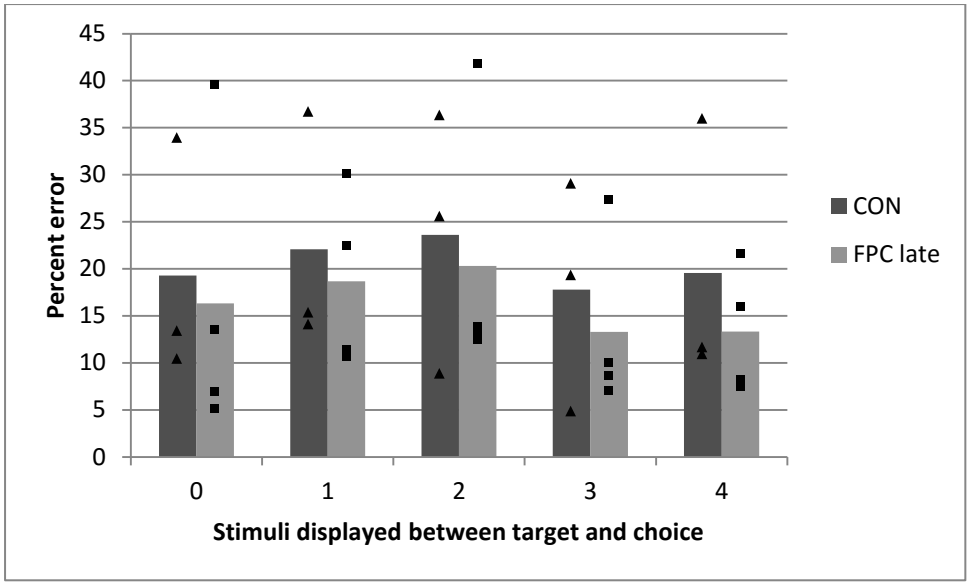


Figure 9 – Percent error of three control animals and four FPC-late animals at each target position (5/5, 4/5, 3/5, 2/5, 1/5).

A two-way repeated measures ANOVA [one within-subjects factor of target position (five levels); one between-subjects factor of group (two levels: control and FPC-late)] conducted on arcsine-transformed percentage error scores at different target positions found no significant effects; the same ANOVA conducted on d' scores at different target positions likewise recorded no significant effects. It was therefore possible to conclude that there was no long-term deleterious effect of lesion on this task. The next analysis looked at short-term effects of lesion to see whether recovery of function may be a confounding factor.

<u>CON vs. FPC-early (within subjects)</u>

Figure 10 shows the pre- and post-operative performance at each sequence length for the three animals that were tested on the task before and two weeks after lesions to FPC. A paired samples t-test was used to compare arcsine-transformed percentage error scores (this was calculated as the mean of the percentage error scores for each sequence lengths) of the CON and FPC-early data; this analysis yielded no significant effect ($t(2)$=1.791, p=0.215). A paired samples t-test which compared the overall d' scores recorded pre- and two weeks post-operatively in these animals also found no significant effect ($t(2)$=2.827, p=0.106).

When a two-way repeated measures ANOVA was conducted on arcsine-transformed percentage error scores [two within-subjects factors of stage of testing (two levels: CON and FPC-early) and sequence length (five levels)], a significant main effect of sequence length ($F(4,8)$=33.950, p<0.001) and a significant interaction effect between sequence length and stage of testing ($F(4,8)$=86.713, p=0.005) was observed. Significant linear ($F(1,2)$=86.339, p=0.011) and quadratic $(F(1,2)$=28.363, p=0.033) components of this interaction were detected. Similarly, when the same ANOVA was conducted on d' scores at different sequence lengths, a significant effect of sequence length ($F(4,8)$=24.253, p<0.001) and a significant interaction effect between stage of testing and sequence length ($F(4,8)$=8.320, p=0.006) were found. A significant linear component of this interaction was detected ($F(1,2)$=102.153, p=0.010); there was also a trend towards a quadratic component ($F(1,2)$=17.805, p=0.052). Paired samples t-tests between CON and FPC-early percentage error scores at each sequence length were conducted in order to isolate the source of this interaction effect; however, these tests found no significant difference which survived correction for multiple comparisons using the Holm-Bonferroni method (the greatest difference was recorded at sequence length 2: $t(2)$=-7.133, p=0.019; this was greater than the significance criterion of p=0.010). Likewise, when d' scores at different sequence lengths were analysed rather than arcsine-transformed percentage error scores, no significant differences were recorded at any sequence length (the greatest difference was observed at sequence length 2: $t(2)$=4.313, p=0.050).

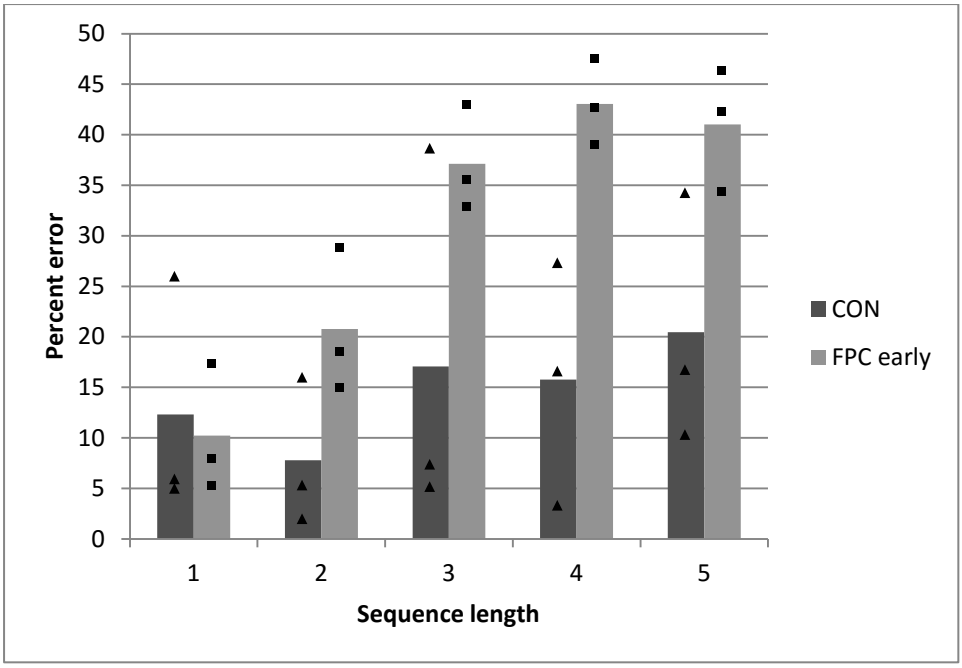


Figure 10 – Percent error of three animals pre- and two weeks post-operatively at each sequence length

As in the previous CON vs. FPC-late analysis, I examined performance at each possible target position at the longest sequence length in order to investigate whether the number of stimuli presented between target and choice modulated the difficulty of this analysis. Only data from the maximum sequence length (five stimuli) was included in this analysis. A two-way repeated measures ANOVA was used to analyse pre- and post-operative data from the animals tested twice on the task at each possible target position [two within-subjects factors of stage of testing (two levels: CON and FPC-early) and target position (five levels)]. No significant effects were observed when either arcsine-transformed percentage error or d' scores were considered, and no significant linear or quadratic components of any effect were detected. Figure 11 shows the pre- and two weeks post-operative performance at the different target positions for the three animals that were tested before and after lesions to FPC.

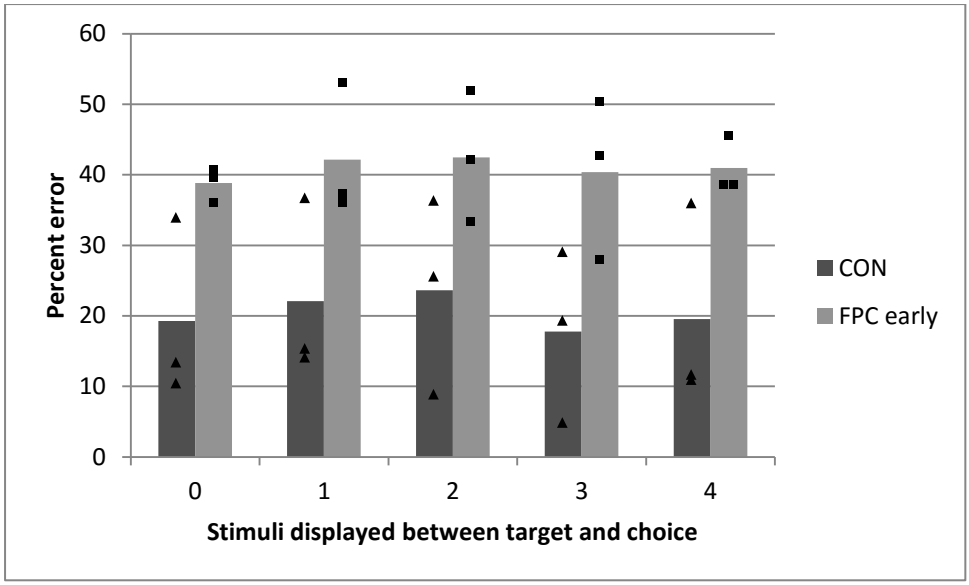


Figure 11 – Percent error of three animals pre- and two weeks post-operatively at each target position (5/5, 4/5, 3/5, 2/5, 1/5).

FPC-early vs. FPC-late (between subjects)

In order to compare overall performance between animals tested two weeks post-lesion and animals tested 32-38 months post-lesion, an independent samples t-test was carried out on the arcsine-transformed percentage error scores averaged across all trials at all sequence lengths; a significant effect of group was found ($t(5)$=2.782, p=0.039; animals in the FPC-late group performed better than animals in the FPC-early group). A similar independent samples t-test carried out on overall d' scores also found that animals in the FPC-late group performed significantly better than animals in the FPC-early group ($t(5)$=-2.724, p=0.042). A two-ways repeated measures ANOVA (one between-subjects factor of group; one within-subjects factor of sequence length) was conducted on the arcsine-transformed percentage error scores in order to compare performance between the FPC-early and FPC-late groups at different sequence lengths. This analysis found significant main effects of sequence length ($F(4,20)$=80.391, p<0.001) and group ($F(1,5)$=6.820, p=0.048) and a significant interaction effect between sequence length and group ($F(4,20)$=16.879, p<0.001), implying that macaques tested on this task 32-38 months post-lesion performed better than macaques tested two weeks post-lesion. Significant linear and quadratic components of the

sequence length*group interaction effect were detected (linear: *F(1,5)*=25.339, *p*=0.004; quadratic: *F(1,5)*=59.783, *p*=0.001). The same analysis conducted on d' scores at different sequence lengths likewise discovered a significant main effect of sequence length (*F(4,20)*=41.320, *p*<0.001) and a significant interaction effect between sequence length and group (*F(4,20)*=3.835, *p*=0.018), although only a trend towards a main effect of group was found (*F(1,5)*=6.087, *p*=0.057). In this analysis, no significant linear component of the sequence length*group interaction effect was detected, but a significant quadratic component was found (*F(1,5)*=9.664, *p*=0.027). In order to investigate the source of this interaction, independent samples t-tests were conducted on percentage error scores relating to each sequence length; however, these tests found no significant differences which survived correction for multiple comparisons using the Holm-Bonferroni method (the greatest difference was detected at sequence length 4 (*t(5)*=3.881, *p*=0.012; this was greater than the significance criterion of *p*=0.010). Similarly, independent samples t-tests were conducted on d' scores at each sequence length found no significant differences which survived correction for multiple comparisons using the Holm-Bonferroni method (the greatest difference was detected at sequence length 4 (*t(5)*=-3.646, *p*=0.015; *p*>0.010). This data is presented in figure 12.

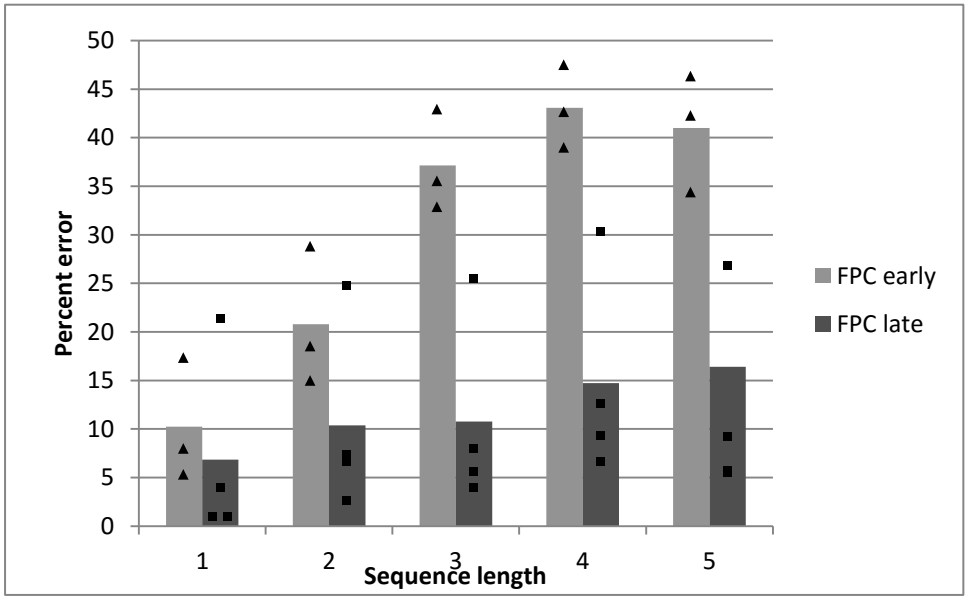


Figure 12 – Percent error of three animals tested two weeks post-lesion and four animals tested 32-38 months post-lesion at each sequence length

As no effect of target position had been noted in the previous CON vs. FPC-late or CON vs. FPC-early analyses, we did not expect to observe any significant effects when comparing performance at different target positions between the FPC-early and FPC-late groups. This was the case: a two-way repeated measures ANOVA was used to analyse pre- and post-operative data from FPC-early and FPC-late groups at each possible target position [one between-subjects factor of group (two levels: FPC-early and FPC-late) and one within-subjects factor of target position (five levels)]. No significant effect of target position or any significant interaction between target position and group were observed when either arcsine-transformed percentage error (main effect of target position: $F(4,20)$= 1.450, p= 0.254; interaction between target and group: $F(4,20)$= 0.631, p= 0.646) or d' scores (main effect of target position: $F(4,20)$= 1.552, p= 0.226; interaction between target and group: $F(4,20)$= 0.649, p= 0.634). were considered, and no significant linear (arcsine performance error, target position: $F(1,5)$= 0.625, p=0.465; arcsince performance error, target position*group: $F(1,5)$= 1.257, p=0.313; d' score, target position: $F(1,5)$= 0.527, p= 0.501; d' score, target position*group: $F(1,5)$= 1.134, p= 0.336) or quadratic (arcsine performance error, target position: $F(1,5)$= 2.972, p=0.145; arcsine performance error, target position*group: $F(1,5)$= 0.938, p= 0.377; d' score, target position: $F(1,5)$= 3.035, p= 0.142; d' score, target position*group: $F(1,5)$= 0.922, p= 0.381) components of any effect were detected.

Discussion

As predicted by the hypothesis, Experiment 1 detected a significant impairment in recency memory in the FPC-lesioned group. No impairment was detected in the control recognition memory task when animals tested a similar period of time post-lesion were compared with controls, indicating that these animals receive an enduring selective impairment in recency memory. Furthermore, this effect may have been underestimated, as only monkeys who completed the pre-training stages of the experiment were tested on the full task. Two FPC-lesioned monkeys did not successfully complete pre-training (although one of these monkeys was tested on the full task after

achieving a score of 2% lower than criterion); these monkeys' difficulty in learning the task may have been due to a failure to learn the rules of the experiment, or a severe deficit in context-learning which meant that they were unable to achieve either the 90% discrimination between a stimulus presented first and a stimulus presented fifth required in order for them to advance beyond pre-training stage two or the 80% discrimination between a stimulus presented first and a stimulus presented fourth required in order for them to advance between pre-training stage three. However, as two control monkeys also failed to complete the pre-training stages, it is not possible to draw any conclusions from the pre-training data in this study. However, other factors which may have led to an underestimation of the effect of lesion in this task included floor effects (both control animals and FPC-lesioned animals performed close to chance at some difficulty levels) and the possibility that, since an extended period of time elapsed between surgery and training on this task (an average of 35 months), some interim restoration of function had occurred (see Chapter 6).

Further analysis of the recency memory impairment on this task indicated that FPC-lesioned animals may have suffered a greater deficit when the target was presented early in the list of stimuli. While this result could support the theory that FPC-lesioned animals are impaired in judging the strength of memory traces (since memory traces relating to the earliest stimulus are those which are likely to be most degraded), it is also possible that this result could support the theory that FPC-lesioned animals are impaired in assessment of temporal cues: the temporal cues surrounding the first item in a sequence are likely to be different from those relating to later items (since it was preceded by an inter-trial, rather than by another stimulus). However, trends towards impairment also existed when targets were presented in later positions in the list of stimuli; since correction for multiple comparisons is likely to produce type II errors, it is not possible to conclude that monkeys in this task were selectively impaired when targets were presented early in the list of stimuli. Floor effects in both FPC-lesioned and control animals may have prevented the observed impairment reaching the significance threshold at a wider range of difficulty levels. However, as a main effect of group was detected, it is possible to conclude that these animals did suffer a deficit on this task. This

deficit may be interpreted as an impairment of temporal context learning, and may exist as part of a more general context-learning deficit: Boschin et al. (submitted) noted deficits in one-trial spatial context learning in macaques with lesions to area 10, although, as these researchers also noted one-trial learning deficits in a context-independent task, they interpreted their findings as evidence of a failure in the evaluation of counterfactuals (this conclusion was reached after examining the performance of FPC-lesioned macaques across a range of tasks). If the primate frontopolar cortex is necessary for maintenance or retrieval of context-specific information, this result may clarify the nature of BA10 association with episodic memory in humans. However, as noted above, the one-trial learning deficit in macaques with frontopolar lesions observed by Boschin et al. was not restricted to situations in which monkeys were required to associate information with contextual information. Furthermore, the deficit observed in this task cannot be interpreted as a one-trial learning deficit; monkeys with FPC lesions did not suffer any lasting impairment on a recognition memory task in which they were required to learn five stimuli over the course of a single presentation. However, it is possible that the deficit observed in macaques in Experiment 1 can be viewed as a selective deficit in judging how recently an object occurred. This ability, while clearly necessary for context-specific memory, is required in order to achieve optimal behaviour in any environment which is subject to change, since it is necessary to establish which outcomes occurred most recently in order to adapt behaviour accordingly. This may provide an explanation for the deficit noted by Kovach et al. (2012) in FPC-lesioned patients; it may also clarify activation observed in human BA10 in tasks in which reward probabilities are continually shifted (e.g. Boorman et al., 2009 and 2011, Daw et al., 2006).

This study also serves to further establish the homology between macaque and human FPC. Activation has been observed in human BA10 while humans carry out tasks which require them to report temporal contexts (Dobbins et al., 2003, Dudukovic and Wagner, 2007); the impairment of macaque monkeys on this task implies not only that these reports of BA10 activation during recency tasks are robust, but also that this region has similar functions in macaques and humans, a conclusion which has been disputed. Neubert et al. (2014) identify a monkey equivalent to human

medial area 10, but argue that there is no monkey equivalent to human lateral area 10. Recency judgements are not clearly associated with either medial or lateral area 10 (Dobbins et al., 2003 and Dudukovic et al., 2007, identify an area which may be localised in the superior frontal gyrus); further research is required to establish whether this functional homology extends to functions associated solely with lateral area 10.

In order to succeed in Experiment 1, it was important that monkeys had an intact ability to remember five stimuli presented sequentially. Experiment 2 tested monkeys' ability to recognise sequentially presented stimuli. Analysis of data from this experiment did reveal a significant interaction between the effects of sequence length and lesion group (control or FPC-lesioned) when control animals and animals tested 32-38 months post-lesion were tested on recognition for stimuli presented in sequences of varying lengths. However, as the largest between-groups difference was enhanced performance in FPC-lesioned animals compared to controls at the shortest sequence length, and no between-groups differences existed when the number of stimuli presented between target presentation and the recognition stage was analysed, it is unlikely that this between-groups difference was robust, indicating that lesions to FPC do not cause any lasting impairments to this task. A significant interaction effect was observed between sequence length and stage of testing when pre- and early post-operative performance were compared in three animals (these animals performed worse on the task when tested post-operatively), although it was not possible to localise the source of this interaction at any sequence length. However, this effect cannot explain the deficit in performance observed in Experiment 1, as all comparisons in that experiment were between control monkeys and monkeys trained and tested on the task post-lesion, a comparison which was not robustly impaired in Experiment 2.

In Experiment 2, a significant difference in performance was noted between animals tested two weeks post-lesion and animals tested 35 months post-lesion; animals tested two weeks post-lesion performed significantly worse than those tested 35 months post-lesion, although it was not

possible to identify an impairment at any individual sequence length. Multiple possible explanations could be proposed to account for this difference: it is possible that the rest period which monkeys received after surgery meant that they were impaired in relation to the general experimental scenario of completing touchscreen tasks in order to earn a food reward; however, the long period of time in which these monkeys were trained on touchscreen tasks prior to this experiment renders this explanation unlikely. It is also unlikely that this result can be explained in terms of monkeys tested in the FPC-late group reacquiring the skills necessary to complete this task in post-operative pre-training, as this task did not require pre-training beyond the delayed match-to-sample task; the delayed match-to-sample task was equivalent to the task tested in Experiment 2 with a sequence length of one, and both post-operative groups performed better than controls at this level (figures 8 and 10). A further possibility is that the length of time which elapsed between surgery and testing on this experiment for monkeys in the FPC-late group (an average of 35 months) allowed these animals to recover cognitive function which was impaired by surgery; this theory is discussed in more detail in Chapter 6. However, since the recency task was impaired when macaques were tested 35 months post-lesion, complete restoration of function did not occur between the FPC-early and FPC-late time-points. It is possible that macaques selectively recovered cognitive function in relation to the recognition memory task, but not in relation to the recency memory task; alternatively, it is possible that recovery occurred in relation to both tasks, but that the deficit on the recency memory task was greater than the deficit on the recognition memory task, such that the recency memory task was still impaired when tested at the FPC-late time-point.

The possible impairment noted in this experiment when animals were tested two weeks post-operatively compared to their pre-operative performance (no significant effect of group was detected, but a significant group*sequence length impairment was recorded; however, it was impossible to localise this to any sequence length) may imply that the macaque frontopolar cortex has some functional input to recognition memory tasks. This is not a function which is associated with human BA10, but is associated with human BA46: visual recognition memory is disrupted by

transcranial magnetic stimulation (Mottaghy et al., 2002) and by lesions to this region (Nielsen-Bohlman and Knight, 1999) in humans. Neubert et al. (2014) suggest that macaque lateral frontal pole may be homologous with macaque area 46. While lesions to area 46 in macaques do not impair basic recognition memory, they do impair monitoring and manipulation of information in working memory (Petrides, 1995). It is possible that the results of this experiment may indicate that the monkey frontopolar cortex is functionally implicated in working memory tasks in which monkeys are required to monitor sets of items in working memory, and that this may indicate that there is some functional homology between macaque areas 10 and 46 (although functional independence between these regions has been noted in other contexts). In order to investigate this further, macaques with lesions to FPC could be tested on a more extensive battery of tasks which involve monitoring and manipulation of information in working memory, including self-ordered and externally-ordered tasks such as those used by Petrides (1995).

CHAPTER TWO

The role of the macaque frontopolar cortex in recollection

Human BA10 is consistently activated by episodic memory tasks, and by tasks which test cognitive functions related to episodic memory, such as context learning and mentalising. In macaque monkeys, lesions to FPC impair performance in one-trial learning and temporal context learning. However, the functional role of this region in episodic memory is unclear. Previous research has used the Receiver-Operating-Characteristics (ROC) technique to study the episodic-like "recollection" component of recognition memory in macaques and other animals. Seven macaque monkeys, four of whom had received lesions to FPC and three of whom remained unoperated controls, were tested on a paradigm designed to produce ROC recognition memory curves. However, the results of this experiment were unclear and it was not possible to conclude whether lesions to this region impaired this type of memory in macaques.

Introduction

Tasks which require human subjects to retrieve information from episodic memory tend to activate FPC among other frontal regions (Duncan et al., 2000) and FPC has been associated with an episodic "retrieval mode" (Lepage et al., 2000). The task demands of these experiments are variable, and it is not always clear that studies of "episodic retrieval" make the necessary distinctions to separate episodic from semantic memory: Fletcher et al. (1998), for example, required subjects to recall items either from a structured list or when presented with a retrieval cue, but did not question subjects about the contextual content of the recalled item. However, there is substantial evidence which associates BA10 with context-specific memory tasks. FPC is activated by source memory tasks (Simons et al., 2005a and 2005b), and by prospective memory tasks (Ramnani and Miall, 2003); continuous theta burst stimulation (cTBS) over FPC disrupts prospective memory in humans (Costa et al., 2011 and 2013); some FPC patients are impaired at prospective memory tasks (Uretzky and Gilboa, 2010; Volle et al., 2011). Furthermore, evidence from macaque monkeys supports the

association of area 10 with cognitive functions related to episodic memory: lesions to this region impair one-trial learning (Boschin et al., submitted) and temporal context learning (see Chapter 1).

The nature of BA10 contribution to episodic memory is debated. Patients with focal damage to the FPC do not suffer from anterograde or retrograde amnesia (Alexander et al., 2003), implying that the contribution of BA10 to context-specific memory cannot be any process which is necessary for the encoding, maintenance or retrieval of items in memory. However, there are many other ways in which BA10 may participate in episodic retrieval. Braver and Bongiolatti (2002), for example, arguing that BA10 "subserves processes related to the monitoring and management of subgoals", provide an account in terms of sub-goal processing: they describe the maintenance of the intention to retrieve an episodic context as the overall task goal and the search of LTM for a match to the retrieval cue as the subgoal task. Ramnani and Owen (2004) believe that the FPC integrates the outcome of multiple cognitive processes and argue that episodic memory should be seen as the integration of retrieved information about an item and retrieved information about the episodic context in which it was encountered. In other studies, Christoff and Gabrieli (2000) argue that FPC contributions to episodic memory are best understood as the evaluation of self-generated information, and Burgess et al. (2007) state that lateral FPC may contribute to episodic memory in directing attention towards stimulus-independent cognition. Koechlin and Hyafil (2007) argue that episodic memory tasks may activate FPC as "the branching process may be involved in performing the judgment task pertaining to each trial while maintaining specified past episodes in a pending state so as to internally retrieve them subsequently for preparing the next memory judgment trial". Alternatively, as medial FPC has been implicated in mentalising, including sensitivity to one's own emotional states (e.g. Ochsner et al., 2004, Hoffmann and Bar-On, 2012), it is possible that the BA10 may contribute to the sense of autonoetic awareness (Tulving, 2002) and mental time travel (Suddendorf and Busby, 2003) which many consider to be a crucial component of this cognitive process. Any of these processes could be damaged while still allowing some episodic memory function.

Neuroimaging has been used in attempts to clarify the contribution of BA10 to episodic memory tasks. Of the theories cited above, Braver and Bongiolatti, Christoff and Gabrieli, Burgess and Koechlin and Hyafil describe processes which they localise in lateral BA10, while mentalising is usually associated with medial BA10 (Amodio and Frith, 2006; Gilbert et al., 2006b). Theoretically, by determining the region of interest activated within BA10 by context-specific memory tasks, it should be possible to determine whether this region is more closely associated with one of those processes located in lateral FPC (subgoal processing, stimulus-independent cognition, evaluation of self-generated information or branching) or mentalising. However, not all of these theories are localised to a specific region in BA10: Ramnani and Owen do not specify the region which they believe contributes to the integration of multiple cognitive processes more precisely than "anterior prefrontal cortex". Moreover, a complex pattern of activation has been observed within BA10 depending on the specific demands of memory tasks: Simons et al. (2005b) found bilateral lateral activation in BA10 in response to a source memory task: when memory for the type of task performed on a stimulus was analysed separately from memory for the list in which a stimulus was presented, only left lateral and medial BA10 activation was observed, with lateral activation occurring earlier than medial activation; when a cue was presented instructing participants to prepare for a task-based source memory judgement but was not followed by a target for retrieval, left lateral (but not medial) BA10 was activated. It therefore seems likely that the different processes involved in episodic retrieval may activate different areas of BA10 at subsequent stages of the retrieval process, and that it may not be possible to give a single account of FPC involvement in episodic memory processes. However, it is difficult to draw any firm conclusions from correlational methods, and it may be necessary to supplement these techniques with evidence from other sources, including animal lesion studies, in order to narrow the wide range of explanations proposed for FPC activation during episodic memory tasks.

There are challenges associated with using non-human primates to study episodic memory in relation to FPC. Most notably, there is controversy regarding the existence of this form of memory

in animals. Human definitions of episodic memory have relied on the subjective experience of recollection (Tulving, 2002), which cannot be evaluated in non-verbal species. Animal research in this area has instead focused on "episodic-like" memory: Clayton and Dickinson (1998) and Eacott et al. (2005) have demonstrated, respectively, that western scrub jays and rats are capable of integrating and using information about the spatial and temporal context in which they encountered an object after a single exposure to that object. While it seems clear that animals, including non-human primates, are able to form complex associations in the course of a single trial (e.g. Menzel et al., 1999), this is not sufficient to establish that these animals have access to a human-like sense of re-experiencing past events. In other experiments, however, it has been established that non-human primates may be aware of the content of their memories (Hampton, 2001; Kornell et al., 2007), indicating that they may have meta-awareness relating to past events.

Another approach which has been applied in investigations of elements of episodic memory in animals is the Receiver-Operating-Characteristics (ROC) technique, used by Fortin et al. (2004) and Eichenbaum et al. (2010) to study rats and Guderian et al. (2011) to study macaque monkeys. This technique depends on a theoretical division of recognition memory performance into two processes, 'recollection' and 'familiarity' (e.g. Yonelinas, 2002). Familiarity is identified with the context-independent sense that an object has been encountered before, while recollection is defined as the explicit memory for an object in the context in which it was previously encountered; that is, the episodic memory for the initial encounter with an object. These processes are assumed to have different characteristics: recollection is considered to be a threshold process which produces high-confidence memories, while familiarity is considered to be a signal-detection process which produces memories of varying confidence-levels. If recognition memory is plotted over varying confidence levels, it should be possible to analyse the shape of the resultant curve to detect the differing contributions of recollection and familiarity. In humans, the confidence-level of each occurrence is measured directly, by asking participants to state their confidence in the accuracy of their judgement that a stimulus is "old"; the proportion of correct "old" judgments ("hits") to

incorrect “old” judgements (“false alarms”) is then plotted cumulatively for each confidence level (i.e. the first data point on the graph will only include all “old” judgements at the highest confidence level; the second data point will include all “old” judgements at the highest confidence level and the second-highest confidence level, and so on). In animals, the level of confidence in a judgement cannot be evaluated directly, but a similar effect is achieved by biasing the animal towards making “old” or “new” responses. If animals are highly biased towards making “new” responses (e.g. by making it easier to make a “new” response than an “old” response or by associating a higher level of reward with correct “new” responses than with correct “old” responses), then an “old” response is likely to be associated with a high confidence-level; if monkeys are highly biased towards making an “old” response, then an “old” response is likely to encompass low-confidence as well as high-confidence judgements that the stimulus has been encountered before.

Figure 1 shows how this data can be plotted in order to estimate the contribution of recollection and familiarity to these judgements. Since recollection is a threshold process, subjects either recollect or do not recollect a stimulus. The effect of this is that the number of hits accrued by the process of recollection is stable so long as the response criterion (i.e. the level of confidence that a subject must have that they have seen the stimulus before in order to report an “old” response) is lower than the confidence that they have in their recollection; as recollection is a high-confidence process, this is likely to be the case at every response criterion. However, as the response criterion for making an “old” response is lowered, the number of hits and false alarm responses continue to increase, as subjects respond “old” to more stimuli on the bases of random guesses; however, as these responses are random, the number of hits and the number of false alarm responses increase at an equal rate; thus a graph of a purely recollection-driven process is linear (as the number of hits and false alarms increase at a constant rate), with a y-intercept greater than zero (as subjects will report “old” to items that they recollect at even the strictest response criterion). Since familiarity is a signal-detection process, when making judgements on the basis of familiarity, subjects must distinguish which of two populations a stimulus is drawn from (“old” or “new”). They do this by

judging the strength of the memory trace associated with these stimuli; “old” stimuli will, on average, be associated with a greater strength of memory trace than “new” stimuli. However, due to random noise, some old stimuli will be associated with a low strength of memory trace, and some new stimuli will be associated with a high strength of memory trace; the strength of the memory trace associated with each item in the two populations follows a Gaussian distribution. Therefore, at the strictest response criterion, no hits are recorded. As the response criterion is lowered, the number of hits initially rises faster than the number of false alarms (as only those “old” responses for which the responder detects the greatest strength of memory trace are recorded); however, as the response criterion is lowered to zero, the proportion of new hits accepted diminishes relative to false alarms (since the majority of hits will already have been accepted at strict response criteria, while the majority of false alarms will not have already been accepted). Thus a graph of a purely familiarity-driven process is curvilinear and symmetrical. Normal recognition memory is driven by both recollection and familiarity, and thus a graph produced by plotting recognition performance at different response criteria will be curvilinear and asymmetrical (since it is produced by addition of the recollection and familiarity curves). It is possible to analyse the shape of a recognition memory curve in order to establish the differential contributions of recollection and familiarity to a task.

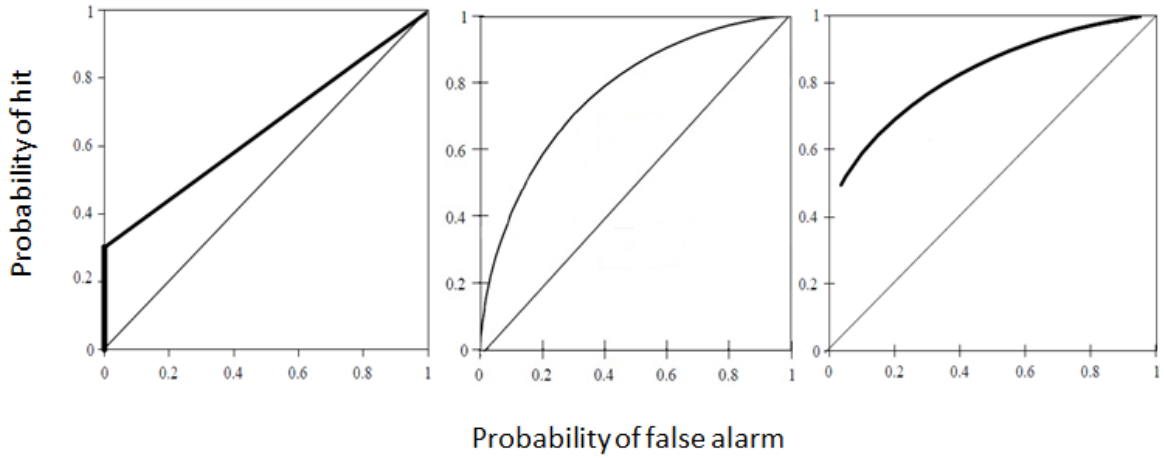


Figure 1 – Receiver-Operating-Characteristics curves in recognition memory. Left, the predicted ROC curve of a pure recollection process; centre, the predicted ROC curve of a pure familiarity process; right, a combined recognition memory ROC curve showing both a recollection and familiarity component. (adapted from Yonelinas, 2001)

This is an especially powerful approach to use in non-verbal animal species, as it allows us to infer information about the way in which these animals experience memory, rather than simply the content of that memory. In particular, the apparent existence of a high-confidence, threshold process in recognition memory in animals, while it cannot be definitively linked with memory for a detailed contextual episode, at least implies that animals experience memory in more than one way, and that a reliably high level of confidence is associated with one particular form of memory. In humans, this reliably high level of confidence is linked with episodic recall of context. Evidence presented by Eichenbaum et al. (2010) suggests that, as in humans, this "recollection-like" component contributes more greatly to associative recognition and that the "familiarity-like" component dominates after hippocampal or prefrontal lesions in rats.

Any attempt to model the contribution of human BA10 to episodic memory in non-human species relies on the functional homology of this brain area between these species. However, it is not clear that the macaque and human FPC are functionally homologous. There are differences in anatomy between macaque and human area 10 (Semendeferi et al., 2001): this region occupies a larger relative brain volume in humans than in other primate species, and the low cell density which

characterises human BA10 is not observed in macaques; cytoarchitecturally, cortical layers in this region are relatively homogenous in humans, while macaques show greater variation in the density of cortical layers. Furthermore, the lateral area of FPC shows a distinctive connectivity profile in humans which is not observed in macaques (Neubert et al., 2014). However, as noted above, macaques with FPC lesions are impaired on tasks that assess at least some elements of episodic memory (one-trial object-in-scene learning; Boschin et al., submitted; temporal context judgements; Chapter 1), indicating that it may be possible to use macaques as a model for FPC contributions to context-specific memory. The pattern of FPC activations in humans during context-specific memory tasks is complex, including medial elements (Simons et al., 2005b); Neubert et al. (2014) and Sallet et al. (2013) both identify a macaque frontopolar region equivalent to human medial FPC. While it is possible that macaque models of frontopolar contributions to context-specific memory may not capture all elements of this process in humans, similar claims could be made regarding any attempts to study human cognitive processes in non-human animals.

The ROC method is therefore suitable for studying the contribution of the FPC to context-specific memory in macaque monkeys, and was used in this experiment. The hypothesis of the experiment was that, while overall accuracy in recognition judgements would not be impaired by FPC lesions, ROC curves plotted for lesioned monkeys would show a greater contribution of a curvilinear familiarity component to recognition memory than an asymmetrical recollection component when compared with those ROC curves plotted for control animals.

Method

Subjects

Seven experimentally experienced female rhesus macaque monkeys (*Macaca mulatta*) were tested in this experiment. Their mean weight at the start of behavioural testing was 6.8 kg (range 4.98–9.01 kg), and their mean age was 6 years and 4 months. They were housed in small socially

compatible groups. There was one group of three, one pair and two monkeys who were housed individually at the start of the experiment (due to a previous fight as part of a larger group) and moved together after recovery from injuries sustained in that fight. They were housed in an enriched environment in which they could forage for small food items once a day. All had automatically regulated lighting and water available *ad libitum*. At the start of behavioural testing, three animals were control animals, and four animals had received FPC-lesions an average of 26.5 months (range 23 – 28 months) earlier. However, only two control animals and three FPC-lesioned animals passed all pre-training stages of this task. After data had been collected in the task described below, the two control animals which had been successfully trained on this task received FPC lesions and were retested on this task. They received a few sessions of testing on this task immediately prior to surgery in order to exclude the possibility that poor performance on the task after surgery was due to forgetting the rules of the task; data from these sessions were not included in any analysis. All monkeys had been pre-trained on a delayed match-to-sample task in the same apparatus that was used in these experiments.

Surgery

Four animals had received bilateral ablation lesions to FPC prior to the start of the experiment; the other three were tested first as unoperated controls and again 14-16 months later after receiving identical lesions. The posterior limit of the lesion was an imaginary vertical line situated 2-3 mm posterior to the anterior tip of the principal sulcus. For details of surgery, see Chapter 1. All licensed procedures were carried out in compliance with the United Kingdom Animals (Scientific Procedures) Act of 1986. Histological analysis confirmed that the lesions were performed as intended, albeit with slight sparing in the most anterior ventral aspect of the right hemisphere of one animal (see Chapter 1, figures 1 and 2).

Apparatus

The apparatus used in this task was identical to that described in Chapter 1.

Stimuli

The visual stimuli presented on the touchscreen were taken from a library of clipart images obtained from commercially available sources. Each image was 128×128 pixels in dimension and comprised a distinct cartoon-like image. The stimuli assigned to each set of trials were chosen at random (without replacement) from a library of over 14,000 stimuli. The resolution of the visual display on the touch-screen was set at 800×600 pixels with the effect that each visual stimulus on the screen subtended approximately 12 to 15 degrees of visual angle from the typical viewpoint of the macaque.

Pre-training

The pre-training procedure for this task is described in Appendix 1.

Task

Trials were presented in blocks of six. Each block consisted of a display stage, in which three stimuli were presented, and a choice stage, in which monkeys were required to make six successive discriminations between a stimulus and a red square; in three of these discriminations, the stimulus was identical to one presented in the display stage (in which case the monkey was rewarded with a banana-flavoured pellet for touching the stimulus) and in three of these discriminations, the stimulus was novel (in which case the monkey was rewarded with a banana-flavoured pellet for touching the red square). Each stimulus presented in the display stage was presented once during the choice stage.

<u>Display stage:</u>

Figure 2 illustrates the screens displayed in the display stage. In this display stage, three stimuli were presented sequentially.Each stimulus was presented in the centre of the screen, against

a grey background. In order to move on to the next screen, monkeys touched the area of the screen in which the stimulus was displayed. There was no delay between the presentation of stimuli. When the monkey touched the third stimulus, it was removed from the screen, leaving a blank grey screen for a 2s delay.

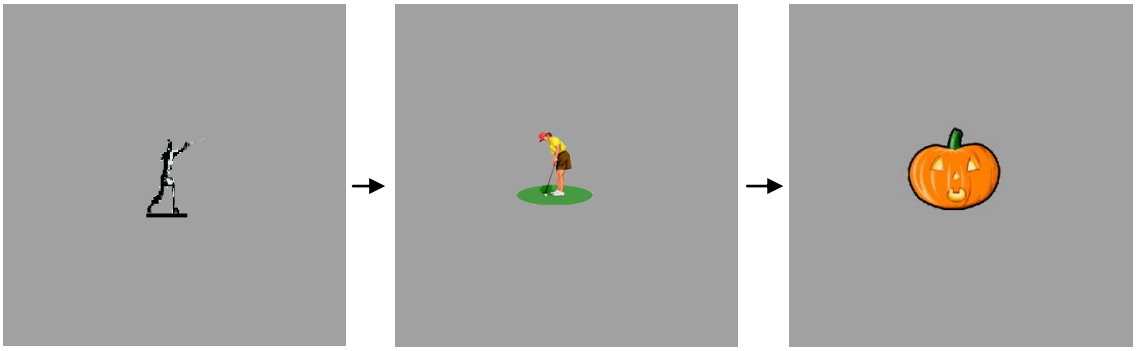


Figure 2 – Illustration of a display stage of a block. When monkeys touched the stimulus at the centre of the screen, each screen was replaced by the next. When monkeys touched the third stimulus, the screen was replaced by a blank grey screen for 2s. Images are derived from free online resources, including Microsoft Clipart.

Choice stage:

In the choice stage, monkeys were required to make six successive discriminations. For each of these discriminations, monkeys were presented with a screen divided into two sections; the bottom half of the screen was outlined with a black bar in order to make it appear a visually separate region. The top half of the screen constituted the "choice" area and the bottom half of the screen constituted the "delay" area. Monkeys were presented with a stimulus and a red square in the top half of the screen, and in the bottom half of the screen, a number of smaller objects (either stimuli identical to that presented in the top half of the screen or red squares) were presented; the number of objects in the bottom half of the screen varied (eight objects were present at level four; four at level three; two at level two and zero at level one); whether stimuli or red squares were presented in

the bottom half of the screen was determined randomly. In three of the discriminations, the stimulus was identical to one of those presented during the delay stage, and in three of the discriminations, the stimulus was novel; the order of these discriminations was determined randomly. Monkeys were rewarded for touching the stimulus in the top half of the screen if it had previously been presented in the display stage (an "old" stimulus) or the red square in the top half of the screen if the stimulus was novel (a "new" stimulus). An illustration of the discriminations made at each level is presented in figure 3.

The length of time which elapsed between monkeys making an old/new discrimination and receiving a reward (if correct) and moving onto the next discrimination (whether or not the monkey was correct) was determined by whether or not the monkey chose the object which was repeated in the bottom half of the screen; if the monkey chose the object which was repeated, a "long" trial occurred; otherwise, a "short" trial occurred. This half of the screen was unresponsive to touch until after the monkey had chosen one of the objects in the top half of the screen; this had the effect of ensuring that, if the monkey was randomly touching the screen, the probability of a "long" trial was 50%; if this was not the case, the probability of a "long" trial would have increased as touching any item in the bottom half of the screen would have triggered the start of a "long" trial. Monkeys were required to touch all of the objects of a particular type on the screen before they received a reward or moved onto the next discrimination. If, for example, the monkey was presented with an "old" stimulus, and the bottom half of the screen contained a number of small red squares, the monkey would receive a reward immediately after choosing the stimulus (a "short" trial). If, however, the bottom half of the screen contained a number of smaller stimuli (each identical to the stimulus in the top half of the screen), the monkey was required to touch all of the stimuli in the bottom half of the screen (a "long" trial) before they received a reward and moved onto the next discrimination. This rule applied whether or not the monkey made a correct discrimination: a monkey which incorrectly touched the red square on presentation of an "old" stimulus would immediately move onto the next discrimination (receiving no food reward) if stimuli were presented in the bottom half

of the screen (a “short” trial), but would have to touch all of the red squares in the bottom half of the screen before moving onto the next discrimination (again receiving no food reward) if red squares were presented in the bottom half of the screen (a “long” trial). The purpose of this manipulation was to bias monkeys towards touching the object in the top half of the screen which was not repeated in the bottom half of the screen (i.e. towards selecting a “short” trial).

After the monkey touched one of the items in the top half of the screen, the next events were determined by whether a “long” trial or a “short” trial had been triggered. If the monkey triggered a “short” trial, the monkey received a reward if they had made a correct “old”/ “new” discrimination and a blank grey screen was presented for either 2s (if the “old”/ “new” discrimination was correct) or 8s (if the “old”/ “new” discrimination was incorrect). The next discrimination was then displayed (i.e. another stimulus and red square was displayed on the top half of the screen, and a number of either stimuli or red squares were displayed in the bottom half of the screen, and the monkey had to make another “old”/ “new” discrimination regarding the stimulus on the top half of the screen).

If the monkey triggered a “long” trial, however, all of the stimuli and red squares were removed from the screen for a period of 10s; the black box remained on the screen. After the delay, the objects in the bottom half of the screen reappeared. The monkey was required to touch every item in the bottom half of the screen; between each touch, the objects on the screen (except the black box) were removed for a period of 10s, after which the objects in the bottom half of the screen which the monkey had not yet touched reappeared. After the monkey touched all the items, a blank grey screen was presented and they received a reward (if the correct “old”/ “new” discrimination had been made). The next “old”/ “new” discrimination was presented after a delay of either 2s (if the monkey had made a correct discrimination) or 8s (if the monkey had made an incorrect discrimination). An illustration of a “long” trial at level 2 is presented in Figure 4.

The effect of a 10s delay between successive touches was to make the delay between a correct touch and a reward much longer for “long” trials than for “short” trials, and this delay increased as

the task level increased and the number of objects in the bottom half of the screen was increased. By varying the number of touches required and delay experienced before receiving a reward for different types of response, we hoped to vary the bias which monkeys experienced towards "old" or "new" responses. Seven bias levels were included:

- Bias level 1 (most biased towards "old") – level 4, 8 squares presented in the bottom half of the screen
- Bias level 2 – level 3, 4 squares presented in the bottom half of the screen
- Bias level 3 – level 2, 2 squares presented in the bottom half of the screen
- Bias level 4 (no bias towards old or new) – level 1; no objects presented in the bottom half of the screen
- Bias level 5 – level 2, 2 stimuli presented in the bottom half of the screen
- Bias level 6 – level 3, 4 stimuli presented in the bottom half of the screen
- Bias level 7 (most biased towards new) – level 4, 8 stimuli presented in the bottom half of the screen

Monkeys received a continuous week of testing at each successive level.

Choice stage: screen layout

Two objects (a stimulus and a red square) were presented in the top half of the screen, equidistant from the horizontal midpoint (whether the red square or the stimulus was presented to the left or the right was determined randomly). These objects were 128x128 pixels in size and subtended a visual angle of 12 – 15 degrees from the typical viewpoint of the macaque. The bottom half of the screen was outlined by a black bar to make it appear a visually separate region. This bar was 1.5 cm wide (subtending a visual angle of approximately four degrees to the typical viewpoint of the macaque). In the bottom half of the screen, between 0 and 8 smaller red squares or stimuli were presented. These squares or stimuli were 64x64 pixels: half the size of the square and stimulus displayed in the top of the screen; they subtended a visual angle of approximately six to eight degrees from the typical viewpoint of the macaque.

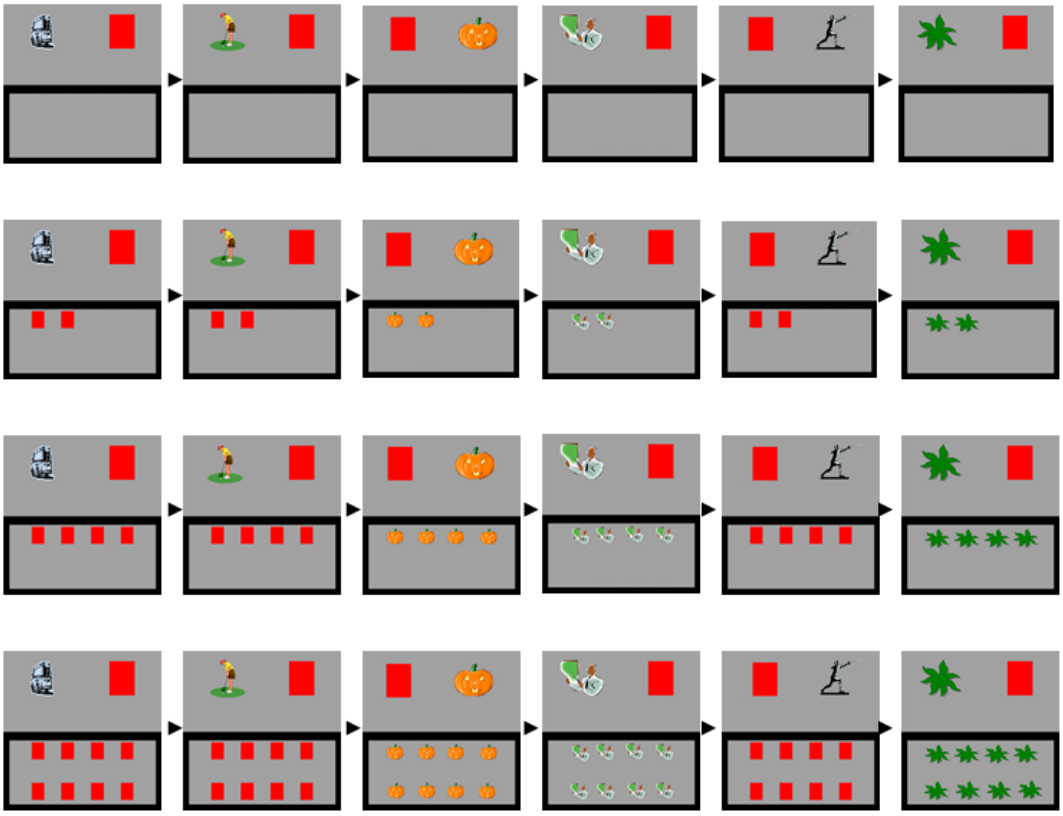


Figure 3 – Illustration of a choice stage of a block at level 1 (top row), level 2 (second row), level 3 (third row) and level 4 (bottom row). Monkeys touched the red square in the top half of the screen to indicate a novel stimulus and the stimulus in the top half of the screen to indicate a previously-displayed stimulus. Images are derived from free online resources, including Microsoft Clipart.

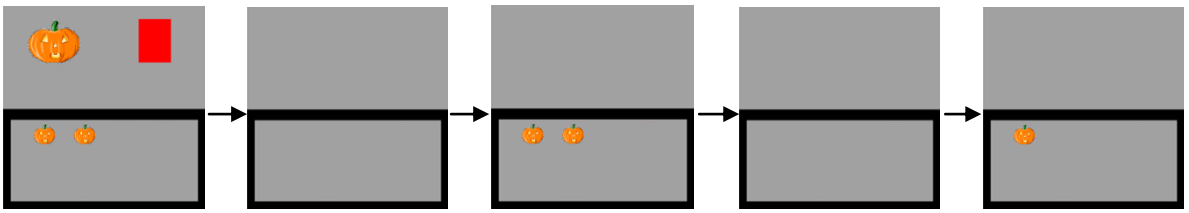


Figure 4 – Illustration of a "long" choice at level 2. The order of events is displayed from left to right: after choosing the stimulus (left), a blank screen was displayed for ten seconds; the two items in the bottom half of the screen then reappeared. After choosing one of the items, a blank screen was displayed for another ten seconds before the monkey was able to choose the final item (right). Image is derived from a free online resource.

After the six discriminations were complete, another display stage resumed, followed by a choice stage, until the monkeys had completed the required number of correct discriminations for

that day (this was determined according to the temperament of each monkey, and varied between 35 and 170).

Data analysis

Yonelinas et al. (1998) provide a description of how estimates of familiarity and recollection may be derived from a recognition memory test. An ROC curve can be fitted to the data by minimising the square of the differences between the predicted model and the data collected. Estimates of recollection and familiarity can be calculated using the following equations:

$P(\text{“old”}|\text{old}) = R + (1 - R)\, F_{old}$

“R” is the probability that a stimulus will be recollected; “F_{old}” is the probability that an old stimulus will reach a level of familiarity exceeding the response criterion at that bias level. The equation therefore states that the probability that a studied stimulus will be identified as old (at a certain bias level) is equal to the probability that it is recollected or that it is not recollected but that its level of familiarity exceeds the response criterion at that bias level.

$P(\text{“old”}|\text{new}) = F_{new}$

This equation states that the probability of a false alarm (an unstudied stimulus identified as old) is equal to the probability that the level of familiarity of the stimulus exceeds the response criterion at that bias level.

The increase in familiarity in old items compared to new items is given by d’. For any trial, the probability that a stimulus will be accepted as old on the basis of familiarity is determined by d’ and by the response criterion at that bias level. The probability that a stimulus will be accepted as old on the basis of recollection is not affected by response criterion, as recollection is a high confidence process.

Since R and d' should remain stable across bias levels while response criterion varies, it is possible to apply these equations to data collected at different bias levels regarding the probability of a hit or a false alarm, and derive an estimate of d' and R. Yonelinas' research group provides an Excel worksheet which will perform these calculations.

It is possible to test whether ROC curves are asymmetric by converting hit and false alarm rates at each bias level into z-scores, and performing a linear regression on these values. The gradient of the line produced will not differ from 1 if the ROC curve for the group is symmetric, but will differ from 1 if the ROC curve for the group is asymmetric; significance is tested with a one-sample t-test (Yonelinas et al., 1998).

Results

Figure 5 shows the mean probability of correct identification of an "old" stimulus plotted against the mean probability of the incorrect identification of a "new" stimulus as old, for control and FPC-lesioned monkeys at each bias level (only data relating to monkeys trained and tested on the task post-operatively is included in the FPC-lesioned group in this figure; data related to monkeys trained and tested pre-operatively and then tested again post-lesion is excluded).

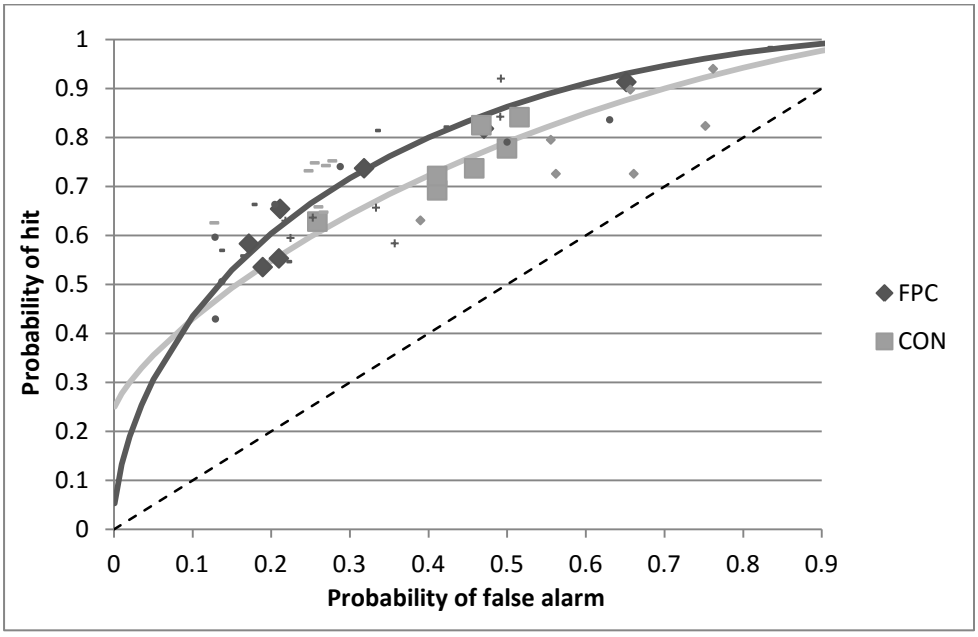


Figure 5 – Mean Receiver-Operating-Characteristics curves for FPC-lesioned and control monkeys (curves fitted to bias levels 1,3,4,5,7 – levels 2 and 6 are shown on the figure for completeness). Small dark markers show probability of false alarm versus probability of hit at different bias levels for individual FPC-lesioned animals; small pale markers show probability of false alarm versus probability of hit at different bias levels for individual control animals.

For FPC-lesioned animals, the contribution of familiarity (d') to this curve was estimated as 1.071; the contribution of recollection (R) was estimated as 0.0313. For control animals, the contribution of familiarity was estimated as 0.589, and the contribution of recollection was estimated as 0.246. An independent samples t-test conducted on the pre-operative data from those animals originally assigned to the control group, and post-operative data from those animals originally assigned to the FPC-lesioned group (i.e. excluding post-operative data from those animals trained pre-operatively on this task) found no significant effect of group for either recollection ($t(3)$=1.640, p=0.199) or familiarity ($t(3)$=-2.489, p=0.089). Similarly, a paired samples t-test examining pre- and post-operative performance in the two animals originally assigned to the control group did not find a significant difference for either recollection or familiarity (recollection: $t(1)$=-0.140, p=0.912; familiarity: $t(1)$=-1.273, p=0.424). Two individual ROC curves are given in figures 6 and 7 in order to show the different effects of biasing in individual animals within the same group.

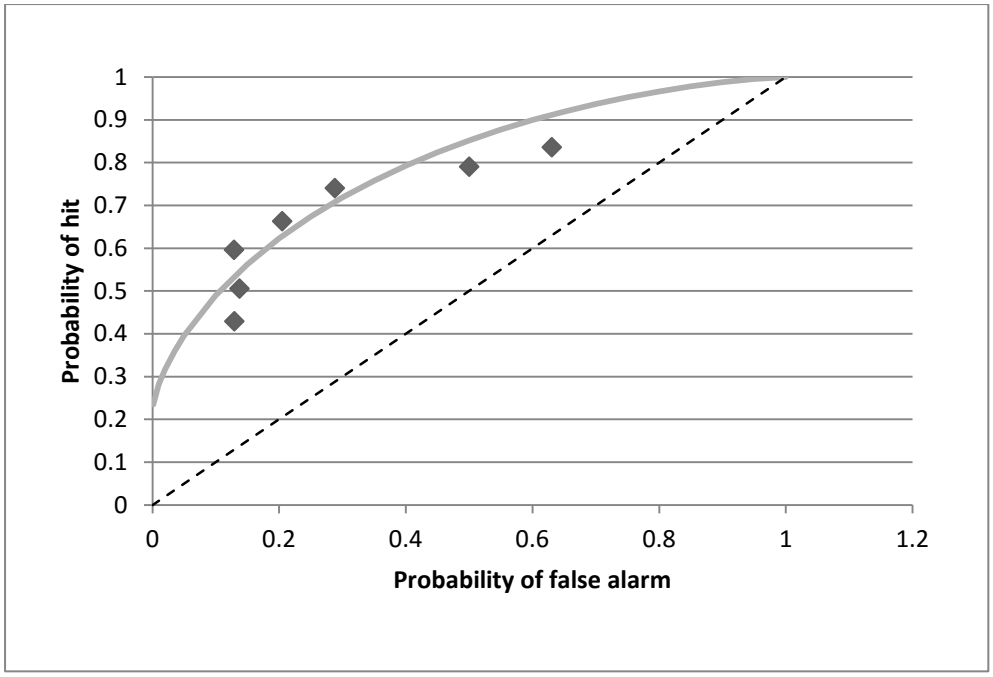


Figure 6 – Receiver-Operating-Characteristic curve for "Monkey A" (group FPC) (curve fitted to bias levels 1,3,4,5,7 – points 2 and 6 are shown on the figure for completeness).

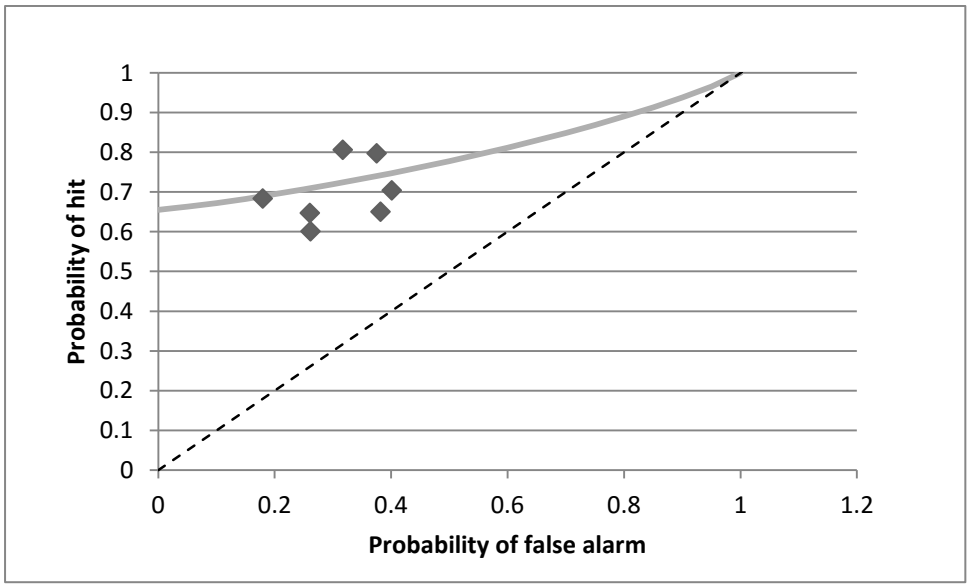


Figure 7 – Receiver-Operating-Characteristic curve for "Monkey B" (group FPC) (curve fitted to bias levels 1,3,4,5,7 – points 2 and 6 are shown on the figure for completeness).

Bias manipulation:

In order to determine whether monkeys were significantly affected by the bias manipulation in this experiment, percent correct scores for two types of trial at level four (the level in which monkeys were subjected to the most extreme bias) were examined. The percentage of trials in

which monkeys made the correct discrimination when to do so would trigger a "short" trial ("bias-plus" trials) was contrasted with the percentage of trials in which monkeys made the correct discrimination when to do so would trigger a "long" trial ("bias-minus" trials). A two-ways repeated measures ANOVA was conducted with one between-subjects factor of experimental group (two levels: FPC-lesioned and control) and one within-subjects factor of bias direction (two levels: "bias-plus" and "bias-minus"). This ANOVA returned no significant main effect of group ($F(1,3)$<0.001, p= 0.990), but did return a significant main effect of bias direction ($F(1,3)$= 23.661, p=0.017), indicating that monkeys were significantly more likely to make a correct discrimination when they were biased towards doing do, and a significant interaction effect between bias direction and group ($F(1,3)$= 16.185, p=0.028).

In order to analyse the source of this interaction effect, paired-samples t-tests were applied to examine whether any effect of bias direction could be detected for either the control or FPC-lesioned groups. No significant effect of bias direction was detected in the control group ($t(1)$= 1.697, p= 0.339). However, a trend towards significance was detected in the FPC-lesioned group, although this did not survive application of the Holm-Bonferroni correction for multiple comparisons ($t(2)$= 5.837, p= 0.028; p> 0.025).

Discussion

This experiment failed to detect a greater contribution of recollection to recognition memory in control animals than in FPC-lesioned animals. The most likely explanation for this result is that the design of the experiment did not elicit sufficiently clear bias curves from control animals in order to accurately estimate the contribution of recollection and familiarity to the ROC curve. While some animals (e.g. "Monkey A" – see figure 6) showed a strong effect of biasing, resulting in a clear ROC curve, other animals showed no clear effect of biasing, and did not return a curve from which it was possible to accurately estimate the contributions of recollection and familiarity to recognition

memory (e.g. “Monkey B” – see figure 7). No significant effect of bias could be detected in control animals, even at the most extreme bias level.

This lack of biasing effect may be attributable to the difficulties animals experienced in learning the experiment. All animals were previously trained on a delayed match-to-sample paradigm and this may have caused interference when training them on this task; even after extensive pre-training, all animals except for one were at least 10% more likely to successfully touch the red square that indicated a “new” response than the stimulus that indicated an “old” response. However, it may also be possible that manipulation of the time between choice and reward is not an effective mechanism in inducing bias in macaque monkeys, and that manipulation of the size or value of the reward (as in the study conducted by Guderian et al., 2011) may be a more effective tool for this purpose. Since the hypotheses of this experiment have not been adequately addressed, it may be worthwhile to train a group of experimentally-naive monkeys on this task, using varying levels of reward as a biasing mechanism. Alternatively, it may be possible to adapt other techniques, such as the process dissociation procedure, in order to study the contribution of context-specific and context-independent processes to recognition memory in macaque monkeys with frontopolar lesions.

This experiment did detect a difference in the sensitivity to bias displayed by control and FPC-lesioned animals. While control animals showed no effect of bias, a clear trend towards an effect of bias existed in the FPC-lesion group. This effect was not predicted by the hypotheses of this experiment, and may have resulted from the small sample size used in the control group; further research is necessary in order to determine whether this is a real effect.

CHAPTER THREE

Monkeys with lesions to anterior cingulate cortex differ from controls and from monkeys with lesions to frontopolar cortex in the influence that the outcomes of previous trials exert on current behavioural choices

Previous studies have indicated that both the anterior cingulate cortex (ACC) and FPC may play a role in determining the influence that the reward history of previous trials exerts on future choices in humans and macaques (Kovach et al., 2012; Kennerley et al., 2006); damage to FPC may impair the ability to privilege the outcome of the most recent behavioural choice in making subsequent decisions, while damage to the ACC may spare this ability, while impairing the ability to make choices based on a longer history of rewarded outcomes. The WCST analogue for monkeys (Buckley et al., 2009), provides a method by which the relative contributions of the outcome of the most recent trial and the outcomes of an extended history of trials to behaviour can be studied in greater detail. Results indicate that lesions to both the ACC and FPC cause abnormal processing of reward history, although the behavioural effects of these lesions differ: monkeys with lesions to ACC are less influenced by the outcome of the most recent rewarded trial when making behavioural choices; the pattern of deficit in monkeys with lesions to FPC is less clear, but these monkeys may be more likely to relatively more influenced by rewarded outcomes in the most recent trials than controls.

Introduction

As discussed in Chapter 1, macaques with FPC lesions are impaired when judging which of two stimuli were encountered more recently. This could provide an explanation for the results of Kovach et al. (2012), who found that frontopolar patients were impaired at making choices based on the outcomes of the most recent trials, and in particular recent trials that had been rewarded (i.e. if the appropriate response was to maintain current behaviour rather than to switch to a new behaviour). In this study, despite no overall impairment (as measured by mean reward per trial) on a

four-armed bandit task, FPC patients differed significantly from controls in the extent to which they prioritised the outcome of recent trials (and, in particular, the difference in outcomes between the most recent two rewards). The best fitting model of the behaviour of healthy participants and brain-damaged controls on this task was one in which, in addition to representing cumulative reward history, a term was introduced which prioritised the outcome of the most recent trial over any other outcomes; however, this term did not improve the fit of this model to the behaviour of FPC-lesioned patients. If these patients were unable to remember which outcome occurred more recently, they would not be able to appropriately weight the outcomes of the most recent trials when making decisions based on the accumulation of evidence regarding rewarded and non-rewarded outcomes in a task with shifting reward parameters. Although Kovach et al. also noted that FPC-lesioned participants differed from healthy controls in that they had a lower mean history of rewards to "bandits" that they continued to select, rather than switching away from (FPC-lesioned patients did not differ significantly from controls on "switch" trials), a difference which could not be explained by this recency effect, it is important to note that FPC-lesioned patients did not differ significantly from brain-damaged controls on this measure. These researchers concluded that lesions to FPC impaired "the ability to extrapolate the most recent trend, despite an intact general ability to learn from past rewards".

Kennerley et al. (2006) used a test in which macaques were rewarded for one of two responses, and in which the rewarded response was shifted periodically throughout the testing session, in order to assess the influence of previous outcomes on behavioural choices in macaques. These researchers found that control animals were influenced by the outcomes of the previous trials (Kennerley et al., 2006, figure 4); the greatest influence was drawn from the immediately previous trial, and influence decreased across preceding trials; in no group did outcomes more than four trials into the past have an effect which was significantly different from zero. The impairment observed by Kennerley et al. in ACC-lesioned monkeys (the influence of the outcome of the most recent trial on behaviour was spared, but the influence of the outcomes of more distant trials declined more

quickly) provides evidence in favour of Kovach's two-process model of the influence of prior trials on behaviour: one which selectively privileges the outcome of the most recent trial and is impaired by frontopolar lesions (Kovach et al.), and one which influences behaviour on the basis of outcomes of more distant trials and is impaired by ACC lesions (Kennerley et al.). Furthermore, as both ACC and FPC have been implicated in studies which emphasise decision-making in uncertain environments (Rushworth and Behrens, 2008, provide a review relating to the ACC; the FPC is activated by tasks in which outcomes in the environment vary e.g. Boorman et al., 2011), this two-process theory may provide a model of how ACC and FPC work together as part of an information-processing network in these situations. However, as different species were used in the Kovach and Kennerley experiments, this potential dissociation between two reward-learning processes has not yet been firmly established. Furthermore, the FPC-lesioned group in Kovach's study had considerable limitations (considerable posterior overlap in lesions existed outside of FPC; lesion volume in the FPC-lesion group was greater than in the brain-damaged control group; 7/8 FPC-lesion patients had bilateral damage, whereas all patients in the brain-damaged control group had only unilateral damage), and it has not yet been established whether the deficits in Kovach et al.'s FPC-group can be attributed to an impairment in function in any other posterior frontal region. If, however, the results of Kovach et al. are reliable and translatable between humans and macaques, we would expect this pattern of prioritising outcomes from the most recent (rewarded) trial in selecting future behaviours to be disrupted in macaques with frontopolar lesions in a task with shifting reward parameters.

The studies described above analysed the effect of the history of rewarded trials on choice behaviour. However, while a rewarded behaviour can be a signal that a particular choice should be maintained, a failure to receive a reward can be a signal that a choice should be changed in order to receive better outcomes in the future. There is evidence that lesions to BA10 impair task-switching (Burgess et al., 2000; Rowe et al., 2007) and that BA10 is activated by task-switching scenarios (Braver et al., 2003). It may therefore be appropriate to examine the influence of non-rewarded

outcomes in addition to the influence of rewarded outcomes on choice behaviour in macaques with frontopolar lesions.

As performance on any task is the result of interacting and complementary information processing, it is useful to assess the differing contributions which various areas of nearby cortex make to one task. If these contributions can be dissociated, it is possible, firstly, to establish the functional independence of a region from its neighbouring areas and, secondly, to analyse the way in which the brain combines the outputs of interconnected and functionally independent regions to complete complex problem-solving operations. These questions of independent and complementary information-processing functions are especially relevant in relation to the monkey frontal lobes as research using different techniques (Semendeferi et al. 2001, Bludau et al., 2013, Sallet et al., 2013, Neubert et al., 2014) has disagreed on the appropriate parcellation of the frontal lobe and, in particular, whether there is a lateral component to macaque area 10 homologous to human lateral FPC or whether this region is functionally identifiable with area 46 in macaques.

The Wisconsin Card Sorting Test (WCST) (Anderson et al., 1991) provides an example of a task in which subjects should shift their responses according to recent outcomes. In this test, subjects are shown a card with certain properties, such as a number of shapes of a certain colour. They are presented with a number of cards which may match the test card on any of these dimensions (number, shape, colour). In order to answer correctly, the subject must discover by trial and error which dimension is currently relevant, and choose the stimulus card which matches the test card on that dimension. They are notified whether their choice is correct or incorrect. The rewarded dimension shifts regularly throughout the experiment (often after a certain number of correct responses to the previous rule). Subjects are not given any notice of this rule change, but must deduce that it has occurred based on trial outcomes. This task tests subjects' ability to change their behaviour in response to a shift in the reward outcomes of the environment; new rewards are always more relevant than past rewards. Additionally, subjects must be able to apply and update

abstract rules, such as “match shape”, rather than attribute a reward to a particular object (“triangles”). A monkey analogue of this task (Buckley et al., 2009) tests the same cognitive capabilities; it is slightly simplified in that animals may only choose between two potential match dimensions (shape and colour). On each trial, a monkey has three options: it may match the test stimulus on the shape dimension, it may match on the colour dimension, or it may choose a stimulus which matches neither shape nor colour. As animals tested on this task choose to match on either shape or colour on the overwhelming majority of trials (Buckley et al. report that, of error trials, only 9% were “non-perseverative” i.e. the monkey matched neither shape nor colour), it is possible to perform a multiple logistic regression in order to assess the effect of outcomes of following a particular rule on previous trials on the response in the current trial. This test therefore provides a mechanism by which the hypothesis described above may be tested. Furthermore, the WCST is useful for addressing the question proposed above regarding the functional independence of the FPC from other regions of the prefrontal cortex, as detailed research has been conducted to examine the contributions of multiple frontal regions to this task (see below). Previous research on whether information-processing in Brodmann’s area 10 underpins functions necessary for the WCST has yielded mixed results. Some researchers (Konishi et al., 2005, Monchi et al., 2001) have identified activity in area 10 which can be correlated with certain events during the WCST task (deactivation on low-conflict trials after dimensional shifts, deactivation to negative feedback), while Nagahama et al. (2005) noted that, in patients suffering from Alzheimer’s disease, decreased cerebral blood flow to FPC is associated with impaired performance on this task; specifically, a greater number of perseverative errors after dimensional shifts. Patients with FPC lesions are not generally impaired on this task: Hoffmann and Bar-On (2012) and Uretzky and Gilboa (2010) provide case studies; in reviews of this topic, Anderson et al. (1991) found that it was not possible to reliably associate impaired performance on WCST with damage to any region of the frontal lobes, including FPC, and Mountain and Snow (1993) did not find that frontal patients were generally impaired on WCST, although this study did not examine evidence relating to focal FPC lesions. However, it is possible

that FPC lesions are associated with subtle deficits immediately following dimensional shifts which do not cause significant enough impairment in overall performance to be detected by these studies.

Buckley et al. (2009) tested macaques with a variety of frontal lesions (orbitofrontal cortex, OFC, principal sulcus, PS, superior dorsolateral prefrontal cortex, sdlPFC, ventrolateral prefrontal cortex, vlPFC, anterior cingulate sulcus, ACC) on the WCST analogue pre- and post-operatively; additional research from this laboratory has tested macaques with frontopolar lesions (Mansouri et al., submitted). These researchers found that OFC, PS and ACC lesions impaired performance on the WCST analogue; no other tested lesion group, including FPC, showed a significant post-operative deficit in performance on this task. Additional information from this Mansouri et al. study (see figure 1) indicates that, contrary to the theory suggested by the neuroimaging results of Konishi et al. and Nagahama et al., FPC-lesioned monkeys show no increase in errors immediately after a task switch. While Buckley et al. (2009) assessed the effect of increasing numbers of consecutive correct responses on the likelihood of making an error on the next trial on PS, OFC and ACC-lesioned animals compared to controls, no research has been conducted which analyses the contribution of recent outcomes to behavioural choices on FPC-lesioned monkeys (or indeed, any other prefrontal or medial frontal lesion) on the WCST analogue, or compares the weighting which FPC-lesioned animals (and other prefrontal and medial frontal lesioned animals) place on recent trials.

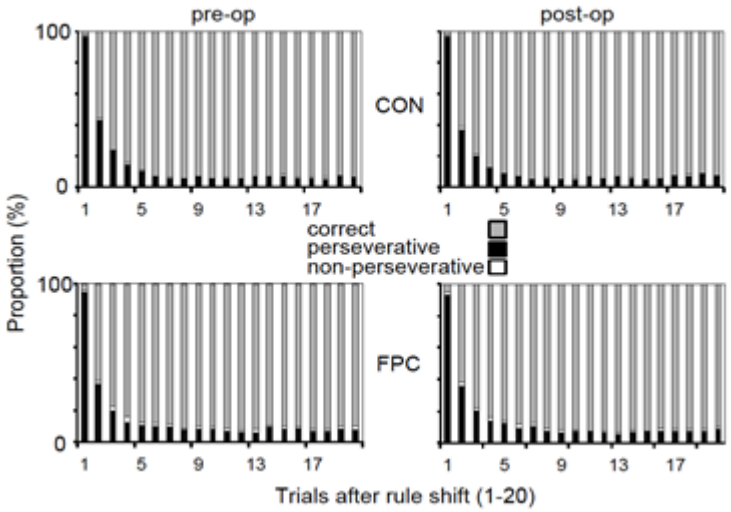


Figure 1 – Proportion of correct responses, perseverative errors and non-perseverative errors in the first twenty trials after rule shifts in FPC-lesioned and control animals (from Mansouri et al., submitted).

Buckley et al. (2009) identified different components of rule-guided behaviour in the WCST analogue which were processed by discrete regions of the macaque frontal lobe (OFC, PS and ACC): they suggested that PS is necessary in order to maintain abstract rules in working memory, OFC is required in order to rapidly update the value of a particular abstract rule, and ACC is responsible for assessing confidence in behavioural choices in uncertain situations and triggering checking processes if necessary (further analysis was presented regarding the role of ACC sulcus in the WCST-analogue task by Tanaka et al., 2013). These suggestions are based on dissociable differences in performance on certain aspects of the task:

1. When the interval between trials was lengthened by five seconds after the monkey attained criterion on a rule (85% correct over twenty consecutive trials), PS-lesioned monkeys scored no better than chance, while OFC- and ACC-lesioned animals showed no decrease in performance.
2. After a single reward, OFC-lesioned animals performed at chance. However, they were not rendered insensitive to reward, because, as the number of consecutive rewards which

animals received increased, the deficit of OFC-lesioned animals on the next trial decreased; hence the OFC group were impaired at 'rapid' reward-based updating of rule value. The deficit observed in PS- and ACC-lesioned animals did not depend on the number of consecutive rewards which animals had received.

3. ACC-lesioned animals had significantly faster response times, in error trials, than PS- or OFC-lesioned animals postoperatively, although response times to correct trials were unchanged; this was not an overall tendency towards disinhibition because no such changes were observed on control tasks; this finding was considered consistent with previous theories regarding the role of the ACC in uncertain environments.

However, it is possible that a more fine-grained analysis of the data from this experiment will enable further examination of the functional role of PS, OFC and ACC in the WCST analogue; in particular, it will be possible to examine whether the findings of Kennerley et al. (2006) regarding the role of ACC in influencing behaviour with relation to reward history were specific to the particular motor task tested in that experiment, or whether they can be observed in other situations. Further analysis of FPC-lesion data from this task will enable assessment of whether any subtle deficits exist in this task when lesions are focally restricted to this region, and if so, how such deficits may be interpreted in the light of the contributions of other frontal regions to this task.

I therefore examined data previously collected by the Buckley (Oxford) and Tanaka (RIKEN) laboratories relating to monkey performance on the WCST analogue. This included pre- and post-operative data from monkeys with lesions inflicted to ACC, PS, OFC, sdlPFC and FPC, in addition to data collected from nine control monkeys, before and after a delay which approximated to the delay in testing resulting from neurosurgery. There were three hypotheses:

1. The influence of the outcome of the most recent trials on the behaviour of FPC-lesioned animals would be reduced.

2. The influence of the outcome of recent trials on the behaviour of ACC-lesioned animals would be abnormal.
3. The deficits observed after ACC lesions would be increased in conditions of greater uncertainty, such as the first few trials of each block.
4. The pattern of influence of outcome of recent trials on the behaviour of FPC-lesioned and ACC-lesioned animals would be dissociable from each other.

Method

In order to avoid ambiguity, I have reported the methods of this study using very similar language to that used by Buckley et al. (2009).

Apparatus

The apparatus used to conduct this study was identical to that described in Chapter 1.

Behavioural Task

The task used was an analogue to the human WCST. In each trial the sample and test-items were randomly (but with the constraints described below) chosen from a large set of coloured shapes (see below). A trial started when the sample appeared in the centre of the touchscreen; the sample remained on the screen until the animal touched it, which led to the appearance of the three test-items (one matching in colour, one matching in shape, and one not matching in colour or shape). The positions of these three test-items in the three available slots (left, right, bottom), was also determined randomly. As the same sample set was employed irrespective of which abstract matching rule was currently reinforced, no cue was available to the animal at the start of each trial as to which rule was currently reinforced. Rather, to choose correctly, the animal had to remember the matching rule applied on the previous trial, and repeat the same rule again on the current trial if the previous decision was rewarded, and change rule if it was not. A visual representation of this task is presented in figure 2.

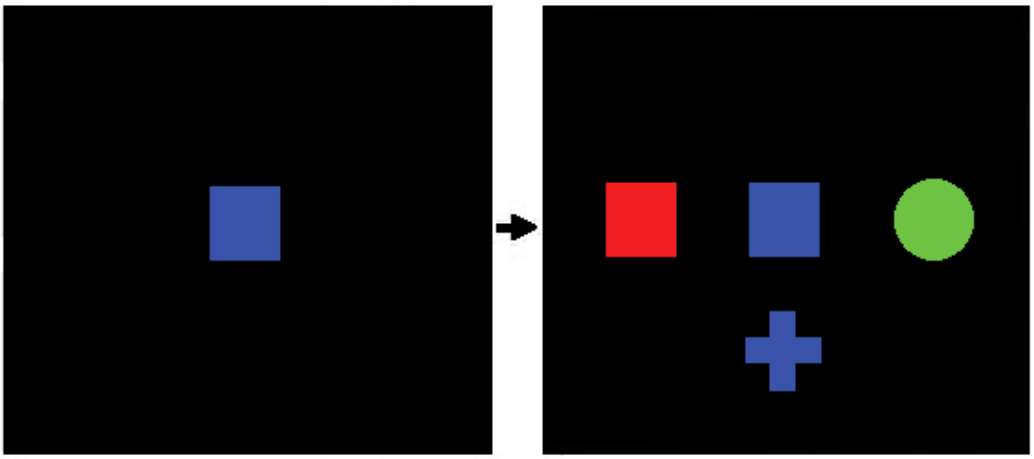


Figure 2 – Display (left) and choice (right) stages of the behavioural task (adapted from Buckley et al., 2009). In the choice stage, the red square matches the target stimulus according to the "shape" rule and the blue cross matches the target stimulus according to the "colour" rule.

The animal had to make its choice within 5s or the trial timed-out and an inter-trial interval began before a new trial was presented. If the animal chose correctly a reward pellet was delivered, the chosen stimuli remained on the screen for 1s, and then an inter-trial interval of 6s commenced; if the animal chose incorrectly, no reward was delivered and an inter-trial interval of 12s followed a salient visual feedback for error (a large white circle displayed for 1s). Each daily session consisted of 300 trials (correction trial procedures were not used) and the rule changed, unannounced, whenever the animal attained a performance criterion of 85 % correct in 20 consecutive trials. The first rule of the day alternated between days.

After preliminary training (see below) the animals continued pre-operative training on the WCST analogue until they become proficient at switching between rules many times per daily session. We then gathered pre-operative data for analysis from a series of control tasks followed by fifteen more consecutive sessions of WCST conducted immediately prior to surgery. After surgery (or an equivalent rest period for controls), comparative post-operative data for each animal was subsequently acquired from fifteen more WCST sessions in addition to control task sessions.

Stimuli

The version of the WCST used in this lesion study utilized 36 different samples comprising of all combinations of 6 distinct colours (red, green, blue, cyan, magenta, and yellow) and 6 distinct shapes (square, circle, triangle, cross, ellipse and hexagon). The sample used in each trial was selected at random from this set of 36 stimuli (without replacement until the entire set had been used). In each trial, the test items were also selected from the same set of 36 stimuli, and at random (with the restrictions imposed by the necessity to generate a trial in which one test item matched the sample in colour but not shape, one other test item matched the sample in shape but not colour, and the remaining test item did not match the sample in either colour or shape). The locations of the three test items (i.e. to the left/right/bottom of the central sample) were also chosen at random. The stimuli were presented on a black background upon a touchscreen monitor placed within arms-reach of the animal. The size of the stimuli was 5-6 cm on the screen and the centre-to-centre distance between the test items and sample was 15 cm.

Preliminary training

Animals were taught the WCST analogue pre-operatively in a well-defined series of stages which introduce the complexities of the task in a gradual manner. Animals proceeded to the final pre-operative scoring stages described only after they completed all the stages described below and had attained a stable level of performance on the WCST analogue with the ability to successfully make many rule changes per session.

Training protocol for WCST analogue:

Stage 1. Macaques were first trained upon a standard battery of shaping tasks that all behaviourally naive animals in the Buckley and Tanaka laboratories are initially exposed to. These familiarise the animals to the testing cubicle which contains the reward-pellet dispenser,

touchscreen, and end-of-session lunchbox containing the animal's daily diet of monkey chow, fruit and other primate treats. By the end of this shaping stage, all animals learn to accurately touch simple stimuli on the screen to obtain a food reward.

Stage 2. In the second stage, macaques were trained upon a delayed matching-to-sample task. In this task the animals learned to match a centrally presented clip-art stimulus to one of two choice stimuli that are presented peripherally after a delay in which the sample stimulus was absent. The aim of this stage was to separate in the monkey's mind the positional attributes of sample and choice items as well as to teaching the matching principle.

Stage 3. The third stage was to gradually reduce the delay, and finally, remove the delay entirely so that the task became a simultaneous match-to-sample task. Experience indicates that simultaneous-matching tasks are learnt faster if the monkey has prior training upon delayed-matching (sample and choice items are more distinct in delayed matching than simultaneous matching tasks and their meanings are more easily acquired in the context of the former).

Stages 4-5. In the fourth stage, the number of test items was increased from two to three. They were arranged in the same relative spatial positions as in the WCST analogue. In the fifth stage, the clipart stimuli used previously were substituted with stimuli akin to those used in the WCST analogue proper, but at this stage only one test item was a match (it matched in both colour and shape attributes).

Stage 6. In the sixth stage, the animals were still required to select the single test item that matched the sample, but the stimuli were manipulated so that the sample and test item only matched in one attribute (colour). After reaching criterion on this stage (85% correct or greater in a single session) the same procedure was carried out for test-items that were engineered so as to only match in shape. The animals continued extensive training on these two rules alternating between 'rules' on the day after they reached criterion on one rule.

Stages 7 and 8. When the animals could reach criterion within a single day's session they moved onto stage seven in which the rule alternated only once within the daily session, at the first

point where criterion is reached (85% in 40 consecutive trials). Animals were required to complete a minimum of 300 trials per day. After sufficient progress was made, animals progressed to stage 8. In this stage, rules alternated whenever the macaque attained performance of 85% correct in 40 consecutive trials. By this stage of training, animals could match according to two different rules but had not been introduced to any conflicting situations (wherein one stimulus matched according to one rule and another stimulus matched according to the other rule) in which they had to choose which rule to apply.

Stages 9 and 10. In stage nine, the animals were presented with conflicting situations: three test items were presented, one of which matched in colour, one of which matched in shape, and one of which did not match either in colour or shape. Firstly, the animals only received reinforcement for matching the item that matched in colour (and ignoring the item that matched in shape). After reaching criterion of 90% in a single daily session (this could take several days), the reinforced matching rule was changed to shape and the animals continued daily testing until they attained 90% in a single daily session. The animals continued training in this manner until they could attain criterion for each new rule change in a single daily session. During this stage, the stricter criterion of 90% was enforced to ensure that the animals attained proficiency at this task prior to each rule change. After this point (in stage 10) we introduced switches within a daily session (initially just one, and subsequently more than one was permitted). Finally, we relaxed the criterion for rule switching to 85% and reduced the minimum number of consecutive trials within which criterion was assessed to 20. It was at this stage of training that pre-operative training was deemed complete.

Subjects

Fourteen macaque monkeys (*Macaca mulatta*) were trained, operated and tested in Oxford, UK, and seven others (*Macaca fuscata*) in RIKEN Brain Science Institute, Wako, Japan. All animal training, surgery and experimental procedures were the same in both laboratories; those conducted in the UK were licensed in compliance with the UK Animals (Scientific Procedures) Act 1986, and

those in Japan were done in accordance with the guidelines of the Japanese Physiological Society, and approved by RIKEN's Animal Experiment Committee.

The animals were housed either individually or in pairs, in rooms with automatically regulated lighting, and they were given water *ad libitum*. Prior to the first stage of surgeries, six macaques were assigned to the CON group (three M. fuscata and three M. mulatta), four macaques were assigned to the PS lesion group (two M. fuscata and two M. mulatta), four macaques were assigned to the ACC lesion group (two M. fuscata and two M. mulatta) on the basis of pre-operative learning scores, so that the mean and range of the numbers of pre-operative rule shifts were comparable between groups, and so that each group consisted of equal numbers of each species. After participating as controls for the post-operative testing period, six animals in the control group were further divided into the OFC lesion group (n=3, consisting of two M. mulatta and one M. fuscata) and the sdlPFC group (n=3, consisting of one M. mulatta and two M. fuscata), so that the mean and range of the numbers of pre-operative rule shifts were comparable between these two equally sized groups. An analysis of variance of the performance data (mean block length) with the between-subject factor 'species', the between-subject factor 'lesion group', the within-subject factor 'stage' (pre-operative and post-operative), and the within-subject factor 'rule' (colour and shape) showed that there were no species differences (Species: $F(1,8)$=5.05, p>0.05) and no species-specific lesion effects in our task, on either rule (Stage x Group x Species: $F(2,8)$=2.60, p>0.1; Stage x Group x Species x Rule: F <1). In a later study, three further animals were assigned to the CON group and four to the FPC-lesion group; all of these animals were M. mulatta. These monkeys were also assigned on the basis of pre-operative learning scores, so that the mean and range of the numbers of pre-operative rule shifts were comparable between groups. In the analyses recorded below, animals later included in the OFC and sdlPFC groups are excluded from the control group when CON vs. OFC and CON vs. sdlPFC comparisons are made in order to preserve the between-subjects nature of the comparison. As a result, nine animals are included in the control group when ACC vs. CON and FPC

vs. CON comparisons are discussed, and six animals are included in the control group when OFC vs. CON and sdlPFC vs. CON comparisons are discussed.

Surgery

Surgery was performed under the same conditions described in Chapter 1. The same surgeon performed the operation in both laboratories. The operated monkeys rested for approximately 14 days after surgery before beginning post-operative training and unoperated control monkeys rested for the same period of time between the two equivalent testing stages.

Histology

After the conclusion of all behavioural experiments, the animals with ablations were sedated, deeply anaesthetized, and then perfused through the heart with saline solution (0.9%), which was followed by formol saline solution (10% formalin in 0.9% saline solution). The brains were blocked in the coronal stereotaxic plane posterior to the lunate sulcus, removed from the skull, allowed to sink in sucrose formalin solution (30% sucrose,10% formalin), and sectioned coronally at 50 µm on a freezing microtome. Every 10th section through the temporal lobe was stained with cresyl violet and mounted. When referring to cytoarchitecturally defined regions in the lesion description below we have adopted the nomenclature and conventions of Petrides and Pandya (Petrides and Pandya, 1994; Petrides and Pandya, 1999) and have reconstructed lesion extents on standard drawings based upon those provided by the Laboratory of Neuropsychology at NIMH .

Principal sulcus (PS) lesion: The intended extent of the PS lesion (Figure 3) included all of the cortex in both banks, and in the fundus, of the PS along its entire anterior-posterior extent; the lesion also extended to include cortex 2-3 mm dorsal and ventral to the lips of the PS. Thus the PS lesion included primarily the middle portion of areas 46 and 9/46. Figure 3 depicts coronal sections

through the area of the intended lesion in the four PS animals (PS1 to PS4). All four of the PS lesions were as intended.

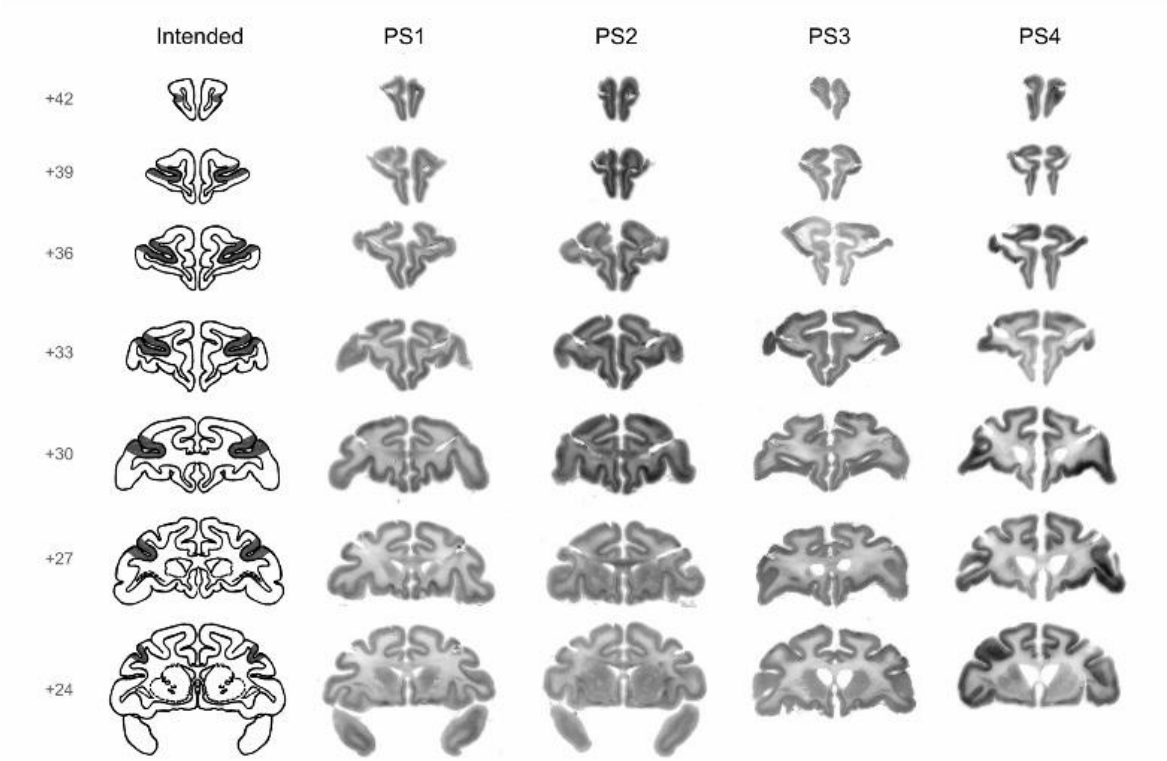


Figure 3 – Photomicrographs of stained coronal sections through the area of the intended lesion in the four PS lesioned animals (PS1 to PS4) alongside drawings of the intended extent of the lesions on drawings of representative coronal sections (left column). Numerals: distance in mm from the interaural plane. (From Buckley et al., 2009)

Anterior cingulate sulcus (ACCs) lesion: The intended extent of the ACCs lesion (Figure 4) included the cortex within the dorsal and ventral banks of the anterior cingulate sulcus (areas 24c, 24c'), with the caudal limit of the lesion in the cingulate sulcus being an imaginary line drawn through the midpoint of the precentral dimple; the lesion extended rostrally for the full extent of the cingulate sulcus. Figure 4 depicts coronal sections through the area of the intended lesion in the four ACCs animals (ACCs1 to ACCs4). In the ACCs group, the lesions were complete and within the intended boundaries apart from in one macaque, ACCs3, whose lesion was larger than intended in one hemisphere, and in another macaque, ACCs4, where the lesion was not extended quite as far

posteriorly as in the other three animals in order to avoid damaging ascending branches of the anterior cerebral artery present at that level of the intended lesion in ACCs4.

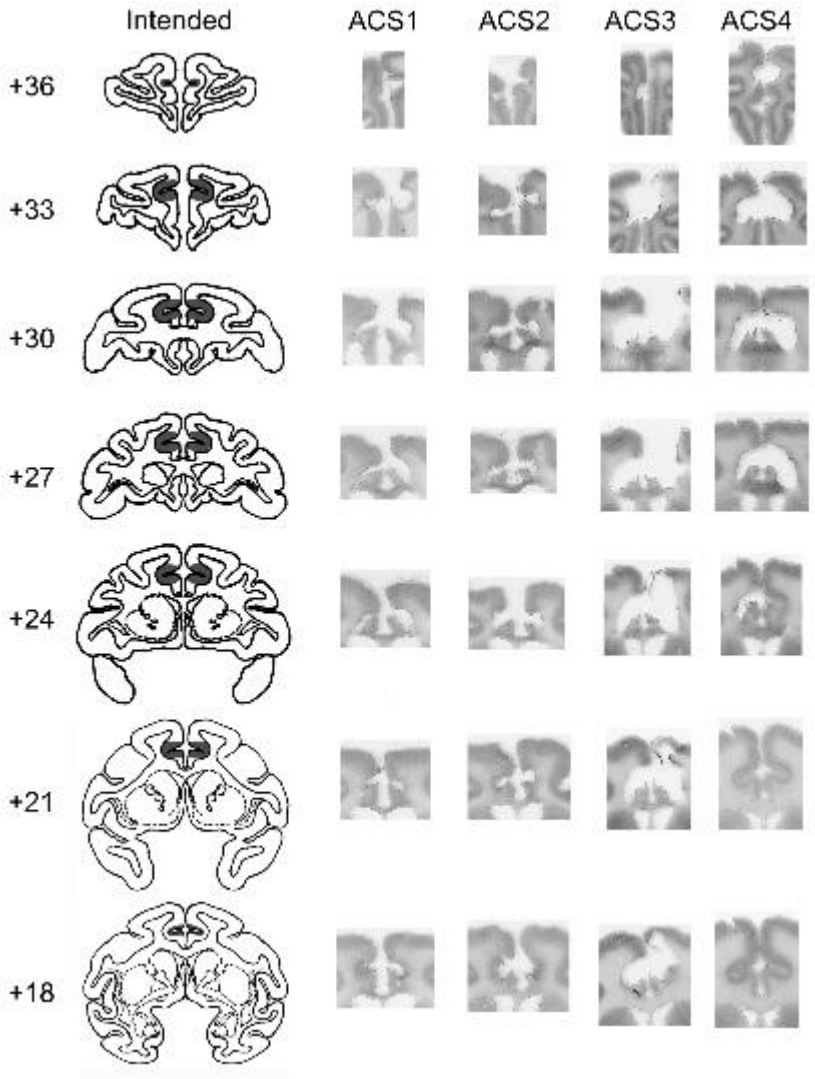


Figure 4 – Photomicrographs of stained coronal sections through the area of the intended lesion in the four ACCs animals (ACs1 to ACs4) alongside drawings of the intended extent of the lesions on drawings of representative coronal sections (left column). Numerals: distance in mm from the interaural plane. (From Buckley et al., 2009).

Orbitofrontal cortex (OFC) lesion: The intended extent of the OFC lesion included at its lateral extent, the cortex in the medial bank of the lateral orbital sulcus; the lesion included all of the cortex between the medial and lateral orbital sulci, and also extended medially until the lateral bank of the rostral sulcus. The anterior extent of the lesion was an imaginary line drawn between the anterior

tips of the lateral and medial orbital sulcii, and the posterior extent was an imaginary line drawn just anterior to the posterior tips of these two sulcii. The intended lesion therefore included areas 11, 13 and 14 of the orbital surface and did not extend posteriorly into the agranular insula. Figure 5 depicts coronal sections through the area of the intended lesion in the three OFC animals (OFC1 to OFC4) in addition to drawing of reconstruction of the actual lesion extent on drawings of standard views of the ventral surface of the macaque brain. None of the OFC lesioned animals sustained any bilateral damage outside the area of the intended region; two animals sustained extremely slight unilateral damage beyond the intended lateral boundary of the lesion OFC2 and OFC3 (Figure 5); and in all three animals the lesions did not extent as far medially as intended.

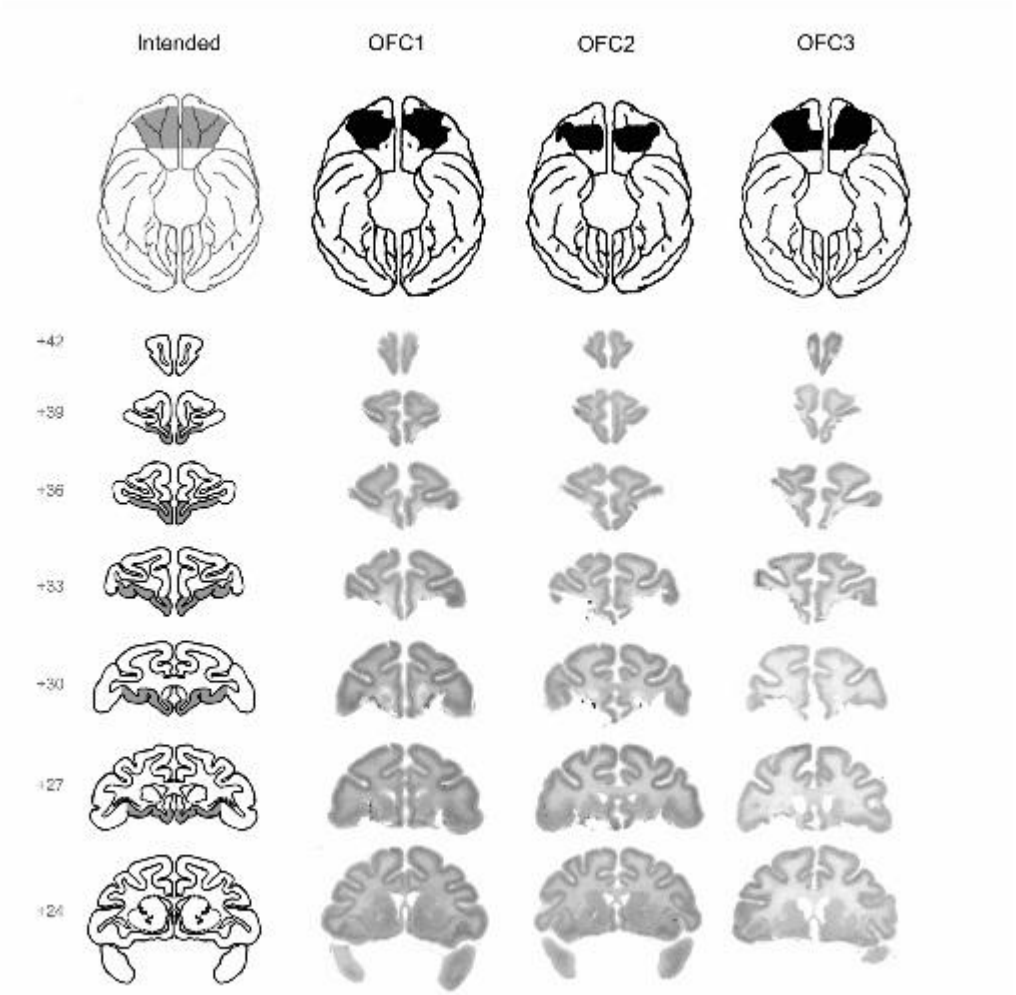


Figure 5 – Photomicrographs of stained coronal sections through the area of the intended lesion in the three animals with OFC lesions (OFC1 to OFC3) alongside drawings of the intended extent of

the lesions on drawings of representative coronal sections (left column). The top row shows reconstructions of the area of cortex lesioned on drawings of a representative ventral surface. Numerals: distance in mm from the interaural plane. (From Buckley et al., 2009).

Superior dorso-lateral prefrontal cortex (sdlPFC) lesion: The intended extent of the sdlPFC lesion was designed to include the cortex on the dorsolateral aspect of the PFC extending up to midline (i.e. lateral area 9 and the dorsal portions of areas 46 and 9/46) but excluding ventrally situated dlPFC cortex that lay within the area of the PS lesion described above; the lesion excluded posteriorly located premotor areas 8A, 8Bd, and 8Bv, nor did it extend anteriorly into area 10. Figure 6 depicts coronal sections through the area of the intended lesion in the three sdlPFC animals (sdlPFC1 to sdlPFC3) in addition to drawings of the actual lesion extent on drawings of standard views of the lateral surfaces of the macaque brain. All three of the sdlPFC lesions were as intended.

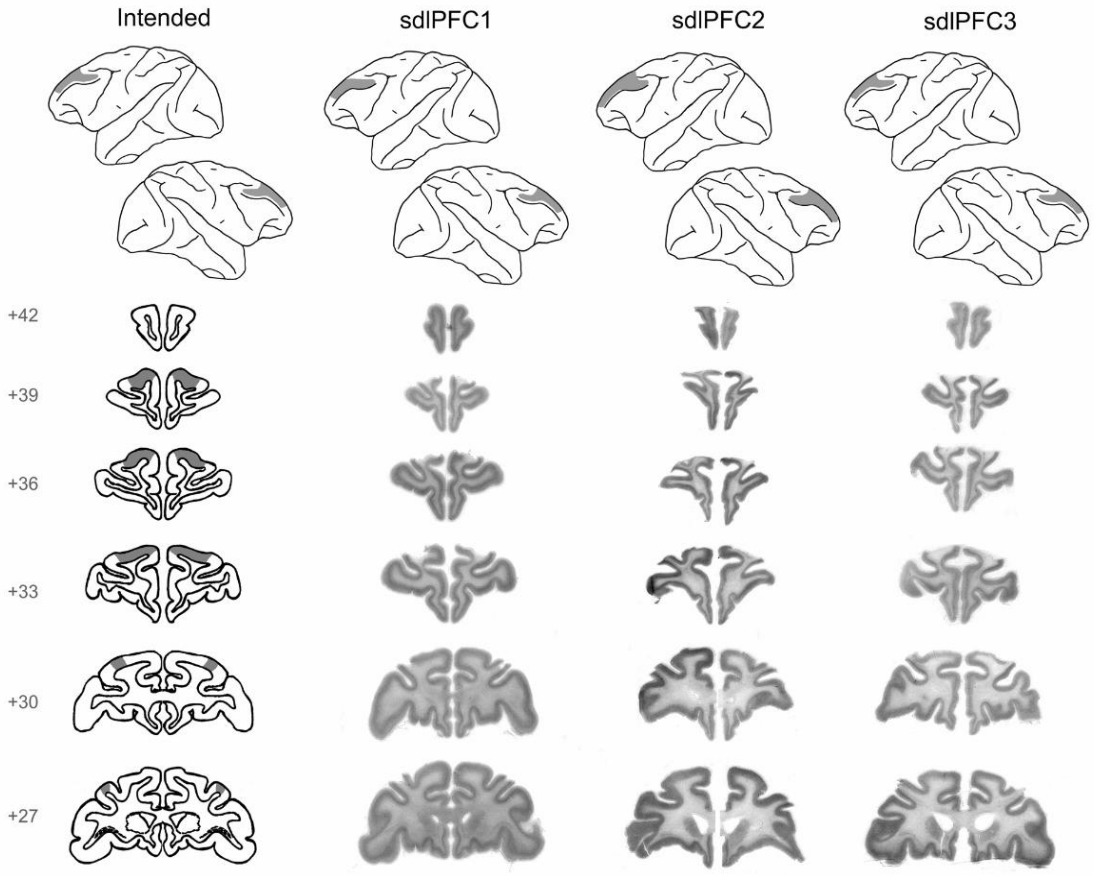


Figure 6 – Photomicrographs of stained coronal sections through the area of the intended lesion in the three animals with sdlPFC lesions (sdlPFC1 to sdlPFC3) alongside drawings of the intended

extent of the lesions on drawings of representative coronal sections (left column). The top row shows reconstructions of the area of cortex lesioned on drawings of representative lateral surfaces. Numerals: distance in mm from the interaural plane. (From Buckley et al., 2009).

Frontopolar cortex (FPC) lesion: The intended extent of the FPC lesion included all cortex anterior to an imaginary line 2mm posterior to the rostral tip of the principal sulcus on the dorsal, orbital and medial surfaces. White matter was spared where possible except in the most rostral part of the lesion. Figure 7 depicts coronal sections through the area of the intended lesion in the four frontopolar animals (FPC1 to FPC4).

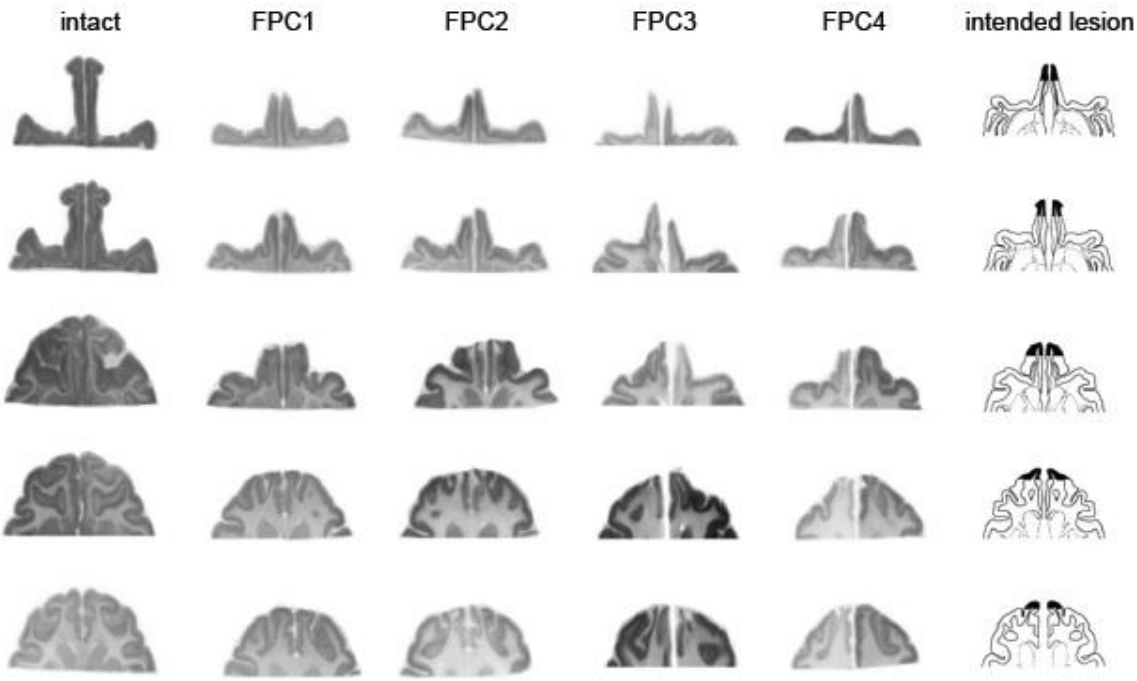


Figure 7 – Photomicrographs of stained coronal sections through the area of the intended lesion in the four animals with FPC lesions (FPC1 to FPC4) alongside drawings of the intended extent of the lesions on drawings of representative coronal sections (right column) and photomicrographs of stained coronal sections through the area of the intended lesion in an intact animal (left column). (From Mansouri et al., submitted).

Data analysis

Overall performance

Planned comparisons were conducted to analyse the differences between post-operative accuracy scores of monkeys in the OFC and CON groups, the ACC and CON groups, and the PS and CON groups (i.e. number of trials answered correctly vs. number of trials answered with an error; trials in which the monkey timed out were excluded). These comparisons were justified, as previously published literature has noted that lesions to these regions in monkeys cause impairments on this task (Buckley et al., 2009). These comparisons confirm that ACC, PS and OFC group were significantly impaired post-operatively compared to controls (ACC: $F(1,21)$=13.301, p=0.002, PS: $F(1,21)$=5.508, p=0.029, OFC: $F(1,21)$=23.773, p<0.001). When these three groups are excluded from the analysis, a one-way ANOVA on post-operative accuracy scores (three levels: sdlPFC-lesioned, FPC-lesioned and control) confirms that no other significant between-groups difference can be found ($F(2,13)$=0.407, p=0.674). Figure 8 shows the overall pre- and post-operative performance for all groups.

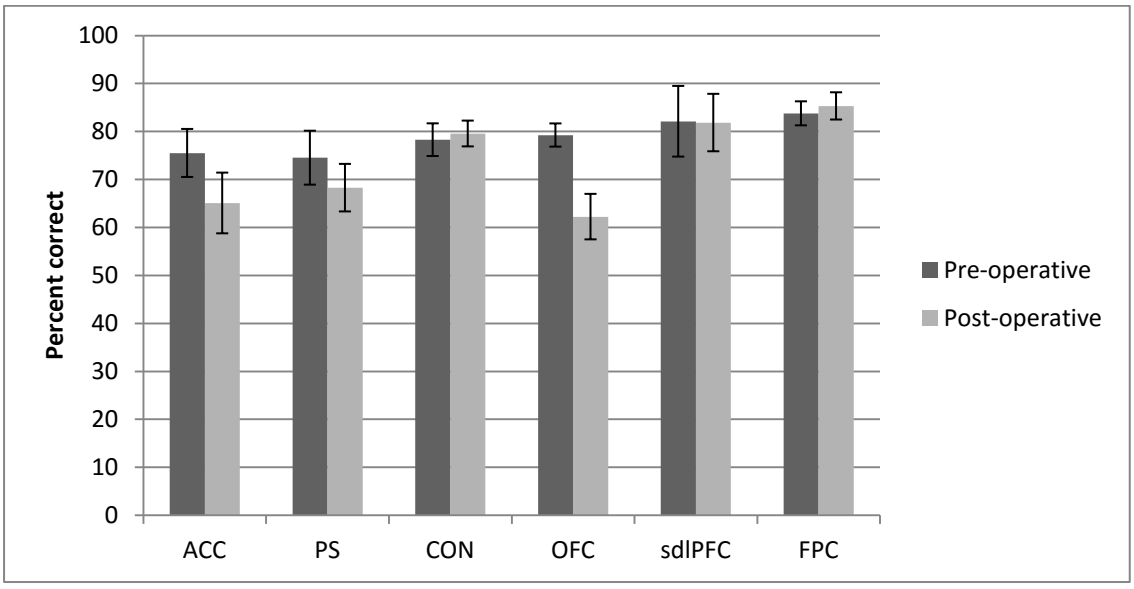


Figure 8 – Pre- and post-operative percent correct scores for all groups, with error bars showing the +/- standard error of the mean.

Logistic regressions

In order to conduct a multiple logistic regression analysis analysing the effect of recently rewarded responses to each rule (colour or shape) on current choice-behaviour, I first excluded a

small minority of trials in which the monkey chose the stimulus which did not match the test stimulus in either shape or colour and trials in which the monkey did not make a response. Error trials in which the stimulus did not match the test stimulus in either dimension made up 1.785% of all trials, and 7.136% of error trials, and no more than 7.630% of all trials and 19.579% of error trials in any monkey. Trials in which the monkey did not make a response made up no more than 0.0315% of all trials in any monkey. The first eight trials of each day were also excluded, so that only trials for which the effect of rewarded responses in all eight of the preceding eight trials on behavioural choice could be calculated were included in the analysis. Data from 15 daily sessions were included for each animal both pre- and post-operatively. Once these trials had been excluded, the pre- and post-operative data from each monkey were subjected to a multiple logistic regression analysis in order to calculate the relative weight that each previous trial outcome had in determining behavioural choices in the current trial. While, due to the nature of the task, a high level of correlation existed between previous trial outcomes at each trial, the high number of trials completed by each monkey (4500 pre- and post-operatively), in addition to pre- and post-operative measurements collected for each animal and the inclusion of multiple animals in each experimental group enabled us to minimise the effect of multicollinearity on our dataset.

Rewarded trials – all trials in block

In order to calculate the influence of the outcome of previously rewarded trials on rule choice on the current trial, I carried out a binary logistic regression analysis on the data from each animal. In this analysis, monkeys' rule choice (regardless of whether or not it was correct) on the current trial was the dependant variable; the possible outcomes were 'chose colour' (coded as "0") or 'chose shape' (coded as "1"). The outcomes of each previous trial (up to the eighth most recent trial, i-8) were covariates. The outcome of the previous trial was encoded in two dummy variables and a baseline: a rewarded shape choice was coded as (1 0), an unrewarded choice (regardless of whether the monkey chose shape or colour) was coded as (0 1), and a rewarded colour choice was

coded as (0 0) (baseline). This coding had the effect of setting colour as the baseline variable, so that the parameters estimated were the increase in likelihood that the monkey chose shape given a previous shape reward versus the monkey choosing shape given a previous colour reward and the increase in likelihood that the monkey chose shape given a previous non-rewarded trial versus the monkey choosing shape given a previous colour reward.

This analysis generated three sets of β values which expressed the weight that the outcome of previous trials had on determining rule choice on the current trial: these allowed the probability that a monkey would choose to follow one rule (arbitrarily set as "shape") rather than the other (arbitrarily set as "colour") to be predicted by the following general formula:

$\log(p(Y_i=1))/\log(p(Y_i=0)) = \beta_1 X_{i-1} + \beta_2 X_{i-2} + \ldots + \beta_8 X_{i-8}$

The arbitrary choice of "shape" rather than "colour" had no effect on the outcome of the analysis; as only two possibilities were available, the probability that a monkey would choose "colour" was equal to 1 – P("shape"). The β values which expressed the weight of a rewarded colour rather than shape response in previous trials on the probability that a monkey would choose to follow the colour rule on the current trial were identical to those which expressed the weight of a rewarded shape rather than colour response in previous trials on the probability that a monkey would choose to follow the shape rule on the current trial.

In the first set of β variables calculated, X expressed the difference in probability between a monkey making a rewarded shape response rather than a rewarded colour response in the relevant previous trial, and the β values give the weight that each rewarded shape response, compared to each rewarded colour response, adds to the likelihood of choosing shape in the current trial ($\beta_{\text{shape vs colour}}$). In the second, X expressed the likelihood that a monkey had made an error rather than a rewarded colour response in the relevant previous trial, and the β values give the weight that each

unrewarded choice, compared to each rewarded colour response, adds to the likelihood of choosing shape in the current trial ($\beta_{error\ vs\ colour}$). Since the likelihood that a monkey would make a rewarded "shape" response rather than a rewarded "colour" response (on any historical trial) can be expressed as the likelihood that a monkey will choose "shape" rather than "error" plus the likelihood that a monkey will choose "error" rather than "colour", these calculated β values allowed me to calculate a third set of β values which expressed the weight of an instance of no reward on the likelihood of choosing colour on the current trial compared to a rewarded shape response ($\beta_{error\ vs\ shape}$): these values were equal to ($\beta_{shape\ vs\ colour} - \beta_{error\ vs\ colour}$).

I compared $\beta_{error\ vs\ shape}$ and $\beta_{error\ vs\ colour}$ for all trial history values (THV) and groups, pre and post lesion. This comparison yielded no statistically significant results that survived correction for multiple comparisons using the Holm-Bonferroni method (Holm, 1979). As a result, subsequent analyses were performed only on the $\beta_{shape\ vs\ colour}$ values, as this value provided a summation of the (rewarded shape vs. no reward) and (rewarded colour vs. no reward) comparisons.

A three-way repeated measures ANOVA was conducted on these β values with two within-subjects factors, namely 'THV' (8 levels: i-1 to i-8) and 'Stage of testing' (two levels: pre-operative and post-operative), and one between-subjects factor of 'Group' (6 levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control). This analysis revealed significant main effects of Group ($F(7,24)$=2.786, p=0.029), THV ($F(7,168)$=159.327, p<0.001) and stage of testing ($F(1,24)$=10.999, p=0.003). Interaction effects were observed between stage of testing and group ($F(7,24)$=3.335, p=0.013), THV and group ($F(49,168)$=4.442, p<0.001), and stage of testing and THV ($F(7,168)$=6.677, p<0.001), and a three-way interaction was observed between stage of testing, THV and group ($F(35,147)$=2.260, p=0.025; the Greenhouse-Geisser correction was applied in order to compensate for a violation in the assumption of sphericity and the associated corrected degrees of freedom were $F(11.456,48.117)$). A graph showing this data is presented in figure 9 (in this graph, pre-operative values for all groups are amalgamated into one series; they are considered separately

in the ANOVA described above). A graph showing the data for only the ACC, FPC and CON groups is presented in figure 10. All nine control animals are included in the data shown in these figures, and all control animals were included in the ANOVA described above.

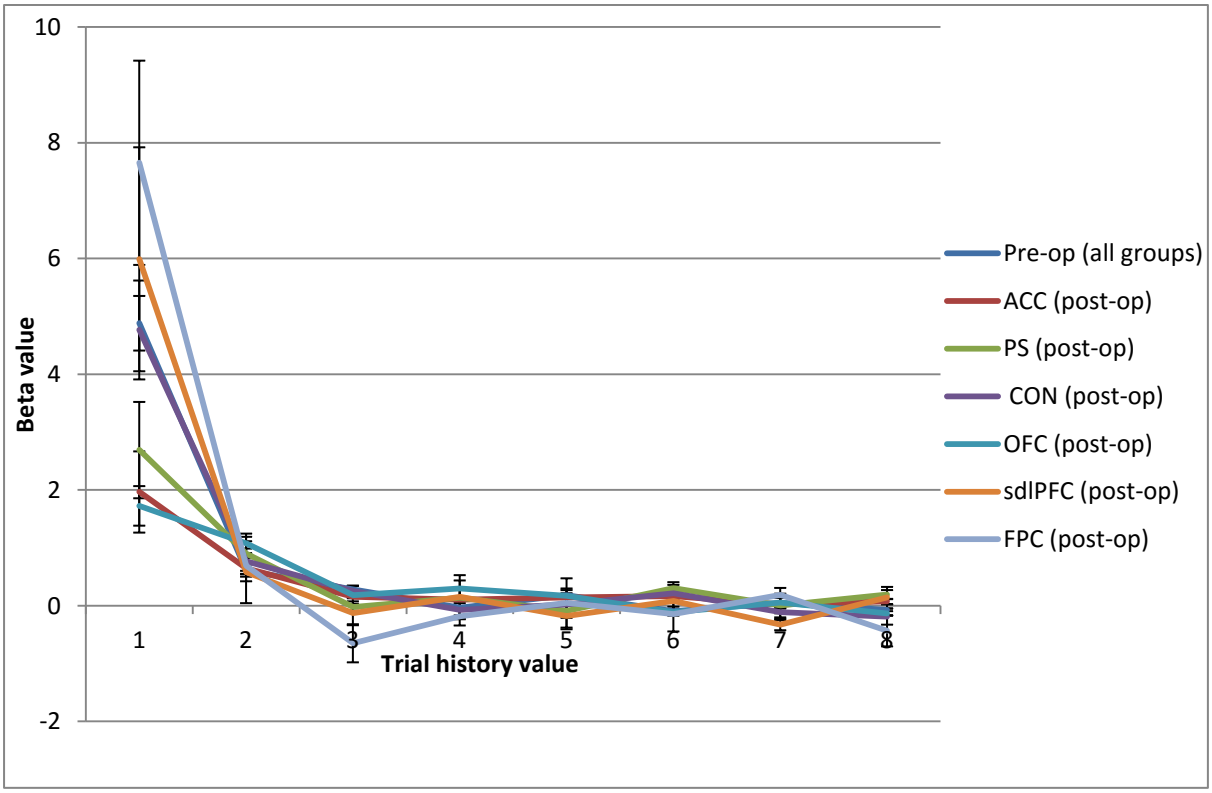


Figure 9 – Pre- and post-operative β values for the eight most recent trials for all groups, with error bars showing the +/- standard error of the mean.

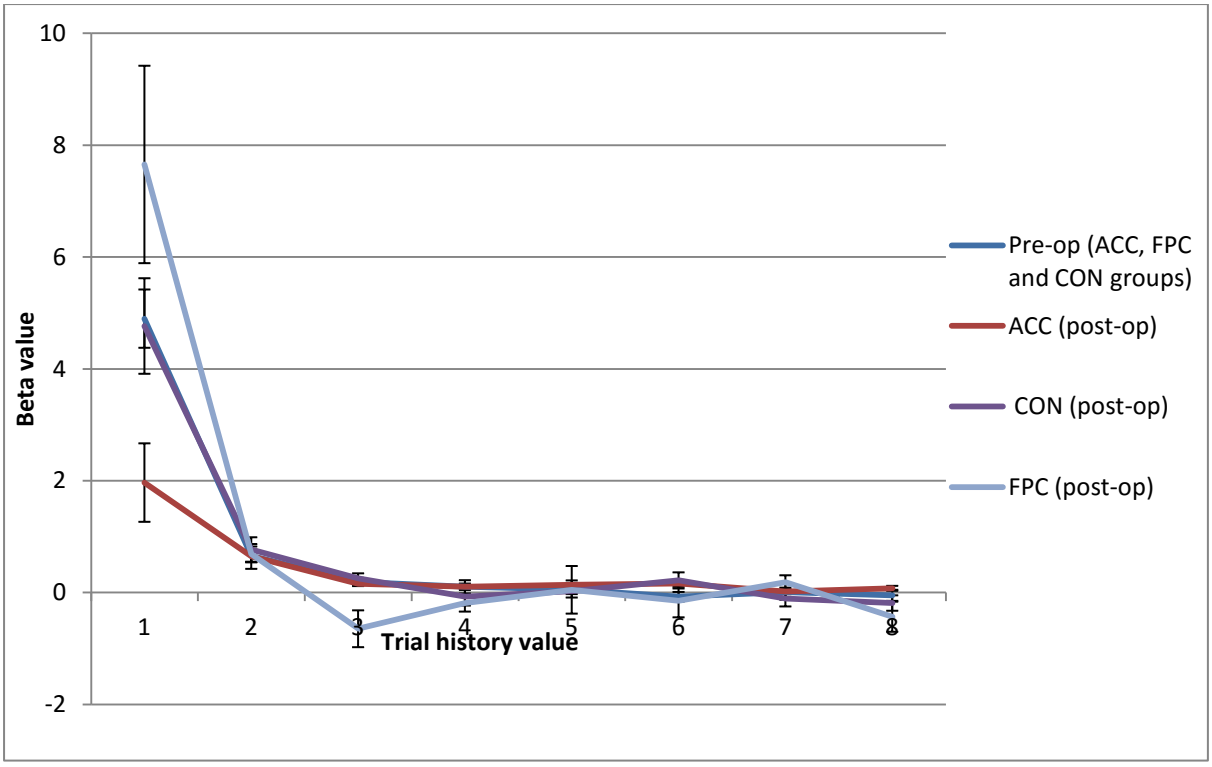


Figure 10 – Pre- and post-operative β values for the eight most recent trials for ACC, FPC and CON groups, with error bars showing the +/- standard error of the mean.

In order to isolate the source of this variance, I conducted one-sample t-tests to identify which β values significantly differed from zero in pre-operative animals (i.e. how many trials in the animal's recent history had a non-zero influence on behaviour). B values were significantly different to zero at THV 1 ($t(26)$=11.233, p<0.001), THV 2 ($t(26)$=6.216, p<0.001) and THV 3 ($t(26)$=5.370, p<0.001); no other THV was significant. Accordingly, THVs greater than three were not included in further analyses. Each of these results survived correction for multiple comparisons using the Holm-Bonferroni method.

A three-ways repeated measures ANOVA was conducted on these β values with two within-subjects factors, 'THV' (3 levels: i-1 to i-3) and 'Stage of testing' (two levels: pre-operative and post-operative), and one between-subjects factor of 'Group' (6 levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control) (i.e. with the same design as that reported above, but including only the three most recent levels of THV). All nine control animals were included in this ANOVA. This analysis identified significant main effects of stage ($F(1,21)$=7.222,

p=0.014) and THV (*F(2,42)*=77.223, *p*<0.001), a significant interaction effect between stage and THV (*F(2,42)*=4.222, *p*=0.042; the Greenhouse-Geisser correction was used in order to compensate for a violation in the assumption of sphericity and the associated corrected degrees of freedom were *F(1.245, 26.135)*), and a significant interaction effect between THV, stage of testing and group (*F(10,42)*=2.653, *p*=0.037; the Greenhouse-Geisser correction was used in order to compensate for a violation in the assumption of sphericity and the associated corrected degrees of freedom were *F(18.932,26.135)*). Further repeated measures ANOVAs were used to examine the results at THVs 1, 2 and 3; these are described below.

A two-way repeated measures ANOVA with within-subjects factor of 'Stage of testing' (two levels: pre-operative and post-operative) and one between-subjects factor of 'Group' (six levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control) was conducted on β values associated with THV 1 (corresponding to i-1, the most recent trial). All nine control animals were included in this ANOVA. There was a significant main effect of group (*F(1,21)*=4.613, *p*=0.044) and a significant interaction effect between stage of testing and group (*F(5,21)*=2.776, *p*=0.045). A two-way repeated measures ANOVA with within-subjects factor of 'Stage of testing' (two levels: pre-operative and post-operative) and one between-subjects factor of 'Group' (six levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control) was conducted on β values associated with THV 2 (corresponding to i-2, the second most recent trial). There were no significant effects relating to either stage or group. A further two-way repeated measures ANOVA with one within-subjects factor of 'Stage of testing' (two levels: pre-operative and post-operative) and one between-subjects factor of 'Group' (six levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control) was conducted on β values associated with THV 3 (corresponding to i-3, the third most recent trial). All nine control animals were included in this ANOVA. There was a significant main effect of stage (*F(1,21)*=14.293, *p*=0.001) and a significant interaction effect between stage of testing and group (*F(5,21)*=3.332, *p*=0.023).

In order to isolate whether the source of the variance detected from THV 1 was consistent with our hypotheses, planned comparisons were conducted between these β values for the PS, OFC and ACC lesion groups with control animals. Planned comparisons were also conducted between the ACC lesion group and the control group with FPC-lesioned animals as warranted by the hypotheses of this experiment. In the PS vs. CON, ACC vs. CON and FPC vs. CON comparisons, all nine control animals were considered; however, in the OFC vs. CON comparison, only six control animals were considered and those animals which were tested originally as controls and then included in the OFC group and retested were excluded. For THV 1, significant between groups differences were detected between ACC-lesioned and control animals ($F(1,21)$=4.779, p=0.040), between OFC-lesioned and control animals ($F(1,21)$=9.319, p=0.006) and between FPC and ACC animals ($F(1,21)$=6.681, p=0.018); this represented a decrease in the influence of a reward at THV1 in ACC-lesioned and OFC-lesioned animals compared with control and FPC-lesioned animals (see figure 11). All nine control animals are included in the data represented in this figure.

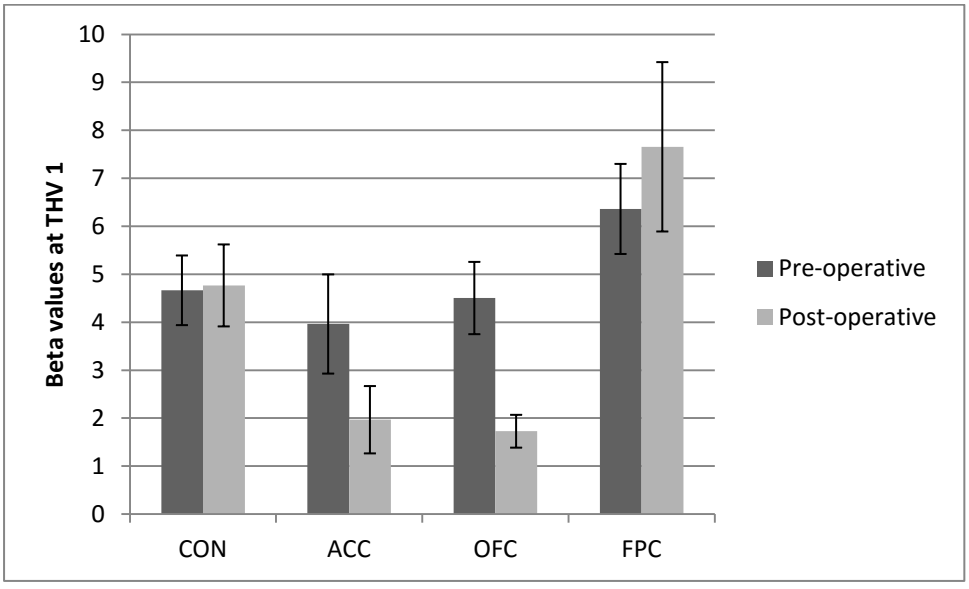


Figure 11 – Pre- and post-operative β values at THV 1 for ACC, OFC and FPC lesion groups and control animals, with error bars showing the +/- standard error of the mean.

As I had no specific hypotheses regarding group differences at THV 3 which would not also hold true at THV 2, planned comparisons were not justified in order to isolate the source of this variance. A post-hoc Tukey HSD test found significant differences between the FPC-lesioned group and the control group (p=0.003) (data from all nine control animals was included in this analysis) and the FPC-lesioned and sdlPFC-lesioned groups (p=0.021). However, further investigation of these differences revealed that the difference detected between the FPC-lesioned and sdlPFC-lesioned groups at THV 3 was due to a large difference in pre-operative β values (FPC mean: 0.208, standard deviation: 0.144; sdlPFC mean: 0.753, standard deviation: 0.482). When a one-way ANOVA comparing the difference between pre- and post-operative β values at THV 3 was conducted, including only FPC-lesioned and sdlPFC-lesioned groups, no significant group difference was found. By contrast, the difference between FPC-lesioned animals and controls at THV 3 did appear to be robust; a one-way ANOVA comparing the difference between pre- and post-operative β values including only the FPC-lesioned and control groups found a significant between-groups difference ($F(1,11)$=7.916, p=0.017). Examination of the data indicates that this difference is due to a significantly negative influence of a rewarded response to a particular rule at THV 3 on rule-choice in the current trial in FPC-lesioned animals, compared to a small positive influence in control animals (see figure 12).

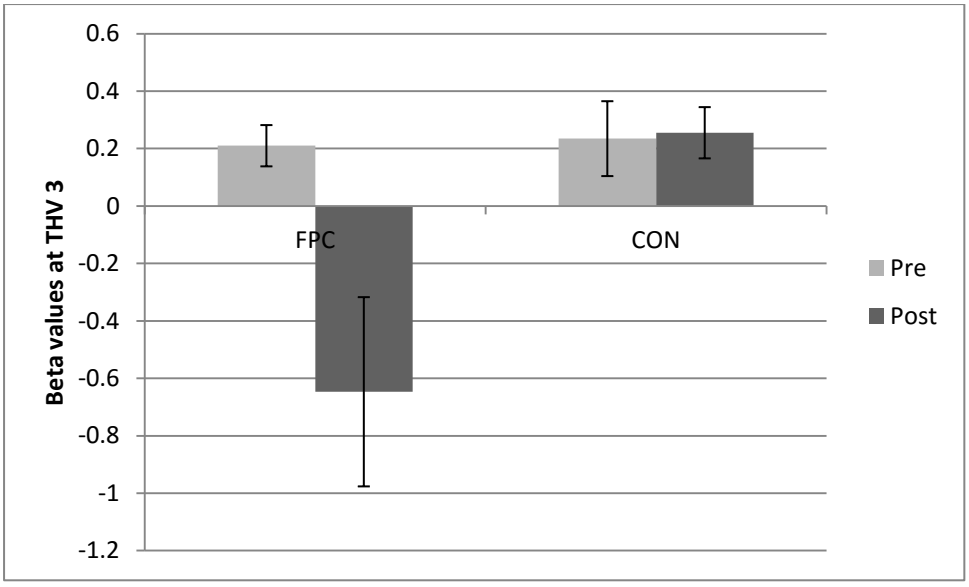


Figure 12 – Pre- and post-operative β values at THV 3 for FPC-lesioned and control animals

Rewarded trials – first 10 trials in block

The linear regression analysis described above included data from every rewarded trial (excluding those in the first 8 trials of a session) in the experiment. However, it may be the case that rewarded trials close to the beginning of a block were more relevant to the hypotheses of this experiment, as in these trials, animals were required to prioritise information from more recent trials (in which one rule had been rewarded) over more distant ones (in which another rule had been rewarded) in order to choose the currently-rewarded rule; this conflict between recent and distant trials did not apply if no rule shift had recently occurred. I therefore conducted a logistic regression analysis identical to the one described above, but including only the first ten trials after each rule shift.

A three-way repeated measures ANOVA was conducted on these β values with two within-subjects factors, namely 'THV' (8 levels: i-1 to i-8) and 'Stage of testing' (two levels: pre-operative and post-operative), and one between-subjects factor of 'Group' (6 levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control). This analysis found significant

main effects of THV (*F(7,147)*=67.892, *p*<0.001; the Greenhouse-Geisser correction was applied in order to compensate for a violation in the assumption of sphericity and the associated corrected degrees of freedom were *F(1.445,30.351)*) and stage (*F(1,21)*=9.541, *p*=0.006) and a significant interaction effect between THV and stage (*F(7,147)*=3.240, *p*=0.025; the Greenhouse-Geisser correction was applied and the associated corrected degrees of freedom were *F(3.160,66.357)*). This analysis did not find a significant interaction effect between THV, stage and group (*F(35,147)*=1.751, *p*=0.072 after the Greenhouse-Geisser correction was applied; the associated corrected degrees of freedom were *F(15.799, 66.357)*). However, a significant stage*group interaction effect was observed (*F(5,21)*=2.918, *p*=0.037). This data is presented in figure 13 (in this figure, pre-operative data from all groups is amalgamated into one series; they are considered separately in the ANOVA described above). Figure 14 presents this data for ACC, FPC and CON groups. Data from all nine control animals is represented in these graphs and in the analysis described above.

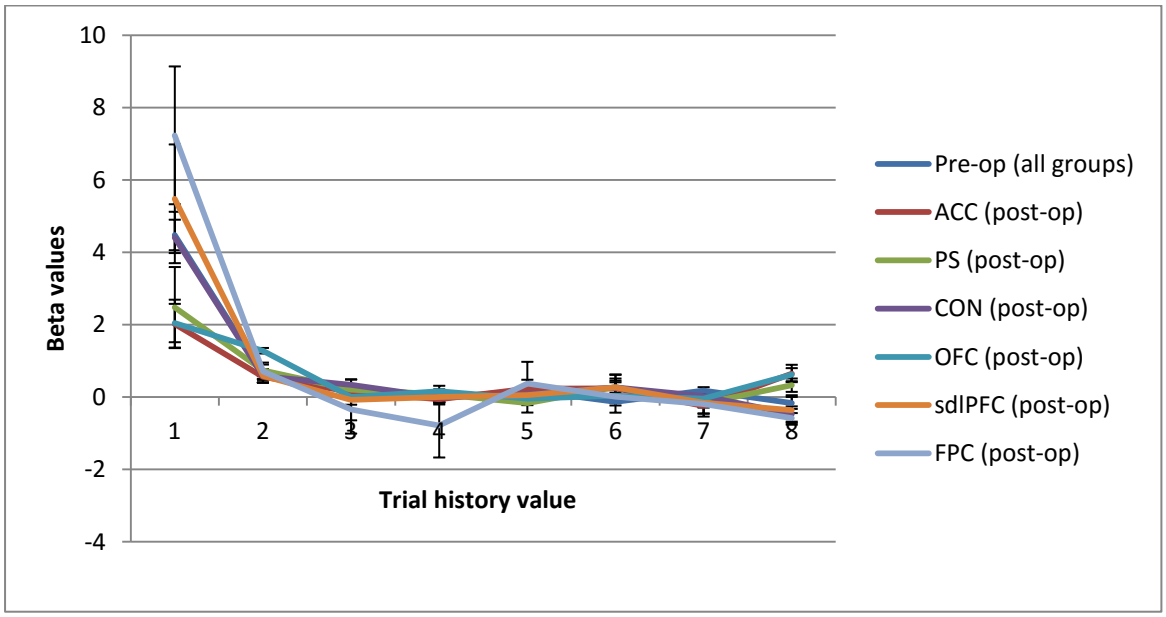


Figure 13 – B values for THV 1 -8 for all groups for the first 10 trials in each block, with error bars showing the +/- standard error of the mean.

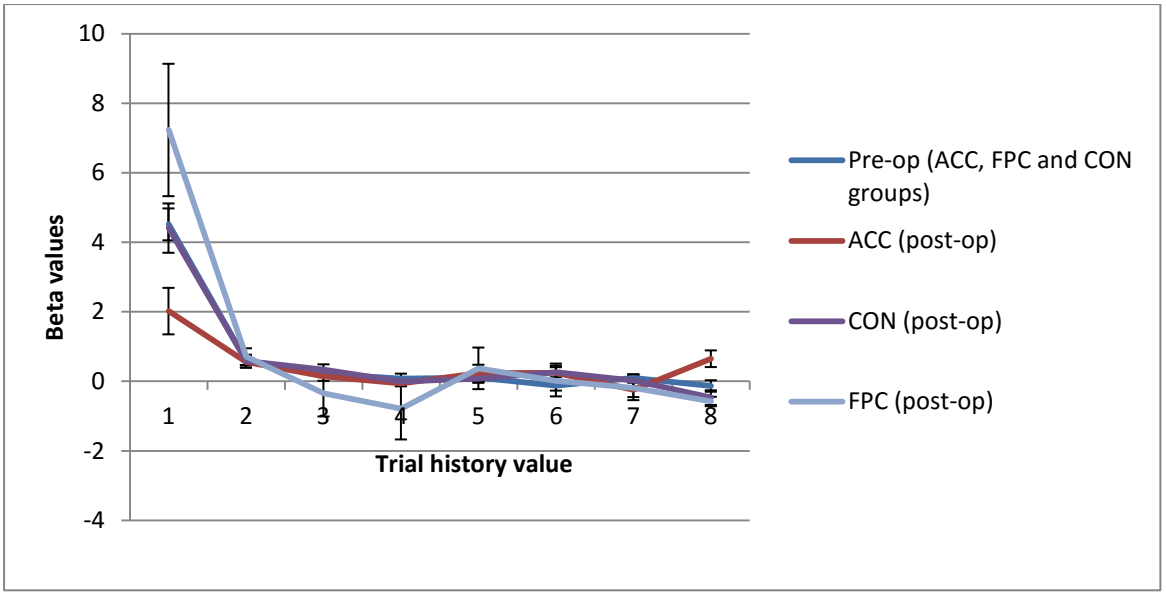


Figure 14 – B values for THV 1-8 for ACC, FPC and CON groups for the first 10 trials in each block, with error bars showing the +/- standard error of the mean.

When the pre-operative β values for all THVs were subjected to a one-sample t-test, only the β values at THV 1, THV 2 and THV 3 differed significantly from zero (THV 1: $t(26)$=11.267, p<0.001; THV 2: $t(26)$=5.868, p<0.001; THV 3: $t(26)$=3.535, p=0.002); all three of these results survived correction for multiple comparisons using the Holm-Bonferroni method. Accordingly, β values relating to THVs greater than three were excluded from further analyses of this data.

A three-way repeated measures ANOVA was conducted on these β values with two within-subjects factors, 'THV' (3 levels: i-1 to i-3) and 'Stage of testing' (two levels: pre-operative and post-operative), and one between-subjects factor of 'Group' (6 levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control) (i.e. with the same design as that reported above, but including only the three most recent levels of THV). This analysis detected a significant main effect of THV ($F(2,42)$=71.789, p<0.001; the Greenhouse-Geisser correction was applied in order to compensate for a violation in the assumption of sphericity and the associated corrected degrees of freedom were $F(1.109,23.281)$), a significant interaction effect between stage and THV ($F(2,42)$=5.294, p=0.009) and a significant interaction effect between THV, group and stage of testing ($F(10,42)$=3.450, p=0.002). When two-way repeated measures ANOVAs (one within-subject factor of

'Stage of testing'; one between-subject factor of 'Group') were conducted examining each THV separately, a significant main effect of stage ($F(1,21)$=6.459, p=0.019) and a significant stage*group interaction effect was detected at THV 1 ($F(5,21)$=3.926, p=0.011). However, no significant stage or group effects were observed at THV 2 or 3. Data from all nine control animals was included in the analyses described above.

I then, as above, conducted planned comparisons between the THV 1 β values of the ACC, PS, OFC and FPC-lesion groups and the control group, and between the ACC- and FPC-lesion groups. These revealed significant differences between the ACC-lesioned group and the control group ($F(1,21)$=5.370, p=0.031), between the OFC-lesioned group and the control group ($F(1,21)$=10.374, p=0.004) and between the FPC-lesioned group and the ACC-lesioned group ($F(1,21)$=9.866, p=0.005). Data from all nine control animals was included in the ACC vs. CON, PS vs. CON and FPC vs. CON comparisons; however, in the OFC vs. CON comparison, only six control animals were considered and those animals which were tested originally as controls and then included in the OFC group and retested were excluded. As in the analysis of the whole dataset, these reflected a significantly reduced influence of a rewarded response to a particular rule in the most recent trial on rule-choice in the current trial in ACC- and OFC-lesioned animals (see figure 15). Data from all nine of the control animals is included in this figure.

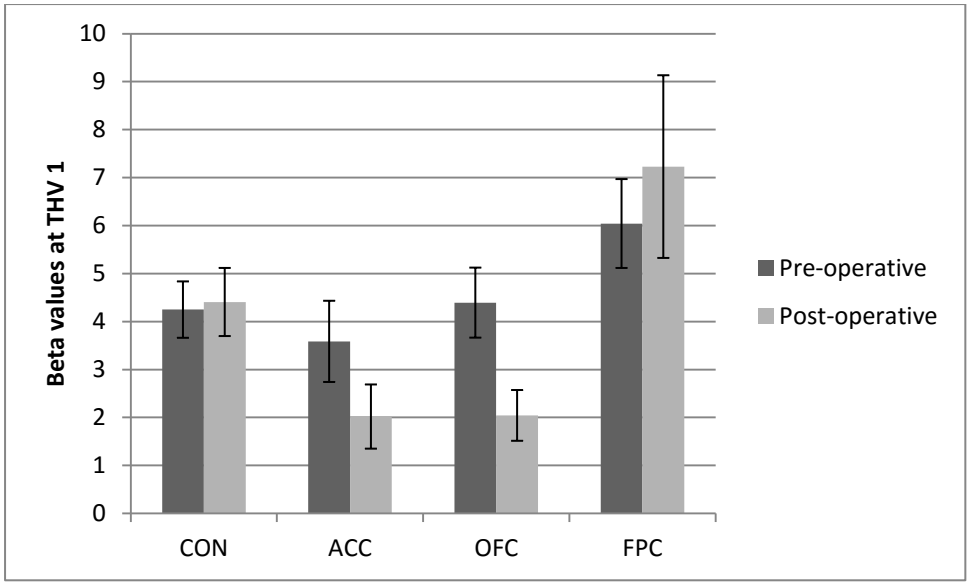


Figure 15 – Pre- and post-operative β values at THV 1 for ACC, OFC and FPC lesion groups and control animals for the first 10 trials of each block, with error bars showing the +/- standard error of the mean.

Overall, outcomes detected when only the first ten trials in each block were included in the analysis were equivalent to the outcomes detected when all trials in each block (excluding the first eight trials in each daily session) were considered, although no significant difference was detected between the influence of outcomes at THV 3 on rule choice in FPC-lesioned animals when compared with rule choice in control animals.

Error trials – all trials in block

The analyses described above relate to the influence that previously rewarded choices to one particular abstract rule exerts on current monkey choice-behaviour compared to the influence of a previously rewarded choice to the alternative abstract rule (there being only two abstract rules to choose between in the WCST analogue). Trials in which animals made a choice which did not receive a reward (i.e. error-trials) were treated as neutral and not considered by the aforementioned analyses. However, a non-rewarded choice of an abstract rule could plausibly serve as a cue to animals that they should shift their behaviour and choose the other rule on future trials. Accordingly,

I carried out a logistic regression similar to that described above, but changing the parameters such that rewarded trials were treated as neutral, so that the analysis focused on the difference between the influence from previous choice-behaviour that resulted in receiving no reward for following a particular abstract rule compared to influence from a previous non-rewarded choice of the other abstract rule on choice-behaviour in the current trial.

In order to calculate the influence of the outcome of previously non-rewarded trials on rule choice on the current trial, I carried out another binary logistic regression analysis on the data from each animal. In this analysis, as above, monkeys' rule choice on the current trial was the dependant variable; the possible outcomes were 'chose colour' (coded as "0") or 'chose shape' (coded as "1"). The outcomes of each previous trial (up to the eighth most recent trial, i-8) were covariates. The outcome of the previous trial was encoded in two dummy variables: a non-rewarded shape choice was coded as (1 0), a correct (rewarded) trial was coded as (0 1) (regardless of the rule which was rewarded), and a non-rewarded colour choice was coded as (0 0) (baseline). Colour was again set as the baseline variable, so that the parameters estimated were the increase in likelihood that the monkey chose shape given a previous shape non-reward versus the monkey choosing shape given a previous colour non-reward and the increase in likelihood that the monkey chose shape given a previous reward (to either shape or colour) versus the monkey choosing shape given a previous colour non-reward.

This analysis generated three sets of β values which expressed the weight that the outcome of previous trials had on determining rule choice on the current trial: these allowed the probability that a monkey would choose to follow one rule (arbitrarily set as "shape") rather than the other (arbitrarily set as "colour") to be predicted by the following general formula:

$$\log(p(Y_i=1))/\log(p(Y_i=0)) = \beta_1 X_{i-1} + \beta_2 X_{i-2} + \ldots + \beta_8 X_{i-8}$$

As above, in the first set of β variables calculated, X expressed the difference in probability between a monkey making a non-rewarded shape response rather than a non-rewarded colour response in the relevant previous trial, and the β values give the weight that each non-rewarded shape response adds to the likelihood of choosing shape in the current trial, compared to each non-rewarded colour response ($\beta_{\text{shape error vs colour error}}$). In the second, X expressed the likelihood that a monkey had made a correct choice (of either type) rather than a non-rewarded colour response in the relevant previous trial, and the β values give the weight that each instance of reward adds to the likelihood of choosing shape in the current trial when compared to each non-rewarded colour response ($\beta_{\text{correct vs colour error}}$). As above, it was possible to calculate a set of β values which gave the weight that each instance of reward adds to the likelihood of choosing colour on the current trial, compared to each instance of a non-rewarded shape response ($\beta_{\text{correct vs shape error}}$). Again, the choice was taken to carry out analyses on the first set of β values ($\beta_{\text{shape error vs colour error}}$).

All analyses described in this section included data from all nine control animals. A three-way repeated measures ANOVA was conducted on these β values with two within-subjects factors, 'THV' (8 levels: i-1 to i-8) and 'Stage of testing' (two levels: pre-operative and post-operative), and one between-subjects factor of 'Group' (6 levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control). This analysis found a significant main effect of THV ($F(7,147)$=168.139, p<0.001; the Greenhouse-Geisser correction was applied in order to compensate for a violation in the assumption of sphericity and the associated corrected degrees of freedom were $F(2.262,47.507)$), a significant interaction effect between stage and THV ($F(7,147)$=7.161, p<0.001; the Greenhouse-Geisser correction was applied and the corrected degrees of freedom were $F(2.802,58.383)$), and a significant interaction effect between group, THV and stage of testing ($F(35,147)$=2.769, p=0.003; the Greenhouse-Geisser correction was applied and the corrected degrees of freedom were $F(14.009,58.838)$). This data is presented in figure 16 (in this figure, pre-operative data from all groups is amalgamated into one series; they are considered separately in the

ANOVA described above). Figure 17 presents this data for ACC, FPC and CON groups. These figures include data from all nine control animals.

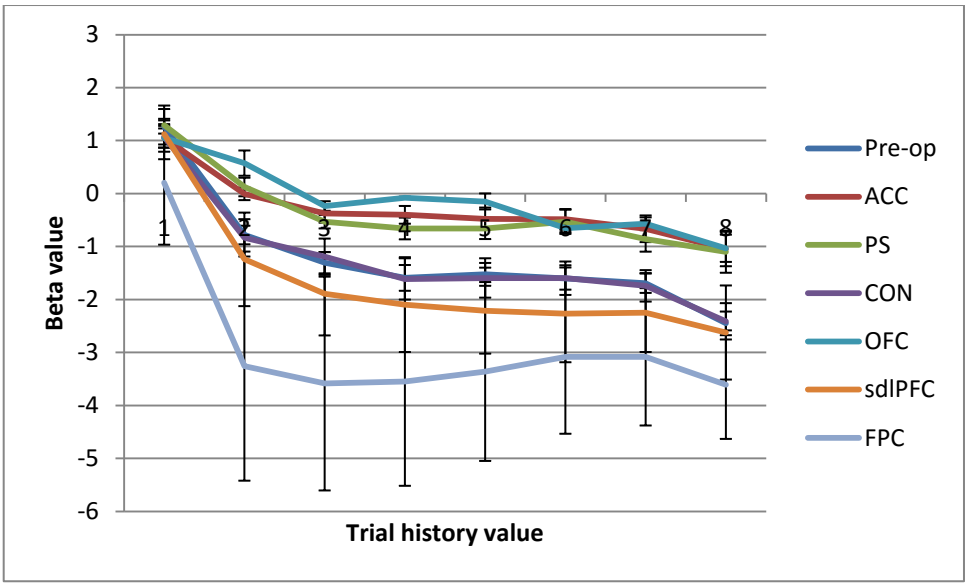


Figure 16 – B values at THV 1-8 for all groups, with error bars showing the +/- standard error of the mean.

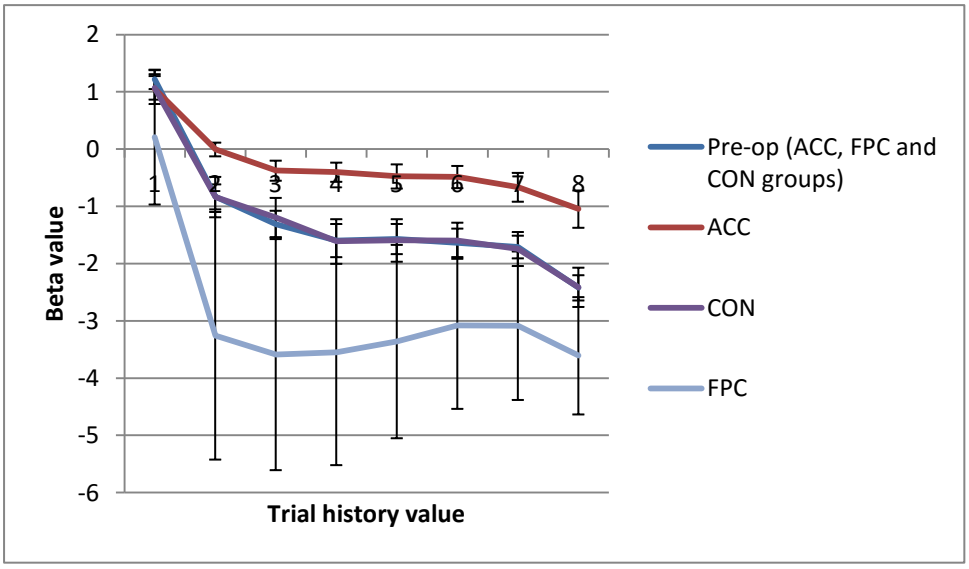


Figure 17 – B values at THV 1-8 for ACC, FPC and CON groups, with error bars showing the +/- standard error of the mean.

In order to analyse which THVs were contributing to this variance, I conducted one sample t-tests on all pre-operative β values. These t-tests found that error outcomes at all THVs significantly influenced pre-operative behaviour (THV 1: $t(26)$=-9.614, p<0.001; THV 2: $t(26)$=4.645, p<0.001; THV

3: *t(26)*=6.776, *p*<0.001; THV 4: *t(26)*=6.902, *p*<0.001; THV 5: *t(26)*=7.444, *p*<0.001; THV 6: *t(26)*=8.121, *p*<0.001; THV 7: *t(26)*=9.785, *p*<0.001; THV 8: *t(26)*=11.752, *p*<0.001). However, since errors at THV 1 had a positive influence on future behavioural choices while all other errors had a negative influence, it is likely that these influences operate by different processes; THV 1 β values were thus analysed separately from β values relating to higher THVs.

A two-way repeated measures ANOVA with one within-subjects factor of 'Stage of testing' (two levels: pre-operative and post-operative) and one between-subjects factor of 'Group' (six levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control) was conducted on β values associated with THV 1 (corresponding to i-1, the most recent trial). This analysis found a significant effect of stage of testing (*F(1,21)*=5.660, *p*=0.027), but no significant interaction between stage of testing and group (*F(5,21)*=1.219, *p*=0.335).

A three-ways repeated measures ANOVA was conducted on β values relating to higher THVs with two within-subjects factors, 'THV' (7 levels: i-2 to i-8) and 'Stage of testing' (two levels: pre-operative and post-operative), and one between-subjects factor of 'Group' (6 levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control) (i.e. excluding the data relating to i-1, the most recent trial, but including all other data). This analysis detected a significant main effect of THV (*F(6,126)*=51.497, *p*<0.001; the Greenhouse-Geisser correction was applied in order to compensate for a violation in the assumption of sphericity and the associated corrected degrees of freedom were *F(1.648,34.598)*) and a significant interaction effect of THV and stage of testing (*F(6,126)*=3.727, *p*=0.025; the Greenhouse-Geisser correction was applied and the associated corrected degrees of freedom were *F(2.366,49.676)*), but no significant main effect of group, nor any significant interaction effect including the factor 'Group'.

Two-way repeated measures ANOVAs with one within-subjects factor of 'Stage of testing' (two levels: pre-operative and post-operative) and one between-subjects factor of 'Group' (six

levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control) were conducted separately relating to data for THV 2, 3, 4, 5, 6, 7 and 8. At THV 2, 3, 4, 5, 6 and 7, these analyses found no significant main effect of group or stage of testing, and no group*stage of testing interaction effect. However, at THV 8, a significant main effect of stage ($F(1,21)$=10.445, p=0.004) was found and a significant interaction effect was detected between group and stage of testing ($F(5,21)$=4.624, p=0.005); this effect survived correction for multiple comparisons using the Holm-Bonferroni method, but was not explored further as it is not plausible to consider an isolated influence of the eighth most recent trial on behaviour.

Error trials – first 5 trials in block

All analyses described in this section include data from all nine control animals. In order to further examine whether any group effects can be discovered relating to the influence of previous error trials on behaviour, a logistical regression analysis was carried out including only data relating to the first five trials in each block. This analysis excluded errors made in the middle of blocks, which were likely to occur due to a lapse in concentration on the part of the animal, and only considered trials in which it was likely that the monkey had yet to adjust to a change in the rewarded rule. This data is presented in figure 18 (in this figure, pre-operative data from all groups is amalgamated into one series; they are considered separately in the ANOVA described below). Figure 19 presents this data for ACC, FPC and CON groups. These figures include data from all nine control animals.

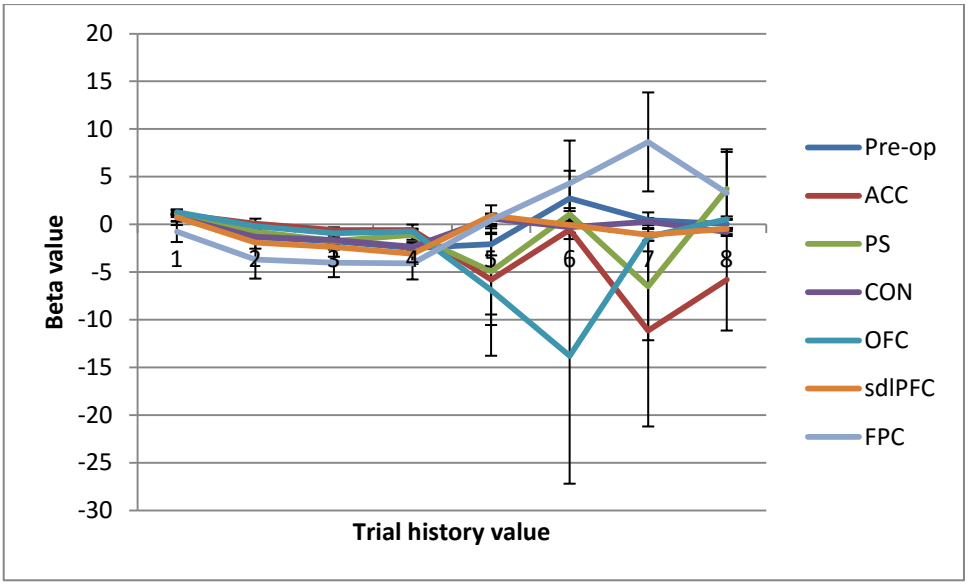


Figure 18 – Pre- and post-operative β values at THV 1-8 for all groups, with error bars showing the +/- standard error of the mean.

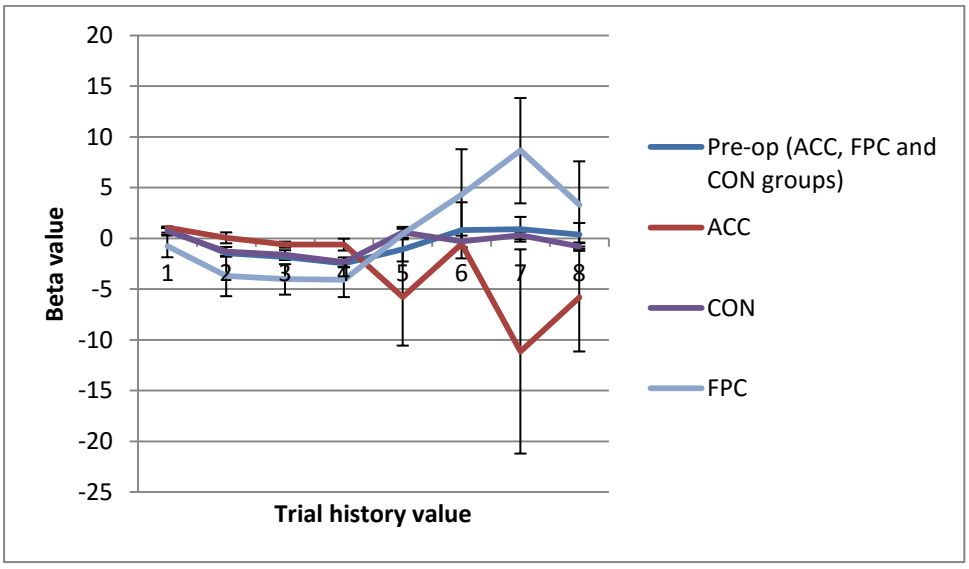


Figure 19 – Pre- and post-operative β values at THV 1-8 for ACC, FPC and CON groups, with error bars showing the +/- standard error of the mean.

A three-way repeated measures ANOVA was conducted on these β values with two within-subjects factors, 'THV' (8 levels: i-1 to i-8) and 'Stage of testing' (two levels: pre-operative and post-operative), and one between-subjects factor of 'Group' (6 levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control). This analysis did not find any significant effects once results were corrected for violations in the assumption of sphericity. However, a one-sample t-test conducted on pre-operative β values for all THVs found that, when only the first five trials of

each block were considered, unoperated animals were significantly influenced only by the four most recent trials (THV 1: $t(26)$=-4.674, p<0.001; THV 2: $t(26)$=6.511, p<0.001; THV 3: $t(26)$=7.489, p<0.001; THV 4: $t(26)$=9.454, p<0.001); each of these results survived correction for multiple comparisons using the Holm-Bonferroni method. As errors at more distant THVs did not significantly affect behaviour pre-operatively (these errors would always have taken place in the preceding block), data relating to these were excluded from further analysis.

A three-way repeated measures ANOVA was conducted on these β values with two within-subjects factors, 'THV' (4 levels: i-1 to i-4) and 'Stage of testing' (two levels: pre-operative and post-operative), and one between-subjects factor of 'Group' (6 levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control). This analysis did not find any significant main effect of group, or any interaction effect between group and stage of testing or group and THV, although a significant main effect of THV ($F(3,63)$=272.163, p<0.001) was found and a significant interaction effect was detected between stage of testing and THV ($F(3,63)$=4.356, p=0.007).

Figure 20 shows the β values for THV 1-4 for all groups (in this figure, pre-operative data from all groups is amalgamated into one series; they are considered separately in the analysis described above). Figure 21 shows this data for ACC, FPC and CON groups.

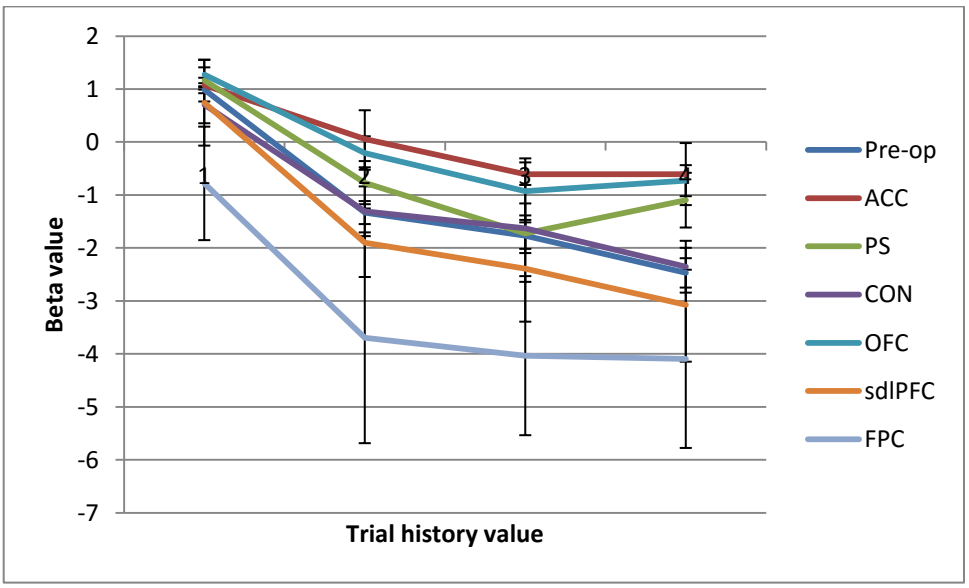


Figure 20 – Pre- and post-operative β values at THV 1-4 for all groups, with error bars showing the +/- standard error of the mean at each data point

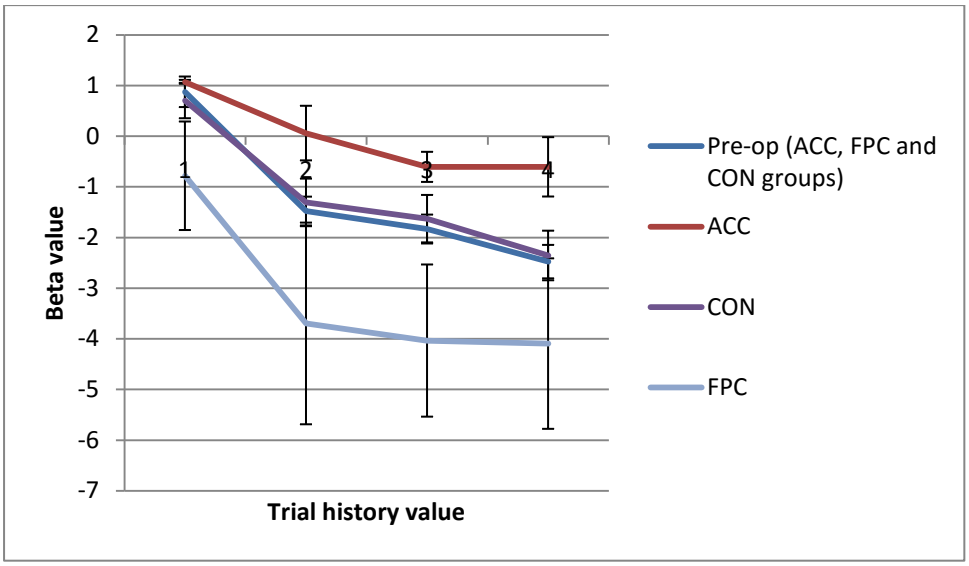


Figure 21 – Pre- and post-operative β values at THV 1-4 for ACC, FPC and CON groups, with error bars showing the +/- standard error of the mean at each data point.

As in the previous analysis, errors made at THV 1 had a positive influence on likelihood of a monkey choosing to follow the same rule again on the current trial, while errors made at higher THVs had a negative influence. Since it was likely that different processes were contributing to these effects, separate analyses were conducted to examine the effect of errors at THV 1 and errors at more distant THVs (2-4).

A two-way repeated measures ANOVA with one within-subjects factor of 'Stage of testing' (two levels: pre-operative and post-operative) and one between-subjects factor of 'Group' (six levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control) was conducted on β values associated with THV 1 (the most recent trials). This analysis found no significant main effect of stage of testing or group, and no interaction between stage of testing and group.

A three-ways repeated measures ANOVA was conducted on β values associated with THVs 2-4 with two within-subjects factors, 'THV' (3 levels: i-2 to i-4) and 'Stage of testing' (two levels: pre-

operative and post-operative), and one between-subjects factor of 'Group' (6 levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control). This analysis found a significant main effect of THV ($F(2,42)$=32.294, p<0.001) and a significant interaction effect between THV and stage of testing ($F(2,42)$=3.842, p=0.029), but no significant effects relating to group. Subsequent two-way ANOVAs including only data relating to THVs 2, 3 and 4 (each considered separately) found no significant effect relating to experimental group, and no significant effect relating to stage of testing which survives corrections for multiple comparisons using the Holm-Bonferroni method.

Logistic regressions – summary

In summary, the following group differences were detected as a result of these logistic regression analyses:

- When all trials are considered, the effect of a rewarded response to one rule in the most recent trial had a large positive influence on the likelihood of a monkey choosing to follow the same rule in the next trial in pre-operative and in control animals. However, this effect was reduced in ACC-lesioned and OFC-lesioned animals.
- When all trials are considered, the effect of a rewarded response to one rule in the third most recent trial had a small positive influence on the likelihood of a monkey choosing to follow the same rule in the next trial in pre-operative and control animals; however, this effect was reversed in FPC-lesioned animals, who were negatively influenced in rule-choice by a rewarded outcome in the third most recent trial.
- When all trials are considered, the effect of a non-rewarded response to one rule was significantly affected by an interaction of stage of testing, group and recency of the response; however, it was not possible to isolate the source of this interaction.

Uncertainty

In order to assess whether any of the impairments identified above were amplified in cases of greater uncertainty, I compared the differences between pre- and post-operative β values calculated between the early-block and full-block analyses, for both rewarded and non-rewarded outcomes.

A three-ways repeated measures ANOVA [(two within-subjects factors of 'Analysis' (two levels: all trials in block and first 10 trials in block) and 'THV' (four levels: i-1 – i-4) and one between-subjects factor of 'Group' (six levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control)] was conducted on the β values following rewarded trials. This analysis included data from all nine control animals. Only the first four levels of trial history value was included, as no values relating to more distant trials had found to be significantly different to zero. This ANOVA found significant main effects of THV ($F(3,63)$=5.517, p=0.010; the Greenhouse-Geisser correction was applied and the associated corrected degrees of freedom were $F(1.760,36.958)$) and group ($F(5,21)$=4.137, p=0.009), and a significant interaction effect between THV and group ($F(15,63)$=3.169, p=0.006; the corrected degrees of freedom were $F(8.799,36.958)$ after the Greenhouse-Geisser correction was applied). However, no significant main effect of 'Analysis', or any interaction effect including this term, was detected. Similarly, a three-way repeated measures ANOVA [two within-subjects factors of 'Analysis' (two levels: all trials in block and first 10 trials in block) and 'THV' (four levels: i-1 – i-4) and one between-subjects factor of 'Group' (six levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control)] was conducted on the β values following error trials. Trial history values greater than four were excluded, as only outcomes at more recent THVs significantly influenced behaviour in the early-block analysis. This ANOVA found a significant main effect of THV ($F(3,63)$=6.033, p=0.001) and a significant interaction effect between THV and group ($F(15,63)$=2.764, p=0.003). However, no significant main effect of 'Analysis', or any interaction effect including this term was detected.

Concrete properties of stimuli

The analysis detailed above assumes that monkey choices are affected by the reward history of previous trials solely in terms of the abstract rule that they applied in that trial. However, it is possible that monkeys are also affected by the concrete properties (i.e. those properties which are available to external observation of a trial, without reference to previous knowledge or reasoning) of the rewarded or non-rewarded stimulus that they chose in the previous trial. These properties varied in three dimensions: shape, colour and location. It is possible that the group effects observed earlier in this study differ in nature due to monkeys responding to concrete properties of stimuli rather than the abstract rule applied in each trial; examining the effects of concrete properties of chosen stimuli may clarify if this is the case.

Information regarding reaction times, the proportion of correct trials, perseverative error trials (i.e. trials in which the monkey touched the stimulus which matched the test stimulus in the wrong dimension) and non-perseverative error trials (i.e. trials in which the monkey touched the stimulus which did not match the test stimulus in either the shape or colour dimension) in shape and colour blocks, and the number of correct, perseverative and non-perseverative error responses to each test stimulus shape had been collected and previously analysed for this data set. However, information regarding the actual shape, colour and location of the stimulus chosen by monkeys for each trial of the experiment had not been collected, so it had not been previously possible to analyse whether the animals were influenced by the concrete properties of the stimuli in each trial. In order to collect this information, I adapted the software used to run the experiment in Oxford and RIKEN so that it was possible to run a simulation of each session performed by each animal. This made it possible to determine the previously unrecorded information as to the location, shape and colour of the correct, perseverative and non-perseverative error responses in each trial; when this information was combined with the data files collected during the experiment, I could establish the concrete properties of each stimulus chosen by monkeys during every trial of the experiment.

Since there were three possible choices of location in each trial, and six possible choices of colour and shape (of which only three were available at each trial), it was not possible to conduct a binary logistic regression on this dataset. However, it was possible to assess whether, for each trial, the monkey chose an object with the same concrete properties as the object on the previous trial, or an object with different concrete properties. The chance of a monkey randomly selecting an object with the same location as that selected in the previous trial was 33%; the chance of a monkey randomly selecting an object with the same colour as the object selected in the previous trial was 16.7%, and the chance of a monkey randomly selecting an object with the same shape as the object selected in the previous trial was also 16.7%. Since the likelihood of a monkey repeating the same colour or shape was low, a multinomial logistic regression approach was not an appropriate way to analyse this data.

Responses were then classified into four categories: a repeated choice of a stimulus with concrete properties rewarded on the previous trial, a repeated choice of a stimulus with concrete properties unrewarded on the previous trial, a non-repeated choice of a stimulus with concrete properties rewarded on the previous trial and a non-repeated choice of a previously non-rewarded concrete property. Trials in which the monkey did not provide a response, trials immediately following trials in which the monkey did not provide a response, and the first trial of each session were excluded. This data was then used to calculate the percentage of rewarded trials which were followed by a repeated choice of a stimulus with the same concrete properties, compared to the percentage of non-rewarded trials which were followed by a repeated choice of a stimulus with the same concrete properties. This was calculated separately for colour, shape and location.

Location

The probability of a monkey randomly selecting the same location on two consecutive trials was 33%. A two-way repeated measures ANOVA [one within-subjects factor of 'Stage of testing' (two levels: pre-operative and post-operative); one between-subjects factor of 'Group' (six levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control)] was conducted on the difference between arcsine-transformed percentages of repeated trials to the same location following rewarded trials and arcsine-transformed percentages of repeated trials to the same location following non-rewarded trials. This analysis found a significant main effect of group ($F(5,21)$=3.889, p=0.012) and a significant interaction effect between stage of testing and group ($F(5,21)$=4.441, p=0.006). This data is presented in figure 22. Data from all nine control animals was included in this analysis, and is included in this figure.

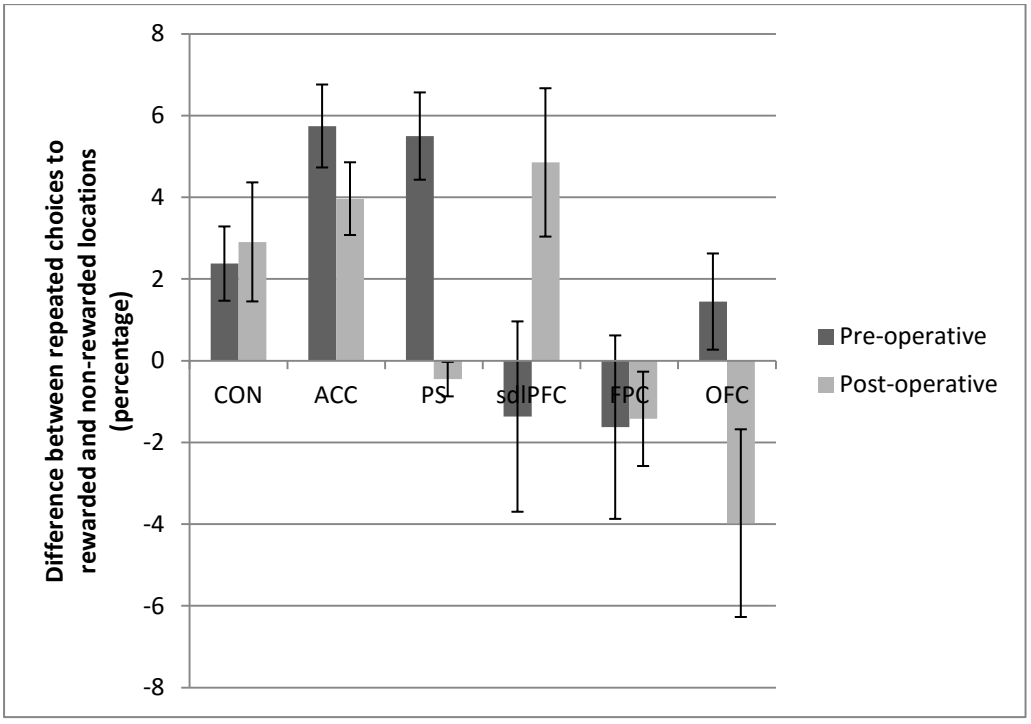


Figure 22 – Difference between percentage of repeated choices to rewarded and non-rewarded locations for all groups, with error bars showing the +/- standard error of the mean.

Planned comparisons between the ACC-, PS-, OFC-, FPC-lesioned groups and control animals, and between FPC-lesioned groups and ACC-lesioned groups revealed significant differences between PS-lesioned animals and controls ($F(1,21)$=7.886, p=0.011) and between OFC-lesioned animals and

control animals (*F(1,21)*=4.347, *p*=0.0495) on percentage of repeated choices to non-rewarded locations post-operatively. Data from all nine control animals was included in the ACC vs. CON, PS vs. CON and FPC vs. CON comparisons; however, in the OFC vs. CON comparison, only six control animals were considered and those animals which were tested originally as controls and then included in the OFC group and retested were excluded. However, a one-way ANOVA conducted on the pre-operative data [one between-subjects factor of 'Group' (six levels: ACC-lesioned, PS-lesioned, sdlPFC-lesioned, OFC-lesioned, FPC-lesioned and control)] found a significant effect (*F(5,21)*=4.426, *p*=0.007), confirming that significant differences were already present between groups pre-operatively (data from all nine control animals was included in this ANOVA). However, when independent samples t-tests were used to compare pre-operative PS and OFC data with pre-operative control data (data from all nine control animals was included in the PS vs. CON comparison; data from the three control animals later retested in the OFC-lesion group was excluded from the OFC vs. CON comparison), neither experimental group was found to differ significantly from controls (PS vs. CON: *t(11)*=2.072, *p*=0.063, OFC vs. CON: *t(7)*=-1.129, *p*=0.296).

It is possible that the difference in likelihood in repeating a choice of location after a rewarded or non-rewarded response to that location is due to differing levels of sensitivity across groups post-operatively to accidentally reinforcement of a location. In order to examine this, I analysed the likelihood of repeating a choice to a location after two consecutive rewarded responses to that location. A two-way repeated measures ANOVA on this data [one within-subjects factor of 'Stage of testing' (two levels: pre-operative and post-operative), and one between-subjects factor of 'Group' (six levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control)] found no significant main effects of either stage of testing or experimental group, and no significant interaction effect between stage of testing and experimental group. This data is presented in figure 23. Data from all nine control animals was included in the above ANOVA and in the following figure.

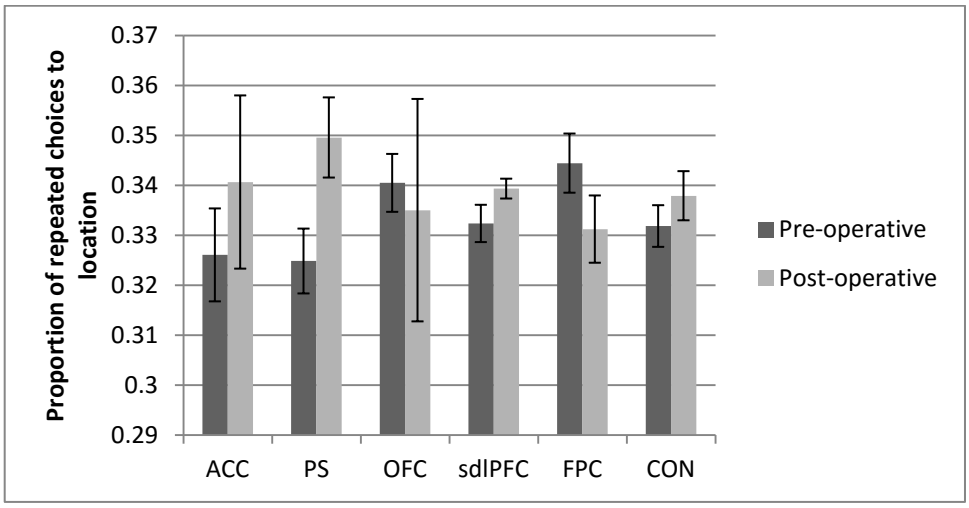


Figure 23 – Proportion of repeated choices to a location after two rewarded choices to that location, pre- and post-operatively for all groups, with error bars showing the +/- standard error of the mean.

Colour

The probability of a monkey randomly selecting the same colour on two consecutive trials was 16.7%. I examined the effect of a previously rewarded or previously non-rewarded choice of a stimulus of a particular colour on the likelihood of a monkey choosing a stimulus of the same colour in the next trial (this was only possible on 50% of trials, on average, as, of the six possible colours of stimuli used in this experiment, only three were used on any one trial). This data is presented in figure 24; this figure includes data from all nine control animals.

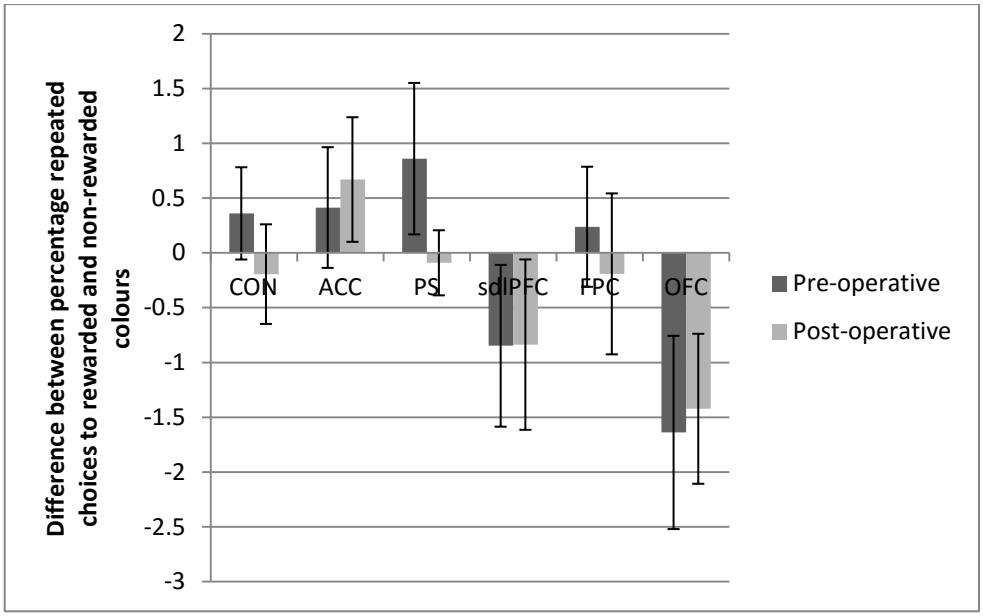


Figure 24 – Difference between percentage of repeated choices to rewarded and non-rewarded colours for all groups, with error bars showing the +/- standard error of the mean.

A two-way repeated measures ANOVA on this dataset [one within-subjects factor of 'Stage of testing' (two levels: pre-operative and post-operative) and one between-subjects factor of 'Group' (six levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control)] found no significant effects. Data from all nine control animals was included in this ANOVA. No further analyses were carried out on this dataset.

Shape

The probability of a monkey randomly selecting the same shape on two consecutive trials was 16.7%. I examined the effect of a previously rewarded or previously non-rewarded choice of a stimulus of a particular shape on the likelihood of a monkey choosing a stimulus of the same shape in the next trial (this was only possible on 50% of trials, on average, as, of the six possible shapes of stimuli used in this experiment, only three were used on any one trial). This data is presented in figure 25. A two-way repeated measures ANOVA on this dataset [one within-subjects factor of 'Stage of testing' (two levels: pre-operative and post-operative) and one between-subjects factor of 'Group' (six levels: ACC-lesioned, PS-lesioned, OFC-lesioned, sdlPFC-lesioned, FPC-lesioned and control)]

found no significant effects. No further analyses were carried out on this dataset. Data from all nine control animals was included in this ANOVA and is represented in figure 25.

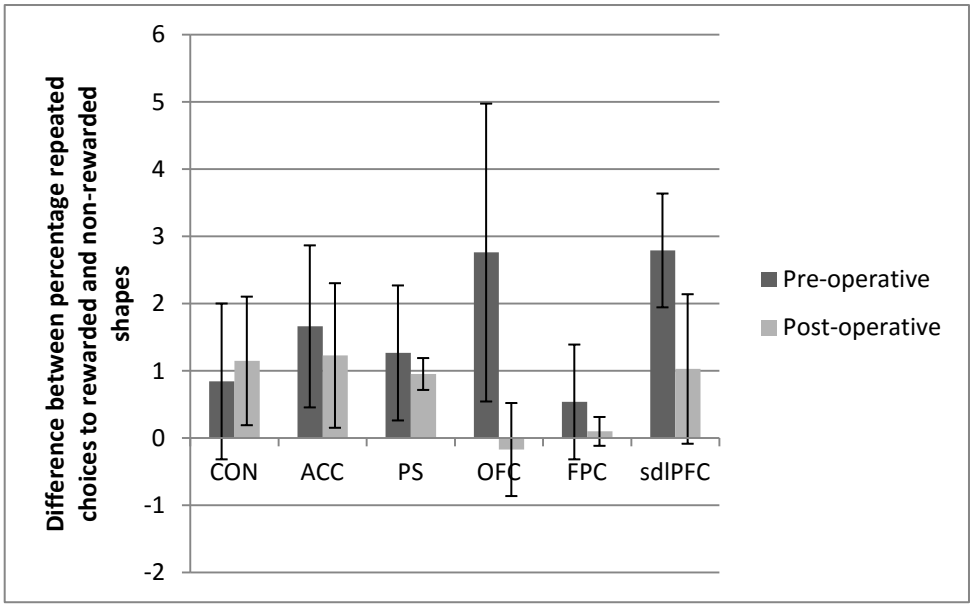


Figure 25 – Difference between percentage of repeated choices to rewarded and non-rewarded shapes for all groups, with error bars showing the +/- standard error of the mean.

Concrete properties – summary

- No statistically significant effects were found relating to monkeys' likelihood of repeating a choice of a particular colour or shape.
- OFC-lesioned animals were more likely to repeat a choice of a particular non-rewarded location relative to preoperative and to control animals.

Discussion

This study repeated the findings of a previous paper (Buckley et al., 2009) in observing that ACC-, PS- and OFC-lesioned animals make more errors on this task than control animals. Lesions to FPC and sdlPFC, by contrast, do not result in a general impairment on this task. However, in general, the first of the hypotheses discussed in the introduction to this chapter was not supported by analyses of this data. FPC-lesioned animals only differed significantly from controls in one analysis: they were significantly less influenced by a rewarded outcome in the third most recent trial than

control animals. Contrary to the theory suggested by Kovach et al. (2012), FPC-lesioned animals did not show a significant reduction in the influence of the most recent trial on behaviour. Moreover, although this difference was not statistically significant, FPC-lesioned animals showed an increase in the influence exerted on behaviour by a rewarded outcome in the most recent trial post-operatively and in comparison to controls (figure 10). This result may suggest an explanation for the finding that the influence exerted on behaviour by a rewarded outcome in the third most recent trial is significantly more negative in FPC-lesioned animals relative to controls and to pre-lesion animals: if animals place a very high weighting on the outcome of the most recent trial, rather than the cumulative reward history in the experiment, this may be reflected in the data by a reduction in the weightings associated with trials further back in the sequence. However, the lack of any significance of the numerical trend associated with the increase in the influence of the most recent trial in FPC-lesioned animals renders this explanation speculative at present.

If this trend is robust, it may help to explain why patients and animals with FPC lesions are not impaired on the WCST: following the rule that the most recent outcome suggests is rewarded is all that is necessary for success on the this task. However, in more ambiguous situations, it is necessary to take into account the outcomes of a more extended history of previous trials in order to calculate the optimal response. It is in these kinds of complicated and ambiguous scenarios that activations to FPC tend to be reported in human subjects (e.g. Boorman et al., 2011, Daw et al., 2006) and human patients tend to experience impairments (Burgess et al., 2000; in this study, patients were required to weigh the rewards associated with completing various tasks and use this information in order to decide how to optimally allocate time). While this explanation is at odds with that the results reported by Kovach et al. (2012), it may be possible to explain the results found by those researchers in a different way: as noted in the introduction, the FPC-lesion group in that study was poorly-controlled. As the lesion overlap in that group included large regions of the prefrontal cortex, it may be the case that the observed deficit in the FPC-lesion group was, in fact, due to an impairment in a function such as credit assignment (associating a rewarded outcome with the choice

which led to it) or rapid updating of rule-value based on rewards, both of which are thought to be dependent on the orbitofrontal cortex (e.g. Walton et al., 2010; Walton et al., 2011, Buckley et al., 2009). If the patients studied by Kovach et al. were delayed in rapid updating of rule-value, they may not have been able to assimilate information relating to the most recent choice outcome before they were required to make a choice in the next trial.

The second hypothesis of this study, that the pattern of influence of recent trials on the behaviour of ACC-lesioned animals would be abnormal, was supported by this data. ACC-lesioned monkeys were significantly less influenced by a rewarded outcome in the most recent trial, relative to pre-operative monkeys and to controls. This evidence supports the conclusions of Kennerley et al. (2006), who found that ACC-lesioned monkeys were less influenced by the reward history of past actions post-operatively and indicates that these results may be generalisable across tasks. While Kennerley et al. (2006) found that ACC-lesioned macaques were not impaired in their ability to respond to the outcome of the most recent trial, but were less influenced by the outcomes of more distant trials, a result which was not replicated in this study, the differing task demands between these experiments may provide an explanation for this variance. In the study described here, macaques performed better if they privileged the outcome of the most recent trial, while in the Kennerley study, macaques performed better if they considered this outcome in the context of a history of reward outcomes. This was reflected in the observation of much higher pre-operative and control β values for the most recent trial in this study than in the Kennerley study (average pre-operative β value at i-1 was 4.88 in this study; in the Kennerley study, these values were in the range of 0.5 – 0.7). It may, therefore, be the case that the special weighting observed in the Kovach et al. (2012) study and here for the most recent outcome in behaviour was not a process which macaques used in order to carry out the behavioural task in the Kennerley study.

Furthermore, this result may clarify the deficit suffered by ACC-lesioned monkeys in this task, as observed by Buckley et al. (2009) and Tanaka et al. (2013). The decreased sensitivity of ACC-lesioned monkeys to the most recent rewarded outcome are not easily explained within the current framework, which states that monkeys with lesions to ACC may be less sensitive to uncertain situations, and thus do not undertake a checking process in order to avoid errors. Similarly, Kuwabara et al. (2014) found that monkeys with ACC lesions did not exhibit slowed responses after error trials, and that neurons in ACC were sensitive to previous error trials. These researchers suggested that the ACC is more active in response selection in cases of elevated error-likelihood (in particular, trials immediately following error trials). If this were the sole cause of the observed deficit on this experiment, we would expect errors to be randomly distributed and for monkeys to be generally less reliable in their responses, especially in conditions of greater uncertainty (after error trials, and at the beginnings of blocks). This would be expressed in generally reduced β values for both rewarded and non-rewarded outcomes (although with a greater effect after non-rewarded outcomes), across all trial history values, and especially in the first few trials of each block. However, data collected from ACC-lesioned animals did not show this pattern of generally reduced β values: when the influence of rewarded outcomes on behaviour was examined, monkeys showed a reduced influence of the most recent trial on behaviour, but did not differ from controls when the influence of more distant trials was examined. Furthermore, the effects which were observed from ACC lesions after rewarded outcomes were not increased when only trials undertaken in greater levels of uncertainty (in the early stages of each block) were considered. In this respect, the observed results were more similar to those of FPC-lesioned patients studied by Kovach et al. (2012), who did not differ from controls in terms of sensitivity to the general cumulative history of reward, but differed from controls in that they did not assign an additional high weighting to the outcome of the most recent trial. The results of this study do not indicate that ACC lesions cause animals to be differentially influenced by error trials and they do not support the conclusion that ACC-lesioned

animals are differentially influenced by the outcome of previous trials in situations in which errors are more likely (i.e. situations of greater uncertainty, such as in the first few trials of blocks).

The third hypothesis of this study, that the sensitivity to reward history measured in ACC- and FPC-lesioned animals would be dissociable, was entirely supported by the results of this study. ACC- and FPC-lesioned monkeys differed significantly in the weight of influence observed in the first trial after a reward, whether the whole data-set or only the first ten trials of each block were considered. In no case did the ACC- and FPC-lesion group share a significant difference from control animals.

Additional differences between groups which were not predicted by the hypotheses of this experiment were observed for OFC-lesioned monkeys. These animals showed a reduced influence of the most recent rewarded trial on behaviour, similar to that observed in ACC-lesioned monkeys. These animals were also more likely to repeat a choice of a non-rewarded location relative to control animals. These results are consistent with the theory proposed by Buckley et al. (2009), which states that OFC-lesioned animals are impaired at rapid updating of rule-value based on rewards received. If monkeys are unable to ascertain which abstract rule is rewarded after one trial, the influence of the most recent rewarded trial on behaviour will be reduced. The other results reported here may indicate that, while monkeys are uncertain of the rewarded abstract rule, they may revert to the application of simpler concrete rules, such as perseveration at one location. A recent study into the role of the OFC in the monkey WCST-analogue task (Mansouri et al., 2014) suggests that monkeys with lesions to this area may be impaired at resolving conflict between abstract rules; this was not assessed in this study, as the level of conflict was not varied across trials (all trials contained one stimulus that matched the target in shape, and one stimulus that matched the target in colour); however, this is a plausible explanation for the deficit observed in OFC-lesioned animals in this study.

This study repeats the findings of a previous experiment (Buckley et al., 2009) in observing that lesions to the ACC, PS and OFC result in deficits to the WCST-analogue task in non-human primates, while lesions to the FPC and sdlPFC cause no general impairment in this task. It also supports the findings of Kennerley et al. (2006), who suggest that monkeys with lesions to ACC may be less influenced by the reward history of previous trials in making responses to a behavioural task. However, it extends the findings of these previous studies by suggesting that ACC-lesioned monkeys are not generally impaired at calculating future responses on the basis of the reward history of previous trials, but that they fail to privilege the outcome of the most recent trial over the outcome of more distant trials when making a response. This study also helps to clarify the role of the FPC in macaque decision-making. Contrary to theories suggested by previous human studies (Kovach et al., 2012), macaques with lesions to this area are not insensitive to the outcome of the most recent rewarded trial. However, they may prioritise information related to these recent outcomes over outcomes from the more distant past in a way which does not cause deficits in a fixed-outcome task such as the WCST, but which may provide an explanation for deficits experienced by FPC patients in more ambiguous tasks, such as the Hotel task (in which patients must sample each of several tasks, and weigh the outcomes associated with each of them against each other in deciding how best to allocate a limited amount of time).

CHAPTER FOUR

The role of the macaque frontopolar cortex in social valuation

Previous studies have suggested that the medial frontopolar cortex (mFPC) has a role in human social cognition. It has been demonstrated that lesions to the anterior cingulate gyrus impair social valuation in macaque monkeys (Rudebeck et al., 2006). The anterior cingulate gyrus is interconnected with the macaque FPC (Petrides and Pandya, 2007); however, no one has as yet tested the role of the FPC in social valuation in macaque monkeys. We tested response latencies of seven macaque monkeys to food rewards while they were exposed to social, fear-inducing and neutral distracting stimuli. After focal lesions to the frontopolar cortex, four monkeys showed shorter reaching latencies for food rewards while being distracted by social stimuli, but not for fear-inducing or neutral stimuli compared to pre-operative reaching latencies; however, this reduction in reaching latencies for food rewards while being exposed to social stimuli was not significantly different to the reaching latencies of control animals tested on this task before and after a delay.

Introduction

Human neuroimaging studies have repeatedly associated the medial area of the FPC with various aspects of social cognition, including Theory of Mind and reporting on one's own and other's opinions (e.g. Oschner et al., 2004, Gusnard et al., 2001, Simons et al., 2005b). Amodio and Frith (2006), in a meta-analysis of studies relating social cognition and the medial frontal cortex, associated activity in medial BA10 with tasks in which participants were asked about their own traits or affective responses ("self-knowledge"), viewed faces or reported whether behaviours or adjectives could relate to people ("person perception"), or represented the psychological perspective of another person ("mentalising"). This correlational evidence has also been supported by evidence that patients with damage to this region perform poorly on some tests of social cognition, such as recognition of socially inappropriate statements (the "Faux Pas" test; Roca et al.,

2011), emotional self-awareness (Hoffmann and Bar-On, 2012) and sensitivity to embarrassing situations (Bird et al., 2004).

Patients with damage to medial BA10 consistently show only a mild impairment in social cognition. Bird et al. (2004) examined a patient with extensive damage to the medial frontal lobe, including medial area 10. This patient scored in the normal range on almost every test of Theory of Mind: for example, arranging pictures into a story (comprehension of which required appreciation of the mental states of the characters); answering questions which required her to infer the thoughts, feelings or motivations of characters in a story; choosing whether to use Theory of Mind or physical descriptions of the interactions of two shapes in an animation; and understanding when a character in a story makes a statement which is socially inappropriate (the "Faux Pas" test). In one test, in which the patient was asked to rate whether situations were or were not embarrassing, the patient rated fewer situations as embarrassing than controls did. However, this was not a large impairment: the patient rated 10 out of 14 potentially embarrassing situations as embarrassing, while two-thirds of controls rated all 14 situations as embarrassing. The results found by Hoffmann and Bar-On (2012) and Roca et al. (2011) were similarly ambiguous. The patient tested by Hoffmann and Bar-On (2012) scored 86 in a test of emotional self-awareness, rather than in the normal range of 90-109, showing only a slight impairment. Roca et al.'s FPC-lesioned group were significantly impaired compared to healthy controls on the "Faux Pas" test, but were not impaired compared to controls with brain damage outside of FPC.

These findings suggest that the impairment suffered by FPC patients is not related to social information processing, as they are unimpaired when asked to analyse social information. Rather, they seem to rate information relating to their own or other's emotions in a social situation as slightly less important: they are less sensitive to embarrassment, less concerned about the giving of offence and less emotionally self-aware. However, it is very difficult to draw conclusions from human neuropsychological data regarding the function of the FPC as the deficits reported so far

have been very mild and the wide-ranging nature of the damage received by patients in these studies limits the conclusions which it is possible to draw regarding the contribution of individual regions of prefrontal cortex to these deficits.

Previous research has established the contribution of the anterior cingulate gyrus (ACCg) to social cognition (Rudebeck et al., 2006) in macaque monkeys. This study found that macaques with neurosurgical ablations to ACCg had shorter reaching latencies to food rewards when these were placed in front of videos of other monkeys compared to reaching latencies in intact controls and monkeys with lesions to orbitofrontal cortex, although monkeys with lesions to orbitofrontal cortex showed a reduced reaching latency when the food reward was presented on a box containing a fear-inducing stimuli (a toy snake). However, this finding of reduced sensitivity to fear-inducing stimuli in OFC-lesioned macaques was disputed by Rudebeck et al. (2013). A subsequent study using the same paradigm (Noonan et al., 2010) found that monkeys' reaching latencies to either social or fear-inducing stimuli did not reduce after lesions to medial orbitofrontal cortex. Projections have been found from the macaque FPC to ACCg (Petrides and Pandya, 2007; Barbas et al., 1999) and from the macaque ACCg to FPC (Barbas et al., 1999), indicating that these areas may interact as part of a frontal network of social cognition. However, no published research has examined the effect of frontopolar lesions in monkeys, probably due to difficulty in surgically targeting this area. There is controversy regarding the extent to which the frontopolar region in monkeys is homologous with the human FPC (Neubert et al., 2014); however, the monkey FPC shares cytoarchitectonic features and connectivity profile with at least the medial area of human FPC (Semendeferi et al., 2001; Neubert et al., 2014).

We used a well-established paradigm (Rudebeck et al., 2006; Noonan et al., 2010) to examine social valuation in four female rhesus macaque monkeys, prior to and after focal FPC lesions, and compared the pattern of behaviour recorded with that observed in three control animals (although one control animal did not undergo the second period of testing on this task; as a

result, only two control animals received both periods of testing on this task). This paradigm assesses sensitivity to distracting social stimuli, but does not require animals to perform any task relating to these stimuli; it therefore examines attention to social information, rather than social information-processing. We predicted, on the basis of the human neuropsychological and neuroimaging evidence, that post-operative monkeys in the lesion group would show less sensitivity to social stimuli compared to their pre-operative behaviour and compared to monkeys in the control group on both the first and second periods of testing.

Materials and method

The materials and method used for this experiment were very similar to those reported in earlier studies using this paradigm (Rudebeck et al., 2006; Noonan et al., 2010) and so is described here using a very similar text in order to avoid ambiguity.

Subjects

Seven female rhesus macaque monkeys (*Macaca mulatta*) aged between 4 years and 1 month and 4 years and 6 months and weighing between 4.4 and 6.1 kg at the start of testing were tested in this study. Four animals received FPC lesions over the course of the experiment, and three were unoperated controls; however, one control animal received only one of the two periods of testing on this task. All seven animals lived together as a social group. All animals were maintained on a 12-h light–dark cycle and had 24-h *ad libitum* access to water, apart from when they were undergoing behavioural testing. All experiments were conducted in accordance with the United Kingdom Scientific Procedures Act (1986).

Surgery and histology

Surgery was conducted as described in Chapter 1. Histology figures for the four FPC-lesioned monkeys in this experiment are included in Chapter 1, figure 1.

Apparatus

All training and testing was conducted while the animals were in a transport cage inside a modified Wisconsin General Testing Apparatus (WGTA). A Plexiglas box measuring 70 × 11 × 11 cm with a hinged back was fixed to the WGTA 20 cm in front of the transport cage. In addition, behind the box, 50 cm from the front of the transport cage, was a PC monitor for presenting visual stimuli. On each trial, stimuli were presented either in the box or on the PC monitor. The stimuli presented in the box could be one of the following: 20 neutral control objects or two fear-inducing stimuli (a static rubber snake or a moving wooden snake). Stimuli presented on the screen were video clips 30s in length and were one of two human stimuli (two staring faces of humans unfamiliar to the animals), one of five social stimuli [a large (11 kg) male macaque staring, a male macaque (8 kg) exhibiting affiliative behaviours (lip-smacking), a male macaque (9 kg) inspecting a transport cage or a male monkey (5 kg) with food, and a female macaque (5 g) with prominent perineal swelling], or a moving, neutral control stimulus (a moving randomly changing coloured object). The video clips had previously been used in investigations of the effects of ACCg and medial orbitofrontal cortex lesions (Rudebeck et al., 2006, Noonan et al., 2010). The social stimuli were chosen because they were expected to elicit varying degrees of interest from control monkeys. Video stimuli were clips taken from longer videos of other monkeys in the colony recorded while they were in either transport cages or primate chairs. All the monkeys in the video stimuli were novel to the subjects. All videos were taken using either a Panasonic or Sony digital camera and edited using VHS video editing apparatus or computer software (Avidemux 2.6) allowing frame by frame analysis. Videos were played using Windows Media Player version 9.0. A camera mounted on top of the PC monitor recorded each monkey's behaviour. The camera faced the whole cage and allowed monkeys' latencies to take food rewards placed at the back of the Perspex box to be measured. Figure 1 shows the apparatus set-up for this experiment; figure 2 shows still images taken from each of the "social" video clips.

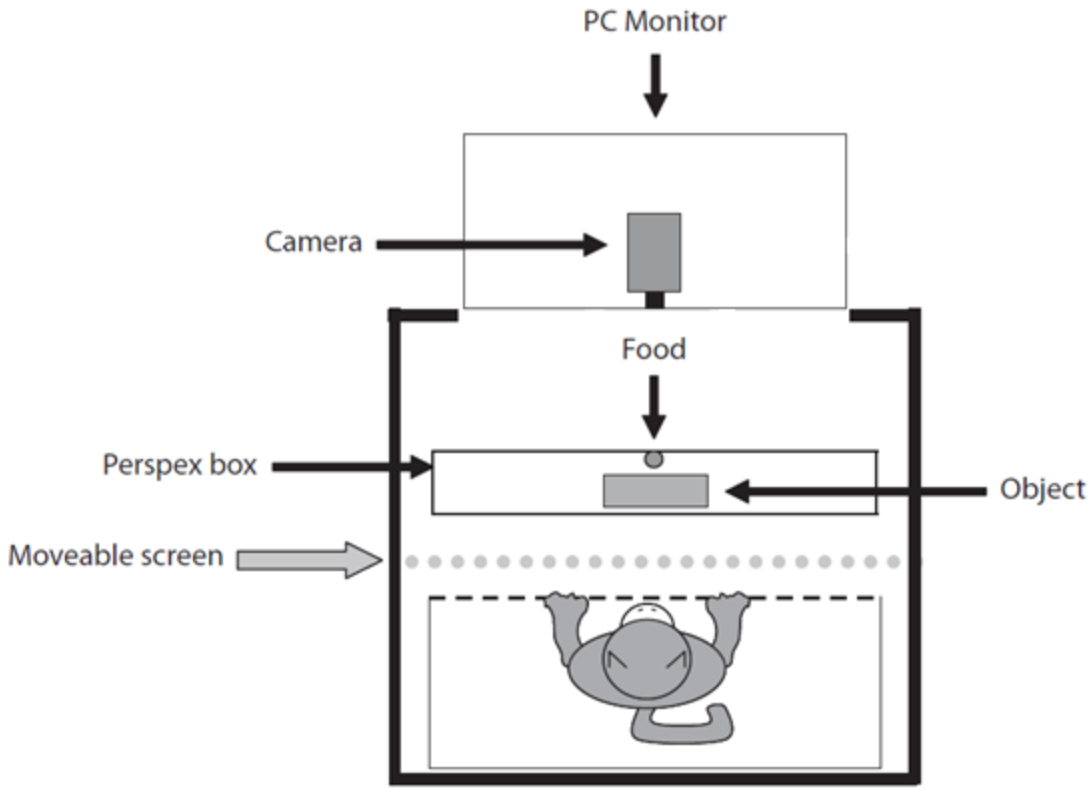


Figure 1 – WGTA testing apparatus. Monkeys sat in a transport cage facing the perspex box and PC monitor. On each trial the moveable screen was raised and animals could take the valued food item (peanut or a quarter of a date) placed at the far edge of the perspex box. After 30 seconds the screen was lowered regardless of whether the animal had taken the food or not. Adapted from Rudebeck et al. (2006).

Figure 2 – Individual frames taken from the five videos used as social stimuli that employed images of macaques. From left to right: monkey inspecting cage, monkey with food, monkey making affiliative gestures, female monkey perinea and staring monkey. From Rudebeck et al. (2006).

Procedure

Four macaque monkeys (*Macaca mulatta*) were tested on a social valuation task (Rudebeck et al., 2006; Noonan et al., 2010) before and after FPC lesions; two control animals were also tested twice on this task; the delay between the two testing periods was approximately three to six months. One further control animal was tested only once on the task. Briefly, animals were tested in the WGTA and on every trial the monkey retrieved a small food item that was placed in a fixed central position on the top of a transparent plastic box. Five short films of other macaques (detailed above) were used to investigate monkeys' responses to social stimuli. Two different emotive toy snakes (static and moving) were used to investigate fearfulness and two videos of humans staring were used to assess monkeys' responses to threatening human behaviour relative to comparable threatening behaviour in monkeys. Finally, responsiveness to neutral control objects was also measured in order to provide a baseline against which to compare any changes in fearfulness and social valuation. On each trial, stimuli were placed in the Perspex box or displayed on a screen behind the box. The food reward was located at the centre of the back edge of the box nearest the PC monitor so that during the actual test the animal would have to reach over anything in the box or as close as possible to the monitor. A small mark on the back of the box meant that the food reward was always put in the same place. Each trial began with the movable screen being raised. The screen stayed up for 30s regardless of whether or not the monkey took the food reward. The animal had 30s to retrieve the food item before an opaque moveable screen was lowered in between the animal and the box for the duration of a 30s inter-trial interval. During this period the experimenter could change the object in the box or image on the monitor and replace the food item. Before the screen was raised for the next trial, a curtain that obstructed the animal's view of the experimenter was fixed to the back of the WGTA. On each day, animals were exposed to ten different stimuli of possible social or emotional importance and 20 neutral objects. The test was repeated over four sessions (with a day of rest in between sessions) and the mean reaching latency for each stimulus per animal was calculated. Each stimulus was presented once per day. Objects in the box and images

on the screen were presented in a pseudo-random order, with the constraint that social stimuli or potentially fear-inducing stimuli were followed by neutral objects.

Habituation and reward preference test

Animals were first habituated to the testing environment and then trained to take food from the top of the Perspex box while it was empty.

For the test to assess emotional and social value of the different stimuli the food rewards had to be motivationally significant. We therefore needed to find a food highly valued by each individual animal. All animals were initially trained to take a single peanut food reward. A food reward was judged as motivationally significant if the animal took the food item from the back of the box in <5 s for 20 consecutive trials. Animals who did not reach this criterion with peanut food reward were trained to criterion with a quarter piece of a date. This food object was then used throughout the rest of training and testing. Over a further 3 days they were then trained to take their preferred food reward from the top of the box while any one of nine novel 'junk' objects were presented inside. Objects were presented in sets of five per day with each object being presented twice (10 trials). These 'junk' objects were not used subsequently during testing and instead further sets of novel junk objects were used in the interleaved control trials in the tests of emotion and social behaviour.

Data acquisition

Each trial was recorded on VHS video and analysed by one of four raters (H.B., C.P., M.N. and J.S.). Reaching latencies were measured from the beginning of the trial, as defined by the raising of the screen, to the time the animals first grasped the piece of food. Three sessions were scored by two scorers, and inter-rater reliability was high ($r=0.998\%$, $p=<0.001$). The average difference between two scores was 0.17s (range: 0 to 0.66s). In the case of a difference, the lower score was taken.

Data analysis

The start of each trial was initiated when the screen was raised above a fixed point marked on the side of the cage at approximately the same height as the top of the Perspex box. For the reaching latency measurement, the response was considered finished at the point just before the animal moved the food object from its initial position. If the animals did not retrieve the food reward within the 30s, a score of 30s was given. Latency measurements were scored to the nearest frame (0.04s).

Results

The results of these tests are shown for all stimuli in figure 3 and summarised across stimulus categories in figure 4:

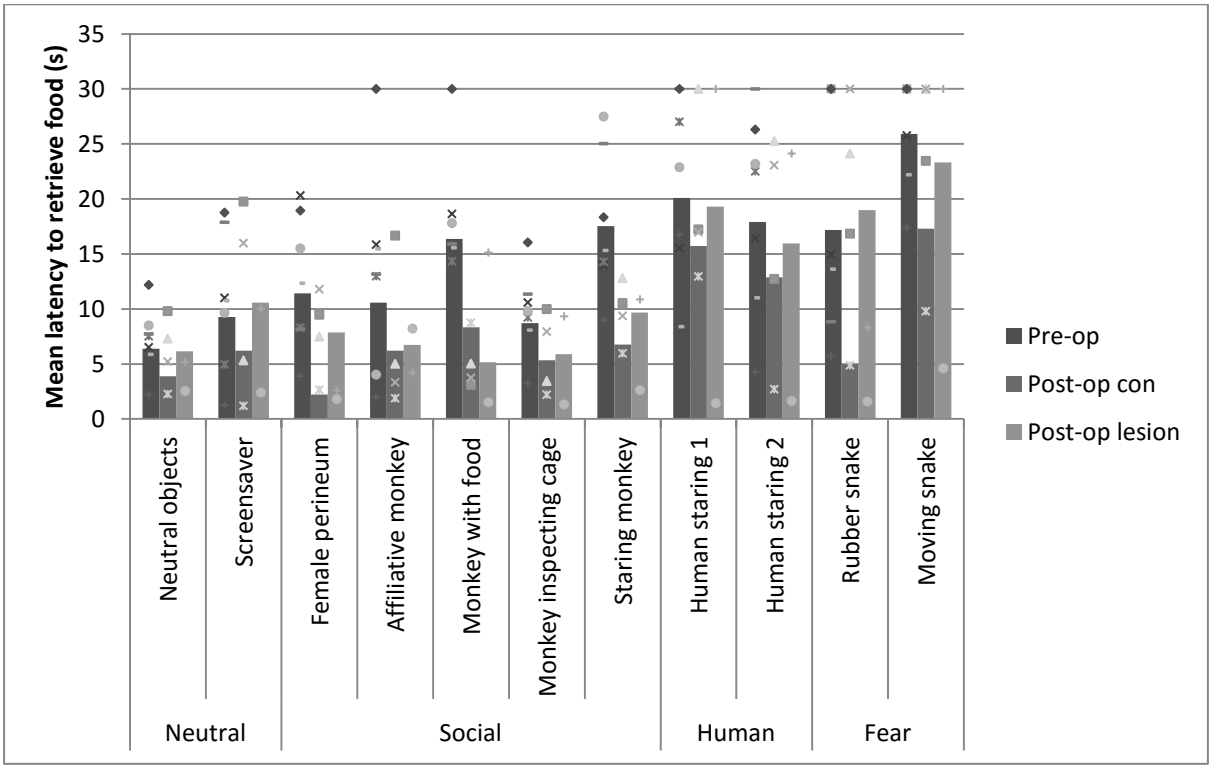


Figure 3 – Pre- and post-operative reaching latencies for FPC-lesioned and control monkeys across all stimuli

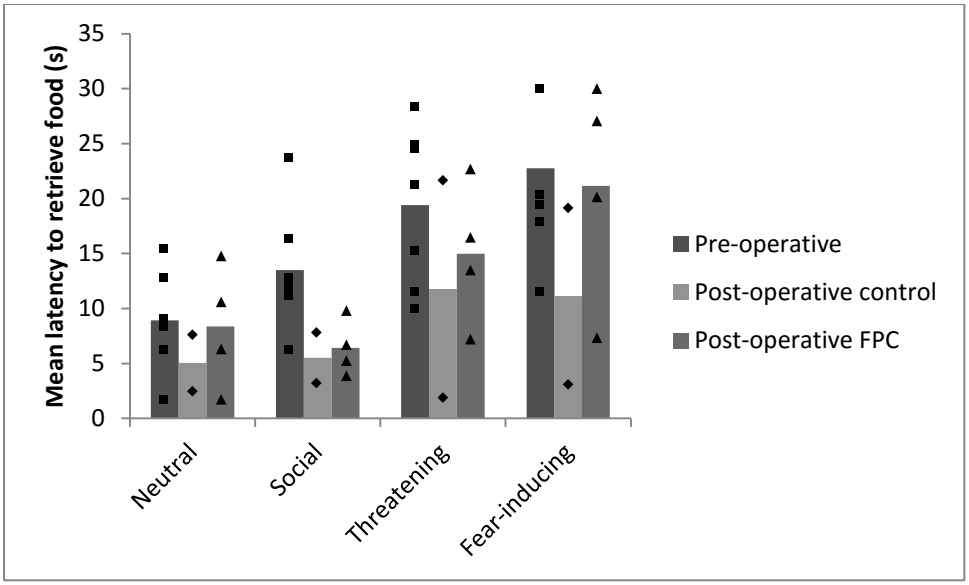


Figure 4 – Pre- and post-operative reaching latencies for FPC-lesioned and control monkeys across stimulus categories

A three-way repeated measures ANOVA was conducted on this data with one between-subject factor of group (two levels: FPC-lesioned and control) and two within-subject factors of testing period (two levels: before and after a 3 - 6 month delay) and stimulus type (four levels: neutral, social, threatening and fear-inducing). The stimulus categories were as follows: social (female perinea, affiliative monkey, monkey with food, monkey inspecting cage and staring monkey), threatening (human staring 1, human staring 2 and staring monkey), fear-inducing (rubber snake and moving snake) and neutral (neutral objects and screensaver). One stimulus (staring monkey) was included in two categories (social and threatening). The mean reaching latency for each monkey for each category and stage of testing was entered in the ANOVA. This ANOVA returned a highly significant effect of stimulus type ($F(3,12)$= 7.914, p=0.004) and a significant effect of testing stage ($F(1,4)$=23.063, p=0.009). However, there was no significant interaction between stimulus type and testing stage ($F(3,12$)= 2.121, p=0.151) and no significant main or interaction effect relating to experimental group could be detected (main effect of group: $F(1,4)$= 0.140, p= 0.727; group*stage of testing: $F(1,4)$= 7.550, p=0.051; group*stimulus type: $F(3,12)$= 0.982, p= 0.434; group*stage of testing*stimulus type: $F(3,12)$= 0.397, p= 0.757), although there was a non-significant trend towards interaction between stage of testing and lesion group (p=0.051).

When the same analysis was repeated with the "staring monkey" stimulus included in only the social category or only the threatening category, the same significant main effects of stimulus type ("staring monkey" as social stimulus: $F(3,12)$=6.597, p=0.007; "staring monkey" as threat stimulus: $F(3,12)$=7.664, p=0.004) and stage of testing ("staring monkey" as social stimulus: $F(1,4)$=16.939, p=0.015; "staring monkey" as threat stimulus: $F(1,4)$=21.430, p=0.010) were observed. However, no significant main or interaction effects relating to experimental group were observed ("staring monkey" as social stimulus: main effect of group: $F(1,4)$= 0.112, p= 0.755; group*stage of testing: $F(1,4)$= 6.232, p= 0.067; group*stimulus type: $F(3,12)$= 0.670, p= 0.587; group*stage of testing*stimulus type: $F(3,12)$= 0.491, p= 0.695; "staring monkey" as threat stimulus: main effect of group: $F(1,4)$= 0.151, p= 0.717; group*stage of testing: $F(1,4)$= 6.967, p= 0.058; group*stimulus type: $F(3,12)$= 0.877, p= 0.480; group*stage of testing*stimulus type: $F(3,12)$= 0.596, p= 0.629). When the ambiguous "threatening" category (it is not clear whether or not human behaviour will be viewed as socially relevant by monkeys) was excluded from the analysis and "staring monkey" was grouped as a social stimulus, significant main effects of stimulus type ($F(2,8)$=10.833, p=0.028; the Greenhouse-Geisser correction was applied in order to compensate for a violation in the assumption of sphericity and the associated corrected degrees of freedom were $F(1.045,4.182)$) and stage of testing ($F(1,4)$=17.064, p=0.014) were again observed. No significant main or interaction effect relating to experimental group could be observed (main effect of group: $F(1,4)$= 0.274, p= 0.629; group*stage of testing: $F(1,4)$= 6.276, p= 0.066; group*stimulus type: $F(1.045,4.182)$= 1.520, p= 0.285 (Greenhouse-Geisser correction applied); group*stage of testing*stimulus type: $F(1.769,7.076)$= 0.583, p= 0.563 (Greenhouse-Geisser correction applied)).

A paired-samples t-test comparing each monkey's pre- and post-operative mean reaching latency confirmed that reaching latencies were significantly reduced post-operatively ($t(5)$=3.332, p=0.021). In order to analyse this result further, a series of paired-samples t-tests were used to compare monkeys' mean reaching latencies for each of the four stimulus categories. After the Holm-Bonferroni correction was applied (Holm, 1979), significant differences were detected in four of the

six comparisons: neutral/social (*t(6)*=-3.642, *p*=0.011), neutral/threat (*t(6)*=-4.181, *p*=0.006), neutral/fear (*t(6)*=-4.981, *p*=0.002) and social/fear (*t(6)*=-3.694, *p*=0.010). Differences in the social/threat (*t(6)*= -2.603, *p*=0.040; *p*>0.025) and the threat/fear (*t(6)*= -1.990, *p*=0.094) categories failed to reach significance. Since each category consisted of a number of different stimuli, the data from these categories were then analysed separately to detect whether any differences between individual stimuli can be observed. For each category, a three-way repeated measures ANOVA was conducted with one between-subjects factor of lesion group (two levels: FPC-lesion and control) and two within-subjects factors of stimulus (number of levels depended on the category) and stage of testing (two levels: before and after a three-six month delay). The results of these analyses were:

- Neutral: a significant main effect of stimulus was detected (*F(20,80)*=1.979, *p*=0.017); no other significant main or interaction effects were observed (main effect of group: *F(1,4)*= 0.110, *p*= 0.756; main effect of stage of testing: *F(1,4)*= 2.389, *p*= 0.197; group*stimulus: *F(20,80)*= 0.872, *p*= 0.621; group*stage of testing: *F(1,4)*= 2.905, *p*= 0.164; stage of testing*stimulus: *F(20,80)*= 1.078, *p*= 0.389; group*stage of testing*stimulus: *F(20,80)*= 1.223, *p*= 0.259).
- Social: a significant main effect of stage of testing (pre/post) (*F(1,4)*= 44.943, *p*=0.003) was observed, reflecting lower reaching latencies post-operatively; no other significant main or interaction effects were detected (main effect of group: *F(1,4)*= 0.006, *p*=0.944; main effect of stimulus: *F(4,16)*= 2.362, *p*=0.097; stage of testing*group: *F(1,4)*= 2.173, *p*= 0.214; stimulus*group: *F(4,16)*=0.530, *p*=0.716; stage of testing*stimulus: *F(4,16)*= 1.911, *p*=0.158; stage of testing*group*stimulus: *F(4,16)*= 1.125, *p*= 0.379).
- Threat: a significant main effect of stage of testing was detected (*F(1,4)*=12.658, *p*=0.024), reflecting lower reaching latencies post-operatively, and a significant interaction effect was observed between stage of testing and stimulus (*F(2,8)*=4.919, *p*=0.040). No other main or interaction effects were detected (main effect of stimulus: *F(2,8)*= 1.856, *p*=0.218; main effect of group: *F(1,4)*=0.005, *p*=0.948; stimulus*group: *F(2,8)*=0.175, *p*=0.842; stage of

testing*group: *F(1,4)*=3.028, *p*=0.157; stimulus*stage of testing*group: *F(2,8)*=0.579, *p*=0.582). In order to investigate the source of the interaction effect observed between stage of testing and stimulus, two-way repeated measures ANOVAs [one between-subjects factor of group (two levels: FPC-lesioned and control) and one within-subjects factor of stimulus (three levels: staring monkey, staring human 1 and staring human 2)] were conducted separately for pre-operative and post-operative data. Neither of these analyses found any significant effects (preoperative: main effect of stimulus: *F(2,10)*=1.100, *p*=0.370; main effect of group: *F(1,5)*=0.446, *p*=0.534; stimulus*group: *F(2,10)*=0.258, *p*=0.778; postoperative: main effect of stimulus: *F(2,8)*= 3.858, *p*=0.067; main effect of group: *F(1,5)*=0.170, *p*=0.702; stimulus*group: *F(2,8)*=0.005, *p*=0.995).

- Fear: a significant main effect of stimulus was observed (*F(1,4)*=10.473, *p*=0.032). There were no other main or interaction effects (main effect of stage of testing: *F(1,4)*=3.949, *p*=0.118; main effect of group: *F(1,4)*=0.895, *p*=0.398; stage of testing*group: *F(1,4)*=1.461, *p*=0.293; stimulus*group: *F(1,4)*=2.249, *p*=0.208; stage of testing*stimulus: *F(1,4)*=0.699, *p*=0.450; stage of testing*stimulus*group: *F(1,4)*=0.074, *p*=0.799). A paired-samples t-test was conducted to follow up this effect, comparing mean response time to the rubber snake with mean response time to the moving snake; a non-significant trend was found (*t(6)*=-2.411, *p*=0.052), reflecting longer reaching latencies to the moving snake.

The inclusion of two types of stimulus within the neutral category (“screensaver” and “neutral objects”) meant that it was possible to analyse whether different modes of presentation (stimuli displayed on the screen versus stimuli presented in the Perspex box) affected reaching latencies; this hypothesis was supported by the finding of a significant main effect of stimulus within the neutral category. When a repeated measures ANOVA was conducted, with one between-subjects factor (group: two levels – FPC-lesioned and control) and two within-subjects factors [stage of testing (two levels: before and after a 3 - 6 month delay) and stimulus presentation type (two levels: box and screen)], no significant effects of stimulus presentation type were found (main effect of

presentation: *F(1,4)*=3.554, *p*=0.132; presentation*stage of testing: *F(1,4)*=0.142, *p*=0.725; presentation*group: *F(1,4)*=0.391, *p*=0.566). However, a near-significant interaction effect of stimulus presentation type, stage of testing and group (*F(1,4)*=7.143, *p*=0.056) was detected, indicating that further research is required in order to clarify whether stimulus presentation type has any effect on reaching latencies in this task. This ANOVA detected a significant interaction between group and stage of testing (*F(1,4)*=12.179, *p*=0.025). However, as no interaction effect between group and stage of testing was observed in any of the more general analyses described above, it is unlikely that this effect was genuine.

In a previous paper (Noonan et al., 2010, Experiment 1, Figure 4), a pre- and post-operative comparison was carried out on lesioned animals, without the inclusion of a control group. In order to facilitate more direct comparison, this analysis was also carried out here, with regard to the two categories (social and threat) in which a main effect of stage of testing had been detected. A two-ways repeated measures ANOVA was carried out on reaching latency data relating to monkeys in the lesion group in response to social stimuli [two within-subjects factors of stage of testing (two levels: before and after a 3 - 6 month delay) and stimulus (five levels: monkey with food, affiliative monkey, female perinea, monkey inspecting cage, staring monkey)]. A significant main effect of stage of testing was detected (*F(1,3)*=23.988, *p*=0.016); no other significant main or interaction effects were detected (main effect of stimulus: *F(4,12)*=1.715, *p*=0.211; stimulus*stage of testing: *F(4,12)*=2.535, *p*=0.095). The same analysis was carried out on reaching latency data relating to monkeys in the lesion group in response to threat stimuli (in this case, there were only three levels of the within-subjects factor of stimulus: staring monkey, staring human 1 and staring human 2). No significant effects were found (main effect of stage of testing: *F(1,3)*=2.039, *p*=0.249; main effect of stimulus: *F(2,6)*=2.762, *p*=0.141; stage of testing*stimulus: *F(2,6)*=2.091, *p*=0.205).

Discussion

Monkeys with lesions to FPC showed significantly shorter latencies before retrieving food rewards when distracted by socially relevant stimuli compared to their pre-operative performance. This finding supports the hypothesis of this study: monkeys with FPC lesions appear to show a reduced sensitivity to distraction by social stimuli post-operatively. This reduction can only be observed for social stimuli; reaching latencies for food rewards are not reduced when neutral, threat, or fear-inducing stimuli are considered. The lack of a significant main effect of stimulus when behaviour in the threat category was considered indicates that monkeys do not respond differently to this type of behaviour in humans and macaques. However, the conclusion that FPC lesions affect monkeys' behaviour on this task was not supported when these results were contrasted with the control group in this study. When repeated measures ANOVAs were used to examine the difference in reaching latencies before and after a delay in FPC-lesioned and control animals with regard to social and threatening stimuli, no significant group effect was observed, although reaching latencies after the delay were significantly reduced. These results therefore imply that the reduction in reaching latency observed in FPC-lesioned animals can be attributed to a statistically significant habituation effect between the two stages of the experiment which could be observed in social and threatening stimuli, but not in neutral or fear-inducing stimuli. This may have been due to animals learning that it is not possible to interact socially with videos presented in this experimental context.

In the study conducted by Rudebeck et al. (2006), monkeys were tested only once on this paradigm, post-operatively. This method meant that animals were not given the opportunity to habituate to any of the stimuli that were presented; differences in reaching latencies to social stimuli were detected between ACCg-lesioned monkeys and control monkeys in their first exposure to these stimuli. The results presented above indicate that control animals and animals with lesions to FPC do not differ significantly with respect to habituation on this experiment. However, it is possible that if animals with FPC lesions are tested on this task for the first time post-operatively, it will be possible to detect a difference in their sensitivity to social stimuli when compared with intact animals tested for the first time on this task. Moreover, this lack of significant difference in pre- and post-operative

behaviour when control animals are compared to FPC-lesioned animals indicates that the lack of inclusion of a control group in the study conducted by Noonan et al. (2010) was a significant drawback; since the results of the study presented here indicate that control monkeys habituate to this task, the lack of habituation displayed by the mOFC-lesioned group between pre- and post-operative exposures to this task may have indicated that lesions to mOFC do influence behaviour on this task. It is likely that the results observed by Rudebeck et al. (2006) are robust (since this study compared the behaviour of control and lesioned animals on their first exposure to the task) and that lesions to anterior cingulate gyrus impair macaques' sensitivity to social stimuli.

Shorter reaching latencies were recorded by monkeys distracted by social and threatening stimuli the second time that they were tested on this task. However, no significant differences in reaching latency were recorded between the first and second times that monkeys were tested on this task when they were distracted by neutral or fear-inducing stimuli. It is possible that this difference in habituation effect can be attributed to differences in the mode of presentation of stimuli in different groups. Both the fear-inducing and the majority of the neutral stimuli (20/21) were presented in a Plexiglas box that the monkey had to reach across in order to access the food reward; the social and threatening stimuli were displayed on a screen positioned behind this box. The fear-inducing and neutral stimuli were therefore closer to the monkey, and the monkey could not retrieve the food reward without observing the contents of the box. The monkey could, however, avoid looking at the screen while still retrieving the reward. Furthermore, there were a greater variety of stimuli presented in the box (22 in total) than on the screen (8 in total), which may have contributed to slower habituation with regard to items presented in the box than on the screen. Similarly, the association of the box with fear-inducing stimuli may have influenced macaques' reactions to objects presented in this way. While habituation is possible to fear-inducing stimuli, repeated exposure to these stimuli may result in sensitisation (Marshall, 1988). Monkeys may not have habituated to stimuli presented in the box if the occasional presentation of the rubber and moving snake objects in this box caused some animals to become sensitised to objects

presented in this way. The inclusion of one neutral stimulus presented on the screen rather than in the box meant that it was possible to contrast the response of animals to stimuli presented on the screen with the response of animals to stimuli presented in the box within one category of stimuli. No statistically significant effect of mode of presentation on reaching latencies in this experiment was found; however, it is not possible to use this result as positive evidence for a lack of effect of the screen versus box presentation on reaching latencies in this task as a near-significant interaction effect between mode of presentation, stage of testing and experimental group was observed.

The lack of observable difference between the experimental and control groups in this experiment may have been caused by a combination of the size of the control sample and the relative subtlety of the deficit. Only two monkeys were included in the control group (a third control monkey was, in fact, included in this condition, but its data were not considered in the analyses because it was only tested once due to factors beyond our control). Of these control animals, one recorded markedly and significantly longer reaching latencies in the second testing period than those recorded by the other animal. This suggests that between-subject variance as a result of temperamental differences between animals may have been an obstacle to detecting lesion-related differences between groups. As results from human neuropsychology suggest that lesions to medial FPC only cause a mild impairment in social cognition, it may require a larger sample size than that employed in this experiment to detect a similar kind of impairment in monkeys.

It is also possible that this task is not sensitive to the particular contribution made to social cognition by the medial FPC. Human patients with damage to this region are impaired at recognising embarrassing situations or socially inappropriate statements and are unimpaired in other forms of social cognition, such as understanding how motivation or belief may drive behaviour. This impairment can be interpreted as a lack of sensitivity to social information, but other explanations are possible: for example, it is possible that damage to FPC impairs second-order social cognition, such as sensitivity to emotions in one person which arise as a reaction to emotions in a second

person. This kind of subtle social cognition deficit would not be detected by this test; it would be very difficult to devise a test which would be sensitive to this kind of deficit in non-verbal animals.

The first priority for further research would be to administer this test to a wider range of control animals in order to increase the statistical power of the comparison of the control group with the experimental group in this study; alternatively, this test could be administered to FPC-lesioned animals for the first time after surgery in order to minimise the effects of habituation. If neither of these tests yields a clear difference between groups, further research could be carried out on FPC patients in order to clarify the nature of their social-processing deficit; in particular, to determine whether they are insensitive to social information per se, or whether their deficit is restricted to second-order social information, such as emotions which are experienced in response to the emotions of a second party.

CHAPTER FIVE

Lasting reductions in resting state functional connectivity after frontopolar lesions in monkeys

Studies in human patients have indicated that traumatic brain injury results in a reduction in functional connectivity in default mode networks (Sharp et al., 2011) – a network which includes FPC (Buckner et al., 2008) – and that human patients with lesions to FPC show reduced network activity in posterior regions when performing behavioural tasks (Rowe et al., 2007). However, no study has yet assessed the effect of focal lesions to FPC on network activity in non-human primates. We performed resting state scans on four monkeys that had received lesions to FPC 40 months previously; in addition, three other monkeys were scanned both pre-operatively and eight weeks after lesions to FPC. Comparison of functional connectivity between groups indicated that resting state network activity was reduced after lesions to FPC, and that this reduction was not constrained to regions known to have direct anatomical connections to FPC. Although a previous chapter of this thesis noted behavioural differences between monkeys tested a short period of time after lesions to FPC and monkeys tested a long period of time post-operatively (Chapter 1), and evidence from human patients indicates that behavioural recovery can be correlated with recovery of functional networks (He et al., 2007), it was not possible to observe any effects of recovery in the networks examined in this study.

Introduction

Previous chapters of this thesis have addressed a number of cognitive areas in which BA10 may play a functional role and have examined changes in behaviour before and after targeted lesions to frontopolar cortex in macaques. However, the role of area 10 as part of a network of regions in the brain has not so far been addressed.

A number of different techniques have been applied in order to identify networks of regions in the brain in which area 10 plays a role, in both humans and macaques. Neuroimaging studies have

been used in resting human subjects to identify those regions in which activations and deactivations are correlated; these regions are thought to be connected in a functional network (e.g. Rosazza and Minati, 2011). Networks identified include a "default mode" network of regions, which includes medial BA10 (Buckner et al., 2008), as well as medial temporal lobe, posterior cingulate cortex, ventral precuneus and medial, lateral and inferior parietal cortex, which can be observed active when subjects are awake but not performing a behavioural task. This technique has also been used to study changes in functional networks in macaque monkeys after targeted neurosurgical lesions were performed (O'Reilly et al., 2013).

Sallet et al. (2013) and Neubert et al. (2014) used diffusion-weighted tractography to parcellate areas of the human dorsal and ventral prefrontal cortex. These researchers then analysed correlations in the BOLD signal in awake resting humans to identify connectivity profiles of these parcellated regions. They identified two "clusters" within human dorsal prefrontal cortex, which they identify with medial and lateral area 10, although Sallet et al. questioned whether this lateral region should properly be thought of as area 10, given its shared connectivity profile with macaque area 46. In humans, activation in dorsal medial area 10 was found to correlate with activation in ventral medial prefrontal cortex, posterior cingulate cortex, rostral anterior cingulate cortex, anterior temporal sulcus, temporal pole and amygdala; activation in dorsolateral area 10 was found to correlate with activation in the precuneus, rostral anterior cingulate cortex, ventromedial prefrontal cortex, caudal ventral prefrontal cortex, both the caudal and mid-section areas of the inferior parietal lobule and the anterior superior temporal sulcus. Two parcellations (medial and lateral) were similarly identified in ventral area 10. As in dorsal medial area 10, activations in ventral medial area 10 correlated with activations in posterior cingulate cortex, dorsal anterior cingulate cortex, ventromedial prefrontal cortex, temporal pole and amygdala; activation was noted in the anterior temporal gyrus rather than anterior temporal sulcus, and these researchers also noted correlations with the parahippocampal place area and area 9m. As in dorsolateral area 10, activations in ventral lateral area 10 correlated with activations in mid-inferior parietal lobule; these researchers also

noted correlations with area PG, areas 8A, 9m, 9/46d and 32. No connections were observed between ventral lateral area 10 and the temporal lobe; Neubert et al. observed that the majority of areas in which activation correlated with ventrolateral area 10 were in the prefrontal cortex.

Networks including BA10 can also be observed in behaving human subjects: for example, Sakai and Passingham (2002 and 2006) noted that correlations in activation between left and right FPC and posterior regions (inferior frontal gyrus, superior frontal sulcus, area 6 and area 47) were modulated by domain and task, even in the absence of an observable difference in FPC activity (Sakai and Passingham, 2002). Furthermore, these networks are disrupted by lesions to FPC: Rowe et al. (2007) found that correlations in activation between five posterior regions (pre-SMA, frontal eye fields, fusiform cortex, parietal cortex, area 8 and area 44) were reduced by lesions to FPC in human patients, and that this reduction in correlation was itself affected by task demands.

In monkeys, neuroimaging and anatomical tracing has been used to identify networks including FPC. Petrides and Pandya (2007), Markov et al. (2014) and studies conducted by Burman et al. in 2011 (Burman et al., 2011a, 2011b) used anatomical tracing to identify cortical and subcortical efferent and afferent connections to and from FPC in monkeys. Despite the different techniques used to study these connections (efferent/afferent) and the different species studies (marmoset/macaque), a very similar range of connections were noted by Petrides and Pandya and by Burman et al.. Both laboratories noted dense interconnections with other areas of the prefrontal cortex (specifically, both laboratories noted connections with areas 8Ad, 9, 11, 12, 13, 14 and 32); in posterior regions, both laboratories identified connections with regions of temporal cortex, including rostral superior temporal gyrus and temporal pole and with retrosplenial cortex. Subcortical connections were identified by both groups with the claustrum and with the mediodorsal thalamic nucleus. Petrides and Pandya observed connections with amygdala, which were not observed by Burman et al.; however, this observation has been described by other researchers (Cavada et al., 2000). As in the human studies described above, sub-regions of FPC were distinguished by differing

network connections: Petrides and Pandya described fibres of the cingulate fasciculus terminating in retrosplenial cortex and the anterior and posterior cingulate cortex as originating only in the dorsal area of FPC; Burman et al. (2011a) detected afferent connections from retrosplenial and parahippocampal regions to only the medial region of area 10. However, while Markov et al. (2014) noted the connections described above between area 10 and other regions of prefrontal cortex, the anterior temporal lobe, and the retrosplenial cortex, this study identified a much wider range of posterior connections with area 10, including connections with parietal and occipital lobes (connections were observed with lateral, medial and posterior inter-parietal and with V2) which have not been observed in other studies.

Neuroimaging studies published by the Rushworth laboratory (Sallet et al., 2013; Neubert et al., 2014) examined correlations in the BOLD signal in anaesthetised macaques which resembled those that they identified in awake human subjects. In dorsal medial area 10, BOLD signal oscillations correlated with BOLD signal oscillations in rostral anterior cingulate cortex, caudal ventral prefrontal cortex, ventral medial prefrontal cortex, anterior temporal sulcus, temporal pole and amygdala. No "cluster" was identified in macaque dorsal lateral area 10, but similarity was noted between the human dorsolateral area 10 connectivity profile and the macaque area 46 connectivity profile. In macaque ventromedial area 10, the BOLD signal time-course correlated with the BOLD signal time-course in face sensitive superior temporal sulcus, temporal pole, ventromedial prefrontal cortex, area 32, area 9m and area 8A. Neubert et al. (2014) state that no macaque area was identified which corresponds with human ventrolateral area 10; the macaque area lateral to the ventromedial area 10 was identified as area 46, on the basis of similarity of BOLD connectivity profile with human area 46. However, the connectivity profile of human ventrolateral area 10 and human area 46 resembled each other; in fact, Sallet et al. (2013) questioned, on the basis of similarity between human dorsolateral area 10 and human dorsal area 46, whether a lateral area 10 region can be firmly established in humans. Activation in the macaque area which most closely matched the

human ventrolateral area 10 correlated with activation in area PG, the mid-inferior parietal lobule, lateral intra-parietal, area 8A and area 9/46d.

However, despite this research into the functional networks including area 10 in humans and monkeys, evidence from human subjects that lesions which include FPC cause changes in network activity in behaving patients (Rowe et al., 2007), and evidence that the default mode network, which includes BA10, is disrupted by brain injury (e.g. Sharp et al., 2011, Mayer et al., 2011), no research has yet been conducted which examines changes in this network in cases of targeted lesions to FPC. We conducted fMRI scans on seven rhesus macaque monkeys: three monkeys received scans pre-operatively and at eight weeks post-lesion; four monkeys did not receive pre-operative scans and were scanned at an average of three years and three months post-lesion (range: 2 years 11 months – 3 years 4 months). This variation allowed us to examine not only the change in connectivity in the brain post-operatively, but also whether recovery could be observed between scans conducted shortly after the lesion was performed, and those conducted after some time had passed (although this analysis was only possible between-subjects). Evidence suggests that spontaneous recovery of function can be observed in both human patients (Conkey, 1938; Bond, 1986) and macaque monkeys (Chapters 1 and 6) after lesion, and that network recovery can be correlated with recovery of function after brain injury in humans (Castellanos et al., 2010; He et al., 2007; Corbetta, 2012, provides a review); this experimental design enabled us to investigate whether recovery of network function in macaque monkeys can be observed after a neurosurgical lesion.

In order to test the hypothesis that network function will be disrupted by lesions to FPC, we examined correlations in BOLD signal between regions which both anatomical and functional connectivity studies consistently identify as strongly connected with FPC: these were mainly identified in the frontal and temporal lobes. Moreover, since functional connectivity is strongly influenced by physical proximity of seeds (Kolchinsky et al., 2014) and local connectivity is likely to be disrupted by anaesthesia (Deshpande et al., 2010; Liang et al., 2012), we only examined

connectivity between seeds in different lobes. We also only examined within-hemisphere connectivity, as inter-hemisphere connectivity may be disrupted by anaesthesia (Peltier et al., 2005; Wang et al., 2011). We therefore examined the correlation coefficients between four frontal regions which every anatomical study has noted as strongly connected with frontal pole in the monkey (areas 9, 13, 14 and 32; these connections have been noted in every subject) and two temporal regions which have similarly been strongly connected with frontal pole in the monkey by every anatomical study (area 22 and the temporopolar proisocortex). Functional connectivity data indicates that BOLD activity is correlated between these regions and frontal pole in healthy monkeys (Neubert et al., 2014; Sallet et al., 2013). In order to determine whether any disruptions are specific to networks which include FPC, or whether these disruptions are widespread, we compared the correlations specified above with two control comparisons. These comparisons were selected on the basis of observed connections between two regions in different lobes, neither of which are strongly connected with FPC. The control comparisons examined were between V1 and MT (Ungerleider and Desimone, 1986, described structural connectivity between these regions in the macaque) and between TE and LIPd (Webster et al., 1994, described structural connectivity between these macaque regions). We did not expect to observe long-term disruption in these networks post-lesion: He et al. (2007) observed disruption to both lesioned and structurally-intact networks in the acute stage post-injury; in the chronic stage, recovery was observed in the structurally-intact network, but not in the lesioned network. This is likely to be due to the fact that functional connectivity between regions strongly depends on structural connectivity (Skudlarski et al., 2008; Greicius et al., 2009), and physical connections between these regions were unaltered by the lesion.

Anaesthesia

Anaesthetic agents cause reduction of functional network connectivity in mammalian species (e.g. midazolam, Greicius et al., 2008; propofol, Boveroux et al., 2010; sevoflurane, Peltier et al., 2005; isoflurane, Liang et al., 2012). Studies comparing resting state networks in awake and

anaesthetised subjects have noted that long-range within-hemisphere cortico-cortical default mode network organisation remains mostly intact when subjects are lightly anaesthetised (isoflurane, Liang et al., 2012, Vincent et al., 2007; midazolam, Greicius et al., 2008; sevoflurane, Peltier et al., 2006, Qiu et al., 2011), although local connectivity, bilateral connections and thalamo-cortical connectivity are disrupted (local connectivity, sevoflurane, Martuzzi et al., 2011, Deshpande et al., 2010; local connectivity, isoflurane, Liang et al., 2012; bilateral connections, sevoflurane, Peltier et al., 2005; bilateral connections, isoflurane, Wang et al., 2011; thalamo-cortical connectivity, isoflurane, White and Alkire, 2003, Arhem et al., 2003; thalamo-cortical connectivity, halothane, White and Alkire, 2003). Additionally, default mode connections with the medial temporal lobe may be selectively decreased by sevoflurane anaesthesia (Martuzzi et al., 2010). The effect of anaesthesia on functional connectivity is critically dependent on the dosage used (isoflurane, Hutchison et al., 2014; sevoflurane, Peltier et al., 2005). Hutchison and Everling (2012), however, note that studies conducted on awake animals and animals anaesthetised with different agents have found convergent results.

The majority of studies examining resting state networks in anaesthetised monkeys have been conducted using isoflurane anaesthesia (e.g. O'Reilly et al., 2013, Sallet et al., 2013, Neubert et al., 2014). However, isoflurane has been associated with less hemodynamic stability and higher rates of ventricular arrhythmia and mortality than sevoflurane, indicating that it may be less preferable on welfare grounds (Regueiro-Purriños et al., 2011). We therefore conducted this experiment using sevoflurane anaesthesia, but compared the correlation coefficients collected from control animals in this study with those collected from a set of forty isoflurane-anaesthetised control animals in order to ensure that the anaesthesia used did not present a confound to the conclusions of this study.

Method

Subjects

Seven female rhesus macaque monkeys (*Macaca mulatta*) were tested in this study. At the start of the study, four animals had received FPC lesions, and three were unoperated controls. Three animals were housed as a social group; the remaining four animals were housed in pairs. They were housed in an enriched environment in which they could forage for small food items once a day. All had automatically regulated lighting and water available *ad libitum*, except for when they were undergoing behavioural testing. All animals had received training on an identical set of behavioural tasks prior to the start of the experiment. Monkeys weighed an average of 7.8 kg (range: 6.98 – 9.35 kg) when scanned pre-operatively; when scanned at the FPC-early time-point, they weighed an average of 8.4 kg (range: 7.11 – 10.44 kg); when scanned at the FPC-late time-point, they weighed an average of 7.2 kg (range: 5.90 – 8.94 kg).

Surgery

Four animals had received bilateral ablation lesions to FPC prior to the start of the experiment; the other three received this surgery over the course of this experiment. The posterior limit of the lesion was an imaginary vertical line situated 2-3 mm posterior to the anterior tip of the principal sulcus. For details of surgery, see Chapter 1. All licensed procedures were carried out in compliance with the United Kingdom Animals (Scientific Procedures) Act of 1986. Histological analysis confirmed that the lesions were performed as intended, albeit with slight sparing in the most anterior ventral aspect of the right hemisphere in one animal (see Chapter 1, figures 1 and 2).

Those animals which received frontopolar lesions prior to the start of the experiment were an average age of 4 years and 4 months at the time of surgery (range: 4 years 1 month – 4 years 6 months). Those animals which received lesions during the course of the experiment were an average age of 8 years and 2 months at the time of surgery (range: 7 years 6 months – 8 years 5 months).

MRI protocol

Three animals were scanned twice: once as unoperated controls and once eight weeks after receiving surgery. Four animals were only scanned once, an average of 40 months after receiving lesions to FPC (range: 35 – 42 months). Monkeys were anaesthetised while scans were conducted. Anaesthesia was induced with intramuscular injection of ketamine (10 mg/kg), xylazine (0.125 – 0.25 mg/kg) and midazolam (0.1 mg/kg) two hours prior to the start of scanning. This delay allowed ketamine to leave the monkey's system before the start of data acquisition. After induction, anaesthesia was maintained with the lowest possible concentration of sevoflurane necessary to maintain a lightly anaesthetised state. Physiological parameters (heart rate and blood pressure) were used to assess the depth of anaesthesia. Exhaled sevoflurane concentration was in the range of 1.4 – 2.5% during the acquisition of EPI data (table 1 gives the minimum and maximum and average expired sevoflurane concentrations for each animal).

Other medications administered were atropine (0.05 mg/kg, i.m.), meloxicam (0.2 mg/kg, i.v.) and ranitidine (0.05 mg/kg). Local anaesthesia (5% lidocaine/prilocaine cream and 2.5% bupivacaine, via subcutaneous injection) was applied around the ears prior to placement of the monkey in an MRI-compatible stereotactic frame (Crist Instruments) in a sphinx position. A constant respiration rate was maintained with intermittent positive pressure ventilation. Physiological parameters of respiration rate, inhaled and exhaled CO_2, inhaled and exhaled sevoflurane concentration, core temperature and SpO_2 were monitored throughout the scan (VitalMonitor software from Vetronic Services Ltd. was used).

The scans were conducted using a horizontal 3T MR scanner with a full-size bore. Data was acquired using a four-channel phased-array coil (Dr. H. Kolster, Windmiller Kolster Scientific, Fresno, CA, USA). A structural scan was acquired for each animal using a T1-weighted magnetization-prepared rapid-acquisition gradient echo (MPRAGE) sequence (128 slices; no slice gap; spatial resolution 0.5 mm isotropic voxels; TR=2500ms; TE=4.01ms). Whole-brain EPI fMRI data were collected for 53 minutes and 20 seconds for each animal in the same session (1600 volumes, of

which the first six were discarded; 36 axial slices; no slice gap; 2mm isotropic voxels; TR=2000ms; TE=19ms).

Monkey	Group	Minimum	Maximum	Average
A	CON	2	2.1	2.02
B	CON	2.2	2.4	2.26
C	CON	2.2	2.2	2.20
A	FPC-early	1.7	1.9	1.76
B	FPC-early	2.3	2.4	2.37
C	FPC-early	2.2	2.3	2.27
D	FPC-late	2.1	2.2	2.17
E	FPC-late	2.3	2.5	2.39
F	FPC-late	1.4	1.9	1.61
G	FPC-late	1.8	2	1.92

Table 1 – Minimum and maximum and average expired sevoflurane concentrations during the acquisition of EPI data.

Data analysis

SPM8 (Wellcome Department of Imaging Neuroscience, London, England) was used to analyse the fMRI data, using a mixture of standard routines and user-generated scripts in MATLAB (MATLAB Release 2013b, The MathWorks, Inc., Natick, Massachusetts, United States).

After non-brain matter was removed from the images, the images were motion-corrected and the data was subjected to Gaussian spatial smoothing with a full-width half-maximum of 3mm. Images were aligned to the McLaren Atlas (McLaren et al., 2009) and temporal band-pass filtered (0.025 – 0.05 Hz). Volumes were segmented according to tissue type (cerebral spinal fluid, white matter and grey matter) and all subsequent analyses were restricted to the portion of the image identified as grey matter.

Regions of interest (ROIs) were selected based on the 56 Brodmann regions of the monkey brain (Brodmann, 1905), from the Paxinos, Lewis and Huang (2000) and from the Lewis and Van Essen (2000) parcellations. These atlas-based ROIs were obtained from masks available in the F99 space (Van Essen et al., 2001) and were aligned to the McLaren Atlas (McLaren et al., 2009).

Different parcellations were chosen in order to accurately capture regions which contain the anatomical connections which were of interest. A relatively larger ROI was chosen in the superior temporal gyrus as FPC connections with this region are relatively widespread. The mean time series of activity for all voxels in these regions was calculated. A subset of seeds was selected (BA10, BA9, BA13, BA14, BA32, BA22, temporopolar proisocortex (TP), V1, MT, TE1-3 and LIPd) and seed-to-seed correlation coefficients were extracted using Fisher's r-to-z transformation. The following set of seed-to-seed correlations were examined: BA9/BA22, BA9/TP, BA13/BA22, BA13/TP, BA14/BA22, BA14/TP, BA32/BA22, BA32/TP, V1/MT and TE1-3/LIPd. BA9/BA22, BA9/TP, BA13/BA22, BA13/TP, BA14/BA22, BA14/TP, BA32/BA22 and BA32/TP were classed as “frontal/temporal” coefficients. V1/MT and TE1-3/LIPd were classed as “control” coefficients. In addition, seed-to-seed correlations between BA10 and each of the other seeds selected were examined in unoperated animals. BA10/BA9, BA10/BA13, BA10/BA14 and BA10/BA32 were grouped as “BA10/frontal” coefficients. BA10/BA22 and BA10/TP were grouped as “BA10/temporal” coefficients. BA10/V1, BA10/MT, BA10/TE1-3 and BA10/LIPd were grouped as “BA10/control” coefficients. Only within-hemisphere correlations were examined; the average was taken of the left and right hemisphere coefficients for each seed-to-seed correlation. Figure 1 shows the BA10 ROI used in this study on horizontal, sagittal and coronal views of a standard T1-weighted brain; figure 2 shows the other frontal, temporal and control ROIs used (larger versions of these images are presented in Appendix 2). Figure 3 shows the connections examined in this chapter.

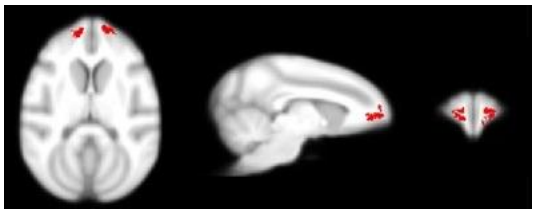


Figure 1 – Mask of BA10 seed region overlaid on horizontal (left), sagittal (centre) and coronal (right) views of a standard T1-weighted brain.

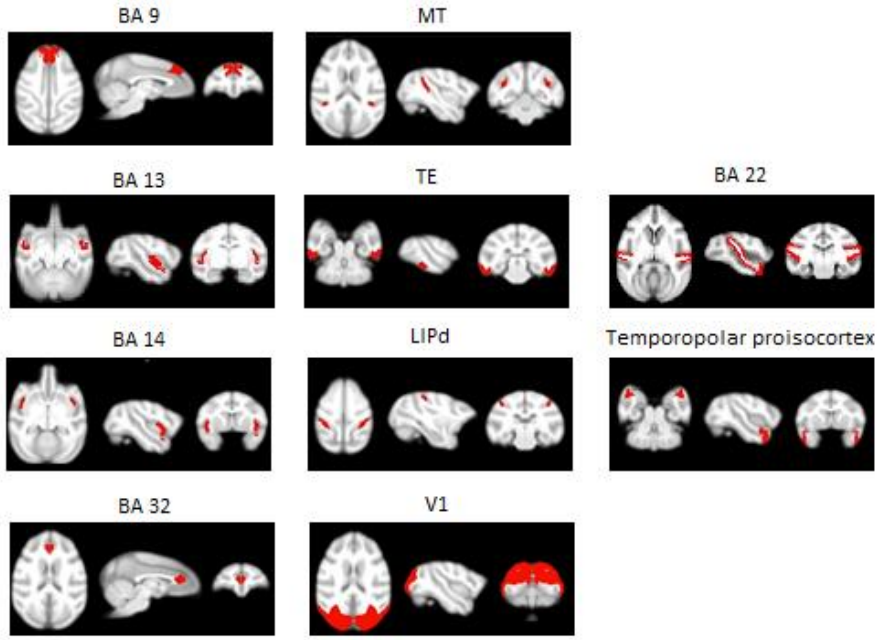


Figure 2 – Masks of frontal (left column), control (centre column) and temporal (right column) seed regions overlaid on horizontal (left), sagittal (centre) and coronal (right) views of a standard T1-weighted brain.

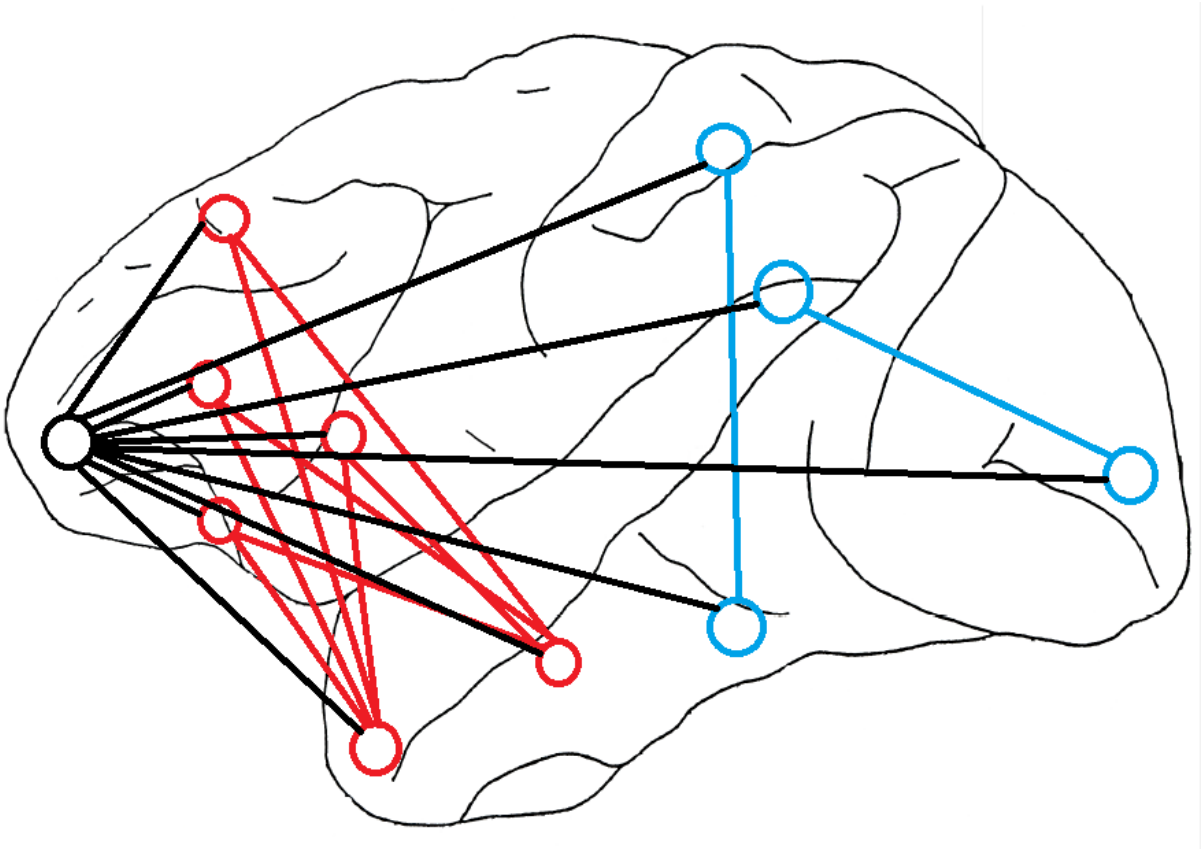


Figure 3 – Functional connections examined in this study, depicted on a drawing of the lateral surface of the macaque brain. Frontal/temporal (excluding BA10) connections are drawn in red; control connections are drawn in blue and connections between BA10 and all other ROIs in this study are drawn in black.

Results

Three sets of data were collected in this experiment: three animals were scanned as unoperated controls and four animals were scanned an average of 40 months after the introduction of a lesion to FPC. FPC lesions were then conducted on the three animals originally assigned to the control group and these animals were scanned again eight weeks post-lesion. This experimental design allowed three comparisons to be conducted: unoperated vs FPC-late (between subjects), unoperated vs FPC-early (within subjects) and FPC-early vs FPC-late (between subjects). The behavioural experience of the unoperated and FPC-late groups was matched, such that the only difference in experience between the two groups was the surgery in which the lesion was inflicted.

The animals originally assigned to the unoperated group received an additional eight weeks of behavioural testing before they were scanned at the FPC-early time-point; however, this was not significant in terms of the overall experimental experience of these animals.

Anaesthesia

We compared the correlation coefficients collected from unoperated animals in this experiment (anaesthetised with sevoflurane) with correlation coefficients between the same regions in forty healthy isoflurane-anaesthetised monkeys. When all correlation coefficients were averaged (including BA10/frontal, BA10/temporal, BA10/control, frontal (excluding BA10)/temporal, V1/MT and TE/LIPd), resting state activity was higher in the sevoflurane-anaesthetised control group (mean correlation coefficient = 0.399) than in the isoflurane-anaesthetised control group (mean correlation coefficient = 0.211), indicating that suppression of resting state networks under sevoflurane anaesthestia was not a concern in this study. Moreover, correlation coefficients between frontal and temporal regions (BA9/BA22, BA9/TP, BA10/BA22, BA10/TP, BA13/BA22, BA13/TP, BA14/BA22, BA14/TP, BA32/BA22, BA32/TP) were also greater in control animals anaesthetised with sevoflurane (mean correlation coefficient = 0.449) than in unoperated animals anaesthetised with isoflurane (mean correlation coefficient = 0.285), indicating that functional connectivity with the temporal lobe was not selectively reduced by sevoflurane anaesthesia (the results reported by Martuzzi et al., 2010, suggested that this may have been a concern).

BA10 connectivity in unoperated animals

The average correlation coefficients between BA10 and frontal regions, between BA10 and temporal regions with anatomical connections to FPC and between BA10 and control regions was calculated in the three sevoflurane-anaesthetised control animals. These values were entered into a repeated measures ANOVA with one within-subjects factor of connection type (three levels: BA10/frontal, BA10/temporal and BA10/control) and one covariate (average end-tidal sevoflurane

concentration). This ANOVA did not return a significant effect of connection type (*F(2,2)*=0.106, *p*=0.904). Average correlation coefficients between BA10 and frontal, anatomically-connected temporal and control regions are presented in figure 4. In order to investigate this surprising lack of effect, the individual correlation coefficients between each seed region and BA10 in each connection type was examined. Repeated measures ANOVAs were conducted for each of the three connection types (BA10/frontal, BA10/temporal and BA10/control) with one within-subjects factor of ROI and one covariate of average end-tidal sevoflurane concentration in order to detect whether significant variation existed between the correlation coefficients between BA10 and the seed regions in each category (frontal/anatomically connected temporal/control). No significant differences were detected in any of these analyses, although a trend effect of ROI was observed when this analysis was conducted on the BA10/control correlation coefficients (*F(3,3)*=8.307, *p*=0.058). The correlation coefficients between BA10 and each of the individual seed regions are presented in figure 5.

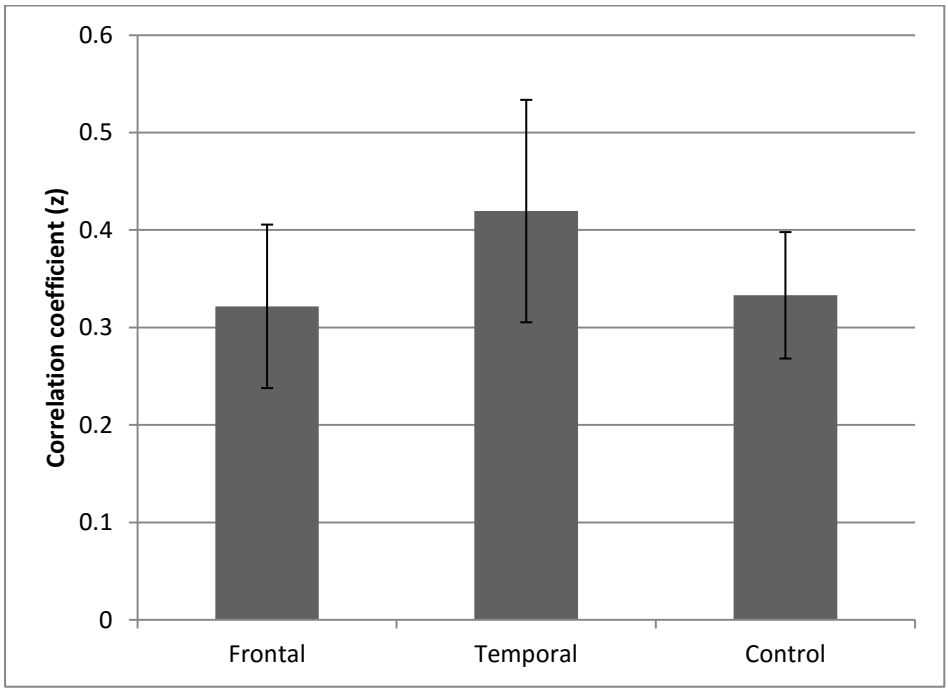


Figure 4 – Average correlation coefficients between BA10 and frontal, anatomically connected temporal and control regions in three sevoflurane-anaesthetised unoperated monkeys, with error bars showing the +/- standard error of the mean.

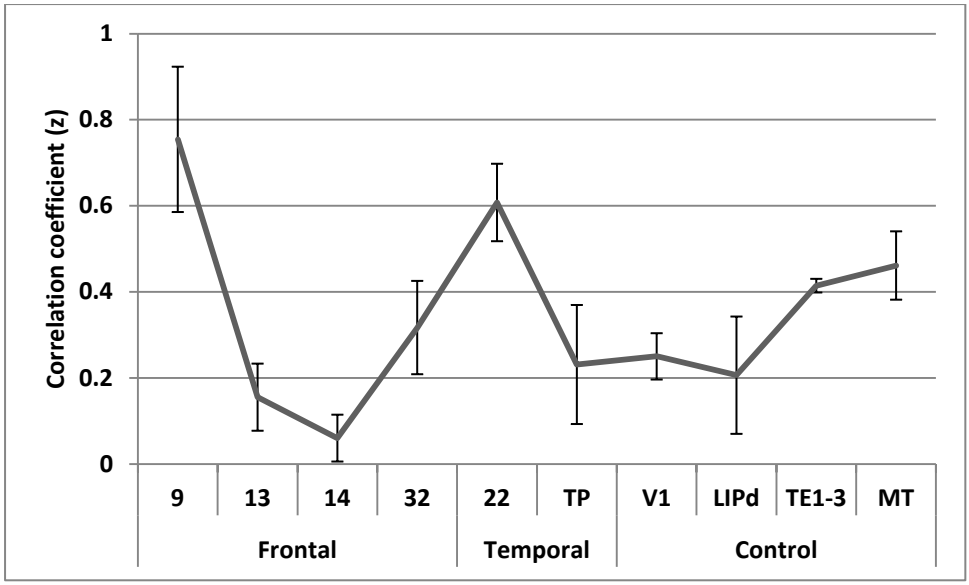


Figure 5 – Correlation coefficients between BA10 and ten ROIs in three sevoflurane-anaesthetised unoperated monkeys, with error bars showing the +/- standard error of the mean.

CON vs FPC-late (between subjects)

The minimum, maximum and average concentrations of expired sevoflurane anaesthesia were compared between control and FPC-late groups using independent samples t-tests. No significant differences were found. The greatest between-groups difference was noted in the minimum concentration ($t(5)$=0.979, p=0.372).

The average correlation coefficient between frontal and temporal regions (BA9/BA22, BA9/TP, BA13/BA22, BA13/TP, BA14/BA22, BA14/TP, BA32/BA22 and BA32/TP) and between control comparisons (V1/MT and TE/LIPd) for each animal were calculated. A two-way repeated measures ANOVA was used to compare correlation coefficients observed between frontal and temporal regions and those observed between control regions in unoperated and FPC-lesioned monkeys [one within-subjects factor of connection (two levels: frontal/temporal and control) and one between-subjects factor of group (two levels: FPC-lesioned and unoperated)]. Average end-tidal sevoflurane concentration was included in this analysis as a covariate. This recorded a significant main effect of group ($F(1,4)$=9.957, p=0.034). A trend towards a significant interaction effect between group and

connection was observed (*F(1,4)*=6.932, *p*=0.058). In order to isolate the source of this interaction, the frontal/temporal correlation co-efficients were compared between groups using a univariate ANOVA with one fixed factor of group and one covariate of average end-tidal sevoflurane concentration; similarly, the control region correlational co-efficients were also compared between groups using a univariate ANOVA with one fixed factor of group and one covariate of average end-tidal sevoflurane anaesthesia. When the frontal/temporal correlation coefficients were compared, there was no significant effect of group (*F(1,4)*=4.525, *p*=0.101). However, when control region correlation co-efficients were compared there was a significant between-group difference (*F(1,4)*=12.379, *p*=0.024) reflecting correlation coefficients between control regions being lower in the FPC-lesioned animals than in unoperated animals. This data is presented in figure 6.

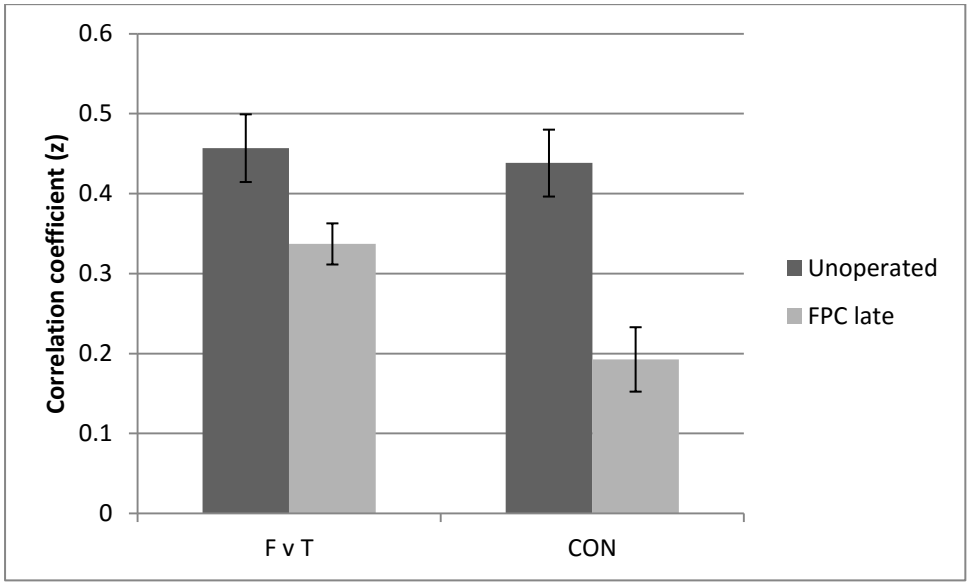


Figure 6 – Correlation coefficients between frontal and temporal regions (F v T) and between control (CON) regions in unoperated and FPC-lesioned animals (40 months post-lesion), with error bars showing the +/- standard error of the mean.

Inspection of the correlation coefficient between each seed region and BA10 in control animals indicated that a low level of functional connectivity was present between some regions in the frontal and temporal lobes and BA10, despite the anatomical connections between these regions. I therefore repeated the analysis described above including data relating to only the two

frontal regions with the highest levels of functional connectivity with BA10 in control animals (BA9 and BA32) and the temporal region with the highest level of functional connectivity with BA10 in control animals (BA22). The average correlation coefficient between BA9 and BA22 and BA32 and BA22 was calculated and a two-way repeated measures ANOVA was used to compare this score with the average correlation coefficient observed between control regions in unoperated and FPC-lesioned animals. There was one within-subjects factor of connection (two levels: frontal/BA22 and control) and one between-subjects factor of group (two levels: FPC-lesioned and control). Average end-tidal concentration of sevoflurane anaesthesia was included in this analysis as a covariate. This analysis returned a significant main effect of group ($F(1,4)$=8.617, p=0.043). No significant main effect of connection or interaction effect between connection and group was observed. This data is presented in figure 7.

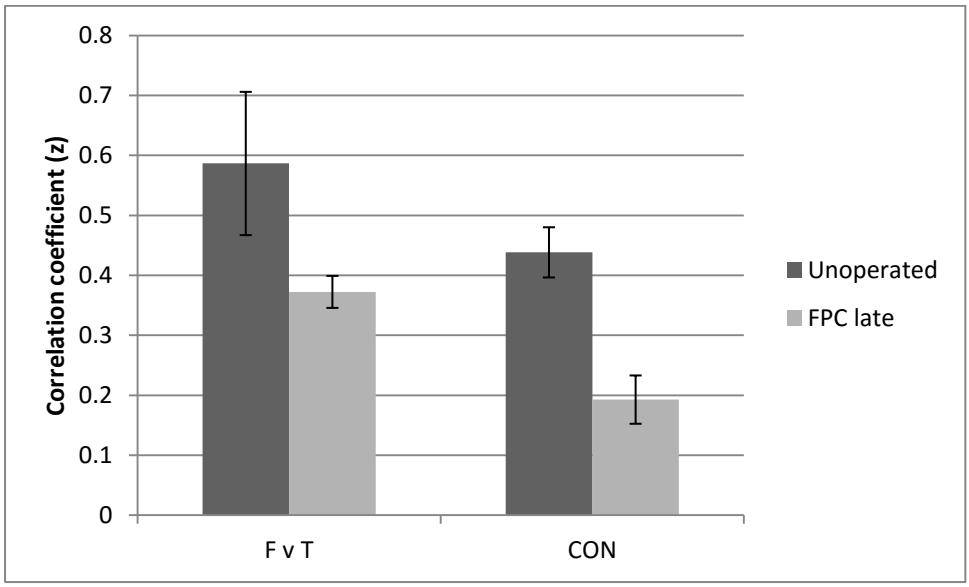


Figure 7 – Correlation coefficients between BA9 and BA32 and BA22 (F v T) and between control (CON) regions in unoperated and FPC-lesioned animals (40 months post-lesion), with error bars showing the +/- standard error of the mean.

<u>CON vs FPC-early (within subjects)</u>

The minimum, maximum and average concentrations of end-tidal sevoflurane anaesthesia were compared between pre-operative and post-operative animals using paired samples t-tests. No significant differences were found, indicating that differences in functional connectivity between groups cannot be attributed to differences in level of anaesthesia. The greatest pre- and post-operative difference was noted in the minimum expired sevoflurane concentration ($t(2)$=0.555, p=0.635).

The average correlation coefficients between frontal and temporal regions and between control regions for each animal were calculated pre- and post-operatively. The correlation coefficients observed between frontal and temporal regions and between control regions in monkeys pre- and post-operatively were analysed using a mixed model with fixed factors of stage of testing, connection and the interaction of stage of testing and connection, a repeated factor of the interaction of stage of testing and connection and a time-varying covariate of concentration of average expired sevoflurane (type: compound symmetry). This analysis recorded no main effect of stage of testing ($F(1,5.757)$=2.195, p=0.191) or connection ($F(1,5.742)$=1.158, p=0.325), and no interaction between stage of testing and connection ($F(1,5.742)$=0.745, p=0.423). This data is presented in figure 8.

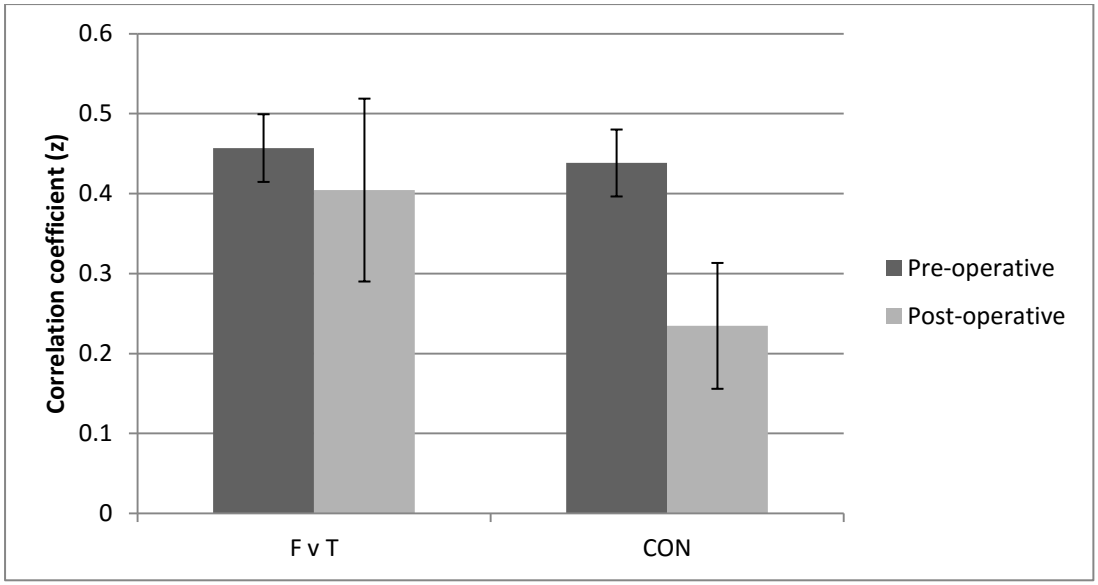


Figure 8 – Correlation coefficients between frontal and temporal (F v T) regions and between control (CON) regions pre- and eight weeks post-operatively, with error bars showing the +/- standard error of the mean.

For the reasons discussed above, this data was reanalysed including only information relating to the correlation in activation between BA9 and BA32 and BA22 and between control regions. The correlation coefficients observed between BA9 and BA32 and BA22 and between control regions in monkeys pre- and post-operatively were analysed using a mixed model with fixed factors of stage of testing, connection and the interaction of stage of testing and connection, a repeated factor of the interaction of stage of testing and connection and a time-varying covariate of concentration of average expired sevoflurane (type: compound symmetry). This analysis recorded no main effect of stage of testing ($F(1,5.857)$=0.968, p=0.364) or connection ($F(1,5.844)$=2.560, p=0.162), and no interaction between stage of testing and connection ($F(1,5.844)$=0.263, p=0.627). This data is presented in figure 9.

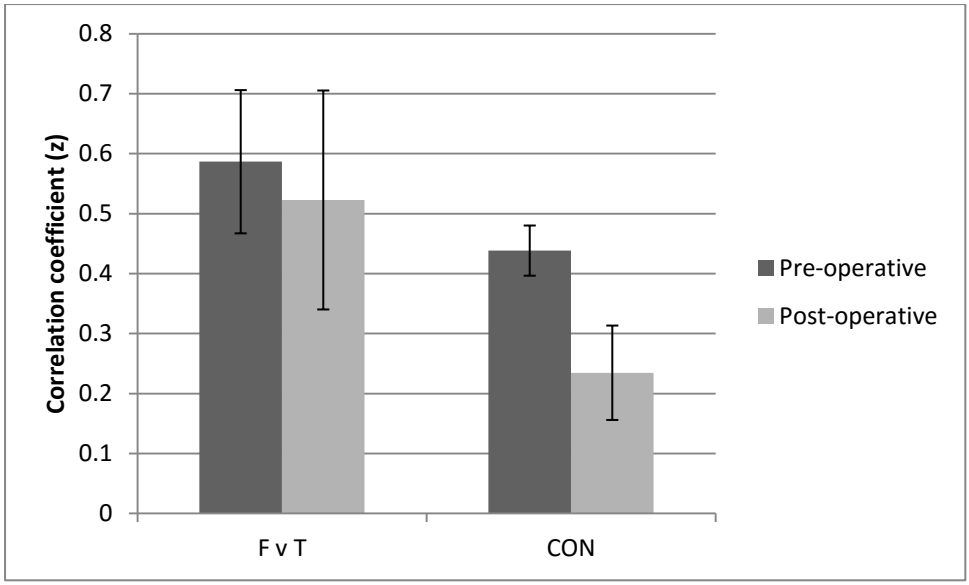


Figure 9 – Correlation coefficients between BA9 and BA32 and BA22 (F v T) and control (CON) regions pre- and eight weeks post-operatively, with error bars showing the +/- standard error of the mean.

FPC-early vs FPC-late (between subjects)

The minimum, maximum and average concentrations of expired sevoflurane anaesthesia were compared between control and FPC-late groups using independent samples t-tests. No significant differences were found. The greatest between-groups was noted in the minimum concentration ($t(5)$=0.598, p=0.576).

The average correlation coefficients between frontal and temporal regions and between control regions for each animal were calculated. A repeated measures ANOVA was used to compare correlation coefficients observed between frontal and temporal regions and those observed between control regions in FPC-early and FPC-late monkeys [one within-subjects factor of connection (two levels: frontal/temporal and control) and one between-subjects factor of group (two levels: FPC-early and FPC-late)]. Average end-tidal sevoflurane concentration was included in this analysis as a covariate. No significant results were observed in this analysis: no main effect was observed relating to either connection ($F(1,4)$=0.819, p=0.417) or group ($F(1,4)$=0.270, p=0.631) and

no interaction effect between connection or group was detected (*F(1,4)*<0.001, *p*=0.992) was detected. This data is presented in figure 10.

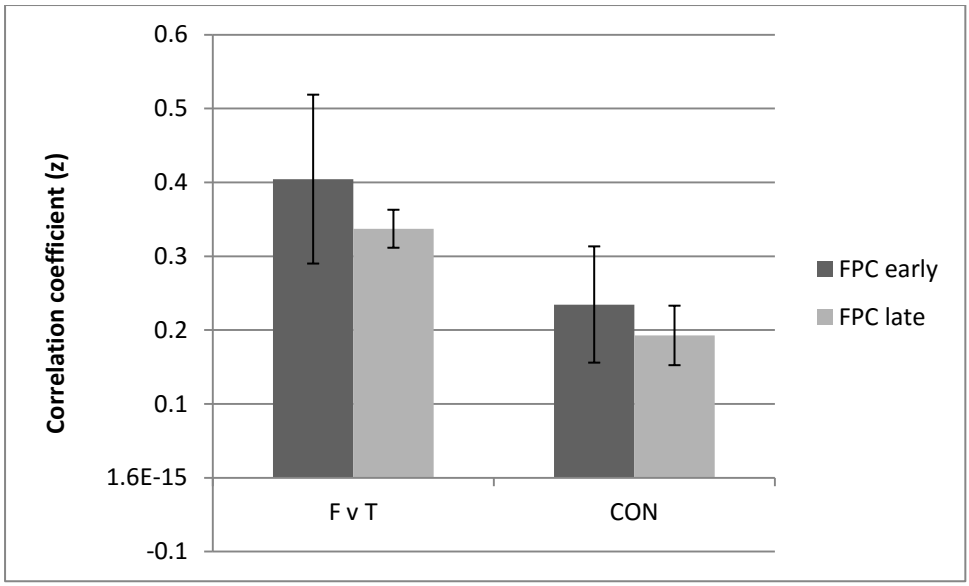


Figure 10 – Correlation coefficients between frontal and temporal regions and between control (CON) regions in animals scanned 40 months after lesions to FPC and animals scanned eight weeks after lesions to FPC, with error bars showing the +/- standard error of the mean.

For the reasons discussed above, this data was reanalysed including only information relating to the correlation in activation between BA9 and BA32 and BA22 and between control regions. A repeated measures ANOVA was used to compare the average correlation coefficients observed between BA9 and BA32 and BA22 and between control regions in FPC-early and FPC-late monkeys [one within-subjects factor of connection (two levels: frontal/temporal and control) and one between-subjects factor of group (two levels: FPC-early and FPC-late)]. Average end-tidal sevoflurane concentration was included as a covariate in this analysis. No significant results were observed in this analysis: no main effect was observed relating to either connection (*F(1,4)*=0.240, *p*=0.650) or group (*F(1,4)*=0.623, *p*=0.474) and no interaction effect between connection or group was detected (*F(1,4)*=0.608, *p*=0.479) was detected. This data is presented in figure 11.

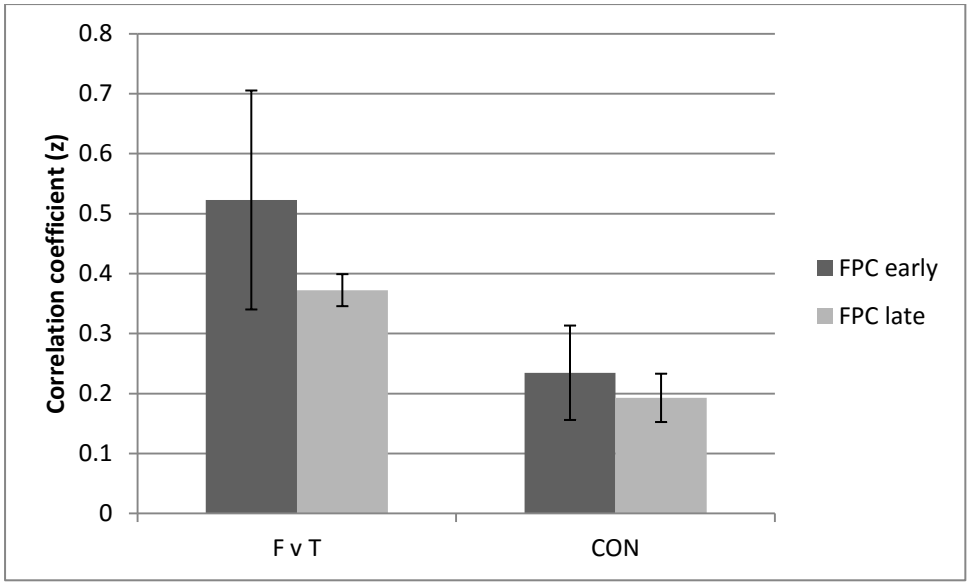


Figure 11 – Correlation coefficients between BA9 and BA32 and BA22 (F v T) regions and between control (CON) regions in animals scanned 40 months after lesions to FPC and animals scanned eight weeks after lesions to FPC, with error bars showing the +/- standard error of the mean.

Discussion

This study examined the functional connectivity of BA10 in healthy monkeys and functional connectivity between areas directly anatomically connected to BA10 and between areas without such direct anatomical connections to BA10 in control animals and FPC-lesioned animals. In our analysis of functional connectivity of BA10 in intact animals, we did not replicate the findings recorded by previous studies which examined the functional connectivity of macaque area 10 (Neubert et al., 2014; Sallet et al., 2013), which found that the BOLD signal in this region correlates with that recorded in frontal and temporal regions, but not with that recorded in most posterior regions (such as the regions included as controls in this study: V1, TE, MT and LIPd). We found no significant difference in functional connectivity with area 10 between regions that are or are not anatomically connected to this region. It is possible that we did not detect some genuine connectivity with BA10: for example, some local connections between BA10 and frontal regions may have been suppressed as a result of anaesthesia, resulting in uneven detection of functional

connectivity within the frontal lobe. Furthermore, the region of the temporal pole which receives inputs from and projects to frontal pole in monkeys is very small (Petrides and Pandya, 2007), and this connection has not been observed in all monkeys in every study (Burman et al., 2011, observed this connection in the majority of animals studied, but not all), although connections between frontal pole and superior temporal gyrus are widespread and have been observed in every monkey studied. It is therefore possible that the temporal pole does not constitute as important a role in networks that include the frontal pole as the superior temporal gyrus; it is also possible that this region failed to be included in our anatomical mask of this area (since Petrides and Pandya do not indicate the area in which the termination was observed on a rostral view of the temporal pole). This may have reduced the detection of functional connectivity between this region and BA10. Likewise, the large cortical mask which was used in the STG may have introduced noise into the analysis by including areas of cortex which are not connected to BA10 in addition to those areas which are connected to this region. However, these explanations cannot account for the positive correlations which were detected between BA10 and posterior regions which are not anatomically connected to this region, such as V1 and MT (figure 2). While this finding may reflect indirect connections between BA10 and these areas, the surprising nature of this result means that it must be carefully examined. Davey et al. (2013) suggest that certain forms of temporal band-pass filtering may artificially induce correlations which are detected as connectivity in resting state data; further analysis of our dataset will analyse whether this may have been the source of the unexpected connectivity detected between BA10 and posterior regions in this study.

The results of this study support the hypothesis that the introduction of circumscribed neurosurgical lesions causes widespread disruptions to functional brain networks in monkeys; monkeys scanned 40 months after receiving bilateral lesions to FPC showed lower correlation in BOLD activation between all sets of regions examined (frontal – temporal, temporal – parietal and temporal – occipital) than was recorded in control monkeys; there was a significant decrease in correlation between those regions defined as controls in this study (TE-LIPd and V1-MT) in

frontopolar-lesioned monkeys relative to controls. This supports the findings of reduced connectivity after brain injury in studies conducted in other species (e.g. humans: Tarapore et al., 2013; rats: Mishra, 2014). Since the same preprocessing methodology was applied to all scans, pre- and post-operative, this finding cannot be explained as an artefact of the analysis method used in this study (since false positive findings of functional connectivity should be introduced equally to all scans). We found no evidence that this disruption was confined to regions which are anatomically connected with the lesioned area. Furthermore, the decrease in correlation was greater in regions that were not anatomically connected to the frontal pole than in regions that were anatomically connected to area 10 (figure 6). A trend in the data suggested that connectivity between posterior regions may have suffered greater disruption as a result of the lesion than connectivity between regions which are anatomically connected with the lesioned area, although this difference did not reach a statistically significant level. If this trend is robust, the results would be very surprising; however, as no difference was observed when the frontal/temporal regions were restricted to those which showed greatest functional connectivity with BA10, it is unlikely that this reflects a genuine difference between the two connection types.

Recent evidence indicates that FPC is anatomically interconnected with a wider range of brain regions than those which have previously been identified (Markov et al., 2014). One of the control comparisons examined in this study included regions which Markov et al. indicated are connected to FPC in monkeys (TE and LIPd; although the connections observed by Markov et al. were relatively weak, and were not observed in all animals in that study); it is possible that the weak connections between FPC and these regions was sufficient that lesions to FPC disrupted activity in this network. However, no study has yet identified anatomical connections between frontal pole and V1 or frontal pole and MT; the results of this study indicate that lesions to FPC nevertheless disrupt functional connectivity between these regions (the average V1-MT correlation coefficient declined by 0.326 between control and FPC-late animals; by contrast, the average TE-LIPd correlation coefficient declined by 0.165 between these groups). As more than three years passed between the

introduction of lesions and scans for the animals tested in the FPC-late group in this study, it is unlikely that this result can be explained by transient traumatic effects of surgery, and the result is more likely to be due to the long-lasting effects of the removal of a part of the cortical network. Furthermore, previous research indicates that disruption to functional networks after brain injury critically depends on the location of the injury (Gillebert et al., 2011), indicating that the disruption in posterior networks observed in this study is unlikely to be due to a generalised disruption in all brain networks. While FPC is relatively structurally isolated from posterior brain regions, it is densely interconnected with other regions of prefrontal cortex and thus is indirectly connected with posterior regions of the brain. Alstott et al. (2009) modelled network connections in the human brain (measured using diffusion-weighted tractography) and predicted the effect of lesions to particular areas on functional connectivity based on the network connections that would be disrupted; they found that lesions to frontal cortex (including frontal pole) would be likely to cause widespread disruption of network connections, although they did not model the effect of an isolated lesion to FPC. The results of this study indicate that area 10 plays a crucial role in normal functional interactions between posterior brain regions, despite the lack of anatomical connections between FPC and some of these regions.

A number of studies have observed recovery in functional brain networks after an insult has been sustained (Tarapore et al., 2013, He et al., 2007, Nakamura et al., 2009). Recovery of levels of functional connectivity was not observed in the current study: in fact, monkeys showed less functional connectivity when scanned 40 months post-lesion than when scanned eight weeks post-lesion; this was the case for both sets of connections (frontal/temporal and control) examined in this study (figure 10), although this effect did not reach statistical significance. The lack of observed recovery in these connections may have been due to functional reorganisation in the brain: if new neural pathways are created which compensate for damaged structures (Hoyer and Celnik, 2011), then it is plausible that these will supersede those which are damaged by lesion, and no recovery of function will be observed in the damaged pathways. Venkatesan et al. (2014) indicate that functional

abnormalities after injury to the brain are long-lasting, and that these abnormalities are not correlated with behavioural outcomes. The addition of more subjects into the FPC-early group may clarify whether activity in this group more closely matched that observed in the control group or in the FPC-late group (connectivity in the FPC-early group did not significantly differ from that observed in either controls or the FPC-late group in this study). The long-lasting effects of the lesion observed in this study may be due to the infliction of bilateral lesions in this study, which show less recovery than unilateral lesions (Kolb and Gibb, 1991, report less dendritic branching in the parietal lobe after bilateral lesions than after unilateral lesions in rats).

Further analysis of this dataset (outside of the scope of this thesis) could take an *a posteriori* approach to identifying regions of interest, rather than the *a priori* approach based on anatomical connectivity taken in this study. The *a posteriori* approach will identify regions which show significant positive correlation (above a defined threshold) with BA10 in the time-course of the BOLD signal. One of the limitations in the approach taken by this study was that it was necessary to specify large regions of interest in the temporal lobes in order to capture the precise regions with which BA10 is anatomically connected. This approach will enable us to define smaller regions which are functionally connected to BA10, thus reducing the noise in the dataset. This may help to clarify whether any reduction in functional connectivity can be observed eight weeks after lesions to BA10, in addition to the reduction which is observed 40 months post-lesion.

CHAPTER SIX

Recovery of function after principal sulcus lesions assessed by longitudinal behavioural testing

Much current animal neuroscience research relies on comparison of performance on tasks before and after surgical infliction of targeted lesions. However, there is reason to believe that plasticity in the brain allows for recovery of function after injury, even in the absence of rehabilitative treatment (Conkey, 1938). This study longitudinally examined performance of two monkeys on behavioural tasks (delayed match-to-position and delayed match-to-sample) before and after a unilateral neurosurgical lesion which was expected to selectively impair function on one task. Performance on these tasks was assessed periodically and without a prolonged interval of retraining. This was combined with periodic functional MRI scans which monitored the recovery of resting state networks, although these MRI results are not discussed in this chapter. The results of the behavioural tasks enabled us to observe recovery of function in one animal. This indicates that researchers should be cautious in interpreting negative results of animal lesion studies, since the magnitude of a behavioural effect of a lesion may vary relative to the passage of time between the introduction of the lesion and the time of testing.

Introduction

The experiments detailed so far in this thesis have relied on the study of non-human primates before and after the introduction of neurosurgical lesions. This experimental technique is powerful because it relies on causation, rather than correlation. If animals are impaired relative to controls on a task after receiving a lesion to a particular brain region, it can be assumed that normal function in this region is essential for some feature of cognitive processing on which this task relies (noting that in the case of aspiration lesions, unintended damage may be caused to white matter tracts, and this damage may be a potential cause of cognitive deficits following these lesions). This technique has some advantages when compared to methods which rely on correlation, such as fMRI studies which examine the fluctuation of the BOLD response while subjects perform behavioural tasks. When correlative methods are used, it is not possible to prove that the activation detected is due to task-relevant processing, as opposed to epiphenomenal activation of brain regions which are

non-crucial to the experimental task, or other incidental features of the experimental set-up (e.g. experimenters may unintentionally convey to subjects whether they are performing an experimental or control task). This is particularly material to examinations of the frontopolar cortex, as a number of tasks induce activation in this region which are not impaired in patients who have received damage to FPC: examples include the WCST (Konishi et al., 2005, note FPC activation during this task; Uretzky and Gilboa, 2010, describe unimpaired performance after an FPC lesion in a human subject), encoding of verbal information (Fletcher et al., 1998, note FPC activation; Alexander et al., 2003, note unimpaired performance after lesions) and Raven's Progressive Matrices (Christoff et al., 2001, describe FPC activation when participants solve "2nd order" problems; Burgess et al., 2000, note no deficit in performance on these problems in FPC patients).

However, lesion studies are limited in a number of ways. Spared performance in an experimental task, despite an injury to a region which is usually activated during that task, can be investigated using functional neuroimaging in order to determine how to interpret this unimpaired performance (possible explanations include: non-necessary input from that brain region to performance, neuronal recovery, cognitive reorganisation and peri-damage activation: Price and Friston, 1999, and Price et al., 1999; Pinsk et al., 2005, extended this research to lesioned monkeys). However, as it is not possible to directly observe brain activity in a lesioned area (since it is absent) during an experimental task, the precise nature of the critical information-processing contribution of the lesioned area to a task which is impaired subsequent to injury cannot be investigated without complementary scanning of healthy controls. Furthermore, brain injury in patients is rarely confined to a single region; while this is less problematic in animal studies, adjacent brain areas or connectivity pathways may be damaged during the surgical creation of an aspiration lesion. While it is possible to evaluate the extent of damage to brain regions and to connectional pathways *in vivo* using structural MRI (Málková et al., 2001), functional MRI (see Chapter 5 of this thesis) and diffusion-tract imaging (Molko et al., 2002), the gold standard test in animal experimentation is histological analysis, which may not, for non-human primates, be available until a number of years

after the lesion is made as these animals are often subjects in multiple experiments (this thesis describes four behavioural studies conducted using the same group of FPC-lesioned macaques), which can be of a long duration.

It is undoubtedly true that traumatic brain injury may cause severe impairments in a range of cognitive functions, and that these impairments may be permanent: patient H.M. received bilateral surgical lesions to the medial temporal lobe in 1953, resulting in near-total anterograde amnesia for semantic and episodic information; he showed almost no recovery over many decades (Corkin, 2002). Severe impairments have been noted in other patients decades after damage was sustained to different areas of the brain: deficits were described 11 years after patient K.F. suffered left parietal-occipital damage (Warrington and Shallice, 1969; Warrington et al., 1971). Patients Beth, Jon and Kate were tested 13-19 years after receiving bilateral damage to the hippocampus in childhood; deficits were observed in all three patients (Vargha-Khadem et al., 1997). Mataró et al. (2001) described cognitive deficits in a patient 60 years after bilateral injury to the frontal lobe.

However, there is also extensive evidence that much function can be recovered after traumatic brain lesions, and this recovery may be enhanced if patients undergo training in their area of deficit (Carney et al., 1999, present a review relating to recovery in human patients; Will et al., 2004, review literature relating to lesions inflicted on rodents; Chapter 1 of this thesis describes improved performance when monkeys are tested 35 months post-lesion compared with performance two weeks post-lesion). This is problematic for research conducted using non-human primates, particularly studies which rely on behavioural tasks on which monkeys are trained after the infliction of lesions (in contrast to experiments which compare pre- and post-operative performance in a task learned before neurosurgery). Monkeys receive extensive and occasionally long-term pre-training on tasks which are designed to test their specific areas of deficit, and which usually continues until they reach a criterion level of performance on pre-training tasks (e.g. previous chapters of this thesis; Hampton et al., 2004; Browning et al., 2013, Buckley et al., 2009;

Bussey et al., 2001). If this training is undergone after the lesion is performed, it is not unreasonable to conclude that this training may have a therapeutic effect: Kleim and Jones (2008), in a review, note the importance of repetition, intensity, specificity and salience of training for neuroplasticity post-lesion; all of these factors exist in the experimental set-up of behavioural training in order to gain a food reward. This may reduce the chances of detecting an effect of a brain lesion on this area of cognitive performance; monitoring the rate of acquisition of these tasks may not be sufficient to control for this effect. Examples of this post-operative training of monkeys in experimental tasks can be seen in a range of studies e.g. Browning et al. (2013), Hampton et al. (2004), Walsh et al. (1993), Alvarado and Bachevalier (2005), Chapters 1 and 2 of this thesis.

Furthermore, as animals may be retained for a long period after neurosurgery and tested on a number of tasks, it is possible that spontaneous recovery (i.e. recovery that occurs without rehabilitative intervention) could occur between the initial post-operative testing and later studies conducted on the same group of animals. While most humans who receive traumatic brain injuries receive rehabilitative treatment, impairing the ability to estimate the extent of spontaneous recovery from injury, research indicates that humans display some recovery even without rehabilitative treatment in at least the first six months after injury (Conkey, 1938) and may continue to recover for up to two years post-injury (Bond, 1986). While it is likely that patients with traumatic brain injuries are able to experience retraining of cognitive functions over the course of normal life (and so it may be inaccurate to describe any recovery as “spontaneous”), the same is true for experimental monkeys that are housed in enriched, social environments. In experimental studies, the typical rest period that monkeys undergo after surgery and before the resumption of testing may be only two-three weeks (e.g. Browning et al., 2013, Buckley et al., 2009, Kennerley et al., 2006). It is likely that this period is of insufficient length to allow spontaneous recovery to occur, and that further recovery over the course of testing may impair the chances of observing a deficit in function in experiments conducted after a period of time has elapsed since surgery.

In order to fully understand the effects that these two considerations may have when experiments are conducted on neurosurgically-lesioned animals, it is necessary first to estimate the effect of spontaneous recovery (without retraining) on performance on an experimental task known to be impaired by lesion to that particular region. Once this effect is known, it will be possible to assess whether training on experimental tasks designed to target areas of cognitive deficit has a therapeutic effect on monkeys who have received these lesions. In order to estimate this effect, the experimental design in this study minimised post-operative retraining (although, as it is not possible to measure task performance without behavioural testing, some retraining necessarily occurred); however, since the primary motivation for conducting this behavioural testing was to correlate behavioural recovery with recovery of functional networks (as measured by fMRI), no retraining control group was included.

For the purposes of the MRI component of this study, we intended to contrast network activity in lesioned and unlesioned brain hemispheres. It was necessary that this experiment target a region which would have a predictable effect on performance on specific behavioural tasks after unilateral lesions. No studies have yet been published which examine the behavioural effects of unilateral FPC lesions. For this reason, we selected the area surrounding the principal sulcus (PS). Lesions or disruptions to this area have been repeatedly shown to cause impairments to spatial working memory in non-human primates (e.g. Mishkin, 1957, Gross and Weiskrantz, 1962, Goldman et al., 1971, Passingham, 1985, Levy and Goldman-Rakic, 1999). Funahashi et al. (1993) observed that unilateral lesions to this region caused impairments in spatial delayed-response tasks when targets were presented contralateral to the lesioned hemisphere; likewise, Stamm (1969) found that performance on a spatial delayed response task was unimpaired by prefrontal lesions ipsilateral to the hand which monkeys used to respond, but that performance was impaired by electrical stimulation of the contralateral principal sulcus. We also tested performance on an object working memory task, which we expected to be unimpaired (Passingham, 1975, Levy and Goldman-Rakic, 1999) or only slightly impaired by PS lesions (Mishkin and Manning, 1978). It was advantageous to

select a region of the prefrontal cortex, as the results of this study can more easily be generalised to the studies described thus far in this thesis which targeted FPC than if a posterior area had been selected.

Furthermore, questions have been raised concerning whether the spatial deficit in working memory observed after lesions to prefrontal cortex is due to a mnemonic failure or an inability to suppress interference between the currently relevant stimulus and previously relevant stimuli (Tsujimoto and Postle, 2012). In order to address this question, we tested animals on a three-choice version of a delayed match-to-position task, in which one choice was always the target location on the previous trial. This experimental design enabled us to assess the proportion of errors made in which monkeys selected locations which had been rewarded on the previous trial.

We collected behavioural data on a spatial working memory task (delayed match-to-position) and a comparable object working memory task (delayed match-to-sample) in two monkeys, pre-operatively, after unilateral (left hemisphere) lesions to PS, and after additional surgery was conducted which introduced symmetrical lesions to right PS. This behavioural data was collected in short bursts, so that monkeys did not receive more than three consecutive days' testing on any task after initial training. This experimental design allowed us to collect data on these tasks without extensive post-operative retraining. The testing was supplemented by resting state fMRI data, which allowed us to analyse the recovery of brain function (measured by the similarity of resting state networks to those detected in the baseline pre-lesion scans) and correlate this with the recovery of cognitive function, as measured by performance in the behavioural tasks. This task design enabled us to contrast the longitudinal effects of unilateral and bilateral lesions to PS on behavioural performance and on functional networks (although only the behavioural effect of these lesions is discussed in this chapter, as MRI analyses were conducted by other researchers).

It was not possible to avoid any retraining on the task while collecting data. In order to assess the effect of this, we collected two days of data from each task during each testing period in

the post-operative stages. This enabled us to compare any improvements in performance between testing days in the same period (which would primarily be due to retraining) with any improvements in performance between testing periods (which would primarily be due to spontaneous recovery). By comparing performance immediately after the first lesion with performance immediately after the second lesion, we were able to assess whether any improvement in monkeys' performance after the first lesion was due to within-hemisphere recovery, or as a result of monkeys learning to rely more on their intact right PS (e.g. by orienting themselves such that the target is presented in the visual field ipsilateral to the lesion). If improvements in performance were due to within-hemisphere recovery, we would expect the second lesion to cause a modest impairment in function (since after the first lesion, monkeys were able to perform the task on the basis of function within the right hemisphere; after the second lesion, monkeys would be able to perform the task on the basis of recovery within the left hemisphere). However, if improvements in performance were due to greater reliance on the intact right PS, we would expect performance on the task to be severely impaired after the second lesion (since monkeys would be impaired in both hemispheres).

Method

Descriptions of the apparatus used to collect behavioural data in this study can be found in Chapter 1.

Subjects

Two experimentally naïve male rhesus macaque monkeys (monkey A and monkey B) were subjects in this experiment. Their mean age at the start of this experiment was 4 years (3 years and 11 months and 4 years and 1 month), and their mean weight was 9.55 kilograms (8.8kg and 10.3 kg). Monkeys were housed together in a pair at the start of the experiment; however, after repeated fights they were housed individually for the remainder of testing for welfare considerations. They were housed in a room with automatically regulated lighting and water available *ad libitum*. All

animal training, surgery and experimental procedures were licensed and conducted in compliance with the UK Animals (Scientific Procedures) Act 1986.

Experimental training

After monkeys had been trained to touch single objects against a black background in order to gain a food reward, training on the experimental tasks began. They were initially trained on the spatial delayed match-to-position task.

Pre-training

In the first stage of pre-training, monkeys were presented with a screen which displayed a red cross on a white background. The width and height of this cross were 35mm on the screen and it subtended a visual angle of approximately 8 degrees from the typical viewpoint of the macaque. It was placed on the circumference of an imaginary circle, the centre of which was the centre of the touchscreen, and the radius of which was 125mm (this imaginary circle subtended a visual angle of approximately 50 degrees from the viewpoint of the macaque). When the monkey touched this cross, it was replaced with a screen displaying a blue square (of sides 25mm; visual angle subtended 6 degrees) at the centre of the touchscreen. After the monkey touched this distractor, there was a delay period of 1s before two red crosses were displayed on the screen (touching this distractor ensured that monkeys were not able to employ a strategy of keeping their hand at the on-screen target position during the delay). These were of identical size to the initial sample stimulus. One was displayed in the same position as the original sample, and one (the foil) was displayed on the opposite side of the imaginary circle (i.e. the angle between the line from the centre of the circle to the centre of the foil cross and the line from the centre of the circle to the centre of the sample cross was 180 degrees). If the monkey touched the cross displayed in the same position as the original sample, a reward pellet was delivered and an inter-trial interval 6s commenced; if the monkey touched the foil cross, no reward pellet was delivered and an inter-trial interval of 12s commenced.

During the inter-trial interval, a blank white screen was displayed. If the monkey touched the screen during the inter-trial interval, the interval time restarted from the time of that touch.

The number of correct trials that monkeys completed each day before being rewarded with a lunchbox was gradually increased. The criterion for moving on to the next stage of pre-training was a score of 90% correct on more than 100 trials. One monkey (monkey B) achieved this score after sixteen days of testing. However, the other monkey (monkey A) was still achieving at chance level after ten days of testing, and took a very long time to complete a short number of trials. In order to make the task easier, a version of the task was introduced in which the correct stimulus flashed twice (a white screen was displayed for 2.5s; then the correct stimulus was displayed for 2.5s; then the white screen, and then the correct stimulus) after an error trial. When this monkey made no improvement after five days of testing on this version of the task, a third version was introduced. In this version, the correct stimulus flashed until the monkey touched this stimulus before the inter-trial interval began. After five days of testing on this version of the task, no improvement had been made, and the monkey returned to testing on the previous version (version 2). After seven days of testing on version 2, the monkey achieved two consecutive days of a score of over 80% correct, and he was returned to testing on the original version.

In the next stage of pre-training, the delay after touching the distractor was varied: the delay was randomly set at 2s, 4s, 8s or 16s, with each delay length comprising 25% of trials. If after three days of testing on this version on the task, monkeys were still achieving >70% correct responses, they progressed to the next stage.

In this stage of pre-training, the angle from the centre of the screen to the two stimuli in the choice phase of each trial was varied randomly between 180 and 45 degrees. Monkeys were tested for one day on this version of the task with a 1s post-distractor delay and for two days on this version of the task with an 8s post-distractor delay.

After reaching this stage of pre-training on the delayed match-to-position task, monkeys began pre-training on the delayed match-to-sample task. In this task, monkeys were presented with a clipart image in the centre of the screen; the background colour of the screen was grey. These images were obtained from commercially available internet sources. Each image was 128x128 pixels (50 – 60mm^2; visual angle subtended 12 – 15 degrees) and comprised a distinct cartoon-like image, superimposed on a grey screen-congruent background. The library used consisted of over 14,000 stimuli, and no stimuli were reused over the course of the task. This stimulus was presented until the monkey touched the stimulus. After this, a blank grey screen was presented for 1s, followed by presentation of two clipart stimuli against a grey background. One of these stimuli was identical to the one initially presented, while the other was a new foil image. One stimulus was presented to the left side of the screen, and the other to the right; the side of the screen that contained the original image was determined randomly on each trial. The stimuli were presented on the horizontal midline of the screen, and were equidistant from the central point. These stimuli were presented until the monkey touched one of the images. If the monkey chose the image which had been presented originally, a reward pellet was delivered and the inter-trial interval was 6s. If the monkey chose the new image, no pellet was delivered and the inter-trial interval was 12s. During the inter-trial interval, a blank grey screen was displayed. If the monkey touched the screen during this interval, the interval time restarted.

The number of trials that monkeys were given on this version of the task was gradually increased. After monkeys achieved a score of >90% across more than 100 trials, they were exposed to the final version of this task for 3-5 days, in which delays between the initial stimulus and the choice screen were varied: in each trial, the delay was randomly set at 3, 5, 9 or 17s, so that there was a 25% chance of each delay. These delays were 1s longer than the comparative delayed match-to-position delays, in order to compensate for the time that monkeys took to touch the distractor in that task. This task is illustrated in figure 1.

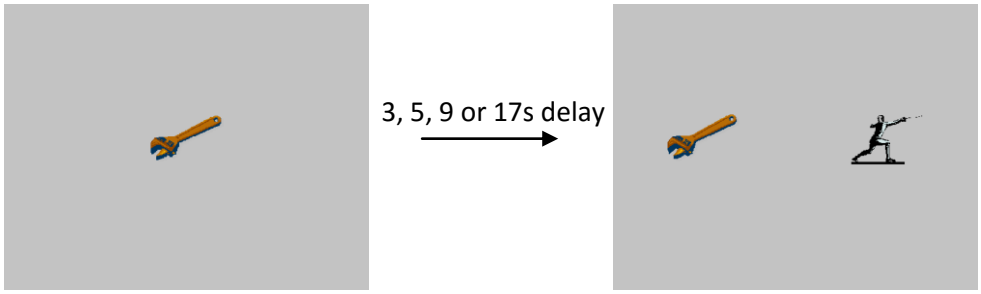


Figure 1 – Display (left) and choice (right) stages of the delayed match-to-sample task. Monkeys were presented with one item; after they touched this, it was removed from the screen. Following a delay (3, 5, 9 or 17s), they were presented with a choice of the previously presented stimulus and a novel stimulus. They were rewarded for touching the previously presented stimulus. Images are derived from commercially available internet resources, including Microsoft Clipart.

After this, monkeys were trained on a 3-choice version of the delayed match-to-position task. This version was identical to that described above, except that in the choice stage of each trial, monkeys were presented with three red crosses, rather than two. These consisted of the correct stimulus, a stimulus positioned in the same location as the target stimulus on the previous trial, and a randomly positioned foil stimulus. The minimum angle between each of these stimuli was 45 degrees. In the pre-training stage of this task, the delay after the distractor was set at 1s; after monkeys achieved a criterion level of 90% over 100 trials, they were moved onto the final version of this task, in which delays were varied over four levels (2, 4, 8 and 16s). This task is illustrated in figure 2.

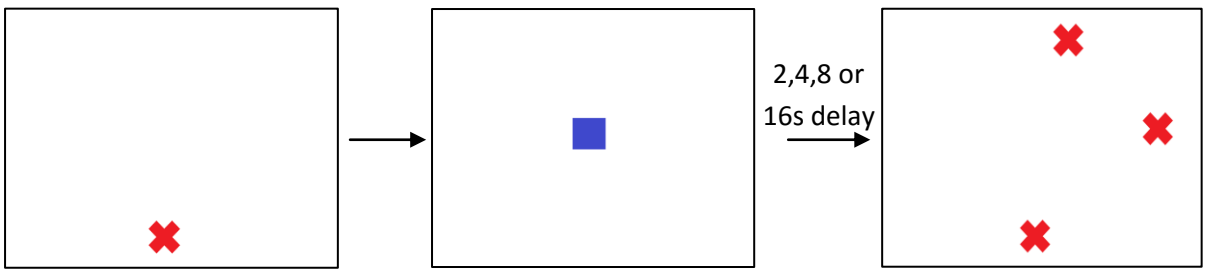


Figure 2 – Display (left), distractor (centre) and choice (right) stages of the delayed match-to-position task. Monkeys were presented with a red cross located at a particular position on the screen; after they touched this, it was removed from the screen and replaced by a distractor in the centre of the screen. After they touched the distractor, this was removed from the screen. Following a delay (2, 4, 8 or 16s), they were presented with a choice of red crosses located on three positions on the screen: the previously presented target location, the target location presented in the previous trial, and a new location. Monkeys were rewarded for touching the most recently presented location.

One monkey (monkey B) was trained on a 3-choice version of the delayed match-to-sample test, which was equivalent to the three choice version of the delayed match-to-position task: in the choice stage of the trial, three stimuli were presented, to the left, right and bottom of the screen; one of the stimuli was identical to the target stimulus presented on that trial; one was identical to the target stimulus on the previous trial, and one was a new foil stimulus. However, as this monkey's performance failed to improve over nine days of testing, it was decided to discontinue training on this version.

Performance tests

After monkeys attained a criterion level of performance on all pre-training levels of both tasks, performance tests were begun. These initially involved six days of testing. On the first two days, monkeys completed a "warm-up" task, in which they were required to touch a single stimulus displayed against a black background in order to gain a reward. On the first "warm-up" day, monkeys completed 50 trials of this task before receiving their "lunchbox" of food; on the second, monkeys completed 100 trials.

On the second two days of testing, monkeys were tested on the delayed match-to-sample task. On the first day, monkeys completed a smaller number of trials on this task (40 for monkey A, 50 for monkey B), and the delay between touching the target stimulus and presentation of the choice screen was 2s. On the second day, monkeys completed a larger number of trials (100 for

monkey A, 120 for monkey B), and the delay between touching the target stimulus and presentation of the choice screen could be 2, 4, 8 or 16s.

On the final two days of testing, monkeys were tested on the delayed match-to-position task. On the first day, monkeys again completed a smaller number of trials on this task (40 for monkey A, 50 for monkey B), and the delay between touching the target stimulus and presentation of the choice screen was 2s. On the second day, monkeys completed a larger number of trials (100 for monkey A, 120 for monkey B), and the delay between touching the target stimulus and presentation of the choice screen could be 2, 4, 8 or 16s.

After the pre-operative stage of testing, the design of the task was altered by introducing a third day of testing on each task in each testing block (i.e. pre-operatively, monkeys received two warm up days, followed by two days of delayed match-to-sample and then two days of delayed match-to-position; post-operatively, monkeys received two warm up days followed by three days of delayed match-to-sample and then three days of delayed match-to-position; in every case the first day of each behavioural task included fewer trials and only short delays between stimulus and test). The number of trials and delay levels were identical on the second and third days of testing. The introduction of a third testing day enabled us to assess monkey performance for evidence of relearning of the task.

After this testing period was completed, monkeys did not perform any more behavioural tasks for approximately 15 days. For those testing periods which were followed by an MRI scan, this was scheduled in the week immediately following testing on the behavioural task (in total, these animals had four pre-operative scans, which did not coincide closely with the testing periods, scans after the first four testing periods after the first lesion was performed, and scans after the first four testing periods after the second lesion was performed); in the second week monkeys were not scanned or tested.

In total, monkeys underwent six testing periods after pre-training was completed and prior to the first lesion. After the first lesion, they received eight testing periods prior to the second lesion. After the second lesion was performed, they received four final testing periods. They received four pre-operative MRI scans, four scans after the unilateral lesion was conducted, and four more scans after the lesion was extended bilaterally.

Surgery

The surgery was carried out as described in Chapter 1. The intended extent of the lesion included both the dorsal and ventral banks and the fundus of the cortex along the entire anterior-posterior extent of the principal sulcus. It also included cortex 2-3 mm dorsal and ventral to the lips of the principal sulcus. This was intended to target the middle portion of areas 46 and 9/46. The lesion was first performed unilaterally in the left hemisphere. After a period of resumed behavioural testing lasting approximately six months, the same lesion was added to the right hemisphere, hence making the lesion bilateral after the second surgery stage.

Data analysis

In order to assess whether these monkeys suffered significant impairments in these tasks after lesions to principal sulcus, and whether they significantly recovered function as time passed after the lesion, only the second day of each testing period was considered (i.e. the first day in which monkeys were tested on 100+ trials, with a range of delay lengths). The data was divided into four testing stages: pre-operative, post-operative 1 (early), post-operative 1 (late) and post-operative 2. The pre-operative stage included the last three pre-operative testing periods; post-operative 1 (early) included the first three testing periods after the first unilateral lesion was performed; post-operative 1 (late) included the last three testing periods of the unilateral lesion state before the second lesion in the opposite hemisphere was added; post-operative 2 included the first three testing periods after the second lesion was added to make the lesion bilateral. Two testing periods

occurred between the end of the post-operative 1 (early) stage and the beginning of the post-operative 1 (late) stage; these were excluded from this analysis in order to balance the number of testing periods included in each stage and to ensure that the time periods considered in the post-operative 1 (early) and post-operative 1 (late) stages were not consecutive. Overall, the period of time from the last post-operative 1 (early) testing period to the first post-operative 1 (late) testing period was nine weeks.

The percentage correct day two score was subjected to an arcsine-transformation before being entered into a three-way between-subjects ANOVA. The low sample size in this experiment meant that it was not possible to use these monkeys as a model for the population as a whole. However, as we made repeated observations of performance in each animal, it was possible to statistically analyse performance across these observations in order to assess whether these particular monkeys significantly recovered after lesion, although it was not possible to analyse whether monkeys in general would follow this trend. In this case, the experimental units were testing sessions, rather than animals. In the ANOVA, there were three between-subjects factors of monkey (two levels: “monkey A” and “monkey B”), task (two levels: object and spatial) and stage of testing (four levels: pre-operative, post-operative 1 early, post-operative 1 late, post-operative 2). This analysis found significant main effects of monkey ($F(1,32)$=64.286, p<0.001), task ($F(1,32)$=224.948, p<0.001) and stage of testing ($F(3,32)$=11.863, p<0.001), and a significant interaction effect between monkey and stage of testing ($F(3,32)$=9.258, p<0.001). A graph of this data is presented in figure 3.

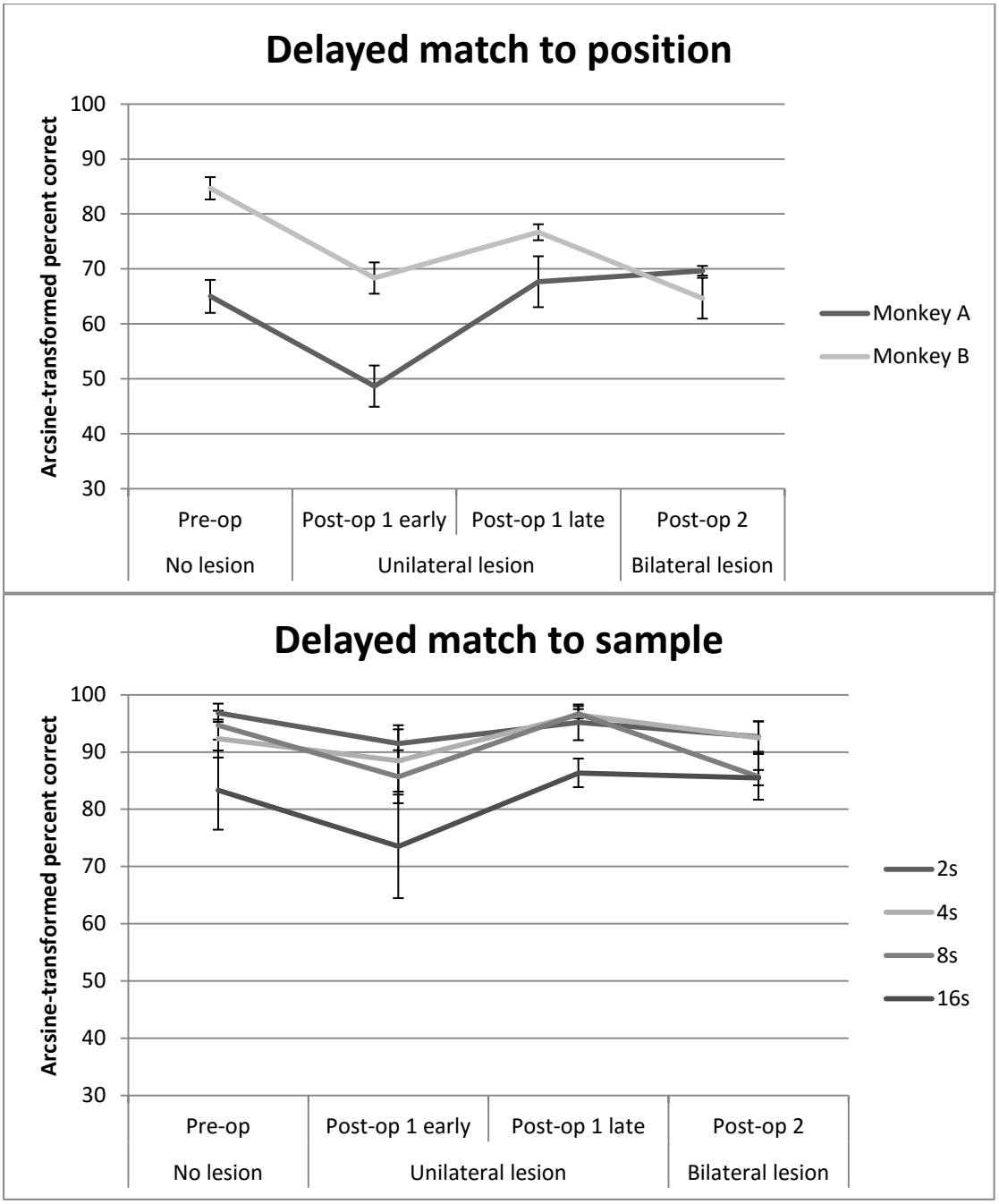


Figure 3 – Percent correct scores on delayed match-to-position (top) and delayed match-to-sample (bottom) tasks for both monkeys across four stages: pre-operative, post-operative 1 (early), post-operative 1 (late) and post-operative 2. Error bars show the +/- standard error of the mean of each monkey's performance across multiple testing sessions in each stage.

In order to investigate the source of this interaction, two-ways between-subjects ANOVAs [two factors of stage of testing (four levels: pre-operative, post-operative 1 early, post-operative 1 late and post-operative 2) and task (two levels: spatial and object)] were carried out separately for data relating to each monkey:

- For monkey A, a significant main effect was detected of both stage of testing ($F(3,16)$=8.792, p<0.001) and task ($F(1,16)$=62.733, p<0.001).
- For monkey B, significant main effects were also detected of both stage of testing ($F(3,16)$=14.771, p<0.001) and task ($F(1,16)$=235.856, p<0.001).

In both monkeys, performance was significantly better on the object task than on the spatial task. In order to clarify the main effect of stage of testing which was observed in both monkeys, planned comparisons were conducted. The comparisons of interest in this study were:

- Pre-operative/post-operative 1 (early) – to investigate whether there was any effect of the first surgery
- Post-operative 1 (late)/post-operative 2 – to investigate whether there was any effect of the second surgery
- Post-operative 1 (early)/post-operative 1 (late) – to investigate whether any recovery could be observed after the unilateral lesion
- Pre-operative/post-operative 1 (late) – to investigate whether function recovered to pre-operative levels
- Post-operative 1 (early)/post-operative 2 – to investigate whether the effect of the bilateral lesion was more severe than the effect of the unilateral lesion

These comparisons were conducted on the spatial task data. As no impairment had been predicted in the object task, planned comparisons on this data were not justified. In monkey A, significant differences which survived correction for multiple comparisons using the Holm-Bonferroni method (Holm, 1979) were detected between the pre-operative and post-operative 1 (early) stages ($F(1,8)$=11.067, p=0.010; this was a reduction in performance), between the post-operative 1 (early) and the post-operative 1 (late) stages ($F(1,8)$=15.369, p=0.004; an improvement in performance) and between the post-operative 1 (early) stage and the post-operative 2 stage ($F(1,8)$=18.609, p=0.003; an improvement in performance). In monkey B, the only significant

difference which survived correction for multiple comparisons using the Holm-Bonferroni method was detected between the pre-operative and the post-operative 1 (early) stage ($F(1,8)$=21.354, p=0.002; a reduction in performance). However, in the same monkey, there was a trend towards impairment which did not survive this correction in the post-operative 1 (late) stage compared to pre-operative ($F(1,8)$=5.884, p=0.042), and in the post-operative 2 stage compared to post-operative 1 (late) stage ($F(1,8)$=9.646, p=0.0145); in these tests, p was greater than the significance threshold of 0.0125.

Separate one-way ANOVAs with one between-subjects factor of stage of testing (four levels: pre-operative, post-operative 1 early, post-operative 1 late and post-operative 2) were used to investigate whether performance on the object task varied by stage of testing for each monkey. In monkey A, no significant effect of stage of testing was detected ($F(3,8)$=3.339, p=0.077). However, in monkey B, a significant effect of stage of testing was present ($F(3,8)$=4.248, p=0.045). A post-hoc Tukey test confirmed that a performance at the pre-operative stage differed significantly from performance at the post-operative 2 stage in this monkey (p=0.030; this represented a reduction in performance in the post-operative 2 stage).

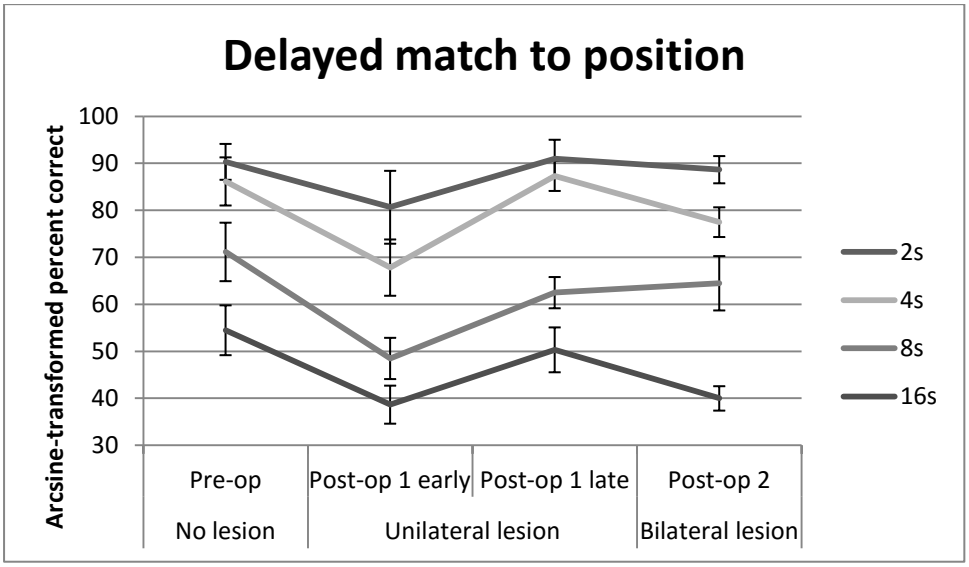
<u>Overall performance – summary</u>

- Both monkeys performed significantly better in the object task than the spatial task.
- Both monkeys suffered a significant impairment in the spatial task after the lesion.
- Monkey A showed significantly improved performance on the spatial task after the unilateral lesion was performed.
- Monkey B was significantly impaired on the object task after the bilateral lesion.

Delay length

It is possible that differences in performance could be observed over greater delay lengths. In order to conduct a post-hoc assessment of the effect of the different delay lengths between

stimulus and test on performance, a four-way repeated measures ANOVA was performed on the arcsine-transformed percent correct scores [one within-subjects factor of delay (four levels: 2s, 4s, 8s and 16s), three between-subjects factors of stage (four levels: pre-operative, post-operative 1 early, post-operative 1 late, post-operative 2), monkey (two levels: “monkey A” and “monkey B”) and task (two levels: spatial and object)]. This revealed significant main effects of delay ($F(3,96)$=152.165, p<0.001), stage ($F(3,32)$=6.705, p=0.001), task ($F(1,32)$=91.137, p<0.001) and monkey ($F(1,32)$=27.613, p<0.001). Interaction effects were detected between delay and task ($F(1,32)$=30.699, p<0.001), delay and monkey ($F(1,32)$=4.196, p=0.008), delay, stage and monkey ($F(3,32)$=2.907, p=0.004) and delay, task and monkey ($F(1,32)$=6.434, p=0.001). This data is presented in figure 4.



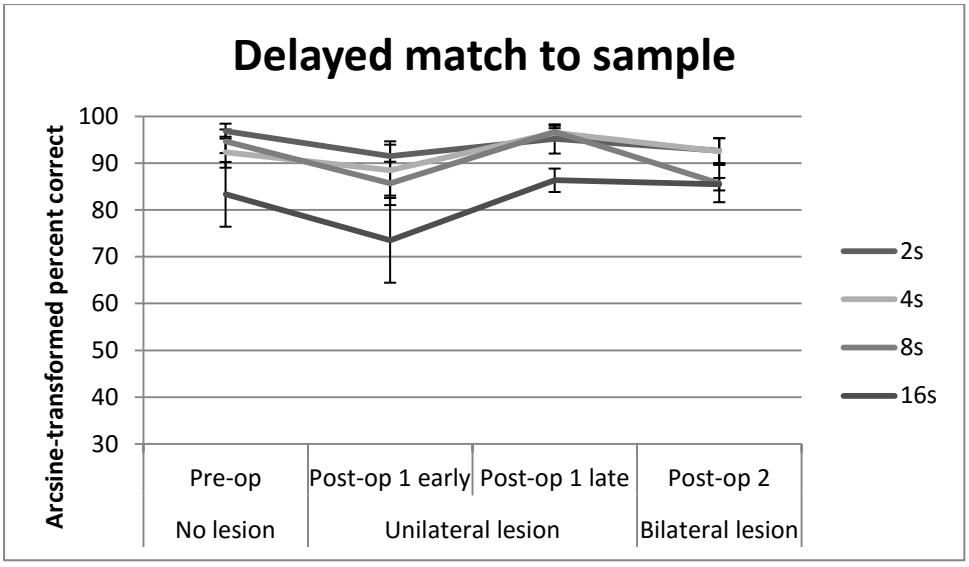


Figure 4 - Percent correct scores on delayed match-to-position (top) and delayed match-to-sample (bottom) tasks for different delay levels across four stages: pre-operative, post-operative 1 (early), post-operative 1 (late) and post-operative 2. Error bars show the +/- standard error of the mean of each monkey's performance at each delay level across multiple testing sessions in each stage.

As I had no hypotheses regarding impairment at different delay levels, it was not valid to conduct planned comparisons on this data. In order to investigate the source of these interactions, separate three-way repeated-measures ANOVAs with two between-subjects factors of stage of testing (four levels: pre-operative, post-operative 1 early, post-operative 1 late and post-operative 2) and task (two levels: spatial and object) and one within-subjects factor of delay (four levels: 2s, 4s, 8s and 16s) were conducted separately for each animal. The results of these ANOVAs are described below.

For monkey A, significant main effects were detected of delay ($F(3,48)$=80.068, p<0.001) and task ($F(1,16)$=30.551). Significant interaction effects were found between delay and task ($F(1,16)$=6.980, p=0.001) and between delay, stage of testing and task ($F(3,16)$=3.247, p=0.004). In order to understand these interaction effects, separate two-way repeated-measures ANOVAs (one within-subjects factor of delay (four levels: 2s, 4s, 8s and 16s) and one between-subjects factor of stage of testing (four levels: pre-operative, post-operative 1 early, post-operative 1 late and post-operative 2) were carried out for the spatial and object tasks.

For the spatial task, significant main effects were detected of delay ($F(3,24)$=73.555, p<0.001) and stage of testing ($F(3,8)$=7.440, p=0.011). The main effect of stage of testing was expected, as this reflects the significant differences already examined above. In order to investigate the main effect of delay, I conducted paired samples t-tests to compare performance at a 2s delay with performance at a 4s delay, performance at a 2s delay with performance at an 8s delay, and performance at a 2s delay with performance at a 16s delay. These t-tests confirmed that performance is significantly different between 2s and 8s ($t(11)$=6.171, p<0.001) and between 2s and 16s ($t(11)$=16.318, p<0.001); no significant difference was detected between performance at 2s and 4s. These comparisons survive correction for multiple comparisons using the Holm-Bonferroni method (Holm, 1979). For the object task, a significant main effect of delay was detected ($F(3,24)$=19.700, p<0.001), in addition to a significant interaction effect between delay and stage of testing ($F(9,24)$=2.703, p=0.025). In order to investigate this interaction effect, paired samples t-tests were conducted between performance at 2s and 4s, at 2s and 8s, and at 2s and 16s for each stage of testing, and the Holm-Bonferroni method was used to correct for multiple comparisons. Performance at 2s and 16s differed significantly ($t(2)$=12.510, p=0.006) in the pre-operative stage; no other significant differences were found which survived correction for multiple comparisons.

For monkey B, significant main effects were detected of delay ($F(3,48)$=77.103, p<0.001), stage of testing ($F(3,16)$=11.602, p<0.001) and task ($F(1,16)$=86.302, p<0.001). A significant interaction effect was observed between delay and task ($F(3,48)$=25.187, p<0.001). In order to investigate this interaction effect, separate two-way repeated-measures ANOVAs [one within-subjects factor of delay (four levels: 2s, 4s, 8s and 16s) and one between-subjects factor of stage of testing (four levels: pre-operative, post-operative 1 early, post-operative 1 late and post-operative 2)] were carried out for the spatial and object tasks.

For the spatial task, significant main effects were detected of delay ($F(3,24)$=146.865, p<0.001) and stage of testing ($F(3,8)$=9.505, p=0.005). A significant interaction was observed

between delay and stage of testing ($F(9,24)$=2.378, p=0.044). In order to investigate this interaction effect, paired samples t-tests were conducted between performance at 2s and 4s, at 2s and 8s, and at 2s and 16s for each stage of testing and the Holm-Bonferroni method was used to correct for multiple comparisons. Significant differences were detected between 2s and 16s at every stage of testing (pre-operative: $t(2)$=14.172, p=0.005; post-operative 1 early: $t(2)$=15.983, p=0.004; post-operative 1 late: $t(2)$=20.457, p=0.002; post-operative 2: $t(2)$=113.190, p<0.001). Additionally, at the post-operative 2 stage, significant differences were detected between 2s and 4s ($t(2)$=20.954, p=0.002) and between 2s and 8s ($t(2)$=22.635, p=0.002). For the object task, a significant main effect of delay was detected ($F(3,24)$=6.571, p=0.002). Paired samples t-tests were used to compare performance between delays of 2s and 4s, 2s and 8s and 2s and 16s. A significant difference was detected between performance at 2s and 16s ($t(12)$=4.216, p=0.001); the significance of this difference survived correction for multiple comparisons using the Holm-Bonferroni method.

Delay length – summary

- Both monkeys were significantly affected by delay in each task.
- In the spatial task, both monkeys performed significantly worse over a 16s delay than over a 2s delay. In the object task, monkey B performed significantly worse over a 16s delay than over a 2s delay; monkey A performed significantly worse over a 16s delay than over a 2s delay in the pre-operative stage.
- Monkey A performed significantly worse at an 8s delay than at a 2s delay in the spatial task; this did not significantly interact with stage of testing.
- Monkey B was significantly more affected by delay length in the spatial task after the infliction of a bilateral lesion; impairments were detected at 4, 8 and 16s delays compared to 2s.

Relearning

The improvement in performance after the unilateral lesion in monkey A could be explained in two ways: the monkey may have relearned the task during testing, or the monkey may have gradually recovered function which was impaired by the lesion. In order to assess this, I compared changes in performance between the two sessions of the testing period (which was likely to be due to relearning) with changes in performance between testing periods (which was likely to be due to recovery). A four-way repeated measures ANOVA was used to assess whether any improvements could be detected between the second and third day of testing of each task within each testing period (i.e. the two days on which monkeys were tested on 100+ trials and with a full range of delay lengths). There was one within-subjects factor of day (two levels: day two and day three) and three between-subjects factors of monkey (two levels: "Monkey A" and "Monkey B"), stage of testing (three levels: post-operative 1 early, post-operative 1 late and post-operative 2) and task (two levels: spatial and object). This analysis detected significant main effects of day ($F(1,20)$=5.372, p=0.031), stage of testing ($F(2,20)$=10.993, p=0.001), task ($F(1,20)$=188.296, p<0.001) and monkey ($F(1,20)$=24.611, p<0.001), and a significant interaction effect between stage of testing and monkey ($F(2,20)$=5.478, p=0.013); there were no interaction effects between day and any other factor in this analysis. There was a mean improvement in performance between the second and third days of testing on each task of 1.737% (arcsine-transformed), with a standard deviation of 4.048. This data is presented in figure 5.

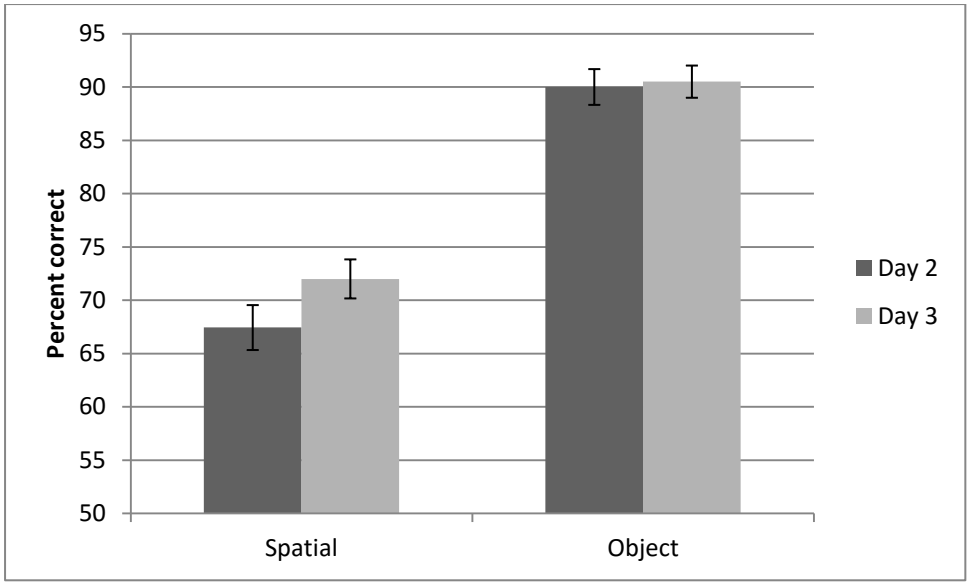


Figure 5 –Percent correct scores on delayed match-to-position and delayed match-to-sample tasks for both monkeys across days 2 and 3 of the testing period. Error bars show the +/- standard error of the mean of both monkeys' performance across multiple testing sessions for each day of testing.

The difference in performance between the second and third days of the session was compared with the average difference in performance between each session and the next (for the purposes of this analysis, the average performance across the second and third days of the session was considered). A paired samples t-test found no significant difference between the change in performance between the two days of the testing period and between the change in performance between each session and the next session ($t(30)$=0.736, p=0.467). The difference in performance between day two of each testing period and day three of the previous testing period was compared with the difference in performance between day two and day three of the same testing period using a paired samples t-test. This also found no significant difference, although there was a trend for there to be greater improvement between the days within a testing period than days between testing periods ($t(30)$=-1.987, p=0.056).

<u>Relearning – summary</u>

- Significant improvement in performance was detected between day two and day three of each testing period.
- This did not significantly differ from improvement in performance between testing periods.

Previous correct location

On each trial in the delayed match-to-position task, it was possible for monkeys to err by choosing either the location which was the correct response on the previous trial, or by selecting a new location. I analysed error responses which were made after correct trials (i.e. after the monkey was rewarded for choosing a particular location) in order to determine whether the proportion of error responses made to the location which was rewarded on the previous trial was affected by the lesion. A two-way between subjects ANOVA was performed, with factors of stage of testing (four levels: pre-operative, post-operative 1 early, post-operative 1 late, post-operative 2) and monkey (two levels: "Monkey A" and "Monkey B"), on the arcsine-transformed error-after-correct data. This did not return any significant effects. This data is presented in figure 6.

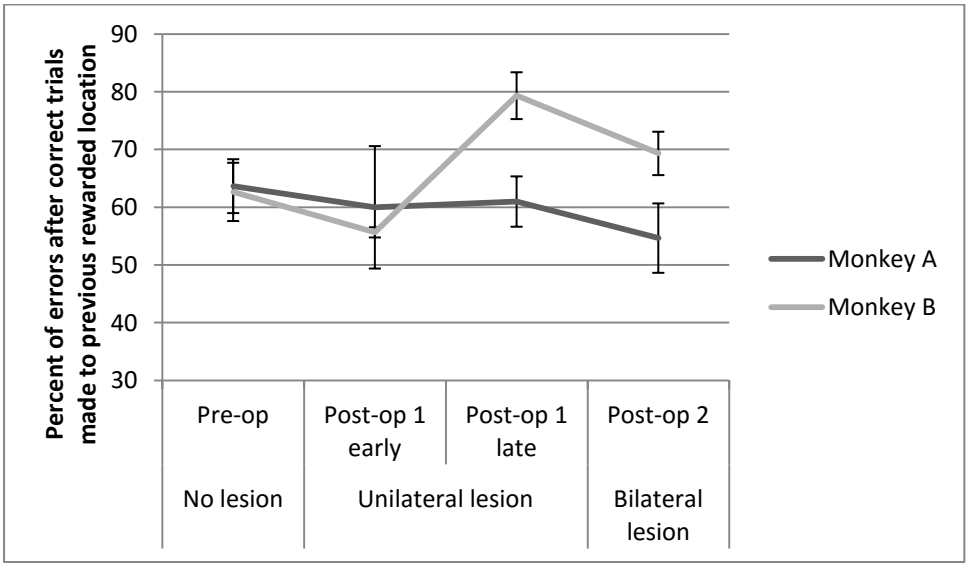


Figure 6 – Percent of error responses following correct trials in which monkeys choose the previously rewarded location, with error bars showing the +/- standard error of the mean for each monkey across multiple testing sessions at each stage.

Discussion

Several conclusions can be drawn from the results of this experiment. It is clear that the two monkeys performed significantly better on the object task than on the spatial task. This is likely to be because, in the spatial task, monkeys had to choose between three possible locations, while in the object task, monkeys had to choose between two possible stimuli. Chance performance was therefore lower on the spatial task (33%) than on the object task (50%). However, it is also clear that monkeys were significantly impaired on the spatial task after the unilateral lesion was performed: a significant reduction in performance was noted for both monkeys between the pre-operative stage and the post-operative 1 (early) stage. This could not be explained by simply forgetting the task over the delay between the last pre-operative testing session and the first post-operative testing session, as both monkeys performed at a high level on the first post-operative day of both tasks, in which monkeys were tested on a low number of trials with short delays between stimulus presentation and test (monkey A scored 71% on the object task and 83% on the spatial task; monkey B scored 100% on the object task and 98% on the spatial task). No evidence was observed to suggest that this deficit was due to an inability to distinguish between stimuli which are relevant on the current and previous trials rather than a mnemonic deficit.

Furthermore, at least one of the animals (monkey A) showed a significant improvement in performance on the spatial task over the course of the six months after the unilateral lesion was performed. This improvement was such that this animal's performance at the post-operative 1 (late) stage no longer differed significantly from his pre-operative performance. In monkey B, no significant difference could be noted between the pre-operative and post-operative 1 (late) stage on the spatial task after correction for multiple comparisons; however, before this correction was applied, the probability that the monkey was significantly impaired at post-operative 1 (late) compared to pre-operative was 0.041; in monkey A, this probability was 0.577. This improvement can be explained in several ways: it is possible that the regular MRI scans under general anaesthetic

which this monkey underwent during the post-operative 1 (early) stage, but not the post-operative 1 (late) stage impaired performance, although no testing period began until more than a week after each MRI scan. It is possible that this monkey had not fully learned the task pre-operatively, and continued to learn the task post-operatively, although monkeys did not begin the cycle of periodic testing sessions until they attained a criterion performance of >90% on the pre-training tasks. However, it is also possible that this improvement in performance is due to gradual recovery of function after the lesion.

In one monkey (monkey B), there was a trend towards impairment in performance between the post-operative 1 (late) stage and the post-operative 2 stage on the spatial task. However, no impairment was detected in either monkey after the bilateral lesion compared to performance after the left hemisphere unilateral lesion. This indicates that monkeys were able to recover function within the left hemisphere which they were able to use after the right hemisphere lesion to complete the task (as they were able to use the spared right hemisphere in order to complete the task after the unilateral left hemisphere lesion), rather than developing cognitive strategies to compensate for the impairment (e.g. orienting their position in the apparatus in order to view the screen with the visual field ipsilateral to the lesion). However, this conclusion may not be supported by evidence from different delay lengths. Increasing the delay between presentation of the target stimulus and the choice phase had a greater effect in the spatial task than the object task for both animals: in the object task, the only effect of delay which could be detected in monkey A was between 2s and 16s in the pre-operative stage, while in monkey B, the only difference which could be detected was a general difference between performance at 2s and at 16s. In the spatial task, however, a significant difference could be observed between performance at 2s and 16s in both animals; in monkey A, a difference also existed between performance at 2s and 8s. In monkey B, significant differences existed between 2s and 4s and 2s and 8s at the post-operative 2 stage. This indicates that monkey B, at least, was more severely impaired after the bilateral lesion than after the unilateral lesion. However, as significant recovery between the post-operative 1 (early) and post-

operative 1 (late) stages was only noted in monkey A, this result does not undermine the conclusion that this monkey significantly recovered left hemisphere function after the performance of the unilateral lesion. Forthcoming neuroimaging data will clarify whether this recovery of function is paralleled by recovery in functional brain networks.

One way in which we assessed whether monkeys displayed significant relearning of the task post-operatively was by testing each task on three consecutive days. The first day was a short session in which monkeys were re-exposed to the behavioural task, but not required to complete a large number of trials or retain information over delays longer than 2s. This ensured that performance on the second day of testing was not impaired by an initial readjustment period, and it is reasonable to assume that performance on the second and third days reflected monkeys' true capacity on the task. Since it is implausible that a significant effect of recovery could be detected in such a short time period, improvement on the task between days two and three can be attributed to relearning. The results of this study indicate that significant relearning did occur between the second and third testing days of each period post-operatively. When difference in performance within testing sessions is compared with difference in performance between testing sessions, no significant difference can be observed, although a trend suggests that there may be greater improvement within sessions than between sessions. It is therefore not possible to conclude that improvement in performance post-lesion is attributable to recovery of function rather than relearning. However, it may not be possible to draw a firm distinction between recovery of function and task learning since the intensive testing with immediate feedback which occurred during testing periods might have itself had a rehabilitative effect.

While the very small sample size tested in this experiment means that it is impossible to conclude whether the results observed here are generalizable to all animals, there is sufficient evidence to demonstrate that at least some animals may differ in task performance depending on the time that they are tested relative to lesion. Although it was impossible to isolate the source of this

improvement, this evidence indicates that the time of testing relative to lesion may be a crucial variable in experiments which rely on animal lesion techniques; if this period is prolonged, it is possible that a reduced impairment may be observed on tasks which do crucially rely on the lesioned region in healthy animals, leading to false negative results. This may be significant for techniques such as the infliction of crossed unilateral lesions (Gaffan and Wilson, 2008, provide a review), which are dependent on a lack of observable impairment after a single unilateral lesion in order to reach valid conclusions. Further research is required in order to determine whether this consideration also applies after bilateral lesions. These lesions, which constitute the majority of surgically targeted experimental lesions, generally result in more severe impairment in function and lead to a deficit which is more resistant to rehabilitation (Kolb and Gibb, 1991) than those impairments which follow unilateral lesions. Evidence considered in Chapter 1 indicates that behavioural recovery can be observed in after bilateral lesions to the frontopolar cortex; however, this recovery has yet to be demonstrated within subjects.

DISCUSSION

This thesis has applied both behavioural and neuroimaging techniques in order to investigate the question of the functional role of the primate FPC. In Chapters 1, 2, 3 and 4, I examined performance on a range of behavioural tasks in unoperated control monkeys and monkeys with lesions to FPC in order to assess the effect which lesions to this region have on behaviour. In Chapter 5, I examined data acquired through magnetic resonance imaging in order to analyse the effect that lesions to FPC have on the functional connectivity of resting state networks in the monkey brain.

A second question which has been examined by this thesis relates to the longitudinal effect of neurosurgical lesions. In Chapters 1 and 2, I compared performance on behavioural tasks between monkeys who had recently received lesions and monkeys who had received lesions several years earlier. In Chapter 5, I compared functional connectivity in resting state networks between monkeys two months and forty months after lesions to FPC. In Chapter 6, I conducted a longitudinal study which examined the effect of unilateral and bilateral PS lesions in two monkeys on performance on behavioural tasks for a period of 6 months post-lesion.

The role of the primate frontopolar cortex

Several changes in behaviour were noticed in macaque monkeys after lesions to FPC. In Chapter 1, I observed an enduring deficit in judgements of recency context and a short-term deficit in recognition memory after such lesions. In Chapter 3, I observed that monkeys with lesions to FPC may place less weight on the outcomes of more distant trials compared to those which occurred more recently. In Chapter 2, I found no clear evidence of a deficit in recollection after lesions to FPC, and in Chapter 4, I found no clear evidence to suggest that lesions to FPC cause changes in social valuation in monkeys. These behavioural results must be interpreted in the light of current knowledge regarding the function of the macaque area 10. However, few studies have yet been

conducted which examine the role of this region in the monkey brain. Of those that have been conducted, one study found that monkeys with lesions to this area are impaired at one-trial learning and at exploring behavioural choices in order to learn about new rules if the opportunity to exploit a previously-learned rule is present (Boschin et al., submitted), and an electrophysiology study found that neurons in this region may have a role in the evaluation of outcomes relating to self-generated actions (Tsujimoto et al., 2010). Macaques with lesions to this region are unimpaired on a range of tasks, including applying more than one abstract rule simultaneously (Boschin et al., submitted), and retaining information relevant to one behavioural task even when interrupted by a second behavioural task (Mansouri et al., submitted); their performance is enhanced relative to controls at performing a behavioural task when distracted by salient events, or when one behavioural task is interrupted by another (Mansouri et al., submitted). While the results described in this thesis do not conflict with these conclusions, it is not clear that all these previous results and the results presented in this thesis can be described by a single, parsimonious theory: for example, Tsujimoto et al. found activity in neurons in the frontal pole relating to monkeys' self-generated "stay" or "shift" decisions at the time of feedback, rather than at the time of decision. However, the deficits in recency context judgement observed in Chapter 1 after lesions to frontal pole cannot be dependent on a general ability to evaluate behavioural choices, as, once monkeys successfully learned the task, the choices made on previous trials were irrelevant to the optimal choice on future trials. Evidence from cytoarchitecture (Carmichael and Price, 1994; Semendeferi et al., 2001) and anatomical connectivity (Petrides and Pandya, 2007) indicates that it may not be appropriate to describe area 10 in macaques as a homogenous region with one associated function; rather, the diversity of results thus far collected regarding the function of macaque area 10 may be explained if different subregions within this area underpin different cognitive functions.

The body of literature relating to the function of the human area 10 is much larger. This region has been associated with many different functions, such as the establishment of task sets, balancing the requirements of multiple tasks, reasoning, episodic memory, attention to internally

and externally generated information, mentalising and decision-making. As in macaques, evidence from cytoarchitecture (Bludau et al., 2013), structural connectivity (as measured by diffusion tractography; Sallet et al., 2013; Neubert et al., 2014) and functional neuroimaging (Gilbert et al., 2006c) suggests that the lateral and medial portions of this region may be heterogeneous, indicating that it may be appropriate to examine the function of subregions of this area independently. However, it is only possible to use information gathered in one species to elucidate the functional role of a brain area in another species if it can be established that these areas are homologous. While there is general agreement that medial area 10 is homologous in humans and monkeys, on the basis of similarity of cytoarchitecture (Semendeferi et al., 2001) and functional connectivity (Sallet et al., 2013; Neubert et al., 2014), less agreement exists relating to whether a brain area exists in macaques which is equivalent to human lateral area 10. Neubert et al. (2014) claim that there is no region in the macaque prefrontal cortex which shares a profile of functional connectivity with human lateral FPC; instead, they believe that the monkey area lateral to the area they identify as medial area 10 is more properly identified with macaque area 46. However, cytoarchitectural similarities exist between the macaque area lateral to medial area 10 and human lateral area 10 (Semendeferi et al., 2001). Furthermore, while Neubert et al. base their claim on the identification of a human lateral frontopolar area which is mainly interconnected with other prefrontal regions, but which also shows coupling with parietal areas (particularly the inferior parietal lobule). This parietal coupling was not observed by Neubert et al. in the macaque lateral area 10. However, Markov et al. (2014) identified anatomical connections between macaque area 10 and parietal areas, suggesting that the distinctive features of the human lateral area 10 may also be observable in the macaque frontal pole. It is not the case, however, that anatomical homology (or a lack of anatomical homology) necessarily indicates that these regions are (or are not) functionally homologous. This must be achieved through comparison of the results of studies which examine frontopolar contributions to behaviour in these species.

The studies described in this thesis go some way towards establishing the functional homology of human and macaque area 10. The recency deficit observed in macaques in Chapter 1 parallels findings in humans which indicate that area 10 is activated when subjects are required to make recency judgements (Dobbins et al., 2003; Dudukovic and Wagner, 2007). Furthermore, there is evidence that humans who have suffered damage to area 10 are impaired at making prospective memory actions when they are required to judge these based on the passage of time (e.g. "in 30 seconds") and that they are impaired at judging the passage of time (Volle et al., 2011). Although no study has yet assessed the performance of human frontopolar patients on recency judgement tasks, it seems clear that both monkey and human area 10 play a role in assessment of the passage of time. This is a function that, in humans, cannot be clearly localised to lateral or medial area 10; as the monkeys tested in this experiment had lesions to both lateral and medial areas of FPC, this study cannot address the hypothesis that only the medial portion of human area 10 has an equivalent region in macaques.

The evidence described in Chapter 3 may also help to establish the functional homology of macaque and human area 10. Like human patients, macaques with lesions to FPC do not suffer a general deficit to the WCST (evidence relating to human patients is described by Anderson et al., 1991, among others; evidence described in Chapter 3 supports the finding of Mansouri et al., submitted, that macaques with lesions to this region do not suffer a general impairment). However, many studies have indicated that FPC may play a crucial role in human decision-making, especially in environments in which the probability of a reward associated with a particular behavioural choice varies over time (e.g. Boorman et al., 2009 and 2011; Daw et al., 2006), and that FPC-lesioned patients are impaired at making decisions in which they must weigh the value of competing tasks and decide the optimal way in which to allocate their time (Burgess et al., 2000). In Chapter 3, I discussed evidence that FPC-lesioned monkeys may prioritise the outcome of the two most recent trials over more distant outcomes when making decisions; this speculation is based on the finding that FPC-lesioned monkeys are less influenced than control monkeys by the outcome of the third-

most recent trial in the WCST task. If this speculation is robust, it may indicate that, in monkeys, the FPC plays a critical role in decision-making in environments in which optimal behaviour cannot be determined by examination of the outcome of the most recent trial. In these non-deterministic environments, the probability that a particular behaviour will lead to a reward must be based on analysis of the outcomes of all trials (with particular weighting to more recent ones, if the environment is changeable). The results of this chapter may indicate that the monkey frontal pole performs a similar role to human BA10 in decision-making in uncertain environments.

In humans, decision-making in uncertain environments has been associated with both lateral and medial FPC. Boorman et al. (2009) describe activation in lateral FPC which correlates with the probability that an unchosen option would have received a reward, and activation in an area that they describe as "ventromedial PFC" which correlated with the relative value of the reward associated with a chosen option: analysis of the coordinates of this "vmPFC" region of interest using MRIcron software (Rorden et al., 2007) indicates that this region may lie within medial BA10. While there is consensus that the macaque FPC contains a region which is anatomically equivalent to human medial FPC, there is less agreement that any equivalent to human lateral FPC may be found in monkeys. However, Boschin et al. (submitted) describe evidence that monkeys are impaired at learning about the value of counterfactuals after lesions to FPC. FPC-lesioned animals were tested on a match-to-sample task that they were already familiar with; they were unimpaired on this task. However, when an additional rule ("smaller than") was introduced to this task, such that monkeys had to combine the two rules ("same as" and "smaller than") in order to correctly identify the rewarded stimulus out of four possible options on the screen (two of which matched the target stimulus, and two of which were smaller than this stimulus), these animals are impaired at learning to apply the new rule without the aid of correction trials (in these trials, if the monkey chose an incorrect stimulus, the same options was presented again until the monkey chose the correct stimulus), implying that they were less likely to explore whether unchosen options on the screen were associated with a reward than healthy monkeys, but that they learned normally when

encouraged to explore through the repeated presentation of the same options and thus discovered that previously unchosen options were associated with a reward. These results imply that monkeys require an intact frontal pole in order to value unchosen options, implying that the monkey frontopolar area may contain a region which is functionally homologous with human lateral area 10.

The evidence described in Chapter 5 may also indicate that the effects of lesions to macaque FPC on functional brain networks are similar to those observed in humans. In Chapter 5, I observed a decrease in functional connectivity in anaesthetised macaques after lesions to the frontal pole, even in connections between regions in which neither region is anatomically connected to area 10. Rowe et al. (2007) observed a decrease in functional connectivity in human brain networks in awake behaving patients with lesions to BA10 and BA46, and this decrease could be observed among a diverse range of regions in the brain (SMA, BA8, BA44, fusiform gyrus, parietal cortex), some of which are not anatomically connected to or have only limited connectivity with area 10 (SMA, fusiform gyrus, parietal cortex).

This thesis has also discussed evidence which may indicate that macaque and human FPC are not homologous. In Chapter 1, I observed a short-term recognition memory deficit in monkeys after lesions to area 10 (this deficit was observed when monkeys were tested two weeks post-lesion, but not when they were tested 32-38 months post-lesion). This may indicate that a dissociation can be observed between human and macaque area 10, as human FPC patients are not impaired in tests of recognition memory (Alexander et al., 2003). However, this recognition memory deficit was only observed a short period of time post-lesion, and did not endure: macaques tested 32-38 months post-lesion were not impaired. Further evidence that macaques with lesions to FPC are not impaired at delayed match-to-sample and delayed non-match-to-sample tasks is described by Boschin et al. (submitted). It is likely that, since this deficit was not enduring, while other differences between FPC-lesioned and control monkeys did not show recovery, FPC was only incidentally, and not critically, necessary for this function. A second functional difference between macaque and human FPC may

be observed when the results of Chapter 3 are contrasted with those described by Kovach et al. (2012). These researchers found that human FPC patients prioritise the outcome of more distant trials over the outcome of the most recent one, which was the opposite pattern to that observed in macaques in Chapter 3; however, as discussed in Chapter 3, the frontopolar lesion group studied by Kovach et al. was poorly controlled, indicating that the conclusions of that study may be unreliable.

The results of this thesis indicate that there are good reasons to believe that the macaque and human FPC do have some homologies, and that it may be legitimate to use the macaque FPC as a model for human area 10. However, further research must be done to fully describe the functional similarities and differences between FPC in macaques and humans, including testing human frontopolar patients on analogues of those tasks which are impaired in macaques by lesions to this area, in addition to tasks which are not impaired in FPC-lesioned macaques (for example, research conducted by Mansouri et al. (submitted) indicates that macaques with lesions to FPC are not impaired in an experiment which requires them to maintain information relating to one task in a pending state while carrying out a second task; this process has been described by Koechlin as "branching", and has been associated with activation in human FPC (e.g. Koechlin and Hyafil, 2007). If the pattern of impairment and spared performance in human patients replicates that observed in macaques, this can be considered strong evidence that FPC is functionally equivalent in humans and monkeys. Furthermore, functional connectivity studies which examined changes in resting state networks in anaesthetised human patients after damage to BA10 or which examined changes in functional networks in awake, behaving macaques after lesions to FPC would clarify whether functional network changes in humans and macaques after lesions to this region are similar in conditions which are more directly equivalent than those examined thus far. A further useful approach would be to conduct macaque lesion studies which specifically target medial and lateral area 10 in order to investigate whether the behavioural results of these lesions can be dissociated, and whether those dissociations replicated the dissociations observed in activations of subregions of FPC in human neuroimaging studies. If any human patients with lesions which are localised to lateral

or medial FPC are found, it would be useful to conduct a case study which compared their pattern of impairment to that discovered in macaques with more targeted lesions.

If we accept the legitimacy of the macaque FPC as a model for human frontopolar function, the results described in this thesis can provide evidence for the function of this brain area in primate species. Evidence from published human studies and from Chapter 1 indicates that FPC is implicated in making judgements about the passage of time in both humans and monkeys, whether this is in judging which of two stimuli was presented more recently (in humans and macaques), estimating units of time, or performing an action after an interval of a particular duration. This observation may clarify the role of FPC in episodic memory: in humans, tasks of this nature consistently activate area 10, but are not impaired by damage to this region. Since episodic memory is defined by recall of the context in which an event occurred, including temporal context, we would expect to observe activation of FPC in situations in which episodic memory is activated; since it is possible to report the past in detail without a specific awareness of when an event occurred in relation to the present, we would not expect frontopolar patients to be impaired in most tests of memory. However, since both lateral and medial activation of BA10 has been observed in source memory tasks, occurring at different stages of task processing (Simons et al., 2005b), it is likely that different areas of FPC contribute to this function in different ways. One possible explanation may be that lateral FPC is activated by recall of temporal context, followed by activation of the medial area of FPC which is implicated in mentalising (since autonoetic awareness and mental time travel are sometimes cited as crucial aspects of episodic memory). Unfortunately, those chapters in this thesis which addressed questions of episodic memory and social cognition most directly failed to yield clear results (Chapters 2 and 4). Further research in this area should continue to address the contribution of macaque BA10 to episodic memory and social cognition; recommendations for implementation of these studies are given in Chapters 2 and 4. It may be useful to test both monkeys with frontopolar lesions and human frontopolar patients on a source memory task.

Lateral FPC is not selectively activated by tasks in which subjects retrieve information from episodic memory. Human neuroimaging studies have also identified activation of this area in relation to task sets, multiple task scenarios, reasoning, decision-making, and internally-generated information. It is possible that the theory described above may account for a wider range of these results: for example, recency memory may contribute to successful processing in decision-making situations. A striking feature of the "bandit" tasks often used to assess decision-making behaviour is that the probability of receiving a reward for making a particular action is varied throughout the task scenario; however, even when an action is associated with a high probability of reward, that probability is rarely deterministic (i.e. it will not equal 0 or 1). In order to achieve optimal behaviour in these situations, the subject must continually update estimated reward probabilities, taking into account an extended history of reward outcomes but giving more weight to recent outcomes over more distant ones. Intact recency memory is required for normal performance on this task, as subjects must be able to judge which outcomes occurred most recently in order to weight them appropriately.

In Chapter 3, I compared the behaviour of monkeys with a range of prefrontal lesions on a WCST analogue and examined the weight that they gave to the outcomes of recent choices in making decisions. I found that lesions to ACC, OFC, PS and FPC influence performance on this task in differing ways. Lesions to FPC may have caused monkeys to prioritise the outcomes of the most recent trials over the outcomes of more distant ones in making decisions. However, this change in behaviour cannot be simply described as a failure of recency memory, since monkeys clearly did prioritise the outcome of the most recent trial (evidence from Chapter 1 did not indicate a selectively spared ability to recognise the most recent stimulus). Furthermore, human neuroimaging studies (Boorman et al., 2009) and monkey lesion studies (Boschin et al., submitted) have indicated that lateral FPC is implicated in assessing the likelihood that an unchosen option is associated with a reward; it is not possible to explain this finding in terms of recency memory. The results of Chapter 3 may indicate that monkeys with lesions to FPC are impaired at switching from a currently-selected

rule onto an unchosen option on the basis of an extended history of trial outcomes. In the WCST task, optimal performance can be achieved through repeating the action chosen in the previous trial (if rewarded) or switching to the other possible outcome if the action chosen in the previous trial was not rewarded; there is no advantage gained by considering the extended history of reward, thus human and monkeys with lesions to area 10 are not impaired on this task. However, human patients with lesions to FPC are impaired when they are required to sample a range of tasks, decide how to optimally allocate their time between these tasks in order to achieve a higher-level goal, and implement this plan, switching tasks multiple times within a short interval. The results of Chapter 3, when combined with human neuroimaging evidence which implicates FPC in tracking the value of counterfactuals, indicate that this impairment may be due to an inability to use the outcomes of past actions in order to estimate the value of actions which differ from the current behavioural choice, leading to a failure to switch from the current action to other, more favourable, alternatives.

Further research is required in order to confirm this theory. It would be advantageous to test both monkeys with lesions to FPC and human frontopolar patients on a "bandit" task in order to confirm whether they are indeed impaired at task-switching in scenarios in which they are required to estimate the value of counterfactual options based on an extended reward history: it seems likely that, in this situation they would either perseverate on one option (since they would be unable to estimate the value associated with other options), or switch behaviours in response to any unfavourable outcome (since they would be driven solely by the most recent outcome, and not by the extended history of reward). Observation of which, if either, of these predictions are borne out might indicate whether subjects are driven to switch behaviours more by the positive counterfactual possibility of a reward from an alternative choice, or the negative experience of not receiving a reward from the current choice.

In Chapter 5, I observed that lesions to FPC cause long-term disruption to widespread functional networks in the brain in anaesthetised monkeys. Connections disrupted include those between

regions in which neither area is functionally connected with FPC, such as V1 and MT, indicating that indirect connections between FPC and posterior regions in the brain have an important role in the functioning of posterior networks. It is implausible that a role encompassing such a large area of the brain is limited to counterfactual evaluation or judgements of recency. Rowe et al. (2007) observed a similar decorrelation in activation in posterior networks in awake frontopolar and principal sulcus patients, which was related to task demands (the reduction in correlation was greatest on trials in which patients did not change their behaviour). It is not possible to link the reduction in functional connectivity observed in Chapter 5 with any one cognitive process, as the monkeys were anaesthetised at the time of scanning. However, the results observed indicate that FPC may play a role in coordinating processing in posterior regions; such a role might be linked to the hypothesised function of the FPC in preparing "task sets" (e.g. Sakai and Passingham, 2002). According to this theory, FPC is necessary in order to represent a goal and all of the cognitive processes which are necessary to achieve that goal, in preparation for undertaking these processes. This role would require FPC to coordinate activity in a wide range of posterior regions. Further experimentation is required in order to clarify this hypothesis, since lesions to FPC clearly do not impair all goal-directed behaviour which requires the coordination of cognitive processes (e.g. macaques with lesions to this area can apply multiple abstract rules simultaneously; Boschin et al., submitted). Since neuroimaging evidence in humans suggests that FPC responds to task conditions that subjects are primed to expect, rather than those which actually occur (e.g. Burgess et al., 2001; Wagner et al., 1998), a task could be designed in which human frontopolar patients or FPC-lesioned monkeys are primed to expect a certain condition (e.g. monkeys could be trained that a recognition task with a blue background implies 70% old stimuli, while a recognition task with a red background implies 70% new stimuli), which is then not met; performance on this task could be compared with performance on blocks in which the conditions which subjects are primed to expect are met.

Further research must also address whether monkeys with lesions to the frontopolar cortex suffer deficits relating to directing attention towards internally-generated or externally-generated

information (Burgess et al., 2006), or in switching between multiple complex cognitive tasks. While the lack of impairment observed in the WCST analogue (Mansouri et al., submitted; Chapter 3) implies that the monkey FPC is not necessary for mediating between attending to internal and external stimuli (since monkeys were required to respond to external stimuli on the basis of an internally-held abstract rule), this task tested macaques' ability to respond on the basis of both internal and external information, but did not directly test their ability to shift attention between these types of information. It may be possible to test whether FPC-lesioned macaques are impaired at making such shifts in attention by, for example, training monkeys on an analogue of the first task used by Gilbert et al. (2005): monkeys could be trained to respond to a rotating stimulus at regular intervals (externally directed attention), which gradually fades until it is invisible; monkeys would then have to direct attention internally in order to maintain regular responses. Similarly, while the enhanced performance in FPC-lesioned animals in an experiment in which macaques alternated between a WCST-analogue task and a face/non-face discrimination task (Mansouri et al., submitted) implies that these animals are not impaired at switching between multiple tasks, the undemanding nature of the face/non-face task may have reduced the chance of detecting a deficit in these situations. Testing FPC-lesioned macaques on an experiment in which they are required to switch between two complex tasks will clarify whether damage to this region impairs performance in such scenarios.

Recovery after lesion

Chapters 1 and 6 of this thesis describe improvements in performance on behavioural tasks when macaques are tested a long period of time after neurosurgical lesions are inflicted. These improvements are described in relation to lesions which are both bilateral and unilateral, and which were performed to different areas of the prefrontal cortex (areas 10 and 46). In Chapter 1, I described performance on a recognition memory task, in which I observed a deficit when monkeys were tested two weeks after lesions to FPC, but in which no deficit was observed when monkeys

were tested 32-38 months post-lesion. However, an enduring deficit was observed in another task, which tested monkeys' ability to judge how recently a stimulus had occurred.

The selective recovery observed in these monkeys could be interpreted in three ways. It is possible that FPC is necessary for performance in both tasks, and that both tasks were impaired after the lesion and that improvement on both tasks recovered after the lesion (Chapter 6 describes mechanisms for potential post-lesion recovery of function); the deficit which was observed at the FPC-late stage in recency memory but not in recognition memory may have been due to a larger recency memory deficit post-lesion that had not fully recovered by the time that monkeys were tested during the FPC-late stage. A second possibility may be that both recency memory and recognition memory were similarly selectively impaired post-lesion, but that recognition memory is in some way more recoverable than the recency memory. A third explanation may be that the FPC is not critically involved in recognition memory, and the infliction of the lesion causes a generalised disruption to all cognitive processes, which, after a certain period of time, recovers, leaving disruption in only those functions which relied on cognitive processing dependent on the damaged area of the brain (in this case, recency memory).

Chapter 5 provides evidence that lesions to FPC may cause generalised disruption in the brain; lesions to this area disrupt connectivity in functional networks, even when the regions in that network are not anatomically or functionally connected with FPC. However, evidence from other studies indicates that patients with lesions to FPC are not impaired on all tests, or even on all tests of memory, even when tested immediately after the lesion is sustained: the patient studied by Hoffmann and Bar-On (2012) was tested on a battery of tasks 24 hours after receiving an isolated lesion to BA10 as a result of an intracerebral haemorrhage; she performed in the normal range of almost all tests administered, including recall of five words after an interval of five minutes (although in this case the lesion was located in the medial, rather than lateral, area of BA10). Boschin et al. (submitted) tested monkeys post-operatively on a task in which they were required to learn which of

two objects in a complex visual scene were rewarded, for 10 scenes, and repeatedly choose this object in order to gain a food reward. Monkeys were tested on this task 3-6 weeks after the lesion was performed. While a one-trial learning deficit was observed on this task, monkeys with lesions to FPC were not impaired in overall performance, indicating that they did not, at this stage post-lesion, suffer from a generalised memory deficit. The third possibility described is therefore implausible. However, since no data was collected in the FPC-early stage on the recency task, it is not possible to conclude whether monkeys showed recovery on this task between the FPC-early and FPC-late stages, and it is unclear whether monkeys experienced selective recovery on the recognition memory task, or whether the recency task was simply more severely impaired by the lesion; a longitudinal study in which monkeys are trained and tested on both of these tasks pre-operatively, and then tested at multiple time-points post-operatively would address this question.

In Chapter 5, MRI scans were performed on three monkeys eight weeks after lesions to FPC, and on four monkeys an average of 40 months after lesions to this area. These scans did not detect any recovery in functional connectivity networks which were disrupted by these lesions; in fact, greater disruption was noted in the FPC-late group than in the FPC-early group (although this difference did not reach statistical significance). This result conflicts with the data collected in Chapter 1, which indicates that it is possible to observe behavioural recovery after lesions to this region. While it is possible that the lack of recovery of functional networks observed is the result of functional reorganisation, which could allow recovery of cognitive functions in the absence of restoration of the networks which are present in healthy subjects, it seems unlikely that this reorganisation would extend to networks which are not connected to the area of damage. It is likely that the recovery observed in the recognition memory task in Chapter 1 indicates that the impairment observed in this task was not due to the widespread functional disorganisation which occurred as a result of FPC lesions, but due to some other effect of FPC lesions. It is possible that this task only marginally relied on processing which occurs in the FPC, and that monkeys were able to learn new cognitive strategies localised in other brain regions that they were able to use to complete

this task after FPC was removed. By contrast, the functional disorganisation observed in Chapter 5 was widespread and long-lasting, and indicates that an intact FPC is necessary for normal function of posterior networks in a way which cannot be compensated by other brain regions. The bilateral lesions inflicted on monkeys in this experiment may have impaired monkeys' ability to recover from these lesions: Kolb and Gibb (1991) observed that rats with bilateral frontal lesions show less dendritic branching in the parietal cortex post-lesion than rats with unilateral lesions.

In Chapter 6, I conducted a longitudinal study which measured changes in performance after unilateral lesions to principal sulcus. This study observed significantly improved performance in a delayed match-to-position task in one animal over the course of six months post-surgery. No evidence could be observed to suggest that this animal was more severely impaired after a second surgery was performed to extend this lesion bilaterally, indicating that this monkey achieved genuine recovery after the unilateral lesion, as opposed to applying a strategy in which the animal relied on the intact hemisphere in order to succeed in the task (e.g. by orienting his body so that the stimulus was displayed in the visual field ipsilateral to the lesioned hemisphere). This recovery occurred without extensive retraining on this task (monkeys were tested on the task for three days in each three week period), although it was not possible to exclude the possibility that this improvement occurred as a result of retraining. This is in contrast to the recovery observed above in FPC-lesioned monkeys as, in this experiment, recovery was observed over a shorter period of time (6 months vs. 40 months), and without extensive retraining; in the frontopolar group, monkeys received long-term post-surgery training on a variety of experimental tasks. Furthermore, in this study, recovery was observed within-subjects, whereas the difference in performance between FPC-early and FPC-late time-points described above was observed exclusively between-subjects. This study validated the hypothesis that recovery from lesion may occur in experimental scenarios, and that this recovery may lead to negative findings if animals are tested a long period of time after lesions are inflicted. This behavioural study was accompanied by an MRI study, in which functional connectivity resting state data was periodically obtained from these monkeys; further analysis of this

data (analysis of this MRI data-set is not part of the work contributing to my thesis) will confirm whether the recovery which was observed in the behavioural data can be correlated with recovery of functional networks.

It is possible that the behavioural recovery observed in Chapters 1 and 6 was due to post-operative training of animals on experimental tasks. Neuroplasticity after lesion is enhanced when training is repeated, intense and specific (Kleim and Jones, 2008): it is possible that experimental testing which targets the cognitive processes in which deficits are experienced post-lesion has a rehabilitative effect in lesioned animals. This question was not specifically addressed by those chapters in this thesis which observed post-lesion recovery in laboratory animals, but should be a priority for future research. This can be addressed by, for example, training a group of lesioned animals on an experimental task in which they are known to suffer a post-operative deficit (such as a group of PS-lesioned monkeys on a delayed match-to-position task) and then testing that group and a group which has received training on a control task on a task which tests the impaired function in another context (another test of spatial delayed response might be an ambulatory spatial alternation task).

Concluding remarks

This thesis has contributed in a number of ways to the understanding of the primate frontopolar cortex. It has helped to establish the functional homology of this region in macaques and humans, by identifying behavioural deficits in macaques with lesions to this region which parallel some deficits noted in humans with lesions to this region, and in tasks which parallel tasks which induce activation in the human area 10. It has furthered our understanding of the functional role of this region in macaques, by indicating that it may play a role in judgements of temporal context and learning from an extended history of rewards, and that it is necessary for the intact functioning of posterior brain networks, even those which are only indirectly connected to FPC. Furthermore, through research into the time-course of recovery of function after lesion, it has also indicated that,

while some cognitive deficits can be observed a long period of time after experimental lesions are inflicted, other deficits may only be observed more transiently; careful longitudinal study is required in order to differentiate the two.

REFERENCES

Alexander, M.P., Stuss, D.T. and Fansabedian, N. (2003). California Verbal Learning Test: performance by patients with focal frontal and non-frontal lesions. Brain, *126(6)*, 1493-1503.

Alstott, J., Breakspear, M., Hagmann, P., Cammoun, L. and Sporns, O. (2009). Modeling the impacts of lesions in the human brain. PLoS Computational Biology, *5(6)*, e1000408.

Alvarado, M.C. and Bachevalier, J. (2005). Comparison of the effects of damage to the perirhinal and parahippocampal cortex on transverse patterning and location memory in rhesus macaques. The Journal of Neuroscience, *25(6)*, 1599-1609.

Amodio, D.M. and Frith, C.D. (2006). Meeting of minds: the medial frontal cortex and social cognition. Nature Reviews Neuroscience, *7(4)*, 268-277.

Anderson, S. W., Damasio, H., Jones, R. D. and Tranel, D. (1991). Wisconsin Card Sorting Test performance as a measure of frontal lobe damage. Journal of Clinical and Experimental Neuropsychology, *13(6)*, 909-922.

Arhem, P., Klement, G. and Nilsson, J. (2003). Mechanisms of anesthesia: towards integrating network, cellular, and molecular level modeling. Neuropsychopharmacology, *28(Suppl. 1)*, S40-S47.

Badre, D. and D'Esposito, M. (2007). Functional Magnetic Resonance Imaging evidence for a hierarchical organization of the prefrontal cortex. Journal of Cognitive Neuroscience, *19(12)*, 2082-2099.

Barbas, H., Ghashghaei, H., Dombrowski, S. M. and Rempel-Clower, N. L. (1999). Medial prefrontal cortices are unified by common connections with superior temporal cortices and distinguished by input from memory-related areas in the rhesus monkey. Journal of Comparative Neurology, *410(3)*, 343-367.

Barbas, H. and Pandya, D.N. (1989). Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. Journal of Comparative Neurology, *286(3)*, 353-375.

Barbey, A.K., Koenigs, M. and Grafman, J. (2013). Dorsolateral prefrontal contributions to human working memory. Cortex, *49(5)*, 1195-1205.

Baxter, M.G., Gaffan, D., Kyriazis, D.A. and Mitchell, A.S. (2008). Dorsolateral prefrontal lesions do not impair tests of scene learning and decision-making that require frontal-temporal interaction. European Journal of Neuroscience, *28(3)*, 491-499.

Baxter, M.G. and Murray, E.A. (2001). Opposite relationship of hippocampal and rhinal cortex damage to delayed nonmatching-to-sample deficits in monkeys. Hippocampus, *11(1)*, 61-71.

Bird, C.M., Castelli, F., Malik, O., Frith, U. and Husain, M. (2004). The impact of extensive medial frontal lobe damage on 'Theory of Mind' and cognition. Brain, *127(4),* 914-928.

Bludau, S., Eickhoff, S.B., Mohlberg, H., Caspers, S., Laird, A.R., Fox, P.T., Schleicher, A., Zilles, K. and Amunts, K. (2014). Cytoarchitecture, probability maps and functions of the human frontal pole. NeuroImage, *93(2)*, 260-275.

Bond, M.R. (1986). Neurobehavioral sequelae of closed head injuries. In I. Grant and K. Adams (Eds.), *Neuropsychological Assessment of Neuropsychiatric Disorders* (pp.347-373), New York: Oxford University Press.

Boorman, E.D., Behrens, T.E.J. and Rushworth, M.F.S. (2011). Counterfactual choice and learning in a neural network centered on human lateral frontopolar cortex. PLoS Biology, *9(6)*, e1001093.

Boorman, E.D., Behrens, T.E.J., Woolrich, M.W. and Rushworth, M.F.S. (2009). How green is the grass on the other side? Frontopolar cortex and the evidence in favor of alternative courses of action. Neuron, *62(5)*, 733-743.

Boschin, E., Piekema, C. and Buckley, M.J. (2014). Essential functions of primate frontopolar cortex in cognition. Manuscript submitted for publication.

Boveroux, P.*,* Vanhaudenhuyse, A., Bruno, M-A., Noirhomme, Q., Lauwick, S., Luxen, A., Degueldre, C., Plenevaux, A., Schnakers, C., Phillips, C., Brichant, J-F., Bonhomme, V., Maquet, P., Greicius, M.D., Laureys, S. *and* Boly, M. *(*2010*).* Breakdown of within- and between-network resting state functional magnetic resonance imaging connectivity during propofol-induced loss of consciousness*.* Anesthesiology, 113(5), 1038-1053*.*

Braver, T.S. and Bongiolatti, S.R. (2002). The role of frontopolar cortex in subgoal processing during working memory. NeuroImage, *15(3)*, 523-536.

Braver, T.S., Reynolds, J.R. and Donaldson, D.I. (2003). Neural mechanisms of transient and sustained cognitive control during task switching. Neuron, *39(4)*, 713-726.

Brodmann, K. (1905). Beitrage zur histologisichen lokalisation der Grosshimrinde. III. Mitteilung: Die rinderfelder der niederen Affen. Journal fur Psychologie und Neurologie, *4*, 177-226.

Brodmann, K. (1909). Vergleichende Localisationslehre der Grosshirnrinde. Leipzig: Barth.

Browning, P.G., Baxter, M. G. and Gaffan, D. (2013). Prefrontal-temporal disconnection impairs recognition memory but not familiarity discrimination. The Journal of Neuroscience, *33(23)*, 9667-9674.

Buckley, M.J., Mansouri, F.A., Hoda., H., Mahboubi, M., Browning, P.G., Kwok, S.C., Phillips, A. and Tanaka, K. (2009). Dissociable components of rule-guided behavior depend on distinct medial and prefrontal regions. Science, *325(5936)*, 52-58.

Buckner, R.L., Andrews-Hanna, J.R. and Schacter, D.L. (2008). The brain's default network: anatomy, function, and relevance to disease. Annals of the New York Academy of Sciences, *1124*, 1-38.

Bunge, S.A., Wendelken, C., Badre, D. and Wagner, A.D. (2005). Analogical reasoning and prefrontal cortex: evidence for separable retrieval and integration mechanisms. Cerebral Cortex, *15(3)*, 239-249.

Burgess, P.W., Dumontheil, I. and Gilbert, S.J. (2007). The gateway hypothesis of rostral prefrontal cortex (area 10) function. Trends in Cognitive Sciences, *11(7)*, 290-298.

Burgess, P.W., Gilbert, S.J., Okuda, J. and Simons, J.S. (2006). Rostral prefrontal brain regions (area 10): a gateway between inner thought and external world? In W. Prinz and N. Sebanz (Eds.), *Disorders of Volition* (pp.373-396). Cambridge, MA: MIT Press.

Burgess, P.W., Quayle, A. and Frith, C.D. (2001). Brain regions involved in prospective memory as determined by positron emission tomography. Neuropsychologia, *39(6)*, 545-555.

Burgess, P.W., Veitch, E., de Lacy Costello, A. and Shallice, T. (2000). The cognitive and neuroanatomical correlates of multitasking. Neuropsychologia, *38(6)*, 848-863.

Burman, K.J., Reser, D.H., Richardson, K.E., Gaulke, H., Worthy, K.H. and Rosa, M.G. (2011b). Subcortical projections to the frontal pole in the marmoset monkey. European Journal of Neuroscience, *34(2)*, 303-319.

Burman, K.J., Reser, D.H., Yu, H.H. and Rosa, M.G.P. (2011a). Cortical input to the frontal pole of the marmoset monkey. Cerebral Cortex, *21(8)*, 1712-1737.

Bussey, T.J., Wise, S.P. and Murray, E.A. (2001). The role of ventral and orbital prefrontal cortex in conditional visuomotor learning and strategy use in rhesus monkeys (Macaca mulatta). Behavioral Neuroscience, *115(5)*, 971-982.

Carmichael, S.T., and Price, J.L. (1994). Architectonic subdivision of the orbital and medial prefrontal cortex in the macaque monkey. Journal of Comparative Neurology, *346(3)*, 366-*402.*

Carney, N, Chesnut, R. M., Maynard, H., Mann, N. C., Patterson, P. and Helfand, M. (1999). Effect of cognitive rehabilitation on outcomes for persons with traumatic brain injury: a systematic review. Journal of Head Trauma Rehabilitation, *14(3)*, 277-307.

Castellanos, N.P., Paúl, N., Ordóñez, V.E., Demuynck, O., Bajo, R., Campo, P., Bilbao, A., Ortiz, T., del-Pozo, F. and Maestú, F. (2010). Reorganization of functional connectivity as a correlate of cognitive recovery in acquired brain injury. Brain, *133(8)*, 2365-2381.

Cavada, C., Compañy, T., Tejedor, J., Cruz-Rizzolo, R. J. and Reinoso-Suárez, F. (2000). The anatomical connections of the macaque monkey orbitofrontal cortex. A review. Cerebral Cortex, *10(3)*, 220-242.

Charles, D.P., Gaffan, D. and Buckley, M.J. (2004). Impaired recency judgements and intact novelty judgements after fornix transection in monkeys. The Journal of Neuroscience, *24(8)*, 2037-2044.

Charron, S. and Koechlin, E., (2010). Divided representation of concurrent goals in the human frontal lobes. Science, *328(5976)*, 360-363.

Christoff, K. and Gabrieli, J.D.E. (2000). The frontopolar cortex and human cognition: Evidence for a rostrocaudal hierarchical organization within the human prefrontal cortex. Psychobiology, *28(2)*, 168-186.

Christoff, K., Keramatian, K., Gordon, A.M., Smith, R. and Mädler, B. (2009b). Prefrontal organization of cognitive control according to levels of abstraction. Brain Research, *1286*, 94-105.

Christoff, K., Gordon, A.M., Smallwood, J., Smith, R. and Schooler, J.W. (2009a). Experience sampling during fMRI reveals default network and executive system contributions to mind wandering. Proceedings of the National Academy of Sciences of the USA, *106(21)*, 8719-8724.

Christoff, K., Prabhakaran, V., Dorfman, J., Zhao, Z., Kroger, J.K., Holyoak, K.J. and Gabrieli, J.D.E. (2001). Rostrolateral prefrontal cortex involvement in relational integration during reasoning. NeuroImage, *14(5)*, 1136-1149.

Christoff, K., Ream, J.M. and Gabrieli, J.D. (2004). Neural basis of spontaneous thought processes. Cortex, *40(4-5)*, 623-630.

Christoff, K., Ream, J.M., Geddes, L.P.T. and Gabrieli, J.D.E. (2003). Evaluating self-generated information: anterior prefrontal contributions to human cognition. Behavioral Neuroscience, *117(6)*, 1161-1168.

Clayton, N.S. and Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. Nature*, 395(6699),* 272-274.

Conkey, R.C. (1938). Psychological changes associated with head injuries. Archives of Psychology, *232*, 1-62.

Corbetta, M. (2012). Functional connectivity and neurological recovery. Developmental Psychobiology, *54(3)*, 239-253.

Corkin, S. (2002). What's new with the amnesic patient H.M.? Nature Reviews Neuroscience, *3(2)*, 153-160.

Costa, A., Oliveri, M., Barban, F., Bonni, S., Koch, G., Caltagirone, C. and Carlesimo, G.A. (2013). The right frontopolar cortex is involved in visual-spatial prospective memory. PLoS One, *8(2)*, e56039.

Costa, A., Oliveri, M., Barban, F., Torriero, S., Salerno, S., Lo Gerfo, E., Koch, G., Caltagirone, C. and Carlesimo, G.A. (2011). Keeping memory for intentions: a cTBS investigation of the frontopolar cortex. Cerebral Cortex, *21(12)*, 2696-2703.

Davey, C.E., Grayden, D.B., Egan, G.F. and Johnston, L.A. (2013). Filtering induces correlation in fMRI resting state data. NeuroImage, *64*, 728-740.

Daw, N.D., O'Doherty, J.P., Dayan, P., Seymour, B. and Dolan, R.J. (2006). Cortical substrates for exploratory decisions in humans. Nature, *441(7095)*, 876-879.

Deshpande, G., Kerssens, C., Sebel, P.S. and Hu, X. (2010). Altered local coherence in the default mode network due to sevoflurane anesthesia. Brain Research, *1318*, 110-121.

Dobbins, I.G., Foley, H., Schacter, D.L., Wagner, A.D. (2002) Executive control during episodic retrieval: multiple prefrontal processes subserve source memory. Neuron, *35(5)*, 989-996.

Dobbins, I.G., Rice, H.J., Wagner, A.D. and Schacter, D.L. (2003). Memory orientation and success: separable neurocognitive components underlying episodic recognition. Neuropsychologia, *41(3)*, 318-333.

Dreher, J., Koechlin, E., Tierney, M. and Grafman, J. (2008). Damage to the fronto-polar cortex is associated with impaired multi-tasking. PLoS ONE, *3(9),* e3227.

Dudukovic, N.M. and Wagner, A.D. (2007). Goal-dependent modulation of declarative memory: neural correlates of temporal recency decisions and novelty detection. Neuropsychologia, *45(11)*, 2608-2620.

Dumontheil, I., Burgess, P.W. and Blakemore, S.J. (2008). Development of rostral prefrontal cortex and cognitive and behavioural disorders. Developmental Medicine and Child Neurology, *50(3)*, 168-181.

Duncan, J. and Owen, A.M. (2000). Common regions of the human frontal lobe recruited by diverse cognitive demands. Trends in Neurosciences*, 23(10)*, 475-483.

Dux, P.E., Ivanoff, J., Asplund, C.L. and Marois, R. (2006). Isolation of a central bottleneck of information processing with time-resolved fMRI. Neuron, *52(6)*, 1109-1120.

Düzel, E., Cabeza, R., Picton, T.W., Yonelinas, A.P., Scheich, H., Heinze, H.J. and Tulving, E. (1999). Task-related and item-related brain processes of memory retrieval. Proceedings of the National Academy of Sciences of the USA, *96(4)*, 1794-1799.

Eacott, M.J., Easton, A. and Zinkivskay, A. (2005). Recollection in an episodic-like memory task in the rat. Learning and Memory, *12(3),* 221-223.

Eichenbaum, H. Fortin, N.J., Sauvage, M., Robitsek, R.J. and Farovik, A. (2010). An animal model of amnesia that uses Receiver Operating Characteristics (ROC) analysis to distinguish recollection from familiarity deficits in recognition memory. Neuropsychologia, *48(8),* 2281-2289.

Fletcher, P.C., Shallice, T., Frith, C.D., Frackowiak, R.S.J. and Dolan, R.J. (1998). The functional roles of prefrontal cortex in episodic memory. II. Retrieval. Brain, *121(7)*, 1249-1256.

Fortin, N.J., Wright, S.P. and Eichenbaum, H. (2004). Recollection-like memory retrieval in rats is dependent on the hippocampus. Nature, *431(7005)*, 188-191.

Fossati, P., Hevenor, S.J., Lepage, M., Graham, S.J., Grady, C., Keightley, M.L., Craik, F. and Mayberg, H. (2004). Distributed self in episodic memory: neural correlates of successful retrieval of self-encoded positive and negative personality traits. NeuroImage, *22(4)*, 1596-1604.

Funahashi, S., Bruce, C.J. and Goldman-Rakic, P.S. (1993). Dorsolateral prefrontal lesions and oculomotor delayed-response performance: evidence for mnemonic “scotomas”. The Journal of Neuroscience, *13(4)*, 1479-1497.

Gaffan, D. and Wilson, C.R.E. (2008). Medial temporal and prefrontal function: recent behavioural disconnection studies in the macaque monkey. Cortex, *44(8)*, 928-935.

Geake, J.G. and Hansen, P.C. (2005). Neural correlates of intelligence as revealed by fMRI of fluid analogies. NeuroImage, *26(2)*, 555-564.

Gilbert, S.J., Dumontheil, I., Simons, J.S., Frith, C.D. and Burgess, P.W. (2007). Comment on “Wandering Minds: the Default Network and Stimulus-Independent Thought”. Science, *317(5834)*, 43.

Gilbert, S. J., Frith, C. D., and Burgess, P. W. (2005). Involvement of rostral prefrontal cortex in selection between stimulus-oriented and stimulus-independent thought. European Journal of Neuroscience, *21(5)*, 1423-1431.

Gilbert, S.J., Simons, J.S., Frith, C.D. and Burgess, P.W. (2006a). Performance-related activity in medial rostral prefrontal cortex (area 10) during low-demand tasks. Journal of Experimental Psychology: Human Perception and Performance, *32(1)*, 45-58.

Gilbert, S.J., Spengler, S., Simons, J.S., Frith, C.D. and Burgess, P.W. (2006c). Differential functions of lateral and medial rostral prefrontal cortex (area 10) revealed by brain–behavior associations. Cerebral Cortex, *16(12),* 1783-1789.

Gilbert, S.J., Spengler, S., Simons, J.S., Steele, J.D., Lawrie, S.M., Frith, C.D. and Burgess, P.W. (2006b). Functional specialization within rostral prefrontal cortex (area 10): a meta-analysis. Journal of Cognitive Neuroscience, *18(6)*, 932-948.

Gillebert, C.R., Mantini, D., Thijs, V., Sunaert, S., Dupont, P. and Vandenberghe, R. (2011). Lesion evidence for the critical role of the intraparietal sulcus in spatial attention. Brain, *134(6)*, 1694-1709.

Goldman, P.S., Rosvold, H.E., Vest, B. and Galkin, T.W. (1971). Analysis of the delayed-alternation deficit produced by dorsolateral prefrontal lesions in the rhesus monkey. Journal of Comparative and Physiological Psychology, *77(2)*, 212-220.

Green, A.E., Kraemer, D.J.M., Fugelsang, J.A., Gray, J.R. and Dunbar, K.N. (2010). Connecting long distance: semantic distance in analogical reasoning modulates frontopolar cortex activity. Cerebral Cortex, *20(1)*, 70-76.

Greicius, M.D., Kiviniemi, V., Tervonen, O., Vainionpää, V., Alahuhta, S., Reiss, A. L. and Menon, V. (2008). Persistent default-mode network connectivity during light sedation. Human Brain Mapping, *29(7)*, 839-847.

Greicius, M.D., Supekar, K., Menon, V. and Dougherty, R.F. (2009). Resting-state functional connectivity reflects structural connectivity in the default mode network. Cerebral Cortex, *19(1)*, 72-78.

Gross, C.G. and Weiskrantz, L. (1962). Evidence for dissociation of impairment on auditory discrimination and delayed response following lateral frontal lesions in monkeys. Experimental Neurology, *5*, 453-476.

Guderian, S., Brigham, D. and Mishkin, M. (2011). Two processes support visual recognition memory in rhesus monkeys. Proceedings of the National Academy of Sciences of the United States of America, *108(48)*, 19425-19430.

Gusnard, D.A., Akbudak, E., Shulman, G.L. and Raichle, M.E. (2001). Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. Proceedings of the National Academy of Sciences of the USA, *98(7)*, 4259-4264.

Hampton, R.R. (2001). Rhesus monkeys know when they remember. Proceedings of the National Academy of Sciences of the United States of America*, 98(9)*, 5359-5362.

Hampton, R.R., Hampstead, B.M. and Murray, E.A. (2004). Selective hippocampal damage in rhesus monkeys impairs spatial memory in an open-field test. Hippocampus, *14(7),* 808-818.

Haynes, J.D., Sakai, K., Rees, G., Gilbert, S., Frith, C. and Passingham, R.E. (2007). Reading hidden intentions in the human brain. Current Biology, *17(4)*, 323-328.

He, B.J., Snyder, A.Z., Vincent, J.L., Epstein, A., Shulman, G.L. and Corbetta, M. (2007). Breakdown of functional connectivity in frontoparietal networks underlies behavioral deficits in spatial neglect. Neuron, *53(6)*, 905-918.

Hoffmann, M. and Bar-On, R. (2012). Isolated frontopolar cortex lesion: a case study. Cognitive and Behavioral Neurology, *25(1)*, 50-56.

Holloway, R.L. (2002). Brief communication: how much larger is the relative volume of Area 10 of the prefrontal cortex in humans? American Journal of Physical Anthropology, *118(4)*, 399-401.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics, *6(2)*, 65-70.

Hoyer, E.H. and Celnik, P.A. (2011). Understanding and enhancing motor recovery after stroke using transcranial magnetic stimulation. Restorative Neurology and Neuroscience, *29(6)*, 395-409.

Hutchison, R.M. and Everling, S. (2012). Monkey in the middle: why non-human primates are needed to bridge the gap in resting-state investigations. Frontiers in Neuroanatomy, *6*, 29, doi: 10.3389/fnana.2012.00029.

Hutchison, R.M., Hutchison, M., Manning, K.Y., Menon, R.S. and Everling, S. (2014). Isoflurane induces dose-dependent alterations in the cortical connectivity profiles and dynamic properties of the brain's functional architecture. Human Brain Mapping, advance online publication, doi: 10.1002/hbm.22583.

Jacobs, B., Schall, M., Prather, M., Kapler, E., Driscoll, L., Baca, S., Jacobs, J., Ford, K., Wainwright, M. and Treml, M. (2001). Regional dendritic and spine variation in human cerebral cortex: a quantitative golgi study. Cerebral Cortex, *11(6),* 558-571.

Kahn, I., Davachi, L. and Wagner, A.D. (2004). Functional-neuroanatomic correlates of recollection: implications for models of recognition memory. The Journal of Neuroscience, *24(17)*, 4172-4180.

Kapur, S., Craik, F.I.M., Jones, C., Brown, G.M., Houle, S. and Tulving, E. (1995). Functional role of the prefrontal cortex in retrieval of memories: a PET study. NeuroReport, *6(14)*, 1880-1884.

Keedy, S.K., Ebens, C.L., Keshavan, M.S. and Sweeney, J.A. (2006). Functional magnetic resonance imaging studies of eye movements in first episode schizophrenia: smooth pursuit, visually guided saccades and the oculomotor delayed response task. Psychiatry research: Neuroimaging, *146(3)*, 199-211.

Kennerley, S.W., Walton, M.E., Behrens, T.E., Buckley, M.J. and Rushworth, M.F. (2006). Optimal decision making and the anterior cingulate cortex. Nature Neuroscience, *9(7)*, 940-947.

Kleim, J.A. and Jones, T.A. (2008). Principles of experience-dependent neural plasticity: implications for rehabilitation after brain damage. Journal of Speech, Language, and Hearing Research, *51(1)*, S225-S239.

Koechlin, E., Basso, G., Pietrini, P., Panzer, S., and Grafman, J. (1999). The role of the anterior prefrontal cortex in human cognition, Nature, *399(6732)*, 148-151.

Koechlin, E., Corrado, G., Pietrini, P. and Grafman, J. (2000). Dissociating the role of the medial and lateral anterior prefrontal cortex in human planning. Proceedings of the National Academy of Sciences of the USA, *97(13)*, 7651-7656.

Koechlin, E. and Hyafil, A. (2007). Anterior prefrontal function and the limits of human decision-making, Science, *318(5850),* 594-598.

Koechlin, E. and Summerfield, C. (2007). An information theoretical approach to prefrontal executive function. Trends in Cognitive Sciences, *11(6)*, 229-235.

Kolb, B. and Gibb, R. (1991). Environmental enrichment and cortical injury: behavioral and anatomical consequences of frontal cortex lesions. Cerebral Cortex, *1(2)*, 189-198.

Kolchinsky, A., van den Heuvel, M.P., Griffa, A., Hagmann, P., Rocha, L.M., Sporns, O. and Goñi, J. (2014). Multi-scale integration and predictability in resting state brain activity. Frontiers in Neuroinformatics, *8*, 66, doi: 10.3389/fninf.2014.00066.

Konishi, S., Chikazoe, J., Jimura, K., Asari, T. and Miyashita, Y. (2005). Neural mechanism in anterior prefrontal cortex for inhibition of prolonged set interference. Proceedings of the National Academy of Sciences of the USA, *102(35)*, 12584-12588.

Kornell, N., Son, L.K. and Terrace, H.S. (2007). Transfer of metacognitive skills and hint seeking in monkeys. Psychological Science, *18(1),* 64-71.

Kovach, C.K., Daw, N., Rudrauf, D., Tranel, D., O'Doherty, J.P. and Adolphs, R. (2012). Anterior prefrontal cortex contributes to action selection through tracking of recent reward trends. The Journal of Neuroscience, *32(25)*, 8434-8442.

Kroger, J.K, Sabb, F.W., Fales, C.L., Bookheimer, S.Y., Cohen, M.S. and Holyoak, K.J. (2002). Recruitment of anterior dorsolateral prefrontal cortex in human reasoning: a parametric study of relational complexity. Cerebral Cortex, *12(5)*, 477-485.

Kuwabara, M., Mansouri, F.A., Buckley, M.J. and Tanaka, K. (2014). Cognitive control functions of anterior cingulate cortex in macaque monkeys performing a Wisconsin Card Sorting Test analog. The Journal of Neuroscience, *34(22)*, 7531-7547.

Lepage, M., Ghaffar, O., Nyberg, L. and Tulving, E. (2000). Prefrontal cortex and episodic memory retrieval mode. Proceedings of the National Academy of Sciences of the USA, *97(1)*, 506-511.

Levy, R. and Golman-Rakic, P.S. (1999). Association of storage and processing functions in the dorsolateral prefrontal cortex of the nonhuman primate. The Journal of Neuroscience, *19(12)*, 5149-5158.

Lewis, J.W. and Van Essen, D.C. (2000). Mapping of architectonic subdivisions in the macaque monkey, with emphasis on parieto-occipital cortex. Journal of Comparative Neurology, *428(1)*, 79-111.

Liang, Z., King, J., Zhang, N. (2012). Intrinsic organization of the anesthetized brain. The Journal of Neuroscience, *32(30)*, 10183-10191.

Logie, R.H., Pernet, C.R., Buonocore, A. and Della Sala, S. (2011). Low and high imagers activate networks differentially in mental rotation. Neuropsychologia, *49(11)*, 3071-3077.

Málková, L., Lex, C.K., Mishkin, M. and Saunders, R.C. (2001). MRI-based evaluation of locus and extent of neurotoxic lesions in monkeys. Hippocampus, *11(4)*, 361-370.

Mansouri, F.A., Buckley, M.J., Mahboubi, M. and Tanaka, K. (2014). Behavioural consequences of selective damage to frontal pole and posterior cingulate cortices. Manuscript submitted for publication.

Mansouri, F.A., Buckley, M.J. and Tanaka, K. (2014). The essential role of primate orbitofrontal cortex in conflict-induced executive control adjustment. The Journal of Neuroscience, *34(33)*, 11016-11031.

Markov, N.T., Ercsey-Ravasz, M.M., Ribeiro Gomes, A.R., Lamy, C., Magrou, L., Vezoli, J., Misery, P., Falchier, A., Quilodran, R., Gariel, M.A., Sallet, J., Gamanut, R., Huissoud, C., Clavagnier, S., Giroud, P., Sappey-Marinier, D., Barone, P., Dehay, C., Toroczkai, Z., Knoblauch, K., Van Essen, D.C. and Kennedy, H. (2014). A weighted and directed interareal connectivity matrix for macaque cerebral cortex. Cerebral Cortex, *24(1)*, 17-36.

Mars, R.B., Neubert, F.X., Noonan, M.P., Sallet, J., Toni., I. and Rushworth, M.F. (2012). On the relationship between the “default mode network” and the “social brain”. Frontiers in Human Neuroscience, *6*, 189, doi: 10.3389/fnhum.2012.00189.

Marshall, W.L. (1988). Behavioral indices of habituation and sensitization during exposure to phobic stimuli. Behavior Research and Therapy, *26(1)*, 67-77.

Martuzzi, R., Ramami, R., Qiu, M., Rajeevan, N. and Constable, R.T. (2010). Functional connectivity and alterations in baseline brain state in humans. NeuroImage, *49(1)*, 823-834.

Martuzzi, R., Ramami, R., Qiu, M., Shen, X., Papademetris, X. and Constable, R.T. (2011). A whole-brain voxel based measure of intrinsic connectivity contrast reveals local changes in tissue connectivity with anesthetic without a priori assumptions on thresholds or regions of interest. NeuroImage, *58(4)*, 1044-1050.

Mason, M.F., Norton, M.I., Van Horn, J.D., Wegner, D.M., Grafton, S.T. and Macrae, C.N. (2007). Wandering minds: the default network and stimulus-independent thought. Science, *315(5810)*, 393-395.

Mataró, M., Jurado, M.A., García-Sánchez, C., Barraquer, L., Costa-Jussà, F.R., Junqué, C. (2001). Long-term effects of bilateral frontal brain lesion: 60 years after injury with an iron bar. Archives of Neurology, *58(7)*, 1139-1142.

Mayer, A.R., Mannell, M.V., Ling, J., Gasparovic, C. and Yeo, R.A. (2011). Functional connectivity in mild traumatic brain injury. Human Brain Mapping, *32(11)*, 1825-1835.

McCaig, R.G., Dixon, M., Keramatian, K., Liu, I. and Christoff, K. (2011). Improved modulation of rostrolateral prefrontal cortex using real-time fMRI training and meta-cognitive awareness. NeuroImage, *55(3)*, 1298-1305.

McLaren, D.G., Kostmatka, K.J., Oakes, T.R., Kroenke, C.D., Kohama, S.G., Matochik, J.A., Ingram, D.K. and Johnson, S.C. (2009). A population-average MRI-based atlas collection of the rhesus macaque. Neuroimage, *45(1)*, 52-59.

Menzel, C. R. (1999). Unprompted recall and reporting of hidden objects by a chimpanzee (Pan troglodytes) after extended delays. Journal of Comparative Pychology, *113*(*4),* 426-434.

Mishkin, M. (1957) Effects of small prefrontal lesions on delayed alternation in monkeys. Journal of Neurophysiology, *20(6)*, 615-622.

Mishkin, M. and Manning, F.J. (1978). Non-spatial memory after selective prefrontal lesions in monkeys. Brain Research, *143(2)*, 313-323.

Mishra, A.M., Bai, X., Sanganahalli, B.G., Waxman, S.G., Shatillo, O., Grohn, O., Hyder, F., Pitkänen, A. and Blumenfeld, H. (2014). Decreased resting functional connectivity after traumatic brain injury in the rat. PLoS One, *9(4)*, e95280.

Molko, N., Cohen, L., Mangin, J.F., Chochon, F., Lehéricy, S., Bihan, D. and Dehaene, S. (2002). Visualizing the neural bases of a disconnection syndrome with diffusion tensor imaging. Journal of Cognitive Neuroscience, *14(4)*, 629-636.

Monchi, O., Petrides, M., Petre, V., Worsley, K. and Dagher, A. (2001). Wisconsin Card Sorting revisited: distinct neural circuits participating in different stages of the task identified by event-related functional magnetic resonance imaging. The Journal of Neuroscience, *21(19)*, 7733-7741.

Mottaghy, F.M., Gangitano, M., Sparing, R., Krause, B.J. and Pascual-Leone, A. (2002). Segregation of areas related to visual working memory in the prefrontal cortex revealed by rTMS. Cerebral Cortex, *12(4)*, 369-375.

Mountain, M. A. and Snow, W. G. (1993). Wisconsin Card Sorting Test as a measure of frontal pathology: a review. The Clinical Neuropsychologist, *7(1)*, 108-118.

Nagahama, Y., Okina, T., Suzuki, N., Nabatame, H. and Matsuda, M. (2005). The cerebral correlates of different types of perseveration in the Wisconsin Card Sorting Test. Journal of Neurology, Neurosurgery and Psychiatry, *76(2)*, 169-175.

Nakamura, T., Hillary, F.G. and Biswal, B.B. (2009). Resting network plasticity following brain injury. PLoS One, *4(12)*, e8220.

Neubert, F.X., Mars, R.B., Thomas, A.G., Sallet, J. and Rushworth, M.F.S. (2014). Comparison of human ventral frontal cortex areas for cognitive control and language with areas in monkey frontal cortex. Neuron, *81(3)*, 700-713.

Nielsen-Bohlman, L. and Knight, R.T. (1999). Prefrontal cortical involvement in visual working memory. Cognitive Brain Research, *8(3)*, 299-310.

Noonan, M.P., Sallet, J., Rudebeck, P.H., Buckley, M.J. and Rushworth, M.F.S. (2010). Does the medial orbitofrontal cortex have a role in social valuation? European Journal of Neuroscience, *31(12),* 2341-2351.

Nyberg, L., Marklund, P., Persson, J., Cabeza, R., Forkstam, C., Petersson, K.M. and Ingvar, M. (2003). Common prefrontal activations during working memory, episodic memory, and semantic memory. Neuropsychologia, *41(3)*, 371-377.

Ochsner,K.N., Knierim, K., Ludlow, D.H., Hanelin, J., Ramachandran, T., Glover, G. and Mackey, S.C. (2004). Reflecting upon feelings: an fMRI study of neural systems supporting the attribution of emotion to self and other. Journal of Cognitive Neuroscience, *16(10)*, 1746-1772.

Okuda, J., Fujii, T., Ohtake, H., Tsukiura, T., Yamadori, A., Frith, C.D. and Burgess, P.W. (2007). Differential involvement of regions of rostral prefrontal cortex (Brodmann Area 10) in time- and event-based prospective memory. International Journal of Psychophysiology, *64(3)*, 233-246.

Öngür, D., Ferry, A.T. and Price, J.L. (2003). Architectonic subdivision of the human orbital and medial prefrontal cortex. Journal of Comparative Neurology, *460(3)*, 425-449.

O'Reilly, J.X., Croxson, P.L., Jbabdi, S., Sallet, J., Noonan, M.P., Mars, R.B., Browning, P.G., Wilson, C.R., Mitchell, A.S., Miller, K.L., Rushworth, M.F. and Baxter, M.G. (2013). Causal effect of disconnection lesions on interhemispheric functional connectivity in rhesus monkeys. Proceedings of the National Academy of Sciences of the United States of America, *110(34)*, 13982-13987.

Osherson, D., Perani, D., Cappa, S., Schnur, T., Grassi, F. and Fazio, F. (1998). Distinct brain loci in deductive versus probabilistic reasoning. Neuropsychologia, *36(4)*, 369-376.

Passingham, R.E. (1975). Delayed matching after selective prefrontal lesions in monkeys. Brain Research, *92(1)*, 89-102.

Passingham, R.E. (1985). Memory of monkeys (*Macaca mulatta*) with lesions in prefrontal cortex. Behavioral Neuroscience, *99(1)*, 3-21.

Paxinos, G., Huang, X.F. and Toga, A.W. (2000). The rhesus monkey brain in stereotaxic coordinates. San Diego: Academic Press.

Peltier, S.J., Kerssens, C., Hamann, S.B., Sebel, P.S., Byas-Smith, M. and Hu, X. (2006). Default mode functional connectivity altered by sevoflurane anesthesia. Proceedings of the International Society for Magnetic Resonance in Medicine, *14*, 3260.

Peltier, S.J., Kerssens, C., Hamann, S.B., Sebel, P.S., Byas-Smith, M. and Hu, X. (2005). Functional connectivity changes with concentration of sevoflurane anesthesia. NeuroReport, *16(3)*,285-288.

Petrides, M. (1991). Monitoring of selections of visual stimuli and the primate frontal cortex. Proceedings of the Royal Society, Biological Sciences, *246(1317)*, 293-298.

Petrides, M. (1995). Impairments on nonspatial self-ordered and externally ordered working memory tasks after lesions of the mid-dorsal part of the lateral frontal cortex in the monkey. The Journal of Neuroscience, *15(1)*, 359-375.

Petrides, M. (2005). Lateral prefrontal cortex: architectonic and functional organization. Philosophical transactions of the Royal Society of London. Series B, Biological Sciences, *360(1456)*, 781-795.

Petrides, M. and Pandya, D. N. (1994). Comparative architectonic analysis of the human and the macaque frontal cortex. In F. Boller and J. Grafman (Eds.), *Handbook of neuropsychology, vol. 9* (pp.17-58). Amsterdam: Elsevier.

Petrides, M. and Pandya, D.N. (1999). Dorsolateral prefrontal cortex: comparative cytoarchitectonic analysis in the human and the macaque brain and corticocortical connection patterns. European Journal of Neuroscience, *11(3)*, 1011-1036.

Petrides, M. and Pandya, D.N. (2007). Efferent association pathways from the rostral prefrontal cortex in the macaque monkey. The Journal of Neuroscience, *27(43),* 11573-11586.

Pinsk, M.A., Moore, T., Richter, M.C., Gross, C.G. and Kastner, S. (2005). Methods for functional magnetic resonance imaging in normal and lesioned behaving monkeys. The Journal of Neuroscience Methods, *143(2)*, 179-195.

Pollmann, S, Weidner, R., Müller, H.J. and von Cramon, D.Y. (2000). A fronto-posterior network involved in visual dimension changes. Journal of Cognitive Neuroscience, *12(3)*, 480-494.

Portrat, S., Barrouillet, P. and Camos, V. (2008). Time-related decay or interference-based forgetting in working memory? Journal of Experimental Psychology: Learning, Memory and Cognition, *34(6)*, 1561-1564.

Preuss, T.M. and Goldman-Rakic, P.S. (1991). Myelo- and cytoarchitecture of the granular frontal cortex and surrounding regions in the strepsirhine primate Galago and the anthropoid primate Macaca. Journal of Comparative Neurology, *310(4)*, 429-474.

Price, C.J. and Friston, K.J. (1999). Scanning patients with tasks they can perform. Human Brain Mapping, *8(2-3)*, 102-108.

Price,C.J., Mummery, C.J., Moore, C.J., Frackowiak, R.S.J. and Friston, K.J. (1999). Delineating necessary and sufficient neural systems with functional imaging studies of neuropsychological patients. Journal of Cognitive Neuroscience, *11(4)*, 371-382.

Qiu, M., Ramami, R., Martuzzi, R., Zhang, X. and Constable, R.T. (2011). Influence of sevoflurane on regional CBF and functional connectivity and implications related to brain/behavior during general anesthesia. Proceedings of the International Society for Magnetic Resonance in Medicine, *19*, 3567.

Ramnani, N. and Miall, R.C. (2003). Instructed delay activity in the human prefrontal cortex is modulated by monetary reward expectation. Cerebral Cortex, *13(3)*, 318-327.

Ramnani, N. and Owen, A.M. (2004). Anterior prefrontal cortex: insights into function from anatomy and neuroimaging. Nature Reviews Neuroscience, *5(3)*, 184-194.

Ramnani, N. and Passingham, R.E. (2001). Changes in the human brain during rhythm learning. Journal of Cognitive Neuroscience, *13(7)*, 952-966.

Ranganath, C., Johnson, M.K., and D'Esposito, M. (2000). Left anterior prefrontal activation increases with demands to recall specific perceptual information. The Journal of Neuroscience, *20(22)*, RC108.

Regueiro-Purriños, M., Fernández-Váquez, F., de Prado, A.P., Altónaga, J.R., Cuellas-Ramón, C., Ajenjo-Silverio, J.M., Orden, A. and Gonzalo-Orden, J.M. (2011). Ventricular arrhythmias and mortality associated with isoflurane and sevoflurane in a porcine model of myocardial infarction. Journal for the American Association of Laboratory Animal Science, *50(1)*, 73-78.

Reinhart, R.M.G., Heitz, R.P., Purcell, B.A., Weigand, P.K., Schall, J.D. and Woodman, G.F. (2012). Homologous mechanisms of visuospatial working memory maintenance in macaque and human: properties and sources. The Journal of Neuroscience, *32(22)*, 7711-7722.

Reynolds, J.R., McDermott, K.B. and Braver, T.S. (2006). A direct comparison of anterior prefrontal cortex involvement in episodic retrieval and integration. Cerebral Cortex, *16(4)*, 519-528.

Ringo, J.L. (1988). Seemingly discrepant data from hippocampectomized macaques are reconciled by detectability analysis. Behavioral Neuroscience, *102(1)*, 173-177.

Roca, M., Torralva, T., Gleichgerrcht, E., Woolgar, A., Thompson, R., Duncan, J. and Manes, F. (2011). The role of Area 10 (BA10) in human multitasking and in social cognition: a lesion study. Neuropsychologia, *49(13),* 3525-3531.

Rogers, R.D., Owen, A.M., Middleton, H.C., Williams, E.J., Pickard, J.D., Sahakian, B.J. and Robbins, T.W. (1999). Choosing between small, likely rewards and large, unlikely reward activates inferior and orbital prefrontal cortex. The Journal of Neuroscience, *19(20)*, 9029-9038.

Rorden, C., Karnath, H.O. and Bonilha, L. (2007). Improving lesion-symptom mapping. Journal of Cognitive Neuroscience, *19(7)*, 1081-1088.

Rosazza, C. and Minati, L. (2011). Resting-state brain networks: literature review and clinical applications. Neurological Sciences, *32(5)*, 773-785.

Rowe, J.B., Sakai, K., Lund, T.E., Ramsøy, T., Christensen, M.S., Baare, W.F.C., Paulson, O.B., Passingham, R.E. (2007). Is the prefrontal cortex necessary for establishing cognitive sets? The Journal of Neuroscience, *27(48)*, 13303-13310.

Rudebeck, P.H., Buckley, M.J., Walton, M.E. and Rushworth, M.F.S. (2006). A role for the macaque anterior cingulate gyrus in social valuation. Science, *313(5791)*, 1310-1312.

Rudebeck, P.H., Saunders, R.C., Prescott, A.T., Chau, L.S. and Murray, E.A. (2013). Prefrontal mechanisms of behavioral flexibility, emotion regulation and value updating. Nature Neuroscience, *16(8)*, 1140-1145.

Rugg, M.D., Fletcher, P.C., Allan, K., Frith, C.D., Frackowiak, R.S. and Dolan, R.J. (1998). Neural correlates of memory retrieval during recognition memory and cued recall. NeuroImage, *8(3)*, 262-273.

Rugg, M.D., Fletcher, P.C., Chua, P.M. and Dolan, R.J. (1999). The role of the prefrontal cortex in recognition memory and memory for source: an fMRI study. NeuroImage, *10(5)*, 520-529.

Rushworth, M.F.S. and Behrens, T.E.J. (2008). Choice, uncertainty and value in prefrontal and cingulate cortex. Nature Neuroscience, *11(4)*, 389-397.

Russell, W.M.S. and Burch, R.L. (1959). The Principles of Humane Experimentation Technique. London: Methuen.

Sakai, K. and Passingham, R.E. (2002). Prefrontal interactions reflect future task operations. Nature Neuroscience, *6(1)*, 75-81.

Sakai, K. and Passingham, R.E. (2006). Prefrontal set activity predicts rule-specific neural processing during subsequent cognitive performance. The Journal of Neuroscience, *26(4)*, 1211-1218.

Sallet, J., Mars, R.B., Noonan, M.P., Neubert, F.X., Jbabdi, S., O'Reilly, J.X., Filippini, N., Thomas, A.G. and Rushworth, M.F. (2013). The organization of dorsal frontal cortex in humans and macaques. The Journal of Neuroscience, *33(30)*, 12255-12274.

Schleicher, A., Morosan, P., Amunts, K. and Zilles, K. (2009). Quantitative architectural analysis: a new approach to cortical mapping. Journal of Autism and Developmental Disorders, *39(11),* 1568-1581.

Schmahmann, J.D. and Pandya, D.N. (2006). Fiber pathways of the brain. New York: Oxford University Press.

Semendeferi, K., Teffer, K., Buxhoeveden, D.P., Park, M.S., Bludau, S., Amunts, K., Travis, K. and Buckwalter, J. (2011). Spatial organization of neurons in the frontal pole sets humans apart from great apes. Cerebral Cortex, *21(7),* 1485-1497.

Semendeferi , K., Armstrong, E., Schleicher, A., Zilles, K. and Van Hoesen, G.W. (2001). Prefrontal cortex in humans and apes: a comparative study of area 10. American Journal of Physical Anthropology, *114(3),* 224-241.

Shallice, T. and Burgess, P.W. (1991). Deficits in strategy application following frontal damage in man. Brain, *114(2)*, 727-741.

Sharp, D.J., Beckmann, C.F., Greenwood, R., Kinnunen, K.M., Bonnelle, V., De Boissezon, X., Powell, J.H., Counsell, S.J., Patel, M.C. and Leech, R. (2011). Default mode network functional and structural connectivity after traumatic brain injury. Brain, *134(8)*, 2233-2247.

Simons, J.S., Gilbert, S.J., Owen, A.M., Fletcher, P.C. and Burgess, P.W. (2005b). Distinct roles for lateral and medial anterior prefrontal cortex in contextual recollection. Journal of Neurophysiology, *94(1)*, 813-820.

Simons, J.S., Owen, A.M., Fletcher, P.C. and Burgess, P.W. (2005a). Anterior prefrontal cortex and the recollection of contextual information. Neuropsychologia, *43(12)*, 1774-1783.

Simons, J.S., Schölvinck, M.L., Gilbert, S.J., Frith, C.D. and Burgess, P.W. (2006). Differential components of prospective memory? Evidence from fMRI. Neuropsychologia, *44(8)*, 1388-1397.

Skudlarski, P., Jagannathan, K., Calhoun, V.D., Hampson, M., Skudlarska, B.A. and Pearlson, G. (2008). Measuring brain connectivity: diffusion tensor imaging validates resting state temporal correlations. NeuroImage, *43(3)*, 554-561.

Spocter, M.A., Hopkins, W.D., Barks, S.K., Bianchi, S., Hehmeyer, A.E., Anderson, S.M., Stimpson, C.D., Fobbs, A.J., Hof, P.R. and Sherwood, C.C. (2012). Neuropil distribution in the cerebral cortex differs between humans and chimpanzees. Journal of Comparative Neurology, *520(13)*, 2917-2929.

Stamm, J. (1969). Electrical stimulation of monkeys' prefrontal cortex during delayed-response performance. Journal of Comparative Physiological Psychology, *67(4)*, 535-546.

Suddendorf, T. and Busby, J. (2003). Mental time travel in animals? Trends in Cognitive Sciences, *7(9)*, 391-396.

Suzuki, M. and Gottlieb, J. (2013). Distinct neural mechanisms of distractor suppression in the frontal and parietal lobe. Nature Neuroscience, *16(1)*, 98-104.

Tanaka, K., Matsumoto, K., Mansouri, F. A. and Buckley, M. J. (2013). Functional division among monkey prefrontal areas in goal-directed behavior. In D.T. Stuss and R.T. Knight (Eds.) *Principles of Frontal Lobe Function, Second Edition* (pp.249-258), New York: Oxford University Press.

Tarapore, P.E., Findlay, A.M., Lahue, S.C., Lee, H., Honma, S.M., Mizuiri, D., Luks, T.L., Manley, G.T., Nagarajan, S.S. and Mukherjee, P. (2013). Resting state magnetoencephalography functional connectivity in traumatic brain injury. Journal of Neurosurgery, *118(6)*, 1306-1316.

Thiebaut de Schotten, M., Dell'Acqua, F., Valabregue, R. and Catani, M. (2012). Monkey to human comparative anatomy of the frontal lobe association tracts. Cortex, *48(1)*, 82-96.

Tsujimoto, S., Genovesio, A. and Wise, S.P. (2010). Evaluating self-generated decisions in frontal pole cortex of monkeys. Nature Neuroscience, *13(1)*, 120-126.

Tsujimoto, S., and Postle, B.R. (2012). The prefrontal and oculomotor delayed response: a reconsideration of the "mnemonic scotoma". Journal of Cognitive Neuroscience, *24(3)*, 627-635.

Tulving, E. (2002). Episodic memory: from mind to brain. Annual Review of Psychology, *53*, 1-25.

Turner, M.S., Simons, J.S., Gilbert, S.J., Frith, C.D. and Burgess, P.W. (2008). Distinct roles for lateral and medial rostral prefrontal cortex in source monitoring of perceived and imagined events. Neuropsychologia, *46(5)*, 1442-1453.

Ungerleider, L.G. and Desimone, R. (1986). Cortical connections of visual area MT in the macaque. Journal of Comparative Neurology, *248(2)*, 190-222.

Uretzky, S. and Gilboa, A. (2010). Knowing your lines but missing your cue: rostral prefrontal lesions impair prospective memory cue detection, but not action-intention superiority. Journal of Cognitive Neuroscience, *22(12),* 2745-2757.

Van Essen, D.C., Drury, H.A., Dickson, J., Harwell, J., Hanlon, D. and Anderson, C.H. (2001). An integrated software suite for surface-based analyses of cerebral cortex. Journal of American Medical Informatics Association, *8(5)*, 443-459.

Vargha-Khadem, F., Gadian, D.G., Watkins, K.E., Connelly, A., Van Paesschen, W., Mishkin, M. (1997). Differential effects of early hippocampal pathology on episodic and semantic memory. Science, *277(5324)*, 376-380.

Venkatesan, U.M., Dennis, N.A. and Hillary, F.G. (2014). Chronology and chronicity of altered resting-state functional connectivity after traumatic brain injury. Journal of Neurotrauma, advance online publication, doi: 10.1089/neu.2013.3318.

Vincent, J.L., Patel, G.H., Fox, M.D., Snyder, A.Z., Baker, J.T., Van Essen, D.C., Zempel, J.M., Snyder, L.H., Corbetta, M. and Raichle, M.E. (2007). Intrinsic functional architecture in the anaesthetized monkey brain. Nature, *447(7140)*, 83-86.

Volle, E., Gonen-Yaacovi, G., de Lacy Costello, A., Gilbert, S.J. and Burgess, P.W. (2011). The role of rostral prefrontal cortex in prospective memory: a voxel-based lesion study. Neuropsychologia, *49(8)*, 2185-2198.

Wagner, A.D., Desmond, J.E., Glover, G.H. and Gabrieli, J.D.E. (1998). Prefrontal cortex and recognition memory: functional-MRI evidence for context-dependent retrieval processes. Brain, *121(10)*, 1985-2002.

Walker, A.E. (1940). A cytoarchitectural study of the prefrontal area of the macaque monkey. Journal of Comparative Neurology, *73(1)*, 59-86.

Walsh, V., Carden, D., Butler, S.R. and Kulikowski, J.J. (1993). The effects of V4 lesions on the visual abilities of macaques: hue discrimination and colour constancy. Behavioural Brain Research, *53(1-2)*, 51-62.

Walton, M.E., Behrens, T.E., Noonan, M.P. and Rushworth, M.F. (2011). Giving credit where credit is due: orbitofrontal cortex and valuation in an uncertain world. Annals of the New York Academy of Sciences, *1239*, 14-24.

Walton, M.E., Behrens, T.E., Rudebeck, P.H., Baxter, M.G., Buckley, M.J. and Rushworth, M.F. (2010). Separable learning systems in the macaque brain and the role of the orbitofrontal cortex in contingent learning. Neuron, *65*, 927-939.

Wang, K., van Meer, M. P. A., van der Marel, K., van der Toorn, A., Xu, L., Liu, Y., Viergever, M. A., Jiang, T. and Dijkhuizen, R. M. (2011). Temporal scaling properties and spatial synchronization of spontaneous blood oxygenation level-dependent (BOLD) signal fluctuations in rat sensorimotor network at different levels of isoflurane anesthesia. NMR in Biomedicine, *24(1)*, 61-67.

Warrington, E.K. and Shallice, T. (1969). The selective impairment of auditory verbal short-term memory. Brain, *92(4)*, 885-896.

Warrington, E.K., Logue, V. and Pratt, R.T.C. (1971). The anatomical localisation of selective impairment of auditory verbal short-term memory. Neuropsychologia, *9(4)*, 377-387.

Webster, M.J., Bachevalier, J. and Ungerleider, L.G. (1994). Connections of inferior temporal areas TEO and TE with parietal and frontal cortex in macaque monkeys. Cerebral Cortex, *4(5)*, 470-483.

Wendelken, C., Nakhabenko, D., Donohue, S.E., Carter, C.S. and Bunge, S.A. (2008). “Brain is to thought as stomach is to ??”: investigating the role of rostrolateral prefrontal cortex in relational reasoning. Journal of Cognitive Neuroscience, *20(4)*, 682-693.

White, N.S. and Alkire, M.T. (2003). Impaired thalamocortical connectivity in humans during general-anesthetic-induced unconsciousness. NeuroImage, *19(2)*, 402-411.

Will, B., Galani, R., Kelche, C. and Rosenzweig, M.R. (2004). Recovery from brain injury in animals: relative efficacy of environmental enrichment, physical exercise or formal training (1990-2002). Progress in Neurobiology, *72(3)*, 167-182.

Wittmann, B.C., Daw, N.D., Seymour, B. and Dolan, R.J. (2008). Striatal activity underlies novelty-based choices in humans. Neuron, *58(6)*, 967-973.

Wood, R.L. and Rutterford, N.A. (2004). Relationships between measured cognitive ability and reported psychosocial activity after bilateral frontal lobe injury: an 18-year follow-up. Neuropsychological Rehabilitation, *14(3)*, 329-350.

Yonelinas, A. (2001). Components of episodic memory: the contribution of recollection and familiarity. The Philosophical Transactions of the Royal Society, Series B, *356(1413)*, 1363-1374.

Yonelinas, A. (2002). The nature of recollection and familiarity: a review of 30 years of research. Journal of Memory and Language*, 46(3),* 441:517.

Yonelinas, A.P., Kroll, N.E.A., Dobbins, I.G., Lazzara, M., Knight, R.T. (1998). Recognition and familiarity deficits in amnesia: convergence of remember-know, process dissociation, and receiver operating characteristic data. Neuropsychology, *12(3)*, 323-339.

APPENDIX

Appendix 1 - R.O.C. pre-training

Delayed match-to-sample: All monkeys were trained on a delayed match-to-sample task prior to the start of behavioural training. In this task, trials were divided into a "display" and a "choice" stage. In the display stage, monkeys were presented with a stimulus, which was a distinct cartoon-like image 128x128 pixels in size (subtending a visual angle of 12 – 15 degrees to the typical viewpoint of the macaque) on a grey background. This stimulus was displayed in the centre of the touchscreen. Stimuli were chosen at random (without replacement) from a library comprising over 14000 distinct stimuli. When they touched this stimulus, it was removed from the screen and a delay period of 2s ensued. At the end of this period, two stimuli were presented on the screen, one of which was the stimulus displayed previously, and one of which was a previously unseen stimulus. These stimuli were presented on the horizontal midline of the screen, equidistant from the central point. Which stimulus was displayed to the left and which was displayed to the right was determined randomly. If the monkey touched the stimulus which was previously displayed, a reward pellet was dispensed and an inter-trial interval of 8s commenced. If the monkey touched the stimulus which had not been previously displayed, no pellet was dispensed, and an inter-trial interval of 16s commenced. During the inter-trial interval, a blank grey screen was displayed. If monkeys touched the screen during this period, the time of the inter-trial interval was reset. Figure 1 illustrates this task.

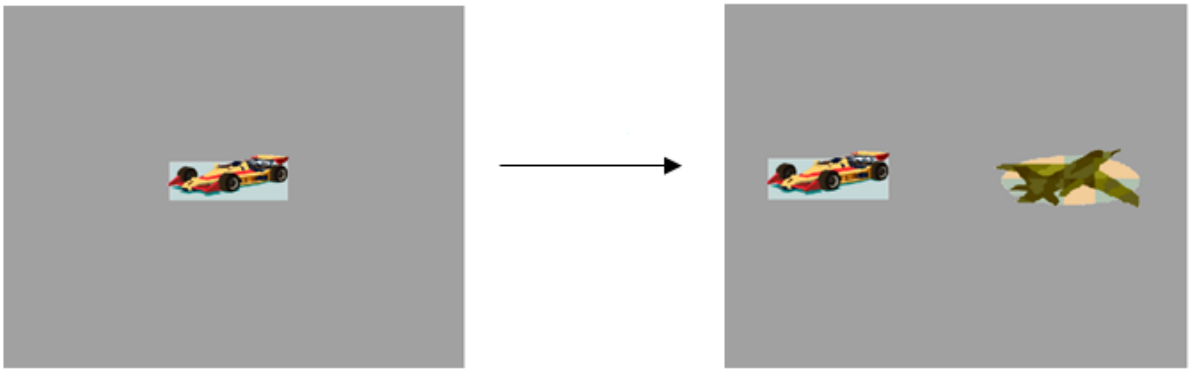


Figure 1 – Illustration of display (left) and choice (right) stages of the delayed match-to-sample task. Images are derived from free online resources, including Microsoft Clipart.

Pre-training stage 1: In the first stage of pre-training for the ROC task, monkeys were trained on an adaptation of the delayed match-to-sample test in which either the old or new stimulus in the choice stage of each trial was replaced by a red square; which stimulus was replaced was determined randomly. Monkeys were rewarded for choosing the "old" stimulus when it was present, or the red square if no "old" stimulus was present. Inter-trial intervals and the delay between display and choice trials were identical to those in the delayed match-to-sample task (these were held constant through all pre-training stages of this experiment). Figure 2 illustrates this pre-training stage. Monkeys were trained on this pre-training stage until they achieved a score of >70% for five continuous days, and scored on average >70% on both "old" and "new" trials across five days.

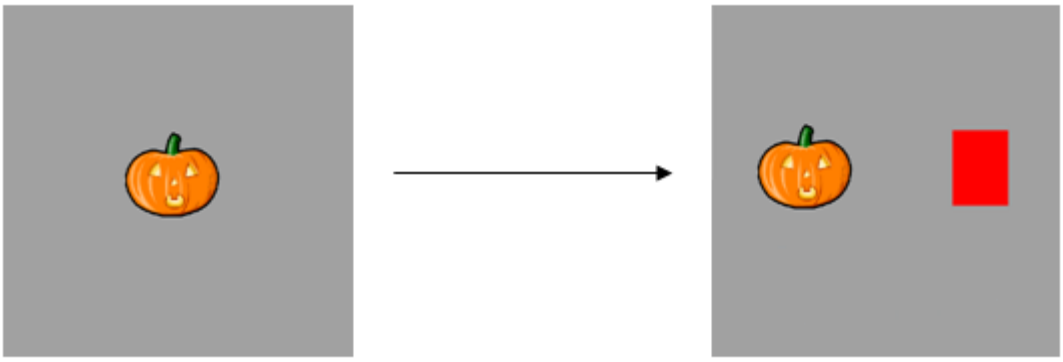


Figure 2 – Illustration of display (left) and choice (right) stages of pre-training stage 1. In this trial, the monkey would be rewarded for choosing the stimulus in the choice stage, as it is identical to the one presented in the display stage. Image is derived from free online resource.

Pre-training stage 2: In the second stage of pre-training, trials were randomly allocated as level 1 and level 2. On level 1, trials were identical to those presented in pre-training stage 1. On level 2, three items were displayed on the screen during the choice phase of each trial. These items were displayed on the horizontal midline of the screen; one item was displayed at the central point, and the other items were displayed at an equal distance from the central point. At least one item was a red square, and at least one item was either the "old" stimulus or a "new" stimulus. The third item was either a second red square (a "square" trial) or a second stimulus (which was identical to that already displayed – a "stim" trial). In a "square" trial, the trial ended if the monkey touched the stimulus; monkeys were rewarded if the stimulus was "old" and were not rewarded if the stimulus was "new". However, if the monkey touched the red square, it was removed from the screen, leaving the second red square and the stimulus displayed. Monkeys then had to touch the second red square in order to end the trial. If, after the monkey touched a red square, the animal touched the stimulus, nothing happened. When the monkey touched the second red square, the trial ended and the monkey received a reward if the stimulus had been "new", and did not receive a reward if the stimulus had been "old". Figure 3 illustrates a trial at level 2 at this pre-training stage. Monkeys were trained on this pre-training stage until they achieved a score of >70% across two continuous days and scored an average >70% on both "old" and "new" trials across two days.

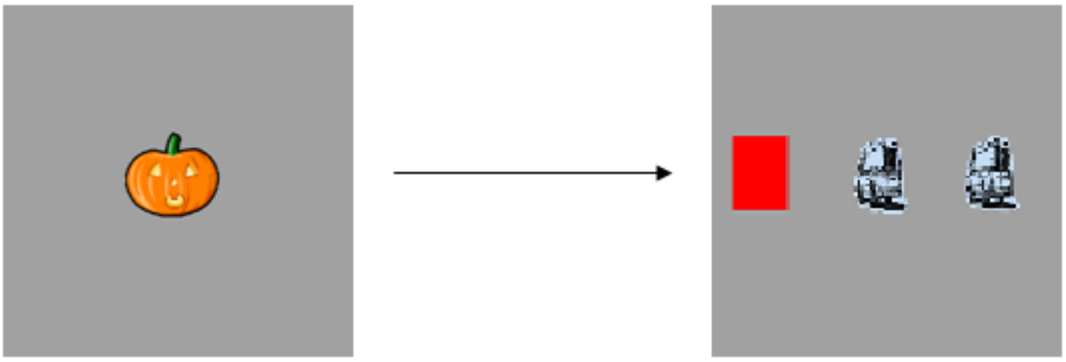


Figure 3 – Illustration of display (left) and choice (right) stages of pre-training stage 2, level 2. In this trial, the monkey would be rewarded for choosing the red square in the choice stage, as the stimulus differs from the one presented in the display stage. Images are derived from a free online resource.

Pre-training stage 3: In the third stage of pre-training, trials were randomly allocated as level 1, level 2 and level 3. Levels 1 and 2 were identical to those presented in pre-training stage 2. Level 3 was identical to level 2, except that, instead of a 2:1 or 1:2 ratio of stimuli:red squares, the ratio was 5:1 or 1:5. At level 3, items on the screen were presented in two rows of three items. Rows were presented equidistant above and below the horizontal midline. Items were horizontally positioned as at level 2. The position of the single red square or single stimulus was determined randomly. Figure 4 illustrates a trial at level 3 at this pre-training stage. Monkeys were trained on this pre-training stage until they achieved a score of >70% on the same day on both “old” and “new” trials.

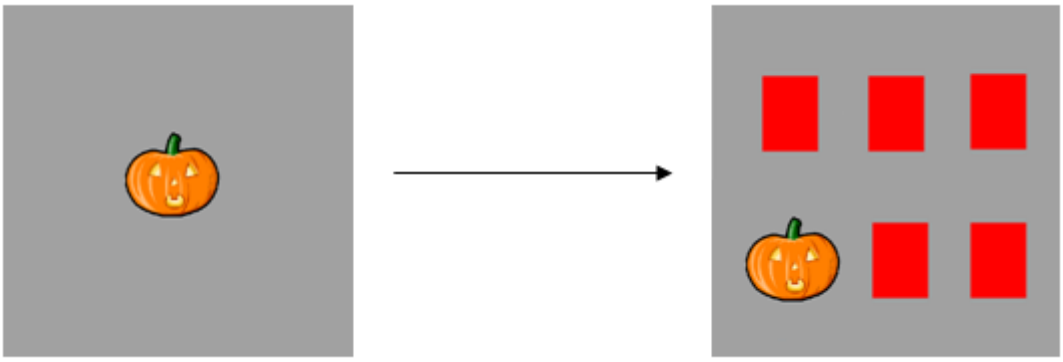


Figure 4 – Illustration of display (left) and choice (right) stages of pre-training stage 3, level 3. In this trial, the monkey would be rewarded for choosing the stimulus in the choice stage, as it is identical to that presented in the display stage. Image is derived from a free online resource.

Task 1: Monkeys were then tested on a version of the task which included trials randomly allocated between level 1, level 2, level 3 and level 4. Levels 1, 2 and 3 were identical to those presented in pre-training stage 3. Level 4 was identical to level 3, except that, instead of a 5:1 or 1:5 ratio of stimuli:red squares, the ratio was 8:1 or 1:8. Items were displayed in three rows and columns. One row was presented at the horizontal midline and the other rows were presented equidistantly above and below this row. One column was displayed at the vertical midline and the other columns were presented equidistantly to the left and right of this column. The position of the single red square or single stimulus was determined randomly. Figure 5 illustrates a trial at level 4 of this task.

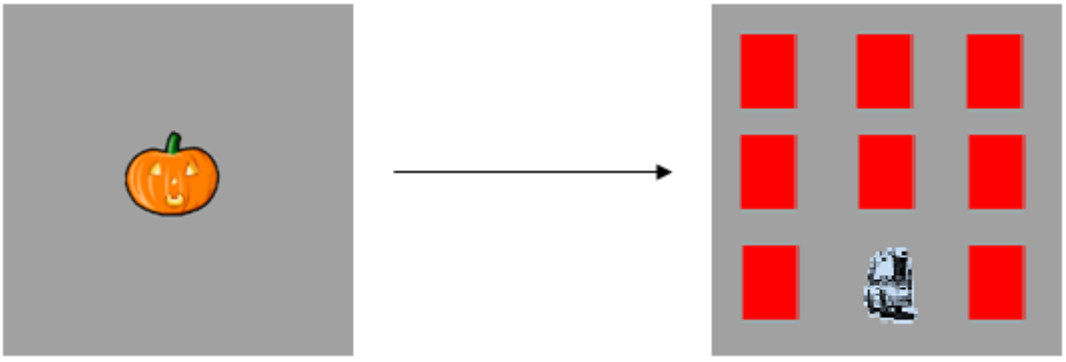


Figure 5 – Illustration of display (left) and choice (right) stages of task 1, level 4. In this trial, the monkey would be rewarded for choosing the red squares in the choice stage, as the stimulus differs from that presented in the display stage. Images are derived from a free online resource.

The decision was taken to abandon this version of the task due to several factors: the short delay between stimulus presentation and choice meant that monkeys correctly recognised a very large proportion of stimuli. Moreover, since, at high levels, the ratio of stimuli and red squares on the screen was unequal, chance performance on these trials was skewed towards the item which made up the majority of items on the screen. Finally, the intermixed presentation of levels may have prevented the monkeys from adjusting their level of bias relative to the ratio of items on the screen.

Task 2, pre-training: Monkeys were then pre-trained on a different version of the task. This version was very similar to the full task described in chapter 2. However, it differed in that two stimuli were presented in the display stage (as opposed to 3 in the full task), and monkeys only conducted one discrimination in the choice stage (as opposed to 6 in the full task). Monkeys were trained at level 1 on this pre-training stage; they were progressed to the full task after achieving a score >75% for three consecutive days. Figure 6 illustrates this pre-training version.

Figure 6 – Illustration of display (left and centre) and choice (right) stages at task 2, pre-training. In this trial, the monkey would be rewarded for choosing the stimulus in the choice stage, as it is identical to one of those presented during the display stage. Images are derived from a free online resource.

Appendix 2 - Horizontal, sagittal and coronal views of seed regions in fMRI analysis, illustrated on a standard T1-weighted image

In the following images, the horizontal view is presented in the left column, the sagittal view is presented in the centre column and the coronal view is presented in the right column.

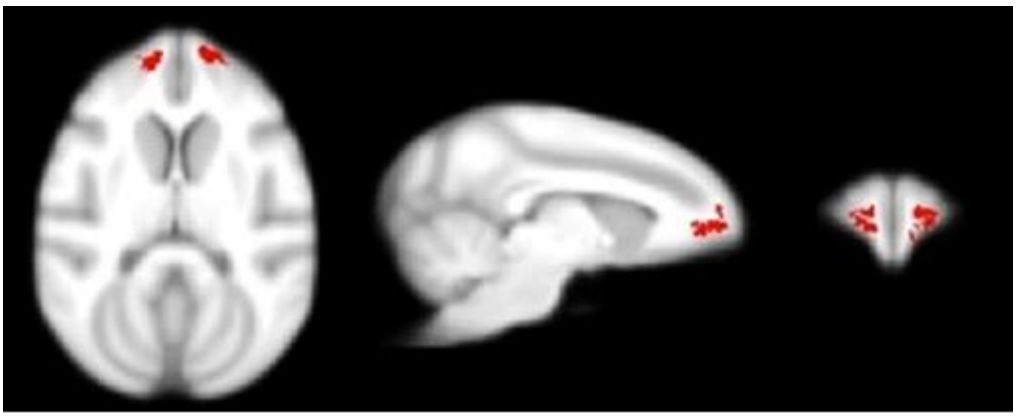


Figure 1 – BA10 seed region

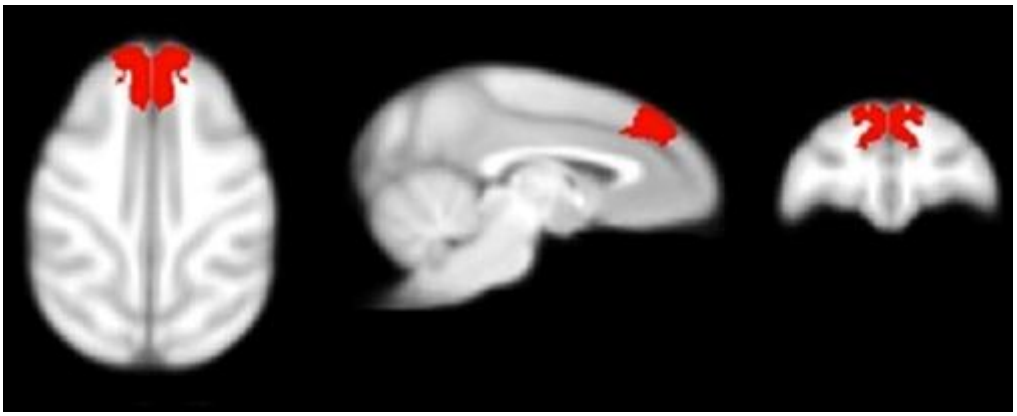


Figure 2 – BA9 seed region

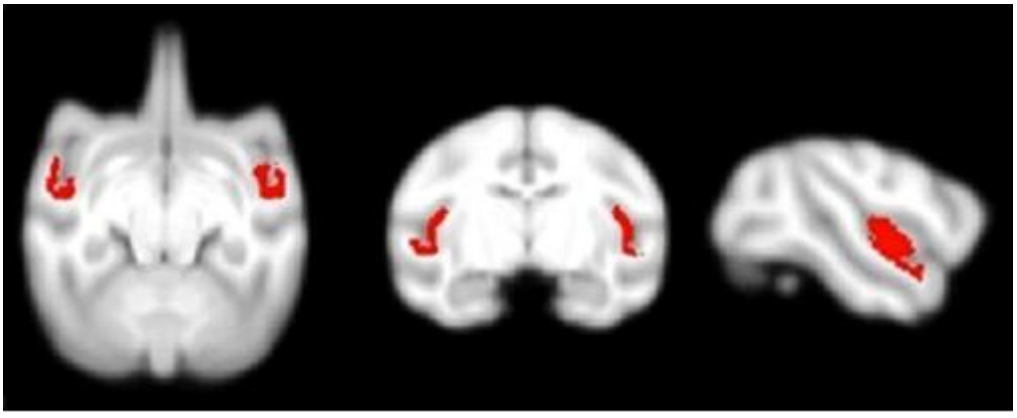


Figure 3 – BA13 seed region

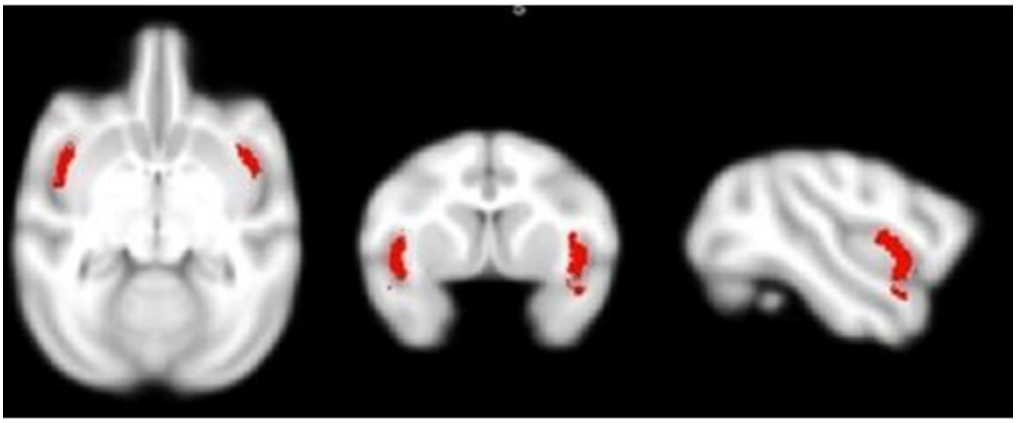


Figure 4 – BA14 seed region

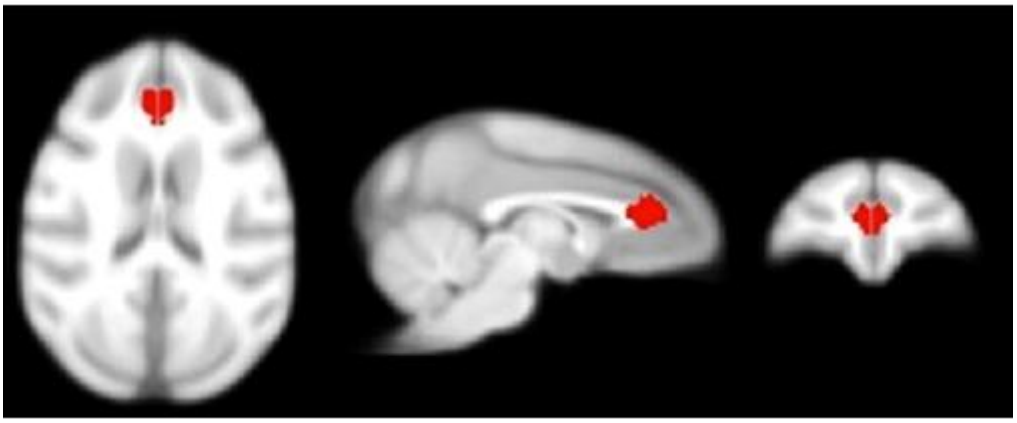


Figure 5 – BA32 seed region

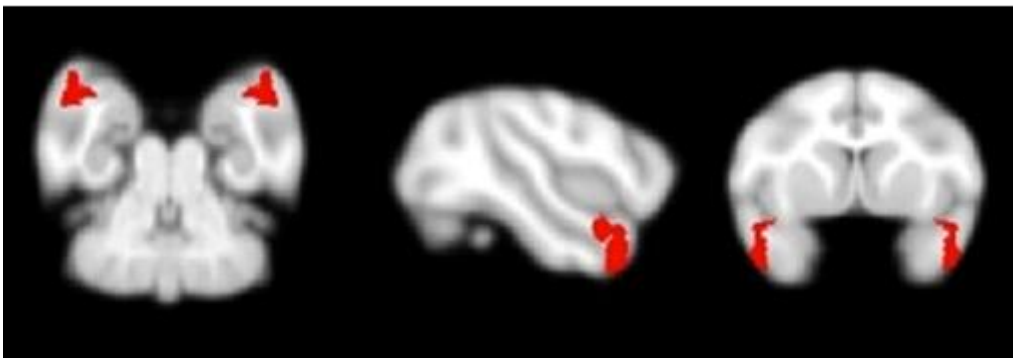


Figure 6 – Temporopolar proisocortex seed region

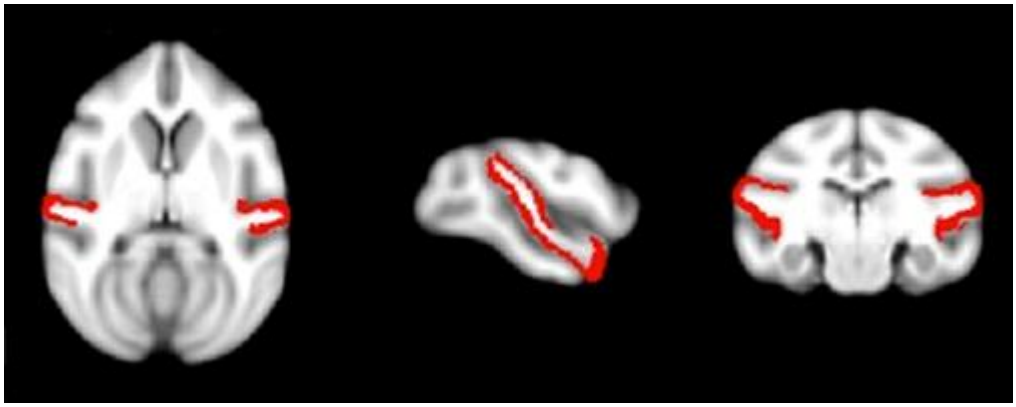


Figure 7 – BA22 seed region

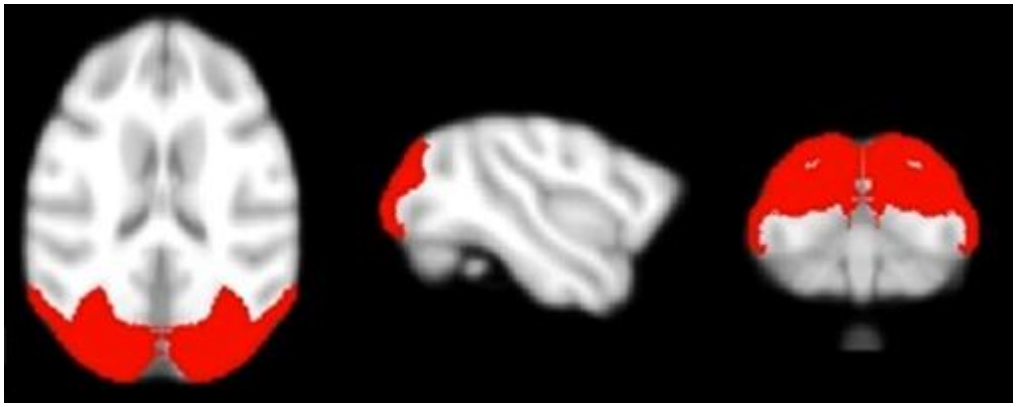


Figure 8 – V1 seed region

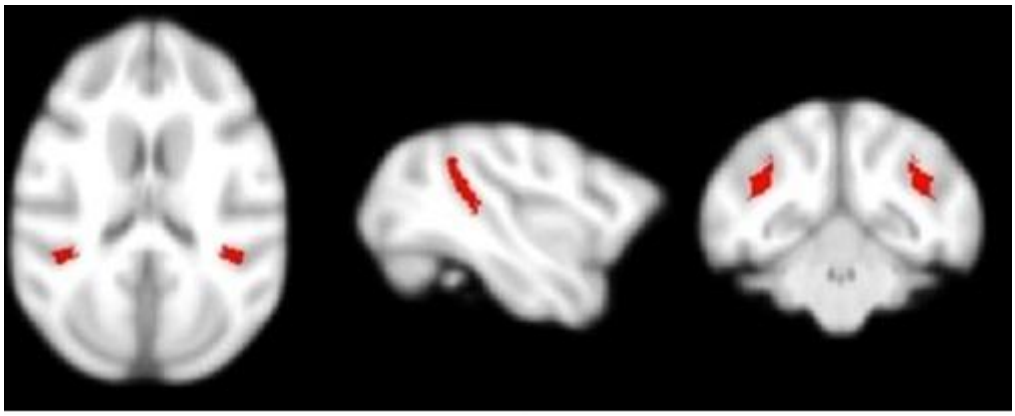


Figure 9 – MT seed region

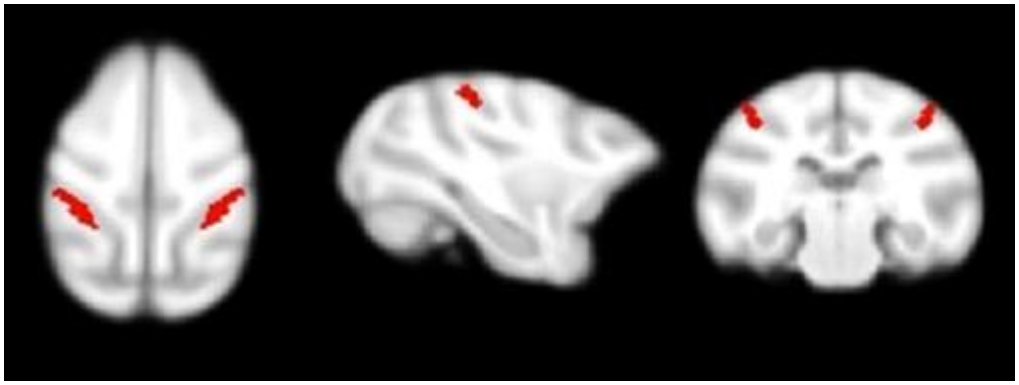


Figure 10 – LIPd seed region

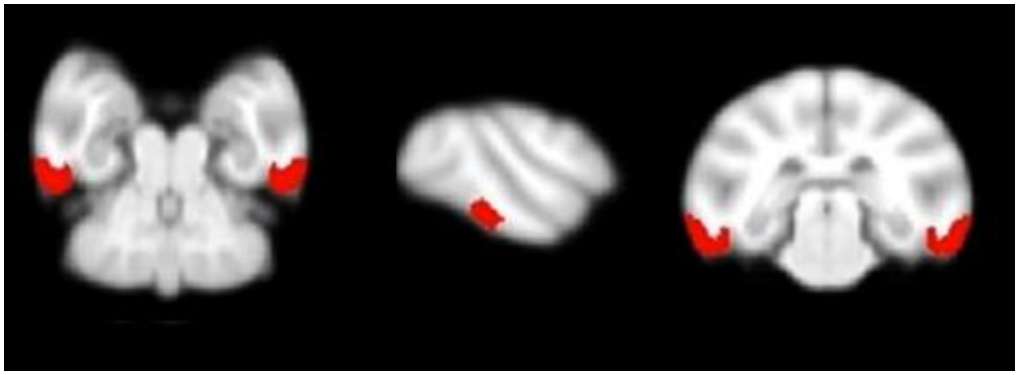


Figure 11 – TE seed region