Medial prefrontal cortex lesions in mice do not impair effort-based decision making.

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Abstract:

The function of the medial prefrontal cortex has previously been determined in the rat to play an important role in effort-based decision making and this, along with functions of other areas, has been assumed largely, to hold true in all rodents. In this study, we attempted to replicate this result in mice and to develop a model for effort-based decision making that could be useful for the study of neurological conditions. Mice were trained on a cost-benefit T-maze paradigm, whereby they chose between a low reward with little effort needed to obtain it or a higher reward, which required increased effort. Following training, the medial prefrontal cortex was lesioned. After surgery, contrary to earlier published rat studies, the performance of the mice did not change. In previous studies, prefrontal cortex lesioned rats chose the low effort/low reward option, but lesioned mice continued to select the high reward/high effort option. However, the other results are in line with previous mouse studies in both the extent of pathology and anxiety-like behaviour. These results illustrate a difference in the functioning of the prefrontal cortex between rats and mice and offer a word of caution on the interpretation of data from studies that employ different species.

Introduction:

The mouse has become the animal of choice for many in vivo studies due to the ease of manipulation of its genome. However, in neuroscience and modelling CNS conditions, most of our knowledge comes from studies using rats. It is therefore important to ensure this knowledge holds true for and is transferrable to mice. Interestingly, species differences have previously been demonstrated. For example, McNamara and colleagues showed both molecular, in the hippocampus, and behavioural differences between rats and mice (McNamara et al., 1996).

Modelling neurological conditions is difficult at the best of times and one has to be cautious not to over-interpret or extrapolate too far from findings. There are transgenic and lesion models for many neurological conditions such as schizophrenia, Alzheimer's disease, depression etc. As a result many specific brain areas have been linked to the pathogenesis of these conditions, such as the hippocampus in Alzheimer's disease (Braak et al., 1993) and the prefrontal cortex to nucleus accumbens projections in schizophrenia (Carr et al., 1999; Csernansky et al., 1991). Indeed the prefrontal cortex has also been shown to have a role in conditions other than schizophrenia, such as drug addiction (Lasseter et al., 2010) and depression (Bennett, 2011).

However, unlike in rats, the role of the prefrontal cortex in mice has had only limited characterisation, though research in this area has recently increased. Similar to rats, the mouse prefrontal cortex is important for spatial working memory, but this is by no means straightforward (Jones, 2002). The role of the mouse prefrontal cortex has been further elucidated by different groups showing, for example, decreased

anxiety-like behaviour (Deacon et al., 2003) and impaired attentional performance (Dillon et al., 2009) following medial prefrontal cortex lesions.

Salamone investigated the role of nucleus accumbens dopamine in rats, using a T-maze cost/benefit paradigm, whereby the rat must distinguish between a high or low reward, irrespective of the amount to effort required to obtain the reward (Salamone et al., 1994). Depletion of the accumbens dopamine caused a reduction in the choice of the high reward, when increased effort was needed to obtain the reward. Work by Walton and colleagues examined lesions in the prefrontal cortex in a T-maze paradigm, based on that of Salamone (Walton et al., 2003; Walton et al., 2002). Similarly, the choice was between a high effort/high reward arm and a low effort/low reward arm. Rats with a lesioned medial prefrontal cortex chose the low effort, low reward arm and it was shown this was as a result of the effort required rather than insensitivity to the level of reward. This showed the importance of the medial prefrontal cortex and, in particular, the anterior cingulate, in effort-based decision making (Walton et al., 2003; Walton et al., 2002).

Since executive decision making is impaired in patients with different cognitive disorders, has been linked to the prefrontal cortex (for review see (Reichenberg and Harvey, 2007)) and these observations are consistent with the rat lesion data, it would be important to develop a mouse model that could be used to further examine this brain area. In addition, a mouse model would be useful to allow examination of the role of risk genes for neurological conditions in conjunction with the role of the prefrontal cortex.

The aim of this study, therefore, was to translate the rat model of an effort-based decision paradigm into mice and to examine anxiety-like behaviour to show that the findings were in line with previous mouse data (Deacon et al., 2003). While in some cases, particular areas of the prefrontal cortex have been studied, we have not restricted ourselves to any one specific area in this case and instead produced a more general lesion of the prefrontal cortex. This is in line with the original rat model in which the prelimbic, infralimbic and cingulate cortices were affected (Walton et al., 2002).

Materials & Methods:

Animals:

Female C57BL/6 mice (Harlan, UK) were group housed (4-6) in plastic cages with wood chip bedding, under a 12 hr light/dark schedule (lights on at 7:00am). All testing occurred during the light phase of the day. During behavioural testing water was available *ad libitum* but they were food restricted to ~90% of their free-feeding weight. The experiments described were conducted under license from the Department of Health and Children in Ireland and were approved by the Royal College of Surgeons in Ireland research ethics committee. The decision to use only female mice for this work was due to the desire to directly compare with the relevant mouse prefrontal cortex studies (Deacon et al., 2003) and through concern for the welfare of the mice that will be group-housed for a long period of time. Inter-male aggression can be a problem in group-housed C57/BL6 mice, especially if they are not littermates (Betmouni, 1999).

Apparatus:

The T-maze used for behavioural testing was based on that described by Walton (Walton et al., 2002), but modified for use by mice. The T-maze is a high-sided wooden T-maze, consisting of a start arm and two goal arms all of which are lined by walls, 20 cm high (Figure 1A). A raised food well was fixed at the far end of each goal arm, equidistant from all sides. The interior walls of the maze were painted black while the floor was grey.

In order to obtain a reward, the mice had to climb over a barrier in the chosen goal arm. The barriers were made out of a heavy wire mesh bent to form a 3 dimensional right-angled triangle. The animals had to scale the vertical side and were then able to descend down the slope to obtain the reward. There were two different height barriers used during testing, namely 5 cm and 10 cm high (Figure 1B). During validation of the experimental protocol, barriers of greater height than 10 cm were used to determine the height that was most effortful and also the threshold height above which the mice would not climb the barrier. It was found that mice would not climb the barriers that were taller than 10cm in height.

<u>Effort-based decision model:</u>

Habituation & Training:

Habituation and training protocols are based on previous work by Walton and are described briefly below (Walton et al., 2002). A summary of the training and surgery timeline is shown in Figure 2. Prior to habituation and training, the mice were put on a restricted feeding schedule. During the first week, upon reaching 90% of their free-feeding weight, the mice were habituated to the maze both in groups and individually. On completion of habituation all mice were freely running on the maze

and eating reward pellets (20mg MLab Rodent Tablets, Test Diet, Richmond IN, USA) from the food wells.

Discrimination training was run in a number of phases. Phase I involved placing 3 reward pellets in the feeding well of one arm [high reward arm (HR)] and 1 reward pellet in the other [low reward arm (LR)]. For half of the mice, the HR arm was to the right, and for the other half, the HR was to the left. Initially, each mouse was placed in the start arm and sampled the food in each arm before being removed from the maze. There were 3 days of phase I discrimination training and each mouse ran 5 trials per day. The mice were cycled in their cage groups, leaving an intertrial interval of approximately 6 minutes. Phase II trials were "forced", when access to one of the goal arms was blocked, thus forcing the mouse to sample pellets for a particular arm on each trial. The LR/HR order of the forced trials was determined pseudorandomly so that the mice never had more than two consecutive turns to either side. Mice ran 10 trials per day for 3 days, to complete phase II. Phase III was the final phase and was very similar to phase I. The first two trials for each mouse were forced and then followed by an additional 10 trials. As in Phase I, the mice were allowed a choice of arms in these trials, but instead of being allowed to sample the pellets in each arm, they were removed from the maze after eating the pellets in the first selected arm. When all of the mice were choosing the HR arm on approximately 85-90 % of trials in training, a 5 cm barrier was introduced to the maze, placed in the centre of the HR arm. For the first five trials with the barrier, none of the mice were removed from the maze until they had climbed the barrier and eaten the food pellets. In all subsequent training, the trials were run on a strict choice basis and the mice were removed from the maze instantly after consuming food in chosen arm. After

three days of 10 trials/day/mouse, the barrier size was increased to 10cm for a further three days.

Surgery:

While in some cases, particular areas of the prefrontal cortex have been studied, we have not restricted ourselves to any one specific area in this case and instead produced a more general lesion of the prefrontal cortex. This is in line with the original rat model in which the prelimbic, infralimbic and cingulate cortices were affected (Walton et al., 2002). Surgery was performed when the mice were ~19 weeks old. They received excitotoxic bilateral mPFC lesions (n=13) or sham surgery (n=11). They were assigned to lesion or sham groups in a counterbalanced way, by virtue of preoperative performance and the right-left orientation of the rewards. All mice were anesthetised with Avertin (2,2,2 tribromoethanol in t-amylalcohol) given at a dose of 0.18g/kg i.p. They were then placed in a stereotaxic frame (Stoelting, Wood Dale, Illinois USA) with the incisor bar set to give an approximately level head between the bregma and lambda. This was subsequently verified. An incision was made along the midline and the skull exposed. A dental drill was used to remove a portion of the bone at the calculated injection sites on each side of the sagittal suture. A 5μl syringe (SGE, Victoria, Australia) with a specially adapted 34 gauge cannula was mounted on the stereotaxic frame in order to administer the injections. Two injections of 0.1μl of 10mg/ml NMDA solution (in phosphate buffered saline (PBS)) were made on each side of the midline to lesion the medial prefrontal cortex at the following anteroposterior (AP), mediolateral (ML), and dorsoventral (DV, from brain surface) coordinates, relative to bregma: AP +2.4, ML ±0.5, DV -1.2; and AP +1.8, ML ±0.45, DV -1.8. These coordinates were chosen with reference to a stereotaxic

axis (Franklin and Paxinos, 2008). Infusions of NMDA were administered at a rate of 0.1μl/min. The cannula remained in place for 1 minute following infusion and was then raised 0.1mm and left for a further minute before being slowly withdrawn. This was to ensure that diffusion from the injection site had occurred. The needle was checked for flow before and after NMDA administration. Sham lesions were produced by following the same procedure, though without the infusion of NMDA. On completion of surgery the scalp was sutured and the mice recovered in a heat-controlled environment until they regained locomotor ability.

Experiment 1:

Following a 10 day surgical recovery period, the mice were returned to a restricted feeding diet to reduce them to 90% of their free-feeding weight. All testing protocols were alike, the exceptions being in the height and/or number of the barriers. The first two trials every day were forced in opposite directions, so that the mice were made to sample the pellets in both arms before beginning the test trials. All mice ran 10 test trials per day for three days, with the 10cm barrier in the appropriate HR arm.

Experiment 2:

In order to determine whether any deficits seen represented a simple motor or spatial impairment or an inability to process reward quantity information, a barrier identical to the one in the HR arm, was placed in the LR arm. This meant that all the mice would have to climb over a barrier in order to receive any reward, and that the effort expended when either arm was chosen was the same. After 10 trials per day for three days, the barrier in the LR arm was removed and the mice were retested with the single barrier in the HR arm.

Anxiety-like behaviour test:

Elevated plus maze:

Anxiety-related behaviour was assessed using the elevated plus-maze test (Pellow and File, 1986). The plus-maze consists of four perpendicular arms: two opposing arms have walls made of clear perspex [closed arms; 15 cm high]; the other two arms do not have walls [open arms]. Clear Perspex was used for the closed arms to make these arms less 'safe' and promote increased exploration by the mice. All of the testing was conducted under dim lighting conditions. Mice were placed individually in one of the closed arms and observed for 5 minutes. The parameters measured included the cumulative time spent in the open arms [aversive], total time in the closed [non-aversive] arms, total number of entries into each arm (defined by all four paws being placed in that arm) and the number of faecal boli. All recordings were made using video tracking and Ethovision software and subsequent analysis was conducted by an observer blind to lesion/control group.

Histology:

Mice were deeply anaesthetised with sodium pentobarbitone (200mg/kg, i.p.) and perfused transcardially with ice-cold phosphate-buffered saline at a rate of 4 ml/min. The brains were then removed, snap-frozen at -60^{o}C in iso-pentane and then stored at -80^{o}C before being sectioned, coronally, at 10µm on a cryostat. Slides were subsequently stained with Haematoxylin before analysis.

Statistical analysis:

Similar to Walton (Walton et al., 2002), for the analysis of results obtained during habituation and training, the data were divided into separate 3 day testing blocks and underwent repeated measures ANOVA with two within-subjects factors

(days of testing and testing block) and with treatment as the single between-subjects factor (sham vs lesion). All results are presented using the conservative Greenhouse-Geisser correction, which overcomes problems caused by violations of the sphericity assumption when using an F test.

For the anxiety-related behaviour, comparison between groups was carried out using either a Student's t-test or Mann-Whitney U test for parametric or non-parametric data respectively. An alpha value of $p<0.05$ was considered statistically significant.

Results:

Pathology

In experimental mice, it has been shown by a number of groups, that over time (<5months), brain tissue surrounding the lesion becomes compressed into the lesion area (Deacon et al., 2003; Passingham et al., 1988) and this was also seen in our case. Therefore, the extent of the initial lesion is more easily determined from sections of representative pilot lesions (n=8), with the brains removed 10 days post surgery. However, both the experimental and pilot lesions were used to determine the extent of the lesion, with the pilots ensuring that the extent of lesion was not underestimated due to compression of the lesioned area. The mice showed bilateral damage to the prelimbic and infralimbic cortices which extended back to include the anterior cingulate cortex (to approximately 0.8mm anterior to bregma, according to the stereotaxic atlas (Franklin and Paxinos, 2008)). As was expected from the delay between surgery and histological processing, the damage in the experimental mice is generally more difficult to visualise, but the extent of lesion does correlate with the

pilot lesions. Figure 3 shows a schematic diagram representing the smallest and largest extent of the lesions of the medial prefrontal cortex. Figure 4 clearly shows the damage to the prelimbic and infralimbic cortices and separately the damage to the anterior cingulate cortex, when compared to the sham lesioned animals. As can be seen in both Figures 3 and 4, there is some damage to the secondary motor cortex, but as evidenced by the performance of the mice, it did not affect their motor ability.

Learning

The responses of the mice over the testing blocks are shown in Figure 5. To determine the effect of learning, the results were split into three pre-surgery testing blocks (Blocks 1, 2, and 3). It can be seen that the mice learned to choose the HR arm without a barrier (Block 1), and when a 5cm (Block 2) and then 10cm (Block 3) barrier was introduced their percentages of correct HR arm choices dropped, but remained above 70% correct. When these blocks were analyzed, there were significant differences between the blocks ($F_{(1.467,\ 32.278)} = 9.310$, $p < 0.01$) and day ($F_{(1.522,\ 33.494)} = 7.817$, $p < 0.02$) but not the two surgical treatment groups ($F_{(1,22)} = 0.507$, NS) or block x treatment interaction ($F_{(1.467,\ 32.278)} = 0.248$, NS). This is as we would expect and is consistent with the results of the mice learning the maze: their initial drop upon the inclusion of a barrier, but subsequent increase of HR choices, then a second drop for 10cm barrier followed by a second increase.

Experiment 1: Lesion Effect

Following surgery, there was no outward difference in performance in either the sham or, more importantly, the lesion groups (Figure 4). Although there was unequal performance between the treatment groups on the first day of testing following surgery, there were no significant differences between the treatment groups in Block 4

($F_{(1,22)}$ = 2.019, NS). Those mice with medial prefrontal cortex lesions showed no significant differences in HR arm choice compared to the sham surgery mice, and in Block 4, both still preferentially chose the HR arm. This is shown in the ANOVA data comparing the final pre-surgery block with the initial post-surgery data where neither the main effect of the surgical group ($F_{(1,22)}$ = 0.213, NS), nor of the block ($F_{(1,22)}$ = 2.65, NS) nor the interaction between these factors ($F_{(1,22)}$ = 2.287, NS) was significant.

Experiment 2: Post-surgery two-barrier test

In experiment 2, a second 10cm barrier was introduced in the LR arm of the maze, (Block 5) and a small increase in HR arm choices in both groups was observed, as seen in Figure 4. This is shown by the results of an ANOVA comparing Block 4 data with that of Block 5. There was a significant effect of block ($F_{(1,22)}$ = 17.576, p<0.001) but not of treatment ($F_{(1,22)}$ = 0.675, NS) or of the interaction between the two ($F_{(1,22)}$ = 3.024, NS). Though the effect of block was significant, that of day was not ($F_{(1.928,\ 42.415)}$ = 0.601, NS). There were no significant effects of block x day ($F_{(1.520,\ 33.449)}$ = 0.621, NS) or block x day x treatment ($F_{(1.520,\ 33.449)}$ = 0.607, NS) interactions. However, there was a significant effect of day by treatment, ($F_{(1.928,\ 42.415)}$ = 3.802, p<0.05). These significant effects can be explained by the difference in performance on the first day of Block 4, where there was seen to be a difference in performance when comparing the sham and lesion groups, though this perceived difference was shown to be insignificant.

When the second 10cm barrier was removed from the LR arm of the maze (Block 6), the performance of the mice in both groups continued to improve, until, at the conclusion of day three, all were choosing the HR arm in >85% of test trials. In

comparing these results with those of the two barrier testing block, the ANOVA showed no significance in the effects of day ($F_{(1.951, 42919)}$ = 1.748, NS) but it did show significant effects of block ($F_{(1,22)}$ = 12.084, p<0.05). All trials reinforced the initial learned behaviour and these results confirm this. There was no significant effect of treatment ($F_{(1,22)}$ = 0.514, NS) or of the interaction of treatment between either day ($F_{(1.951, 42.919)}$ =0.108, NS) or block ($F_{(1,22)}$ = 0.845, NS) and nothing in the interaction of all three ($F_{(1.394, 30.661)}$ = 0.396, NS).

Elevated plus maze:

Lesioned mice displayed a significant reduction in anxiety-like behaviour, manifesting as an increased number of entries into the open arms (p<0.05), reduced latency to enter the open arms (p<0.01) and increased duration in the open arms (p<0.05) when compared to controls, as illustrated in Figure 6. Furthermore, neither the number of entries into the closed arms (often used as a measure of activity rather than anxiety (Lister, 1987)) nor the velocity of mice in the maze showed any group differences (p>0.05 in each case). There was negligible defaecation and urination during testing (often used as an additional measure of anxiety-like behaviour in maze based tests) and so could not be used for group comparisons in this case.

Discussion:

Mice with medial prefrontal cortex lesions did not exhibit any change in their ability to distinguish between reward size or in the effort required to obtain this reward. This work demonstrates that mice with mPFC lesions show an altered behavioural phenotype to rats with an approximately equivalent lesion in the effort-based decision making paradigm. However, our data shows, similar to other mouse

data, that lesions of the mPFC produce a mild anxiolytic effect and do not impair motor function or coordination (Deacon et al., 2003; Etkin et al., 2006; Touzani et al., 2007).

Contrary to the existing rat data (Walton et al., 2002; Walton et al., 2009), the behaviour of mPFC lesioned mice was not impacted in an effort-based decision-making paradigm. As Frick and colleagues stated, mice are not little rats and species differences have been shown in, for example, water maze performance and corticotrophin releasing factor (CRF) input to the amygdala (Asan et al., 2005; Frick et al., 2000). However, there are studies showing consistency in behavioural effects between rats and mice following an equivalent lesion in each (Bissonette et al., 2008). Most of the data available on the connections between brain regions has been obtained from rats. Comparative studies between rats and mice have found most connections to be present in mice, though the extent can differ and some may be absent (Yilmazer-Hanke, 2008). Therefore, caution is needed when making comparisons and interpretations between rats and mice.

Effort-based decision

During the pre-surgery, training phase the mice learned to choose the high reward arm, climbing a high barrier to obtain the reward. Following a lesion of the prefrontal cortex, unlike the rat data (Walton et al., 2002), there was no difference in the performance of our mice. Other studies, using different paradigms, have shown results that reinforce our own. For example, Touzani concluded that based on published data, lesions of the mPFC of mice do not result in motivational deficits, which correlates with our observations (Touzani et al., 2007).

Studies examining mPFC lesions in rats have shown impairment of delayed spatial working memory (Floresco et al., 1997; Otani, 2003; Sloan et al., 2006) which may be due in part to disturbance of the global functioning of this brain region. In contrast, lesions of the mouse mPFC have produced conflicting results. Some studies showed no impairment of working memory on Y- or T-mazes (Deacon et al., 2003; Jo et al., 2007) whereas others showed significant impairment using a radial maze (Bach et al., 2008; Touzani et al., 2007), which may be due to the mnemonic load in the different tasks employed. There is a greater consensus regarding the effects of these lesions on reference memory, which appears to remain intact in both rats and mice (Deacon et al., 2003; Jo et al., 2007; Sloan et al., 2006). Our results are consistent with these studies.

It has been suggested that the mPFC may not be required for the retrieval of information, necessary when animals are re-exposed to an experimental situation (Botreau et al., 2004), therefore, lesions of this area would not be expected to affect reference memory. It has been proposed that this goal-directed information storage is due to a functional interaction between the PFC and other cerebral structures, including the hippocampus (Costanzi et al., 2009). These hippocampal-prefrontal interactions may also play a role in spatial and non-spatial memory (Costanzi et al., 2009). Lesions of the PFC have been shown to cause changes in the neuronal activity of the hippocampus, particularly that which is associated with spatial localisation (Del Arco and Mora, 2009). Those changes of the PFC which affect its dopamine receptors, will cause alterations in the memory processes associated with the hippocampus (Del Arco and Mora, 2009). Research has shown that impairments due to hippocampal lesions are overcome by rats that use contextual cues to retrieve

encoded information on the reward arm in which to find the larger and smaller rewards (Mariano et al., 2009).

In our study the lesion did not detrimentally affect motor skills as the mice were still able to climb the barrier and this finding is consistent in a number of mouse studies (Deacon et al., 2003; Etkin et al., 2006; Touzani et al., 2007). Similarly, in rats, lesions of the prefrontal cortex have not been shown to cause impairment of motor activity (Botreau et al., 2004; Walton et al., 2002; Walton et al., 2009).

As stated above there is conflicting data on the role of the mPFC in spatial memory in mice. This may also be due to the extent of lesioning as, for example, Bach and co-workers showed their lesioned mice were only able to acquire a conditional associative learning task when the lesion did not extend to the anterior cingulate cortex (ACC) (Bach et al., 2008). However, this is in contrast to the behaviour shown by Deacon, where there was no spatial learning impairment even though the lesion centred on the ACC (Deacon et al., 2003). Throughout our study the performance of the mice continued to improve demonstrating no impairment.

Anxiety-like behaviour

While the effects of mPFC lesions on anxiety are less well documented in mice than rats, the work of Deacon showed that mPFC lesioned mice displayed significantly less anxiety-like behaviour in the elevated plus maze and successive alleys tests (Deacon et al., 2003). Our results are consistent with these, demonstrating the comparability and effectiveness of the lesions. Furthermore, our results are also consistent with the majority of the available rat data, showing that excitotoxic mPFC

lesions resulted in attenuated fear-responses in the plus maze (Lacroix et al., 2000; Maaswinkel et al., 1996; Shah and Treit, 2003).

General Discussion

The experimental design and hypothesis used here was based on the work of Walton who demonstrated that, in Lister Hooded rats, lesions of the mPFC led to a reduced preference to exert effort in order to obtain a larger reward (Walton et al., 2002). Other studies have similarly shown the importance of the mPFC on work versus reward decisions and the neurochemical interactions between the mPFC and other cerebral structures (Rudebeck et al., 2006; Walton et al., 2003; Walton et al., 2002). In clinical studies, patients with similar lesions of the prefrontal area, demonstrate a loss of spontaneity and initiation of movement (Fuster, 2001).

Goal directed behaviours are influenced and controlled by the PFC and cerebral areas connected to the PFC by numerous projections including dopaminergic and glutamatergic neurons. The PFC can modulate the NAc directly through action of these inputs from the ACC into the core of the NAc, or indirectly via excitatory inputs to GABAergic neurons (Brog et al., 1993; Del Arco and Mora, 2009; Walton et al., 2009). The cells of the ACC were completely lost in the mPFC lesions of Walton and co-workers (Walton et al., 2002), and the importance of the ACC in motivation was further illustrated by later work where lesions of the ACC resulted in demotivation that was reversible by D2 antagonism (Walton et al., 2009). Other studies have used T-maze paradigms to produce similar results (Rudebeck et al., 2006; Schweimer and Hauber, 2005; Walton et al., 2003). Interestingly, the same study by Walton also showed NAc lesions failed to affect the choice of the high reward arm, as, it was conjectured, this structure may be unnecessary for evaluating effort versus reward (Walton et al., 2009). It must be noted however, that all these

studies were carried out in rats. In line with our work, and as mentioned above, Touzani and co-workers, noted that the effect of mPFC lesions in mice on radial arm maze performance was not as a result of a motivational deficit (Touzani et al., 2007). Interestingly, a recent paper by Pardo and colleagues demonstrated that D2 antagonism, in mice, resulted in a decrease in the choice of the high reward arm, a result opposite to that shown by Walton in mice (Pardo et al., 2012).

As described above the extent of lesioned area can contribute to inconsistent behavioural performance. Bach and co-workers discovered that lesioned mice that were unable to acquire a conditional associative learning task had lesions extending to the ACC (as in (Walton et al., 2002), were more caudally extended and there was Cg2 and increased Cg1 damage (Bach et al., 2008). These mice had deficits in executive function. Interestingly, mice that acquired the task had lesions restricted to prelimbic and infralimbic cortices with little ACC damage. However, these mice showed significant impairment of working memory (Bach et al., 2008). The lesions produced in our study were designed to be anatomically comparable to those induced by Walton et al., who showed excitotoxic loss of the prelimbic, infralimbic and cingulate cortices. Their pathology showed a majority of cell death to have occurred in the most anterior sections of the frontal lobe (Walton et al., 2002). The lesioned area in our study corresponds closely to this and also to earlier mouse mPFC work (Deacon et al., 2003), primarily affecting the prelimbic, infralimbic and the anterior cingulate cortices. Indeed, while we have lesioned the prelimbic, infralimbic cortices, our study can also be compared to the work of Bach and also of Walton due to the extent of our effects on the anterior cingulate cortex (Bach et al., 2008; Walton et al., 2003). Therefore, the mice in our study show a different behavioural phenotype to the rats in

the Walton study, further strengthening the evidence of a species difference in behaviour (Walton et al., 2002).

Despite this, we cannot completely rule out that the lesion did not affect the most appropriate sites. In rats, the nucleus accumbens is important for effort based decisions and as shown by Walton the MFC which projects to the NAc is also involved (Walton et al., 2009). As we do not have a separate cohort of mice with lesions centred on the NAc, we cannot eliminate the possibility that the difference in behaviour results from the role of the MFC projections to the NAc having a different role. Interestingly, this same study by Walton, also showed that NAc lesions failed produce a behavioural deficit, as, it was conjectured, this structure may be unnecessary for an evaluation of effort versus reward (Walton et al., 2009), so the contribution of the different areas clearly needs to be further elucidated.

As this report only utilises female mice, any conclusion must be made with the caveat that it may only represent a sex difference rather than a species difference. However, while there are reports detailing sex differences in behaviour, oftentimes these differences are minor when compared to the differences caused by prior treatment or even strain (Brown et al., 2000; Rodgers and Cole, 1993; Rogers et al., 1999). Indeed, it has also been shown that the oestrus cycle may have less of an impact on behaviour as previously thought (Parra et al., 1999).

There is still much work to learn about the role of the prefrontal cortex in mice and as shown here, results of studies conducted using rats cannot always be directly extrapolated. The contribution of different areas, such as the anterior cingulate cortex and nucleus accumbens, needs to be clarified using selective lesioning techniques to

overcome the sometimes contradictory results seen to date. However, our results show that a lesion of the mPFC in mice does not lead to any alteration of behaviour in effort-based decision making. Their executive function and memory, in this paradigm at least, remain intact. This highlights the need for more research into the differences that can occur between species and provides a word of caution for the development of a mouse model based on the results of previous work carried out using rats.

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Figure legends:

Figure 1: Pictorial description of the apparatus used in the study. (A) a view from above of the high-walled T-maze used throughout testing, showing where the food rewards are located and where the barrier can be placed. (B) A side-view illustration of the barrier as it would appear in the arm of the T-maze. In order to obtain the reward, mice have to climb the vertical wall of the barrier and descend the slope on the other side.

Figure 2: Schematic illustration of the timeline of the training, surgery and testing stages of this study. The duration and barrier sizes used are shown at each stage of the experiment.

Figure 3: Reconstruction of the medial prefrontal cortex (mPFC) lesions. Coronal sections adapted from (Franklin and Paxinos, The Mouse Brain in Stereotaxic Coordinates. 2007: Academic Press) and illustrate the greatest extent of lesion (light grey area) and the smallest extent (black) at 2.46 mm, 1.7 mm and 0.98 mm anterior to bregma..

Figure 4: Photomicrographs of coronal sections of control/sham (A, C) and lesioned (B, D) mouse brain. The sections approximately correspond to 1.8 mm and 1.2 mm anterior to bregma (according to the atlas of Franklin & Paxinos (2007)). The lesioned sections show damage to the cingulate cortex (cg1), the pre-limbic and infralimbic cortices and small damage to the edge of the secondary motor cortex **(B)**

and to the cingulate cortex (areas cg1 & 2) and extending to the fringes of the secondary motor cortex **(D)**. The Scale bar = 1 mm.

Figure 5: Percentage of trials (Mean ± SEM) in which lesioned and sham mice chose the high reward arm. The presence or absence of one or two barriers in the arms is detailed on the x-axis. Each testing block consisted of three days of testing with 10 choice trials per day.

Figure 6: Anxiety-like behaviour on the plus maze. (A) The latency to enter the open arm, in seconds. (B) Number of entries into open arm. Data are means ± SEM for mPFC and sham lesioned mice; ** $p<0.01$, * $p<0.05$ mPFC vs sham.