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# The role of the orbitofrontal cortex in the cognitive flexibility of rats assessed using novel touchscreen-based tasks

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# Content

# Abstract

The orbitofrontal cortex (OFC) is critical for cognitive flexibility that aids organisms to adapt their behavioural within the ever-changing environments. Its role in flexible behaviour is typically assessed using tasks in which subjects must change an established behavioural response in order to adapt to new contingencies. In the present study, two tasks were performed to investigate the role of the OFC in rats. This was assessed by using novel touchscreen-based tasks. One of these tasks is the serial reversal learning, a test that was performed to explore the role of the OFC when the contingencies of the visual stimuli are reversed, specifically, when the rats receive different doses of the 5-$HT_{2c}$ receptor antagonist SB242084. This task used two visual cues, one positive (CS+) and one negative (CS-). Touching the screen was required for reward delivery. After training, rats received intra-OFC infusions via cannulae during the reversals. In the rats, reversal learning was significantly enhanced when receiving the 1.0 µg/ml dose, but not when receiving the vehicle or 3.0 µg/ml. This effect was shown only during the perseverative phase and thus seems phase-specific. The second experiment that was performed is the intra-dimensional/ extra-dimensional (IDED) set shifting task. This is a test composed of different stages to measure rule acquisition and reversal. Again the task started with two visual cues during training. After that, the rats received exocitotic lesions in the medial orbitofrontal cortex (mOFC), the lateral orbitofrontal cortex (lOFC) or sham lesions. The rats were able to perform intra-dimensional set-shifting stages and therefore were able to form a dimensional set. These set shifting results also yielded differences between the lateral and medial OFC lesioned animals. Lesions in the mOFC showed enhanced intra-dimensional set shifting compared to the lateral and sham lesion groups. The reversal results, on the other hand, showed no clear differences between any of the groups. These results provide the first direct evidence from touchscreen-based tasks for the involvement of the 5-$HT_{2c}$ receptor in enhancing the perseverative phase of reversal learning. Moreover, they provide evidence for dissociable functional roles of the mOFC and lOFC during the performance of intra-dimensional set shifting.

***Keywords:***
*cognitive flexibility; reversal learning; intra-dimensional/extra-dimensional set shifting; medial orbitofrontal cortex; lateral orbitofrontal cortex; perseveration; translation*

# Introduction

The orbitofrontal cortex (OFC) is an important brain area implicated in cognitive flexibility that aids organisms to interact successfully with their ever-changing environments (Clarke et al. 2011; Clarke and Roberts 2011; Schoenbaum et al. 2009; Wallis 2007). The anatomy of the OFC is particularly suited for the modulation of behavioural flexibility. It uniquely receives information from all sensory modalities, via direct and indirect connections as illustrated in figure 1(Hooker and Knight 2006; Rolls 2000; Wallis 2007). In addition, the OFC has direct projections to the primary and secondary sensory cortices, and can modulate the strength of the neural signal coming from the sensory cortex, regulating the influence of that sensory signal on the rest of the brain, and, ultimately, on behaviour (Hooker and Knight 2006; Kringelbach and Rolls 2004). The OFC also has immense reciprocal connections with subcortical structures, such as the amygdala, thalamus, striatum and periaquaductal gray area (Kringelbach and Rolls 2004). Especially the subcortical connections the OFC has with the amygdala and the striatum and the interconnection between these latter areas are important for behavioral flexibility(Schoenbaum et al. 2007), as all three are involved in reversal learning. The orbitofrontal cortex (OFC) and amygdala are necessary for decisions based on expected outcomes during reversal learning(Burke et al. 2014) and the connections via striatum to motor areas are important for the action selection component of reversal learning(van der Schaaf et al. 2013).

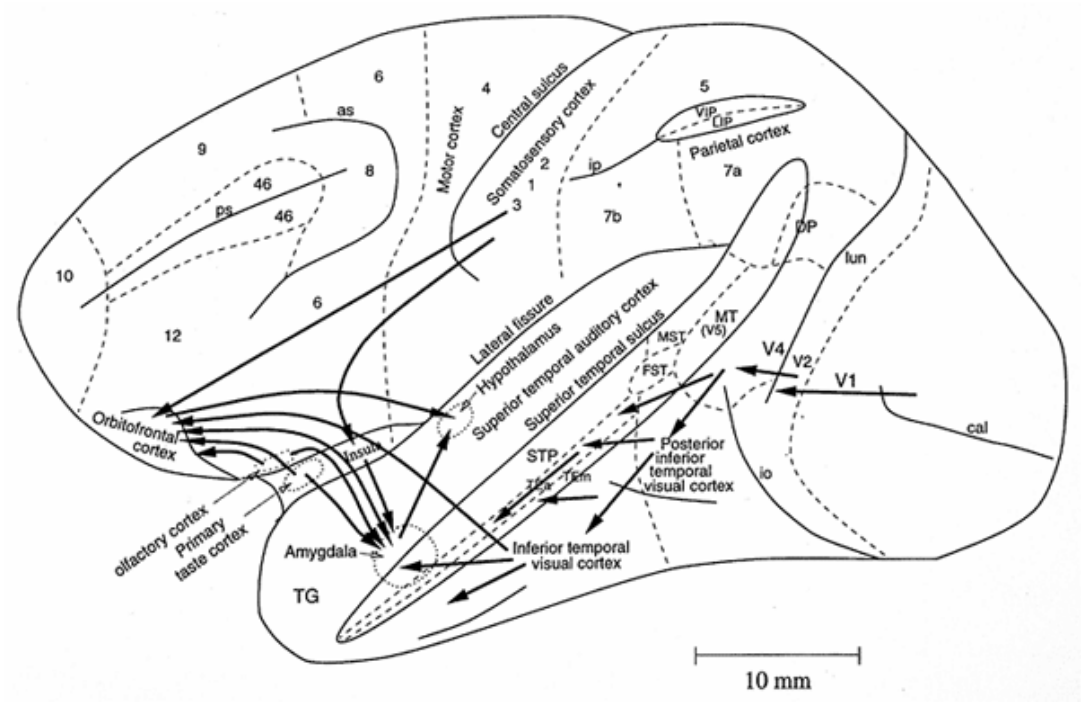


**Figure 1: Connections between the orbitofrontal cortex (OFC) and other major regions in the brain. Source: The New York Academy of Science.**

Part of the connections of the OFC belong to the cortico-striato-thalamic (CST) circuitry that are anatomical substrates for many facets of cognition, including working memory, cognitive control and reward informa-

tion(Alexander et al. 1986; Honey et al. 2003). It is hypothesised that, due to an altered balance in the flow of information between different CST loops, these circuits are implicated in diverse neuropsychiatric disorders (Alexander et al. 1986; Clarke and Roberts 2011; Honey et al. 2003; Robbins 1990). As mentioned, the OFC has been particularly implicated in cognitive processes relating to behavioural flexibility. Dysfunction in the OFC leads to behavioural inflexibility, which is a prominent symptom in a variety of neuropsychiatric disorders including schizophrenia, Huntington disease and Parkinson's disease (Clarke and Roberts 2011; Di Giovanni et al. 2011). The OFC is an area that is believed to integrate expected and actual outcomes to signal which cues in the environment are most relevant for predicting reward (Chase et al. 2012; Roberts 2011; Schoenbaum et al. 2009; Wallis 2007). Additionally, when an expected reward does not materialise, the OFC uses information from preceding trials to update the reward-prediction, thus facilitating stimulus- reward associations and learning .

As there is still much unknown about the specific functions of the OFC, its role in flexible behaviour is typically assessed using tasks in which subjects must change an established behavioural response in order to adapt to new contingencies (Schoenbaum et al. 2009). One of these tasks is serial reversal learning, a test that will be performed in this study on a touchscreen to explore the specific role of OFC when the contingencies of the visual stimuli are reversed. During this experiment intra-OFC infusions will be given with different doses of the 5-$HT_{2c}$ receptor antagonist SB242084. A second experiment that has changing contingencies is the intra-dimensional/ extra-dimensional (IDED) set shifting task. This is a test of rule acquisition and reversal. It features discrimination and attentional set formation maintenance, shifting and flexibility of attention. The task will be used to examine the role of OFC subregions on the formation of an attentional set and reversal learning and is also performed on a touchscreen using visual modality.

<u>Reversal learning and the OFC</u>

Organisms need to be able to flexibly adjust their behaviour when faced with changing environments or rules. Appetitive reversal learning procedures are widely used assays for such flexibility(Mar et al. 2013). Reversal learning has been investigated using similar tasks in humans (Fellows and Farah 2003; Izquierdo and Jentsch 2012), non-human primates (Clarke et al. 2005; Clarke et al. 2007; Lee et al. 2007) and rats (Birrell and Brown 2000; Boulougouris et al. 2008; McAlonan and Brown 2003). In reversal learning tasks, subjects initially need to learn to discriminate between two stimuli (CS+ and CS-) and choose the stimulus that is associated with reward (CS+). The stimuli used can be in any modality, but visual or olfactory stimuli are used most often. After the subject fully acquires which stimuli is the CS+, a reversal stage is implemented in which the previous CS+ becomes unrewarded (CS+ → CS-) and the previous CS- now becomes associated with reward delivery (CS- → CS+). The subject therefore needs to relearn the altered associations and inhibit choosing the previous CS+ and learn to respond to the previous avoided CS- that has become the new CS+. This

alternation of acquisition and reversal can be repeated many times within the same set of stimuli, and the ability of a species to adapt to this regimen has been considered as an indication of behavioural flexibility (Bond et al. 2007; Boulougouris et al. 2008). Using the serial version of the reversal task in animal research has the advantage that it is designed for the subjects to reverse in a small number of sessions, making it possible to perform manipulations, e.g. intracranial infusions using a within-subject design in which each animal is their own control and prevents (de)sensitization of the receptor or other changes in the brain. This is simply not possible in the "standard" version of the task, where a reversal sometimes can take up to 2 weeks or more. In classic reversal tasks specific cognitive operations are recruited e.g. detection of the shift in contingency during reversal;  inhibition of a prepotent, latent response (stop responding to the previously CS+) ; overcoming "learned irrelevance (CS- will be CS+ after reversal); and new associative learning (new CS+ is rewarded)(Boulougouris et al. 2008). Performing these operations for the first time makes the task difficult. Especially very young children and laboratory animals have negative transfer effects that are substantial when reversing for the first time (Kendler 2014). By performing multiple reversals it may recruit different mechanisms that makes it less difficult and learning occurs faster.

Converging evidence from numerous studies in humans, monkeys and rodents has consistently shown that lesions of the OFC selectively disrupt efficient reversal learning(Boulougouris and Robbins 2010; Brigman et al. 2010; Chase et al. 2012; Clarke et al. 2011; Di Giovanni et al. 2011; Roberts 2011; Rygula et al. 2010; Walker et al. 2009) while leaving intact the processes required for the initial discrimination. Moreover, the deficits associated with disruption of OFC function have been most often linked to a particular period following reversal - the perseverative phase. This phase is typically defined as occurring immediately following the contingency reversal in which the animal will not be rewarded anymore for the previous CS+. The perseverative deficits due to OFC dysfunction during reversal learning on a serial reversal task have been shown to be due primarily to a failure to inhibit responding to the previously rewarded stimulus, as opposed to overcoming the avoidance of the previously unrewarded stimulus (Roberts 2011; Rolls 2000; Rygula et al. 2010).

Just as there have been numerous studies that have documented deficits in the perseverative phase of a serial reversal task using different modalities and species (Boulougouris and Robbins 2010; Clarke et al. 2011; Roberts 2011), also the specific role of serotonin is investigated in more depth (Boulougouris and Robbins 2010; Nilsson et al. 2012). Clarke et al. (2004) suggests an important role for 5-HT neurotransmission within the OFC, as tasks depending on function of the OFC often also need intact 5-HT transmission (Clarke et al. 2004). Indeed, experiments with selective local depletion of serotonin in non-human primates and monkeys showed an impairment on a serial visual discrimination reversal learning task, mainly during the perseverative responses (Clarke et al. 2004; Di Giovanni et al. 2011). Subsequent work has established that this deficit was specific to serotonin and not dopamine in reversal learning and did not affect the per-

formance on set-shifting, which is the ability to move back and forth between tasks, operations, or mental sets in response to changing goals or environmental experiences (Clarke et al. 2005; Walker et al. 2009; Wallis 2007). It has also been investigated which specific receptor is involved in the perseveration and for the function of the OFC in reversal learning it is known that the post-synaptic receptor 5-$HT_{2c}$ plays an important role (Boulougouris and Robbins 2010; Clarke et al. 2011; Lapiz-Bluhm et al. 2009). Boulougouris et al. (2008) performed an instrumental "spatial" serial reversal task in which the animals needed to choose between two levers. They received SB242084, a 5-$HT_{2c}$ receptor antagonist, by systemic administration and showed a significant enhancement of spatial reversal learning (Boulougouris et al. 2008). Another lever pressing "spatial" reversal learning task was performed by Boulougouris and Robbins (2010) in which the role of the 5-$HT_{2c}$ was investigated neuroanatomical more specific by using infusions in the OFC (Boulougouris and Robbins 2010). As the enhancement effect is present when administered systemically and via infusions of a low dose (0.1,0.3 or 1.0 µg/ml) of SB242084, this receptor might be a possible target for diminishing the impairments during reversal learning of the mentioned disorders(Roth et al. 2004). However, so far most rodent research has focused on different modalities compared to human work. To minimize the effect of the difference in paradigms and improve the translational value of the results, it would be important to work with a paradigm that is more comparable between the species. Izquierdo et al and Brigman et al. did this by using a touchscreen-based operant reversal task for mice (Brigman et al. 2010; Izquierdo et al. 2006). Hence, the present study uses the recently newly developed touchscreen-based visual discrimination serial reversal learning task for rats that makes it possible to use infusions into the OFC during the experiment to follow up on the experiments by Boulougouris et al. from 2008 and 2010. By assessing the role of serotonin, and more specific the 5-$HT_{2c}$ receptor in the visual modality, it reveals if the visual reversal uses different neural and behavioural mechanisms within a touchscreen setting using 2D visual stimuli, when receiving infusions of the selective receptor antagonist SB242084 in a low (1.0 µg/ml) or high dose (3.0 µg/ml) compared to the simple "spatial" or response using scent and lever pressing tasks to measure behavioural flexibility. Therefore, this experiment will be important for the translation between modalities, but even more importantly, between species.

Intra-dimensional/extra-dimensional (ID/ED) set shifting and the OFC

In the literature it is often reported that besides discrimination learning, other types of learning important for adapting to a changing environment can be measured(Leeson et al. 2009). These include learning to change responses when stimuli are no longer relevant (reversal learning), learning to generalize responses from a particular stimulus to others in the same dimension (rule abstraction or intra-dimensional set shifting), and shifting attention to a different contingency when the current dimension is no longer fruitful (attentional extra-dimensional set-shifting) (Leeson et al. 2009). Studies in non-human primates and humans have shown that these different forms of learning are mediated by different neural processes. A double-

dissociation in humans, non-human primates and rodents has been demonstrated implicating lateral prefrontal cortex (lPFC) to mediate shifts in attention and strategy transfer between perceptual dimensions of complex stimuli, which requires updating of attentional biases or rules, and orbitofrontal cortex in reversal learning(Birrell and Brown 2000; Boulougouris and Robbins 2010; Dias et al. 1996, 1997; Hampshire and Owen 2006; Lapiz-Bluhm et al. 2009; Owen et al. 1991; Rogers et al. 2000; Rygula et al. 2010).

Both of the functions mentioned in the double dissociation can be measured during the intra-dimensional/extra-dimensional (IDED) set shifting task. This task is part of the Cambridge Neuropsychological Test Automated Battery (CANTAB)(Owen et al. 1991; Robbins 2007) and is developed to mimic tasks as the Wisconsin Card Sorting Task (WCST)(G.B. Bissonette et al. 2013; Chase et al. 2012). The intra-dimensional/ extra-dimensional task is designed in a way that the subject is required to switch dimensions and progress through different stages in which responses can be modified by feedback. Therefore, it can be used to form cognitive profiles relevant to schizophrenia, Huntington's disease, Parkinson's disease, and Alzheimer's disease (Lawrence et al. 1998; Owen et al. 1991).

During this task, subjects initially need to learn to discriminate between two stimuli (CS+ and CS-) within a certain dimension (in this study shapes or lines) and choose the stimulus that is associated with reward (CS+). When the subject fully acquires which stimuli is the CS+, a second dimension is added. The previous dimension that contains the CS+, called the relevant dimension, will be overlaid with this irrelevant, second dimension. This irrelevant dimension does not contain any CS+ stimulus. The animal should keep responding to the relevant dimension. After that it is possible to insert a reversal stage or start an intra-dimensional set shifting stage. When choosing to insert a reversal stage, the animal needs to keep responding to the same dimension but as with the serial reversal task, the previous CS+ becomes unrewarded and the previous CS- now becomes associated with reward delivery. Instead, when choosing to start with an intra-dimensional stage the animal receives new stimuli in the relevant and irrelevant dimension. The same dimension is still relevant and the animal should find out which stimulus is the new CS+ and which one is the CS-. This intra-dimensional stage can be repeated a couple times or another reversal can be initiated. If it is performed multiple times, the animal should stay focussed on the same dimension and still ignore the irrelevant one. Therefore, the animals forms a set in which they learn about learning, and acquire new stages more efficiently when consistent dimensions. Without set formation, each new problem is a blank slate, and thus no improvement should be seen over the course of the stages. Thereafter, another stage can be performed in the IDED task: the extra-dimensional set shifting. The animal will again receive four new stimuli in total, but now the CS+ will be a stimulus within the previously irrelevant dimension. The subject therefore needs to switch between attentional sets and show the ability to switch between arbitrary internal rules guiding behaviour (Garner et al. 2006).

Although many studies investigated the role of the OFC with relation to the double dissociation, recent work from Chase et al. is challenging the assumption that the contribution of the OFC to behavioural flexibility is limited to reversal learning(Chase et al. 2012). By using two different IDED set shifting paradigms they investigated the role of the OFC. The 4ID task, without any reversals, showed that the animals with lesions in the OFC were slower to form attentional set. When they did, they required more trials to complete the extra-dimensional shift stage. A standard '7-stage task' that includes reversal learning stages after each compound acquisition, revealed impairments in reversal learning and reduced shift-costs(Chase et al. 2012). As the lesioned group had problems in both the acquisition of the ID stages in the 4ID task and during the reversal stages of the 7-stage task the authors concluded that the role of the OFC might not be limited to reversal learning. Because of these findings, the role of the OFC in dimensional set shifting is investigate in more depth in this study. Another reason to perform research on the specific role of the OFC is that several articles have suggested that certain parts of the OFC have different connectivity patterns that suggest that different regions of the OFC may be specialized for distinct functional roles(Blair 2007; Elliott et al. 2000; Fuchs et al. 2004; Hampshire et al. 2012; Mar et al. 2011).

With rare exceptions, the precise parts of the OFC responsible for subserving its various functions have been largely ignored. This omission is surprising given that different anatomical connections of the mOFC and lOFC are well document, while evidence of their different behavioural roles has been lacking(Noonan et al. 2010; Rudebeck and Murray 2011b). Only a few studies have attempted to dissociate the function of its medial and lateral subregions that are revealed by cytoarchitectonically and connectionally studies(Walton et al. 2010). In human, primate and rodent studies, the medial subdivision of the OFC has its strongest connections with the hippocampus and associated areas of the cingulate, retrosplenial and entorhinal cortices, anterior thalamus and septal diagonal band (Elliott et al. 2000). This region is associated with making stimulus-reward associations and in the reinforcement of behaviour. Therefore this subregion functions in responding to, monitoring, or adjusting the incentive value of stimuli (Elliott et al. 2000; Iversen and Mishkin 1970; Kringelbach and Rolls 2004). The lateral OFC can be divided into three sectors in primates and strong anatomical and functional parallels appear to exist between rodents and primates(Schoenbaum et al. 2009). The most caudal part is characterized by strong connections with the amygdala, midline thalamus non-isocortical insula and temporal pole. The most anterior sector has more pronounced connections with the granular insula in primates, which is agranular in rats, the association cortex, mediodorsal thalamus, inferior parietal lobe and dorsolateral PFC, involved in higher-order cognition (Elliott et al. 2000). This subregion seems to be involved in stimulus-outcome associations like with punishment-reward stimuli and the evaluation and possibly reversal of behaviour when the contingency change and the previously rewarded response requires suppression (Elliott et al. 2000; Iversen and Mishkin 1970; Mar et al. 2011; Walton et al. 2010).

That there is a real dissociation between the two subregions of the OFC has already been shown by Iverson and Mishkin in 1970, who studied lesions in monkeys. They lesioned discrete parts of the prefrontal cortex in different monkeys and showed convincingly that when parts of the lateral orbitofrontal cortex were lesioned, the monkeys became significantly impaired with respect to object reversal learning. Specifically, they continued to respond much longer than controls to an object that was no longer rewarded on the first reversal trial. This was not the case for monkeys who had lesions to the medial parts of the OFC. These monkeys were not completely unaffected by the lesion, but showed moderate impairment on all but the first of the object discrimination reversals. These results strongly suggested a differential role for the lateral and medial parts of the orbitofrontal cortex and it also demonstrated the importance of the orbitofrontal cortex in reversal learning .

Since then only a few studies worked clearly on this dissociation. Most of these studies are performed on non-human primates. Only recently a study was performed on rodents. Mar et al. who performed a delayed discounting lever pressing experiment on rats with specific lesions in medial and lateral OFC and found a dissociation in function between the subregions of the OFC as mentioned above(Mar et al. 2011). An IDED design is, however, never performed using lesions to OFC or subregions of the OFC and also a touch screen operant task has only recently been developed for the rodent. With both human and monkey versions of IDED implemented using a touchreen method, and touchscreen task batteries being developed to characterize the cognitive profiles of rodents including genetically modified mice, a rodent touchscreen IDED task would be a useful tool to characterize models and translate results between human and rodent data. Therefore using this touchscreen operant task for the rats with lesions of medial and lateral OFC or sham lesions it is possible to investigate if the rats can learn attentional set in a touchscreen setting using 2D visual stimuli and to find out if there are distinctive roles in the OFC subregions during the different stages. The touchscreen task was inspired after the stages used in the research performed by Chase et al. (2012), however instead of two different tasks this study uses simple and compound discrimination followed by several intra-dimensional stages, reversals and extra-dimensional set shifting stages in one visual discrimination task in which the stimuli were designed to use the same dimensions (lines and shapes) as in the human and monkey IDED tasks.

Overall, this study investigated the influence of disruption of the OFC and specific subregions of the OFC on the cognitive flexibility of rats during the performance of two touchscreen tasks. During a serial reversal task the rats are required to switch their responses between two stimuli on each reversal, which allows us to investigate the function of the 5-$HT_{2c}$ receptor of the OFC in set shifting during a visual discrimination task. Performing this experiment with a vehicle, low (1.0 µg/ml) and high (3.0 µg/ml) dose of infusions of the 5-$HT_{2c}$ receptor antagonist SB242084, it will follow up on the results of Boulougouris et al. in 2008 and 2010 and because of this prior animal data, we predict that the first experiment would confirm the previous re-

sults. At least the low dose of the 5-$HT_{2c}$ antagonist will "enhance" the level of postsynaptic serotonin in the OFC as the same dose had the strongest effects in the study of Boulougouris and Robbins (2010). This dose will therefore improve the reversal learning by diminishing perseverative errors. The higher dose is expected to have a much more lenient effect, if any, while there seem to be a decreased effect of the drug when getting more remote from the 1.0 µg/ml dose. This experiment will also validate the use of the touchscreen task and therefore at the same time it will show if it makes translation easier between species by using the visual discrimination paradigm, as the modality corresponds to human and primate research.

In the second experiment animals with medial OFC, lateral OFC or sham lesions (medial sham or lateral sham) are tested on an IDED paradigm as the results from Chase et al. (2012) raises questions about the function of the OFC. Their finding that the role of the OFC might not be limited to reversal learning will therefore be investigated in this study. In total this IDED paradigm was designed to directly assess the formation of dimensional set, in addition to set-shifting. It will contain simple discrimination (SD), a compound discrimination (CD), several intra-dimensional (ID), reversal (rev), and extra-dimensional (ED) shifting. These stages of a visual discrimination touch screen task will therefore provide information about the role of the OFC in cognitive flexibility.

Furthermore, as results of the dissociation between the medial and lateral OFC is shown in monkeys and non-human primates in object reversal paradigms (Iversen and Mishkin 1970) and recently also in rats using a delay-discounting paradigm (Mar et al. 2011), this study will investigate the same specific lesion of the OFC subregions within a touchscreen visual discrimination paradigm for the first time. Therefore, this study will continue rodent research within a different modality, adding the ability for translation to other species including humans. The expectations are that the different specific lesions would have different effects on the stages of the IDED task. Specifically, lateral PFC lesions including the lOFC have been previously linked to problems with reversal learning, while the medial PFC lesions including mOFC seem to be more involved in the dimensional set shifting. Thus, the lateral lesioned animals are expected to have problems on the reversal stage in both the intra- and extra-dimensional stages as this subregion seems to be involved in stimulus-outcome associations like with punishment-reward stimuli and the evaluation and possibly reversal of behaviour when the contingency change and the previously rewarded response requires suppression. While lesions in the medial OFC would not affect the reversal learning. For the formation of an dimensional set the lateral OFC is not expected to show any deficits, as previous research keeps showing the double dissociation with the prefrontal cortex that does show problems with forming an attentional set when lesioned. The mOFC are, however, expected to be affected within the dimensional set shifting in the IDED paradigm by showing slower learning over the course of the intra-dimensional set shifting as this region is involved in making stimulus-reward associations and with the reinforcement of behaviour.

# Materials & Methods

## 1. Serial Reversal Learning

### Animals

In total twenty male, Lister Hooded rats were obtained from a registered breeder (Charles River, UK). Rats were housed in groups of 4 in enriched cages measuring 40x23x19 cm in a colony room on an artificial 12-h light/12 hours dark cycle (lights on at 7 pm). To increase their motivation on work for food reward, rats were placed on a moderately restricted diet (15-20g of standard lab diet per rat per day) with water always freely available. Rats maintained on 90% of their free-feeding weight; at the completion of testing the range was 430-470g.

Training commenced when the rats were approximately 3 months old. Starting with touchscreen training in the Med Associate box (figure x), visual discrimination, acquisition of serial reversal, surgery, recovery and re-baseline. After that, when the rats were around 4-5 months of age, the animals started actual testing which was completed over 2 months. The experimental and welfare practices described herein complied with the Animals (Scientific Procedures) Act 1986, and were carried out under the authority of a Project License (70/7548) approved by the UK Home Office and the University of Cambridge Animal Welfare and Ethics Committee.

### Surgery

Rats were anaesthetised using 5% isofluorane in oxygen during induction and 2-3% isofluorane during maintenance. Baytril, an antibiotic for veterinary use with the active ingredient enrofloxacin, was given in the drinking water for one week after surgery to prevent infection.

The rats were secured in a stereotaxic frame fitted with atraumtic earbars. Bilateral stainless-steel guide cannulae (22 gauge;plastic ones) were aimed dorsal to the target brain structure (OFC) using standard stereotaxic techniques. The sites were calculated from Paxinos& Watson (1998, figure 2) and, with respect to the Bregma, were Anteroposterior (AP) +3.7 mm, Mediolateral(ML) +/- 2.5 mm and dorsoventral (DV) -3.6 mm (from skull surface). The incisor bar was set at -3.3 mm relative to the interaural line for a flat skull position. Four small screws and cranioplastic cement secured the guide cannulae to the skull. Removable stylets (Plastics One) were placed into the guide cannulae to prevent occlusion and were held in place with a screw-on dust cap. After surgery, the animals were housed individually. Behavioural and physiological evidence suggested that all rats recovered well, with normal eating and pre-surgery weights returning within 48 h. Testing began no fewer than 7-9 days after surgery.

## Intracranial drug infusions

Infusions took place before the experimental session. The rats were held gently and habituated to the infusion procedure 1 day before behavioural training, where they were lightly restrained and the stylet was removed and then replaced. The following day, the behavioural task was introduced: rats received a vehicle infusion, which contained 20% PEG400 and 10% HPB in sterile saline, immediately before behavioural testing. On the third day the actual testing commenced and the animal received the vehicle or drug infusion before behavioural testing. The schedule which animal received what dose first was based on the performance before surgery and balanced between the three groups.

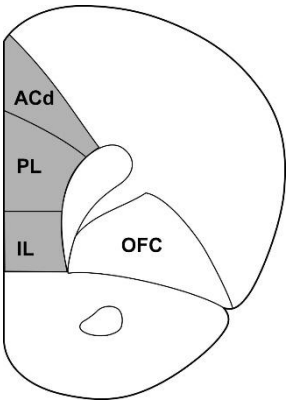


**Figure 2: A coronal section at approximately 1.7 mm to 3.7 mm from bregma through the rat brain illustrating the mPFC (shaded area) and the OFC. ACd: dorsal anterior cingulated cortex; PL: prelimbic cortex; IL: infralimbic cortex; OFC: orbitofrontal cortex. Modified after Paxinos and Watson 1998.**

During intracranial infusions, rats were gently restrained while stainless-steel injectors (28 gauge; Plastic One) extending 2 mm below the length of the guide cannulae were inserted into the OFC. The injectors were attached by polyethylene tubing (Portex) to 10 µl Hamilton syringes that were mounted on an infusion pump (Harvard Apparatus). One minute elapse after inserting the injectors to the relevant brain structure before beginning the infusions of vehicle or drug (0.5µl) over two minutes, after which the injector was left in place for one min to allow diffusion of the drug into the tissue surrounding the injector. The injector was then slowly removed and the stylet was replaced. Five minutes after the infusion the rat started the behavioural task.

## Drugs

6-Chloro-5-methyl -1-[2(2methylpyridyl-3-oxy)-pyrid-5-yl carbarnoyl] (SB 242084; Solvay) was tested in this experiment which is an selective antagonist for the $5HT_{2C}$ receptor. SB 242084 was dissolved in PEG400 and prepared in a final solution of 20% PEG400, 10% HPB in sterile saline.

Before drug infusions, animals were divided in groups, matched for their performance during the discrimination phase before surgery. In total there were 3 cycles of reversal. Each animal received infusions of SB 242084 (1.0 and 3.0 µl) and a vehicle in a separate cycle. All drugs were infused daily immediately before the start of the behavioural task.

## Histology

After the completion of behavioural testing, animals were given a lethal dose of sodium pentobarbitone (1.5 ml per rat; Euthanal, 200 mg/ml; Genus Express) and perfused transcardially with 0.01 µM PBS followed by 4% paraformaldehyde. The brains were removed, postfixed in 4% paraformaldehyde for 24 hours, and dehydrated in 20% sucrose in 0.01 µM PBS overnight. Coronal sections of 60 µm were cut on a freezing microtome and mounted on double-subbed glass slides. They were then stained with cresyl violet and cover-

slipped with DePeX mounting medium (BDH). The sections were then used to verify cannulae placement. The location of the cannulae was mapped onto standardized sections of the rat brain, the cytoarchitectonic borders and nomenclature of which were taken from Paxinos and Watson (1998).

## Behavioural testing – general protocol & the task

*Pretraining in MedAssociate testing box*

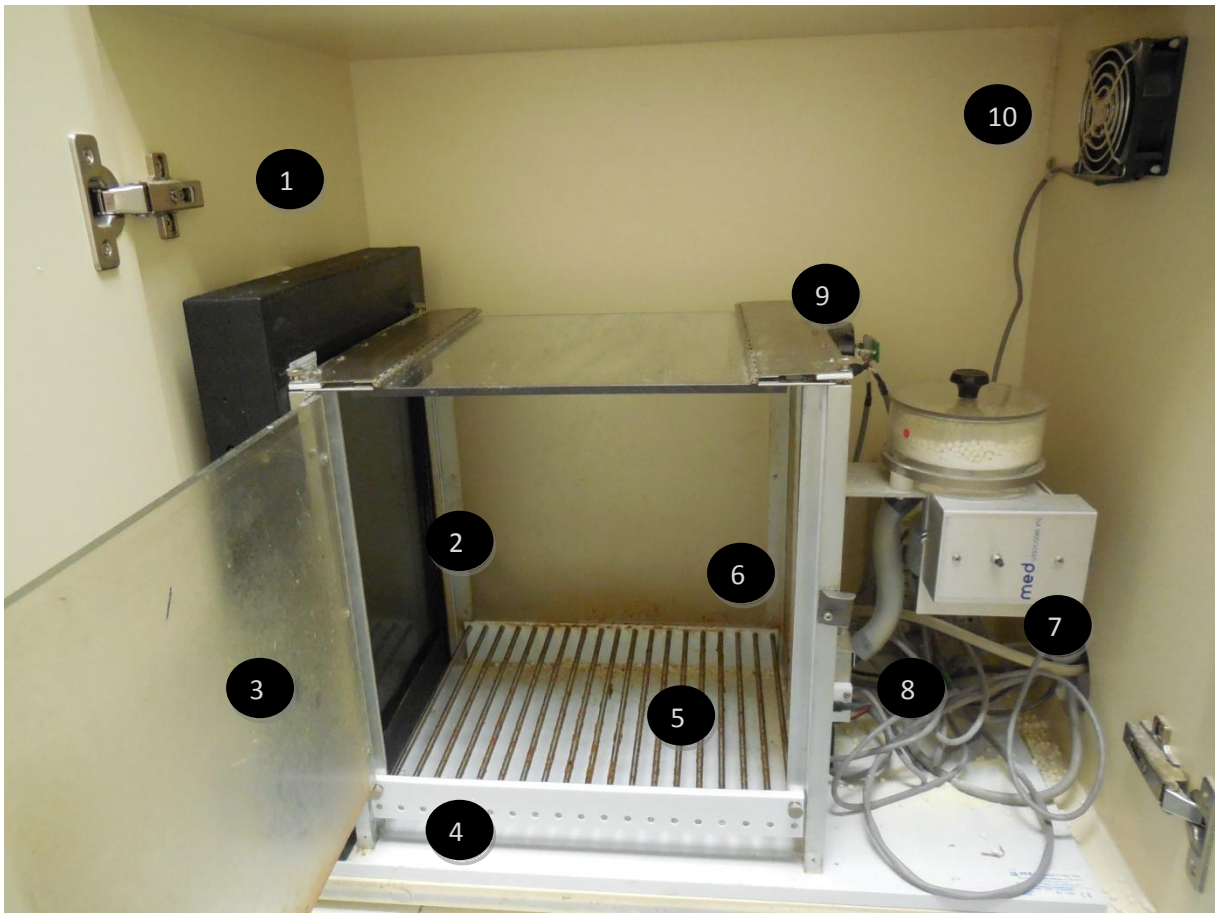


**Figure 3: The MedAssociate apparatus is enclosed within a sound-attenuating cubicle (1). On the front wall of the chamber (2) is the touchscreen equipped with IR detectors. To close the box, a transparant polycarbonate door is hinged at the front of the apparatus (3). Underneath the floor of the chamber (5), that consists of stainless steel rods, a removable tray could be placed (4). At 6, IR photo beams are present inside the food magazine. The pellet dispenser (7) is located outside the box and automatically delivers food pellet to the magazine through a plastic tube (8). On the opposite wall of the touchscreen the house light is locate (9). An electric fan (10) provides ventilation and low background noise.**

Initially, rats were pretrained in the Med Associate box (figure 3) which contained a touchscreen with a resolution of 1024 x 756 pixels. The touchscreen chambers were controlled by a Dell OptiPlex computer running a custom program developed in Microsoft Visual Basic 6. The animals were required to collect pellets at the magazine that were delivered every time they touched the big white bar on the screen (figure 4a)that had the coordinates of 100 pixels from the left, 0 pixels from the top, a width of 600 pixels and height of 100 pixels. The delay and intertrial interval (ITI) were both 0 and the time-out was set to 3 seconds. When the rats were reliably retrieving 100 pellets in 45 minutes, they were trained on a smaller white bar with the

coordinates of 250 pixels from the left, 0 from the top, a width of 300 pixels and height of 100 pixels (figure 4b). Again they were required to touch the bar to earn and consume 100 pellets in 45 minutes. Once criterion was reached, a final reduction on the size of the white bar was used. The white bar, now called the start button, had the coordinates of 325 pixels from left, 0 from the top, a width of 150 pixels and a height of 100 pixels (figure 4c). When the rat was able to obtain 100 pellets within a 45-minutes sessions, training proceeded to the visual discrimination task that consisted of a maximum of 250 trials per session.


**Figure 4: Example of long white bar (a; 3x18 cm) the shorter white bar (b; 3x9 cm) and start button (c; 3x4.5 cm) during pretraining with a black background.**

A white square was randomly presented in either the left or the right side of the touchscreen window (figure 5a). The second stimulus was a black square which was invisible against the black background. The stimuli remained on the screen until the rat responded to it by touching it with a nose poke or other touching response. Following the response, the rat was rewarded with a pellet concomitant with illumination of the food magazine. After 5 sessions there was a 5-second intertrial interval (ITI) introduced (figure 5b). When the rat choose the right stimulus (white square), it flashed. Picking up the reward, by making an entry in the food magazine, the animal initiated the next trial. If choosing the wrong stimulus (black square; not visible on screen), the house light went on for 5 seconds during which the stimulus will flash on the screen. If the animal didn't choose the trial is counted as an omission and the ITI will begin.

*Acquisition of visual discrimination*

Half of the animals were presented with a discrimination task in which the white square was replaced by vertical bars. The other half of the animals received the horizontal bars. They needed to do the same as with the first visual discrimination and if they got the maximum amounts of rewards the animal got the opposite stimuli. The animals that got the vertical bars first, now got the horizontal and the other way around. Again the animals needed to receive the maximum number of rewards.

After that the animals were presented with both horizontal and vertical stimuli at the same time in a trial. They needed to choose between the horizontal and the vertical bars and only one of them is rewarded (figure 5c).

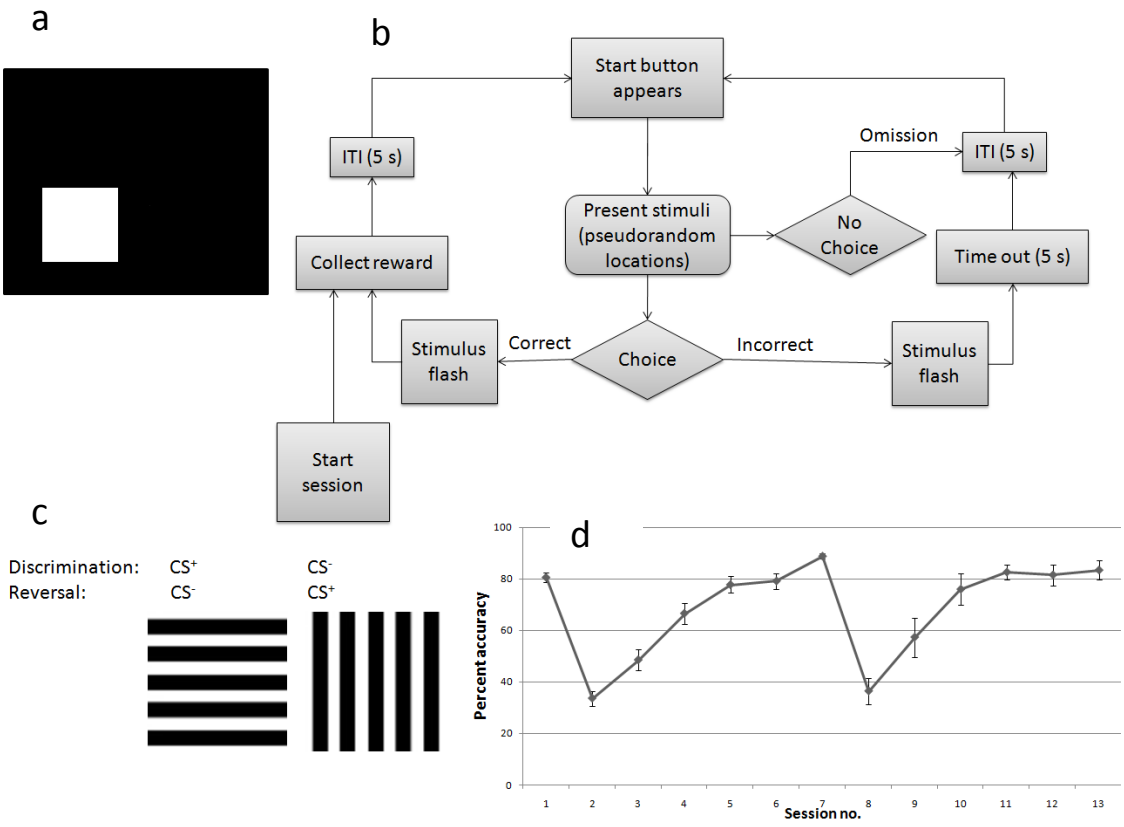


**Figure 5: a) During the reversal task the animal needs to choose between the horizontal or vertical bars as stimuli. b) A flow chart of the task, further described in the text. c) During the serial reversals the positive stimuli (CS+) and negative stimuli (CS-) change each reversal. d) Typical serial reversal data.**

*Pre-surgery and post-surgery Reversals*

When the animal reached criterion a retention session followed. Thereafter the new reversal was started. The animal got several reversals. These serial reversals within the task were designed for the rats to reverse in a small number of sessions (ideally 3 or less), this way it was possible to make use of intracranial infusions in a within-subject design (each rat is their own control). With the "standard" version of the task, where a reversal sometimes can take up to 2 weeks or more the amount of infusions would not have been feasible. Reaching the criterion in maximum 3 sessions made the animal ready for surgery.

After recovery the animals got baseline training on the same stimulus as the one before surgery. When they reached criterion the first post-surgery reversal was started. During this reversal the animals received intra-OFC infusions of SB242084 (1.0 or 3.0 µl) or vehicle infusions just before they started the task and it was repeated every day until they made criterion. After that there was a day of retention without any infusion. Following this day was the next reversal stage in which the animal received a different dose than before. Each animal got three reversal cycles in total (vehicle, low and high dose).

## Statistical Analyses

In the serial reversal learning task, the animals received three reversal learning cycles/stages in total in which they received a vehicle, a 1.0 μg/ml and a 3.0 μg/ml dose of the 5-$HT_{2c}$ receptor antagonist SB242084. Thea mount of errors and trials to criterion for each dose was analysed through a two-way repeated measures ANOVA (SPSS, version 22.0) in which the drug was the first variable. The second factor was the phase. As shown in table 1 the learning during reversal could be split into four phases, when assuming a binomial distribution. The first phase, which was called the perseverative phase, occurred immediately after the animals were reversed. With scores representing less than 11 correct responses out of the last 30 within a moving window. This corresponds to an accuracy of less than 37.5% and is significantly lower than chance level. The second phase, when the animals had between 11 and 19 correct responses out of the last 30 was called the random phase in which performance was around chance level. When reaching scores between 20 and 23 correct out of the last 30 in the moving window, the animals were performing above chance level within the learning phase (phase 3). After phase 3, which is a statistically significant bias, was completed the animals reached criterion and scored a minimal of 24 correct responses out of the last 30. This minimum of 80% accuracy showed that the animal truly learned to discriminate between the stimuli.

**Table 1: Overview of the four phases for the serial reversal learning based on a binomial distribution.**

| Phase | Description | Score |
|---|---|---|
| **1 Perseveration** | reversal performance significantly worse than chance | < 11/30<br>< 37,5% |
| **2 Random** | reversal performance around chance level | Between 11/30 and 19/30<br>Between 37.5% and 62.5% |
| **3 Learning** | reversal performance above chance | Between 20/30 and 23/30<br>Between 67% and 77% |
| **4 Criterion reached** | reversal performance far above chance | ≥24/30<br>≥ 80% |

The assessments of the different treatments was done based on within-subject results. Significant interactions ($P<0.05$) and trends ($0.10<P<0.05$) were followed-up by paired samples t-test. These t-tests were also performed when prior results indicated a possible difference. Behavioural analyses only included the eight animals finishing all reversal cycles of drug treatment.

## 2. Intra-dimensional/extra-dimensional (ID/ED) set shifting

### Animals

Thirty two male, Lister Hooded rats were obtained from a registered breeder (Charles River, UK). Rats were housed in groups of 4 in enriched (with cardboard tunnel) cages measuring 40x23x19 cm in a colony room on an artificial 12-h light/12 hours dark cycle (lights on at 7 pm). To increase their motivation on work for food reward, rats were maintained on a moderately restricted diet (15-20g of standard lab diet per rat per day) with water always freely available. Before surgery the weight range was 300x 400gand at the completion of testing the range was 550-700g.

Testing commenced when the rats were approximately 3 months old. Starting with touchscreen training in the Med Associate box (figure x), visual discrimination, undergoing surgery, recovery and re-baseline. Then actual testing started. The experimental and welfare practices described herein complied with the UK Animals (Scientific Procedures) Act 1986, and were carried out under the authority of a Project License (70/7548) approved by the UK Home Office and the University of Cambridge Animal Welfare and Ethics Committee.

### Surgery

In the IDED experiment the twenty-five rats that made criterion were allocated to three groups matched for simple discrimination performance and received bilateral lesions of lateral OFC (n=8), medial OFC (n=8), or sham surgery (n=9; from which 5 mOFC and 4 lOFC). Rats were anaesthetised using 5% isofluorane in oxygen during induction and 2-3% isofluorane during maintenance and are secured in a stereotaxic frame (David Kopf Instruments) with atraumatic ear bars and the nose bar set to -3.3mm to achieve a level skull. Excitotoxic lesions were made using 0.1 M NMDA dissolved in 0.1 M phosphate buffer (vehicle), with pH adjusted to 7.4. Bilateral infusions were made via 31 gauge stainless-steel injector (Coopers Needlework) attached to a Hamilton microinfusion pump by polyethylene tubing using the following parameters (Fuchs et al. 2004; Mar et al. 2011): lOFC: AP ± 3.2 mm, ML ± 2.5 mm, DV ± 3.6 mm, 0.3 µl over 3 min; mOFC: AP± 4.2 mm,ML±0.6 mm, DV ± 4.3 mm, 0.2 µl over 2 min.The AP, ML, and DV coordinates were taken from bregma, midline, and dura, respectively; the incisor bar was 3.3 mm below the interaural line. Injectors were left in place for a time period equivalent to the duration of infusion before removal. Sham surgeries (n = 9) were performed in similar manner, but with infusing vehicle alone.

Rats were single housed for the 24 h after surgery. Behavioural and physiological evidence suggested that all rats recovered well, with normal eating and pre-surgery weights returning within 48 h. Baytril, an antibiotic for veterinary use with the active ingredient enrofloxacin, was given in the drinking water for one week after surgery to prevent infection. Testing began 7 - 10 days after surgery.

## Histology

After the completion of behavioural testing, animals were given a lethal dose of sodium pentobarbitone (1.5 ml per rat; Euthatal, 200 mg/ml; Genus Express) and perfused transcarcially with 0.01 ᴍ PBS followed by 4% paraformaldehyde. The brains were removed, postfixed in 4% paraformaldehyde for 24 hours, and dehydrated in 20% sucrose in 0.01 ᴍ PBS overnight. Coronal sections were of 60 µm were cut on a freezing microtome and mounted on double-subbed glass slides. They were then stained with cresyl violet and coverslipped with DePeX mounting medium (BDH). The sections were then used to verify lesion locations. The location of the lesions was mapped onto standardized sections of the rat brain, the cytoarchitectonic borders and nomenclature of which were taken from Paxinos and Watson (1998).

## Behavioural testing– general protocol & the task

*Pretraining*

Rats were given some "free" pellets in their home cage to habituate them to the taste of the reward to counteract neophobia. Rats were then trained in the Med Associate box (figure 3) the same way as described in the first experiment. The difference with the first experiment was the background colour. Instead of a black screen the rats were trained on a grey coloured background. Besides that, the animal still started with a long white bar on the screen (figure 6a) that disappeared and resulted in reward delivery when the animal touched it. When the rats were able to obtain 100 pellets within 45-minutes session, they proceeded in pretraining to the smaller white bar(figure 6b) and got a final reduction on the size of the white bar (now called start button; figure 6c) when reaching criterion. When this criterion was reached, the rat continued to the visual discrimination task.


**Figure 6: Example of long white bar (a; 3x18 cm) the shorter white bar (b; 3x9 cm) and start button (c; 3x4.5 cm) during pretraining with a grey background.**

*Visual discrimination*

After this the animals proceeded to their first visual discrimination. Following a touch on the start button, the start button was removed and a white square was randomly presented in either the left or the right side of the touchscreen window. The second stimulus was a black square which was visible in this experiment against the grey background. The stimuli remained on the screen until the rat responded to it by touching it with a nose poke or other touching response. Following the response, the rat was rewarded with a pellet

concomitant with illumination of the food magazine. When the rat picked the correct stimulus (e.g., white square) the stimulus flashes and he initiate the next trial by making an entry in the food magazine. If the subject chose the wrong stimulus (e.g., black square) the house light went on for 5 seconds during which the stimulus will flash on the screen.

**IDED-stages**

*Simple discrimination (SD)*

After pretraining the animals were presented with a simple discrimination (SD1) between either two shapes or two lines, e.g. figure 7a and 7b. A second simple discrimination (SD2), with a new pair of stimuli followed when the animal reached a given criterion.

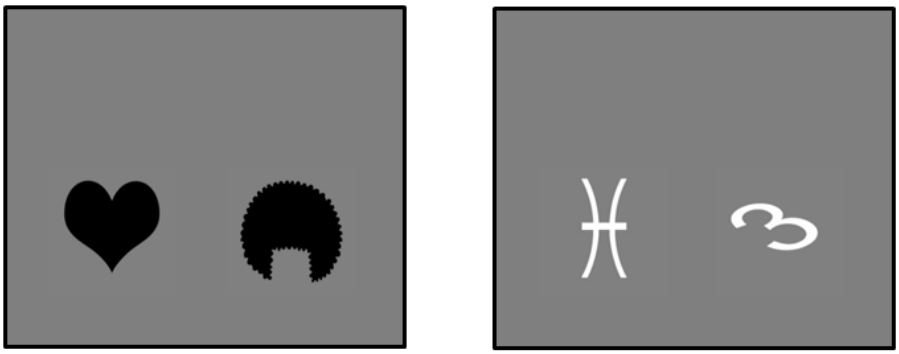

**Figure 7: Simple discrimination stage in which the animal received stimuli within the shapes (a) or lines (b) dimension.**

This criterion was set to 30 correct responses out of 40 trials within a moving window. All animals stayed on the same contingency of the discrimination, so the same sixteen animals received stimuli from the shape dimension as in SD1, but with a new pair of stimuli for SD2 (table 2). Also the stimuli were counterbalanced between the thirty two animals, which meant that each stimulus was used as CS+ and CS-. Counterbalancing across all stimuli is a useful control for ensuring that the results are not influenced by a specific stimulus at any given stage of the experiment.

After reaching criterion for SD2 the animals underwent surgery. Upon recovery, they were retested to criterion on SD2 in order to assess retention (memory) for the previously learned discrimination and to re-establish pre-surgical performance levels. This was called SD2 baseline.

**Table 2: All used stimuli pairs in the simple discrimination stages SD1, SD2 and SD2 baseline.**

| STIMULI | SD SHAPES | | SD LINES | |
|---|---|---|---|---|
| PAIR 1 |  |  |  |  |
| PAIR 2 |  |  |  |  |
| PAIR 3 |  |  |  |  |
| PAIR 4 |  |  |  |  |
| PAIR 5 |  |  |  |  |
| PAIR 6 |  |  |  |  |
| PAIR 7 |  |  |  |  |
| PAIR 8 |  |  |  |  |

*Compound discrimination(CD)*

After attaining criterion each animal proceeded to the next stage – compound discrimination (CD) – in which an irrelevant dimension was added. This means that instead of two shapes or lines, the animal now has both dimensions overlaid on the screen (figure 8a and 8b). In total there are four stimuli on the screen. Two shapes (in black) and two lines (in white). Again the contingencies stayed the same as the SD stages. The animals that were trained on the shapes dimension still needed to choose within the same dimension and the same applied for the animals trained on the lines. The combination of the stimuli changed randomly between. When reaching the same 30/40 criterion the animals were ready for the next stage.

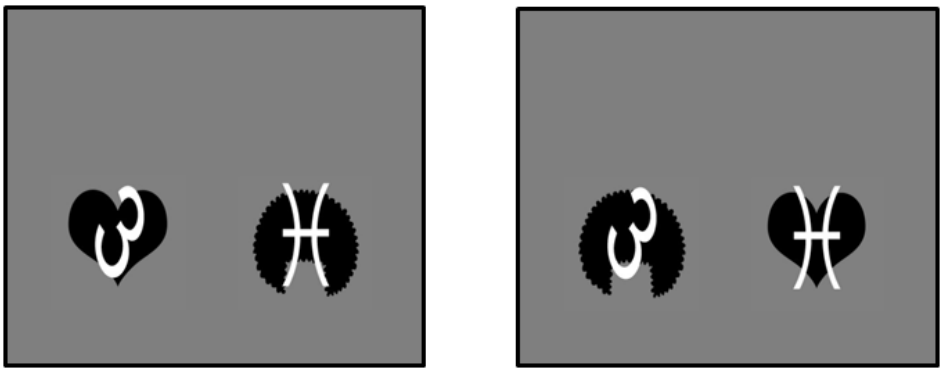

**Figure 8: From the compound discrimination stage onwards the animals saw four different stimuli on the screen. Two shapes overlaid with two lines in multiple combinations (a and b).**

*Intra-dimensional set shifting (ID)*

The next four stages of the task were intra-dimensional set shifting acquisitions (ID1-ID4; table s) in which different stimulus pairs where presented at each stage (table 3). The relevant dimension was held constant during all these stages and the stimuli were counterbalanced again with each stimulus occurring as CS+ and CS-. As shown in table 3, new stimulus pairs were introduced in ID4, that were counterbalanced as well. These four ID stages provided a direct measurement of the formation of a dimensional set.

*Intra-dimensional reversal (ID-rev)*

When subject completed these four intra-dimensional acquisitions, there was an intra-dimensional reversal (table 4). In reversal learning the individual animals first learned to make a discrimination between the two stimuli within a dimension (shapes or lines) and were then supposed to reverse the choice within the same dimension. Such reversals tend to be difficult for most subjects since the animal tends to persist responding to the previously rewarded stimulus (CS+) and avoiding the previously unrewarded stimulus (CS-). With this stage the ability to flexibly adapt to the new rules was tested. In this experiment, in contrast to the serial reversal, a single reversal was performed only. With no infusions to give to the animals, there was no problem when animals needed more than three sessions to reverse. The difference in sessions, errors and trials between the ID4 stage and the reversal are called switch-costs and showed the reversal effect. The study used the last intra-dimensional acquisition contingency to reverse the animals in. Therefore, the animals received the same stimulus pairs as in ID4, but now the previously incorrect CS- became the rewarded CS+ and the opposite for the previously rewarded stimuli in ID4, which was the CS- in the reversal stage. As in all intra-dimensional stages so far, the irrelevant stimulus was held constant. All animals still attended the same dimension as were they were trained on in SD1, SD2, CD and the four ID stages.

*Extra-dimensional shift (ED)*

Thereafter an extra-dimensional shift (ED) was planned for the rats (table s). For an extra-dimensional shift, the initially irrelevant dimension (lines or shapes) is given relevance by rewarding selection of one of its alternatives (line CS+) and by failing to reward choices for the other (line CS- and both shape stimuli, when trained on shape dimension previously). The animals, therefore, needed to attend to a new stimulus dimension and develop a new dimensional set.

In effort to minimize any effects from the reversal, an ID5 stage was presented first. In ID5 the same sixteen animals needed to acquire an attentional set in the shape dimension as they were trained in before. Thereafter ED was started with the rats. As for all stages, the criterion was set to 30/40 in a moving window to make sure the animals reached an accuracy that is not possible by chance.

*Extra-dimensional reversal (ED-rev)*

Having formed the new ED attentional set, a reversal was scheduled in this previously irrelevant dimension. This extra-dimensional reversal (EDR) stage was, similar as with the intra-dimensional reversal, used to test the animals' ability to flexibly adapt to the new rules. Unfortunately, due to the length of training at each stage in the current experiment, the EDR was not performed on this group of animals.

**Table 3: The stimulus pairs with two dimensions - shapes and lines used from the compound discrimination stage onwards. Each stimulus pair is used for the mentioned stages.**

| Stimuli | Stimulus 1 | Stimulus 2 | Stimulus 3 | Stimulus 4 |
|---|---|---|---|---|
| Pair 1 for CD, ID1, ID2, ID3, ID4, ID5 |  |  |  |  |
| Pair 2 for CD, ID1, ID2, ID3, ID5, ED |  |  |  |  |
| Pair 3 for CD, ID1, ID2, ID3 |  |  |  |  |
| Pair 4 for CD, ID1, ID2, ID3, ID4, ID5, ED |  |  |  |  |
| Pair 5 for CD, ID1, ID2, ID3, ID4, ID5 |  |  |  |  |
| Pair 6 for CD, ID1, ID2, ID3, ID5, ED |  |  |  |  |
| Pair 7 for CD, ID1, ID2, ID3 |  |  |  |  |
| Pair 8 for CD, ID1, ID2, ID3 |  |  |  |  |
| Extra pair for ID4, ID5, ED |  |  |  |  |
| Extra pair for ID4, ID5, ED |  |  |  |  |

**Table 4: Overview of all stages scheduled for the Intra-dimensional/Extra-dimensional set shifting task.**

| Stages | |
|---|---|
| SD1 | **Simple discrimination 1: animal discriminate between 2 stimuli in the shapes or lines dimension** |
| SD2 and baseline | **Simple discrimination 2: animal discriminate between 2 stimuli in the shapes or lines dimension** |
| CD | **Compound discrimination: animal discriminate between same 2 stimuli as SD2 with a second dimension added to the stimuli** |
| ID1-ID5 | **Intra-dimensional stimulus 1-5: animal discriminate between 2 stimuli in the shapes or lines dimension with a second dimension added** |

| | |
|---|---|
| IDR | **INTRA-DIMENSIONAL REVERSAL: ANIMAL DISCRIMINATE BETWEEN 2 STIMULI IN THE SHAPES OR LINES DIMENSION WITH A SECOND DIMENSION ADDED AND THE PREVIOUS POSITIVE STIMULUS (S+) IS NOW THE NEGATIVE STIMULUS (S-)** |
| ED | **EXTRA-DIMENSIONAL SHIFT: ANIMAL DISCRIMINATE BETWEEN 2 STIMULI IN THE OPPOSITE DIMENSION AS BEFORE** |
| EDR | **EXTRA-DIMENSIONAL REVERSAL: ANIMAL DISCRIMINATE BETWEEN 2 STIMULI IN THE OPPOSITE DIMENSION AND THE PREVIOUS POSITIVE STIMULUS (S+) IS NOW THE NEGATIVE STIMULUS (S-)** |

### Statistical Analyses

All data from the stages was collected into files. During each stage of the IDED the animals needed to reach a criterion of 30 correct responses out of the last 40 trials. This criterion was used with a moving window and corresponds to 75% percent correct responses. Based on prior experiments, this level of accuracy was only reached when the animal truly learned to discriminate between the stimuli. When this criterion was reached, the animals moved on to the next stage and the data was included. The files, including only the lesion groups (lOFC, mOFC and medial/lateral shams), per stage were used to extract the main measurements: number of sessions, errors and total trials needed to reach criterion. It appeared, however that the medial and lateral sham lesioned animals did not differ from each other. Therefore, they were combined to one sham group during analyses. The animals that did not receive any lesion (N=5) as well as the two animals (rat nr. 1 and 8) that died during the study were all excluded completely from the data analyses. Due to occasional individual differences in learning particular stimulus discriminations, extreme results in any of the measurements were examined using the interquartile range (IQR) for each lesion group separately. By measuring the lower (Q1) and upper quartiles (Q3) one could define an outlier to be any observation outside the range: Q1-1.5 (Q3-Q1), Q3+1.5 (Q3-Q1). Boxplots were made to show the range and distribution along a number line. An example of these can be found in supplementary information at the end of this thesis together with a table in which is listed which animals were extremes how many animals were included per stage. The results of the two extra-dimensional stages (ED and ED-reversal) were excluded from the results as only a limited amount of animals completed them.

Investigating the distribution of the data revealed a non-normal distribution within the stages. Therefore, with SPSS, version 22.0, a non-parametric Kruskal-Wallis H test was performed for each of the stages (SD1 and 2, CD, ID1-5, and REV).Also for the cumulative scores of the four ID stages preceding the reversal stage were subjected to this test. The output of this test gave a chi-value ($X^2$) and a P-value. Differences were marked as significant when $P \leq 0.05$ and trends were defined as having a value between $0.10<P<0.05$. Significant effects and prior expectations of the measurements were followed up with independent two-tailed Mann Whitney U t-tests. All mean effects were marked with an asterix (*) in the graphs and text.

Comparing between stages of the task was done with a non-parametric repeated -measures ANOVA, which is called the Friedman's test. This test was performed to compare SD1 to SD2 and SD2 re-baseline to CD. A final comparison was made between the fourth intra-dimensional set shifting stage (ID4) and reversal. Again a P-value identical or less than 0.05 was suggested significant and a trend lied between 0.05 and 0.10. Independent two-tailed paired Mann-Whitney U tests (non-parametric) followed any significant difference or prior expectation. Mean differences were noted with asterix (*) sign or a different sign, which is mentioned in the text.

# Results

## 1. Serial Reversal learning

### Histology results

Figure 9 shows a schematic reconstruction of the position of injector tips in the OFC together with a photomicrograph of a coronal section taken from a representative rat. It shows that all cannulae entered the lateral or ventrolateral subregion of the OFC. In total, 12 animals were excluded from data analyses due to the cannulae falling off before finishing the end of the third reversal cycle.

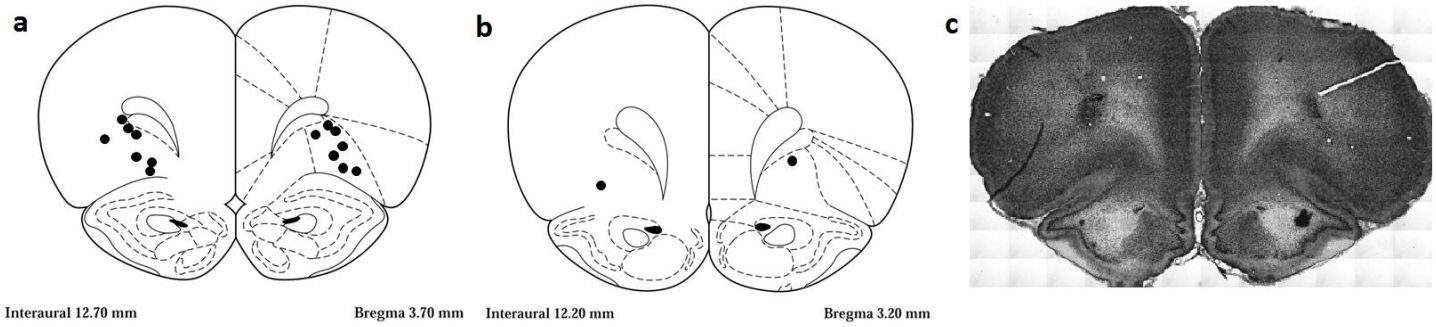


**Figure 9: Schematic diagrams (a and b) and photomicrograph of a coronal section taken from representative rat (c) showing the location of the injector tips in the OFC. Data are reconstructed from Paxinos and Watson (1998).**

### Behavioural results

**Performance with drug infusions: effects of intra-OFC infusions of SB242084 in serial reversal learning**

*Dose effects: Number of errors and trials to criterion*

The mean number of *errors* made to reach criteria is shown in figure 10a. The repeated measures ANOVA with dose as the factor shows that the errors to criterion were not significantly different between different dose levels ( $F_{2.14}$=1.409 , p=0.277). Repeated-measures comparing the total trials to criterion across all phases showed that subjects did not make significantly more total errors during the cycle when receiving the different doses of SB242084 ($F_{2.14}$=1.118, p=0.354 ; figure 10b).The doses of SB242084 used in this experiment did not appear to alter the subjects' main indices of performance on the serial reversal task.

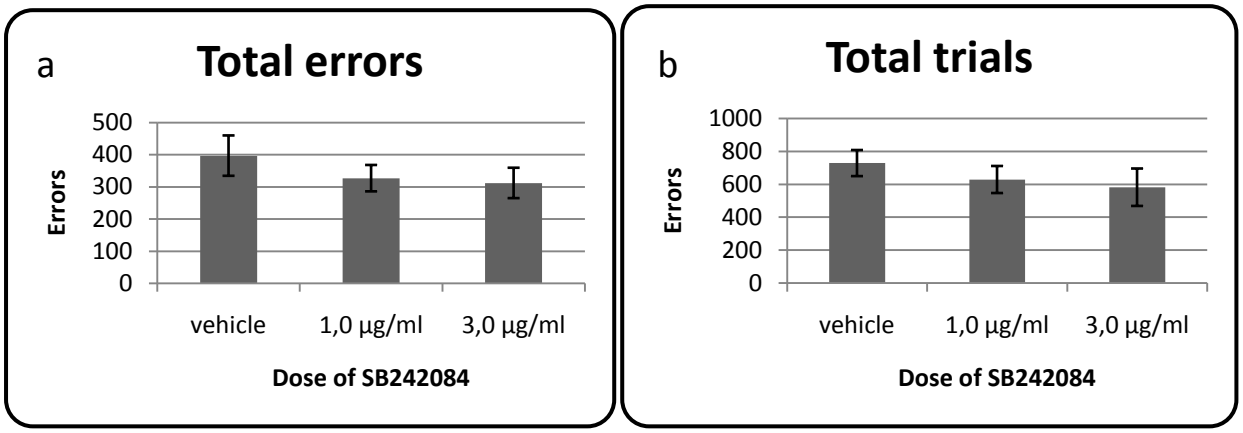


**Figure 10: a. Mean number of the total amount of errors made in a reversal cycle under different treatments of SB242084. b. Number of trials necessary to reach criteria under different treatments of SB242084 in a reversal cycle.**

*Phase effect: Perseverative, random and learning errors*

While no overall significant differences between the drug groups were noted on the reversal learning on total mean errors or trials to criterion, if the reversal learning during the cycle is broken down to analyse the total perseverative (<11/30), random (between 11/30 and 19/30) and learning errors (between 20/30 and 23/30; see method section for details) made during the whole reversal, certain significant effects were found.

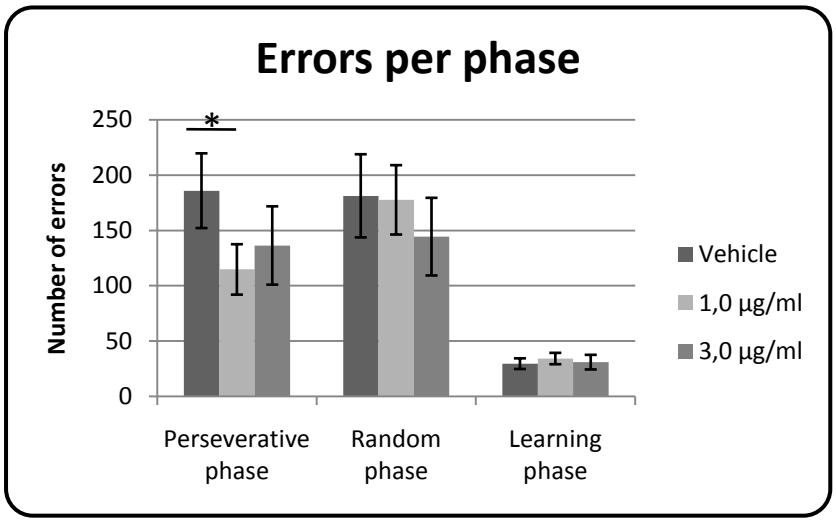


**Figure 11: The mean number of errors during the perseverative, random and learning phases of the reversal cycle for all SB242084 doses infused intracranial in the OFC.**

For perseverative errors and the perseverative trials to criterion a repeated measures ANOVA was performed to compare performance at each dose level of SB242084 (vehicle, 1.0 and 3.0 µg/ml) and showed that subjects did not make significantly more or less perseverative errors during the reversal learning( $F_{2.14}$ =2.510, p=0.117; figure 11).

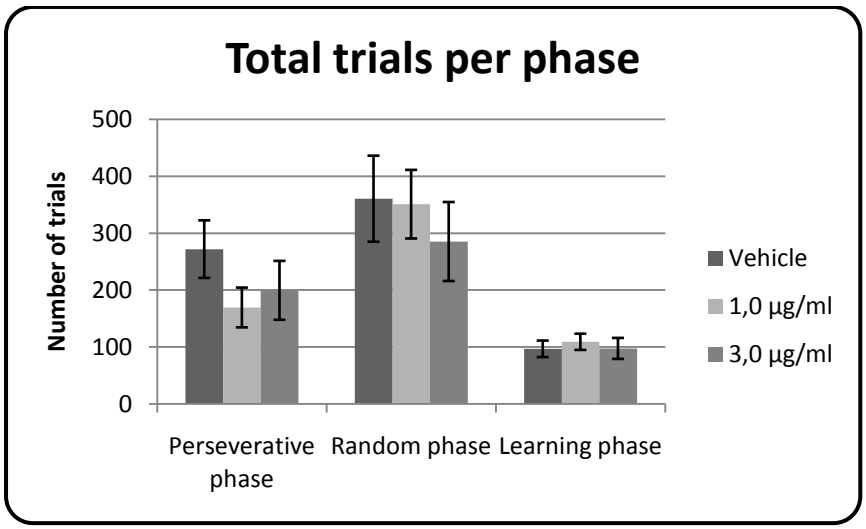


**Figure 12: The mean number of errors during the perseverative, random and learning phases of the reversal cycle for all SB242084 doses infused intracranial in the OFC.**

Also the trials to criterion for this phase did not reveal a statistical difference ($F_{2, 14}$ =2.410, p=0.126; figure 12). Based on our priori hypotheses, each dose was compared to the vehicle with a t-test as well. The t-scores revealed that the perseverative errors made when receiving the lower 1.0 µg/ml dose was significantly different from the vehicle dose (t(7)=2.389, P=0.048). This lower dose improved the number of errors to criterion compared to treatment with vehicle. The vehicle vs. 3.0 µg/ml did not show significance ( t(7)= 1.569, p=0.161).The t-tests performed on the trials to criterion showed scores of t(7)=2.295, P=0.055 and t(7)= 1.581, p=0.158 for vehicle vs. 1.0 µg/ml and vehicle vs. 3.0 µg/ml respectively. The comparison of 1.0 µg/ml to vehicle dose level was close to significance. This suggests a strong trend in which fewer trials are needed during the perseverative phase of the reversal cycle when rats were infused with the low dose of SB242084 as compared to vehicle-treated animals.

ANOVA of the random (phase 2) errors and trials to criterion across SB242084 dose showed that the number of errors made in this phase of the reversal learning cycle were not significantly different ($F_{2.14}$ =0.351, p=0.710). The number of trials to criterion during the random phase were also not significantly different( $F_{2.14}$ =0.367, p=0.699).

The number of errors and trials to criterion made during the learning phase of the reversal cycle (phase 3) were also not significantly affected by the dose of SB242084($F_{2.14}$ =0.180, p=0.837 and $F_{2.14}$ =0.184, p=0.834 respectively).

## 2. Intra-dimensional/extra-dimensional set shifting

### Behavioral results

#### Performance before surgery

Prior to surgery the animals had two stages of simple discrimination learning, namely SD1 and SD2. When comparing the results of the animals within these IDED stages the groups did not differ in the number of sessions, erroneous responses or total trials to reach performance criterion (figure 13 and 14).

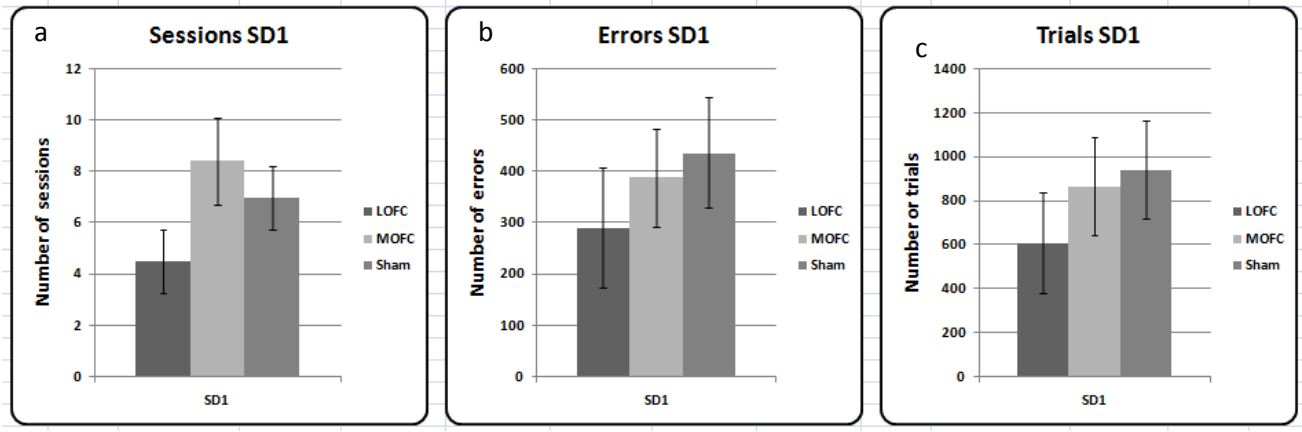


**Figure 13: Number of sessions (a), errors (b) and trials (c) to criterion for the first simple discrimination stage (SD1).**

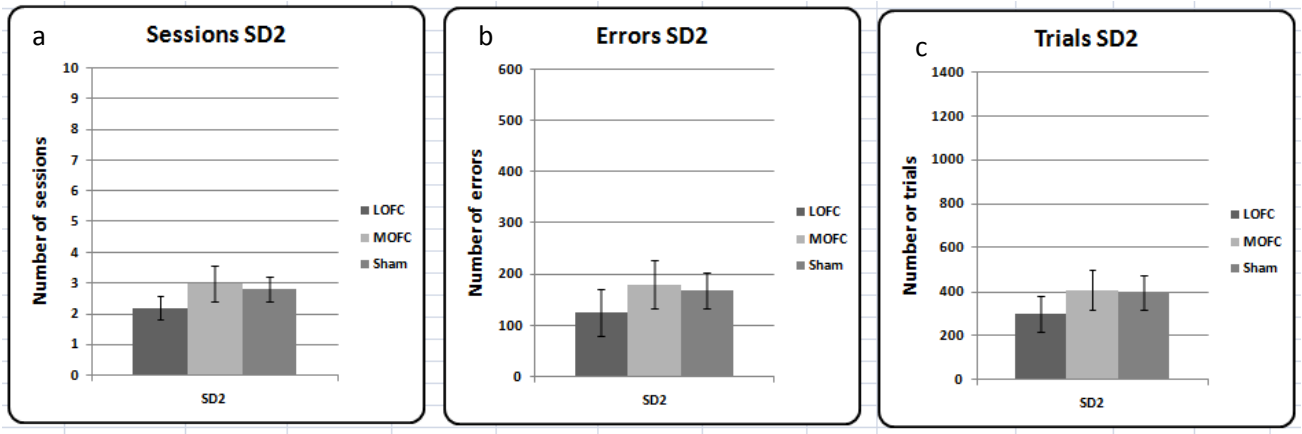


**Figure 14: Number of sessions (a), errors (b) and trials (c) to criterion for the second simple discrimination stage (SD2).**

For the number of sessions, errors and trials to reach criterion in SD1, the non-parametric Kruskal-Wallis H test indicated no significant difference in performance between the lOFC, mOFC and the sham lesion groups ($X^2$= 3.004,P=0.223; $X^2$= 1.059, P=0.589 and $X^2$= 1.237,P=0.539; respectively).

For the SD2 stage the Kruskal-Wallis H test showed that there were no difference in number of sessions, errors and trials to criterion between the lesion groups either ($X^2$ = 1.809, P= 0.405; $X^2$= 0.903, P=0.637; $X^2$= 1.005, P=0.605; respectively).

Simple discrimination comparison

A within-subjects analysis of the simple discrimination data for the number of sessions, errors and trials to criterion between SD1 and SD2 stage was performed to examine whether any transfer or rule learning had occurred (figure 13 and 14). The repeated measures Friedman's two way ANOVA showed that there was a strong difference between the sessions ($X^2(1) = 10.286$; P=0.001).The comparison of the number of errors and trials to criterion between the simple discrimination data of SD1 and SD2 stage using the same non-parametric Friedman's ANOVA revealed that there is also significant difference between the stages in number of errors ($X^2(1)=4.765$; P=0.029), but only a trend for the trials to criterion($X^2(1)=2.882$; P =0.090). The results suggest that there was a general transfer of learning between the SD1 and SD2 stage, with significantly fewer number of sessions and errors required to reach criterion in SD2 relative to SD1.

**Re-baseline performance after surgery**

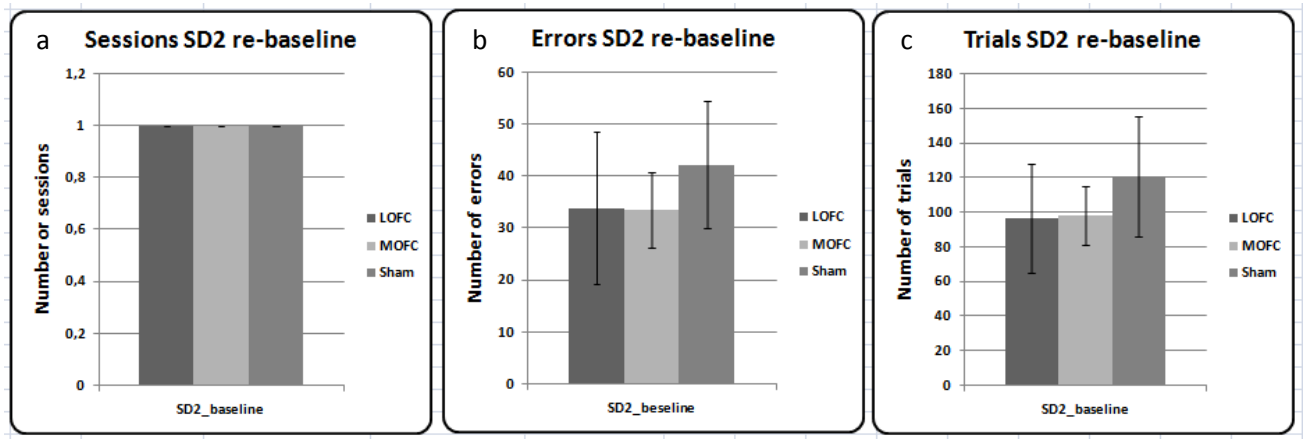


**Figure 15: Amount of sessions (a), errors (b) and trials (c) to criterion for the simple discrimination re-baseline stage after recovery from surgery.**

After recovering from the surgery the animals received the same stimulus as inSD2 and all animals reached criterion in one session (figure 15a). When performing the Kruskal-Wallis H test on the errors of this stage no significant difference was found between the groups as shown in figure 15b and with the $X^2 = 0.500$and a P-value of 0.779. For the total trials to criterion the statistical test showed that there is no significant effect of the lesion in this re-baseline performance between lOFC, mOFC and sham ($X^2 = 0.512$; P= 0.774; figure 15c).

**Performance with lesions: effects of lateral, medial or sham lesions in Intra-dimensional/extra-dimensional set shifting**

Compound Discrimination (CD)

*Numbers of sessions, errors and total trials to criterion*

During the CD stage the animals had both the shape and line dimensions combined for each image, with only one dimension being relevant. The number of sessions to criterion did not appear to differ significantly between lesion groups when performing a non-parametric Kruskal-Wallis H test ( $X^2 = 4.211$; P=0.122; figure

16a). After performing the same test on the errors and trials (figure 16b and c), no statistical differences were shown( $X^2$ = 2.352; P=0.308 and $X^2$= 3.643; P= 0.162, respectively).

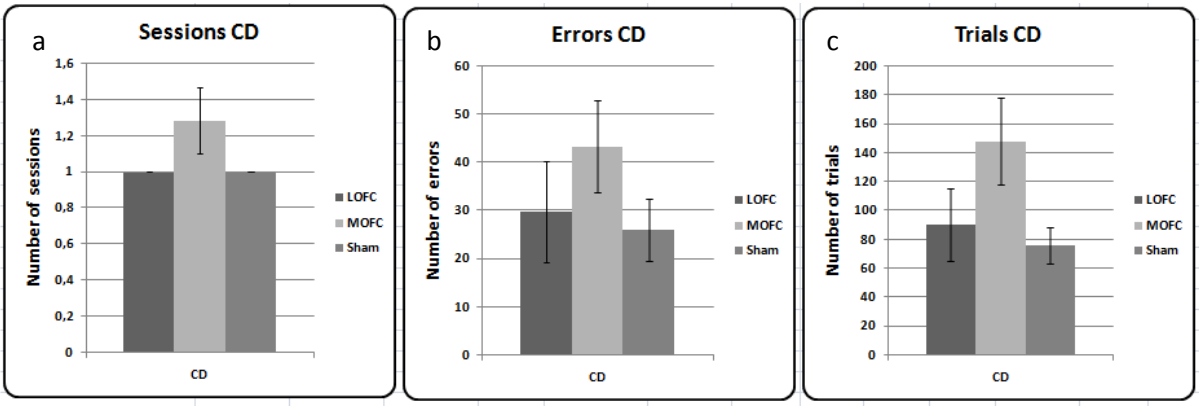


**Figure 16: Amount of sessions (a), errors (b) and trials (c) to criterion in the compound discrimination stage.**

Re-baseline and CD comparison

The results above showed that medial or lateral OFC lesions do not seem to affect the amount of sessions, errors or trials to criterion during the SD2 re-baseline and the CD stages. To test if there was a difference on how much the animals remembered after recovery relative to how much they were distracted by the introduction of a new dimension that was added in the CD stage, a Friedman's two-way ANOVA was performed to compare these stages. As in both stages the average number of sessions was one, there was no significant difference in the number of sessions to criterion between the stages. The errors and trials overall showed no significant effects of improvement or impairment between the stages either ($X^2(1)$=0.474; P = 0.491 and $X^2(1)$= 0.200; P = 0.655). This showed that none of the groups differ between these two stages.

Intra-dimensional (ID) discrimination – analysis across stages

There are four different ID set shifting stages before the reversal learning which makes it possible to test how the acquisition of learning proceeded throughout the intra-dimensional stages in which each animal had four different pairs of stimuli in total. Due to the counterbalancing of images at each stage and potential for increased variability due to idiosyncratic carry-over effects between stages, taking the cumulated scores across several intra-dimensional stages can help reduce or stabilize the variability and make a more reflective average ID performance score. A non-parametric Kruskal-Wallis H test was performed on the cumulated scores to reveal effects on number of sessions, errors and trials over the course of the intra dimensional set shifting stages. When performing the independent pair wise comparisons on each of the cumulative scores, there were clear significant differences between certain groups (figure 17).

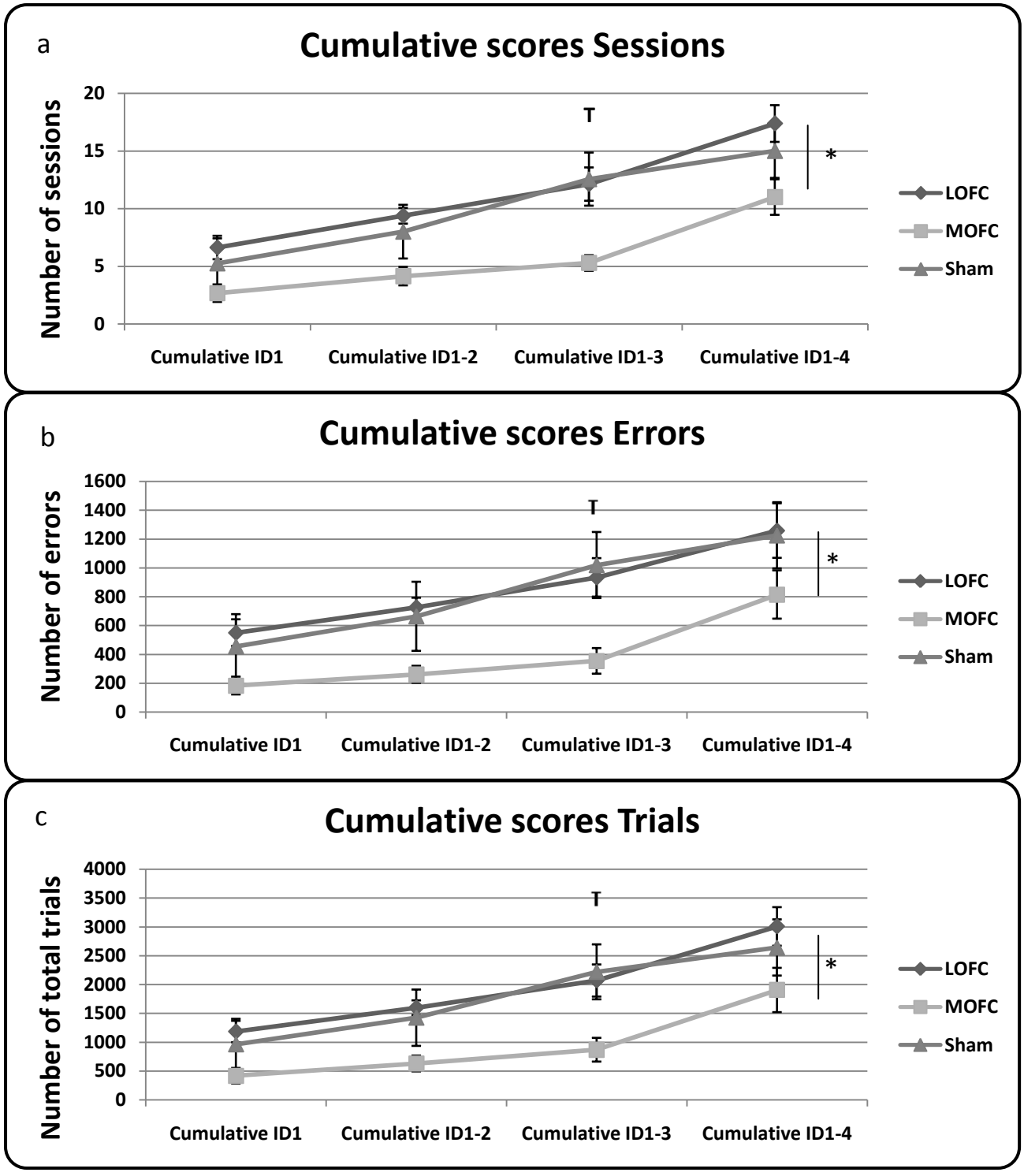


**Figure 17: Cumulative scores of numbers of sessions (a), errors (b) and trials (c) to criterion of ID1, ID1-ID2 together, ID1-ID3 together and all four ID stages together (ID1-ID4). The (*) sign shows an overall difference between lOFC and mOFC over all scores and the (T) is shows an statistical effect between the mOFC and the sham groups.**

The ID1 stage revealed a significant difference between the number of sessions, errors and trials of lOFC and mOFC when performing the paired comparisons (see * in figure 18). Combining the results of the ID1 and ID2 the Kruskal Wallis H test was performed that revealed that sessions, errors and trials to criterion were significant different overall with scores of $X^2$=8.665;P=0.013, $X^2$=8.473;P=0.014, $X^2$=8.451;P=0.015 respectively. All measurements were therefore followed up by paired comparisons. The difference between lOFC and

mOFC seems to be significant (sessions: U=2.5;P=0.003, errors: U=1.0;P=0.002 and trials: U=2.0;P=0.003;see *). The lOFC and sham group did not show any statistical effects on any of the measurements (sessions: U=19.5;P=0.111, errors: U=21.0;P=0.149, trials: U=21.0;P=0.149). mOFC and sham lesioned animals also yielded no significance difference (sessions: U=19.0;P=0.180, errors: U=21.0;P=0.266, trials: U=19.0;P=0.186).

Cumulative score for ID1, ID2 and ID3 together showed the same pattern, with a Kruskal Wallis H test revealing a significance for sessions, errors and trials to criterion overall(sessions: $X^2$=10.25;P=0.006, errors: $X^2$=7.695;P=0.021, trials: $X^2$=7.404;P=0.025). The lOFC and mOFC showed again statistical effect when comparing lOFC and mOFC for all three measurements (sessions: U=1.5;P=0.002, errors: U=5.0;P=0.008, trials: U=5.0;P=0.008). The lOFC versus the sham lesion group did not show a difference at all (sessions: U=34.0;P=0.847, errors: U=35.0;P=0.923, trials: U=34.0;P=0.847) and the mOFC compared to the shams revealed a statistical effect on sessions, errors and trials (U=8.0;P=0.012, U=11.0;P=0.030,U=12.0;P=0.039 respectively).

For the final score all four intra-dimensional stages are accumulated. The Kruskal Wallis H test showed that overall the sessions, errors and trials to criterion do not show a statistical effect ($X^2$=5.151;P=0.076, $X^2$=3.308;P=0.191, $X^2$=4.323;P=0.115 respectively). The difference between the lOFC and the mOFC lesion group showed a significant difference for the sessions and trials, but only a trend for the errors (sessions: U=7.5;P=0.017, errors: U=13.0;P=0.083, trials:U=9.0;P=0.028). For the lOFC and sham comparison, no differences were found (sessions: U=27.0;P=0.384, errors: U=35.0;P=0.923, trials: U=28.0;P=0.441) and also the mOFC was not different from the sham lesion group (sessions: U=20.0;P=0.221, errors: U=18.0;P=0.153, trials: U=21.0;P=0.266).

<u>Intra-dimensional (ID) discrimination - analyses per stage</u>

*Number of sessions, errors and trials*

The IDED-task included five different ID discrimination stages (figure 18-22). Four of these occurred before the reversal and the final fifth ID stage after the reversal. The Kruskal-Wallis H test analysing lesion group performance on the number of sessions, errors and trials to criterion provided the following results.
For the first ID stage (ID1) there were no measurements that yielded significance. There was, however, a trend towards significance for the errors and trials to criterion ($X^2$ = 4.365,P=0.113; $X^2$ = 5.043,P=0.080; $X^2$ = 5.137; P=0.077). Based on prior hypotheses that predict differences during the groups over time, the ID1 stage was subjected to individual paired statistical tests. The Mann-Whitney U test, that compared each group against the others, showed that all three measurements reached significance between the lOFC and the mOFC (sessions: U=7.0,P=0.026; see * figure 18a; errors: U=5.0; P=0.014; see * figure 18b; trials: U=5.0;

P= 0.014; see * figure 18c). When comparing the lOFC and the mOFC with the sham group no statistical effect was found (sessions: lOFC vs. Sham U=20.5; P= 0.223 and mOFC vs. Sham U=20.5; P = 0.643; errors: U=19.0; P= 0.172 and U=23.0; P=0.897 and trials: U=19.0; P= 0.172 and U=22.0; P= 0.796).

In ID2 the lesion group performances in number of sessions, errors and trials to criterion were also analysed by the use of the Kruskal-Wallis H test. There was no significant difference found for the measurements ($X^2$ = 3.100,P=0.212; $X^2$ = 3.532; P = 0.171; $X^2$ = 3.161; P= 0.206, respectively). As for the same prior hypotheses as for ID1, the Mann-Whitney U test was performed on the groups. As shown in figure 19 none of the comparisons showed statistical differences. There were only trends towards significance between the mOFC and the shams in number of errors and trials (sessions: lOFC vs. mOFC U= 23.0,P = 0.892; lOFC vs. sham U=19.5, P=0.180; mOFC vs. sham U=11.5,P=0.091; errors: lOFC vs. mOFC U=23.0,P = 0.897; lOFC vs. sham U=20.0, P=0.208; mOFC vs. sham U=9.0,P=0.053; trials: lOFC vs. mOFC U =22.0,P = 0.795; lOFC vs. Sham U=20.0, P=0.207; mOFC vs. Sham U=10.0,P=0.71).

The third intra-dimensional stage (ID3), that was also subjected to the Kruskal-Wallis H test to analyse the lesion group performances on number of sessions, errors and trials to criterion, showed no statistical differences for any of the measurements ($X^2$ = 2.710,P=0.258; $X^2$ = 3.268; P=0.195; $X^2$ = 3.039; P= 0.219, respectively). The performed Mann-Whitney U test, comparing each group against each other because of prior hypotheses, did on the other hand show significance between certain groups. Figure 20 shows that there were no differences between the sessions (lOFC vs. mOFC U=12.5,P = 0.108; lOFC vs. sham U=25.5,P = 0.768; mOFC vs. sham U=17.5,P = 0.198), nor for the number of errors or trials (errors: lOFC vs. mOFC U=12.0,P = 0.110; lOFC vs. sham U=25.0,P=0.728; mOFC vs. sham U=15.0,P=0.132; trials: lOFC vs. mOFC U=14.0,P = 0.179; lOFC vs. sham U=26.0, P=0.817; mOFC vs. sham U=9.0,P=0.105).

In the ID4 stage, performed before the reversal, the performance of the lesion groups has been analysed using the Kruskal-Wallis H test again. No significant difference were revealed for the number of sessions, errors or trials to criterion ($X^2$ = 1.175,P=0.556; $X^2$ = 1.692,P=0.429; $X^2$ = 2.823,P=0.244; figure 21). The individual statistical tests performed based on prior hypotheses showed that during the ID4 stage the number of sessions, errors and trials to criterion did not differ between any of the groups (sessions: lOFC vs. mOFC U=24.5,P = 0.682; lOFC vs. sham U=23.5,P = 0.363; mOFC vs. sham U=20.5,P = 0.375; errors: lOFC vs. mOFC U=25.0,P = 0.728; lOFC vs. sham U=28.0,P=0.674; mOFC vs. sham U=22.0,P=0.487; trials: lOFC vs. mOFC U=25.0,P = 0.728; lOFC vs. sham U=24.0, P=0.401; mOFC vs. sham U=20.0,P=0.355).

ID5, as the final intra-dimensional discrimination, was performed after the reversal learning. It was analysed with the non-parametric Kruskal-Wallis H test to show differences in performance between lesion groups in

number of sessions, errors and trials to criterion (figure 22). There were no significant differences found ($X^2$=1.435,P=0.488; $X^2$ = 0.360,P= 0.835; $X^2$ = 0.548,P= 0.760). Based on prior hypotheses, this stages were also subjected to the independent Mann-Whitney U test. The sessions did not differ between any of the groups (lOFC vs. mOFC U=10.0,P = 0.521; lOFC vs. sham U=10.0,P = 0.577; mOFC vs. sham U=7.5,P = 0.238), nor for the number of errors or trials (errors: lOFC vs. mOFC U=7.0,P = 0.251; lOFC vs. sham U=12.0,P=0.917; mOFC vs. sham U=11.0,P=0.754; trials: lOFC vs. mOFC U=7.0,P = 0.251; lOFC vs. sham U=12.0, P=0.917; mOFC vs. sham U=10.0,P=0.597).

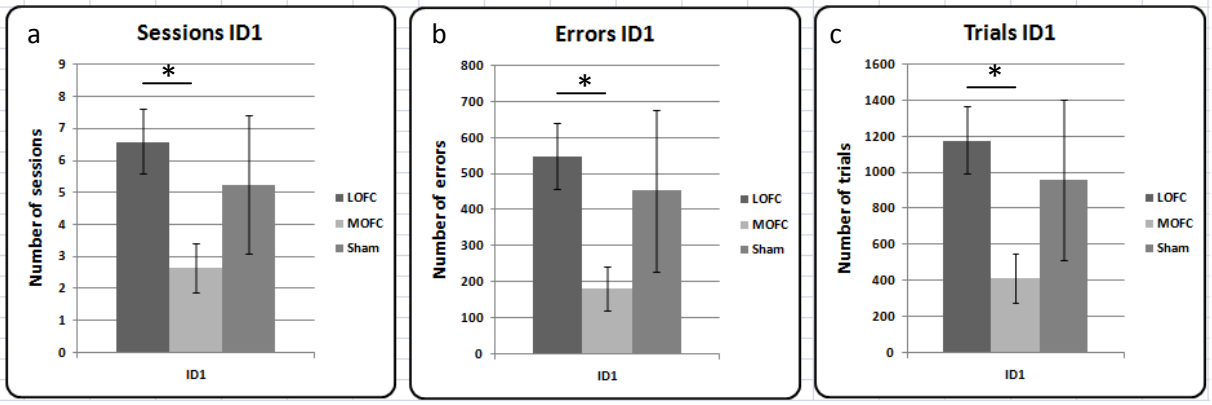


**Figure 18: Amount of sessions (a), errors (b) and trials (c) to criterion of the first Intra Dimensional set shifting stage.**

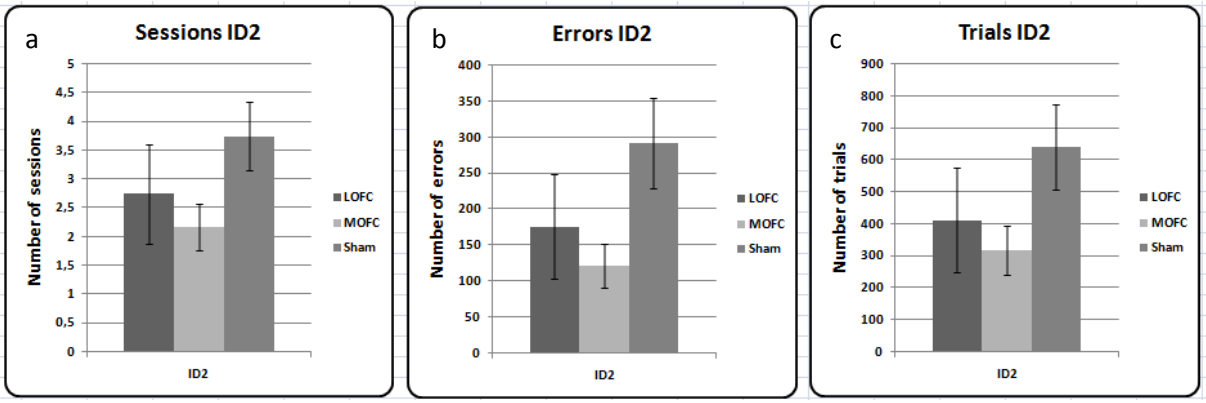


**Figure 19: Amount of sessions (a), errors (b) and trials (c) to criterion in the second Intra-dimensional set shifting stage.**

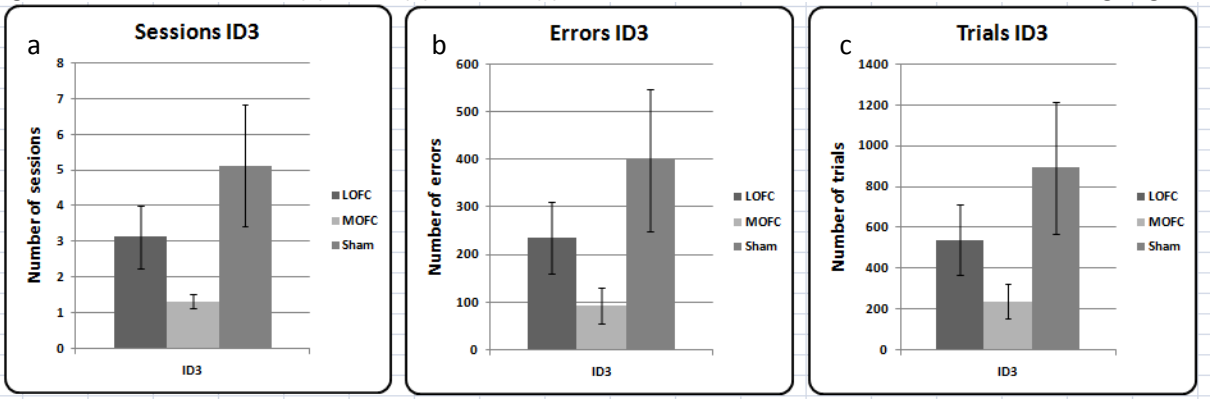


**Figure 20: Amount of sessions (a), errors (b) and trials (c) to criterion for the third Intra-dimensional set shifting stage.**

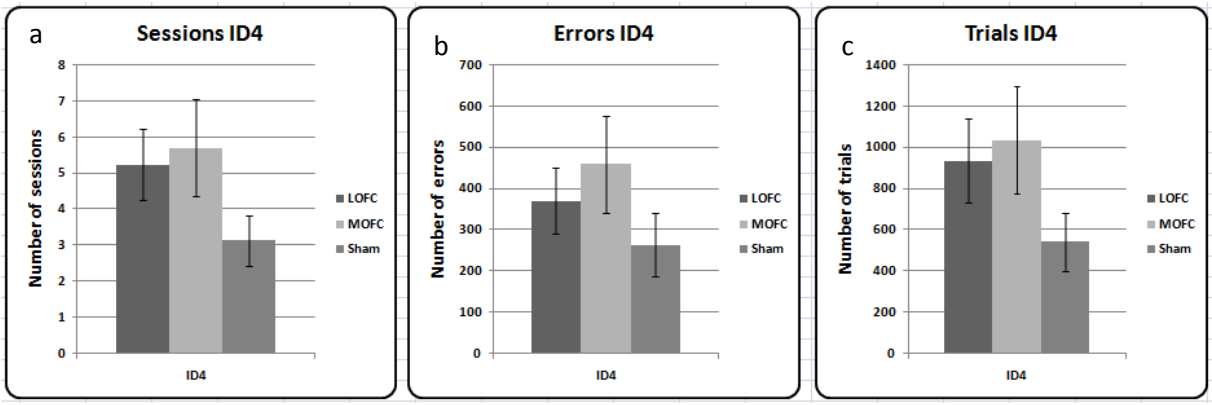


**Figure 21: Amount of sessions (a), errors (b) and trials (c) to criterion of the fourth Intra-dimensional set shifting stage.**

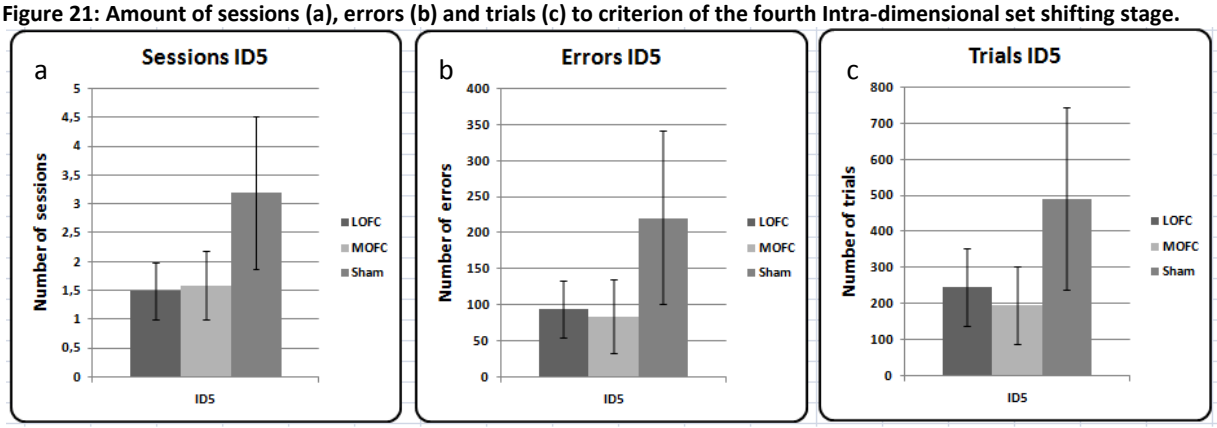


**Figure 22: Amount of sessions (a), errors (b) and trials (c) to criterion in the fifth Intra-dimensional set shifting stage.**

Reversal learning

After ID4 the reversal stage was performed. The Kruskal-Wallis H test performed on this stage showed no significant differences in sessions, errors or trials overall, which is shown in figure 23 ( $X^2$ = 2.009,P= 0.366; $X^2$ = 2.423, P=0.298; $X^2$ = 2.344,P=0.310; respectively). As there is a prior expectation of difference in this stage between the lesion groups, Mann-Whitney U tests are performed on all measurements. For the lOFC vs. mOFC lesioned groups sessions (U= 27.5; P = 0.954), errors (U=27.0; P= 0.908) and trials (U= 27.0; P= 0.908) did not show a statistical effect. lOFC and mOFC relative to sham revealed no differences either. Only a weak trend appeared when comparing lOFC with sham errors (sessions lOFC vs. sham U=11.0,P = 0.185; mOFC vs. sham U=8.5,P = 0.142; errors lOFC vs. sham U= 8.5,P = 0.092; mOFC vs. sham U= 8.0,P = 0.123; trials lOFC vs. sham U=9.0,P = 0.107; mOFC vs. sham U= 8.0,P = 0.123).

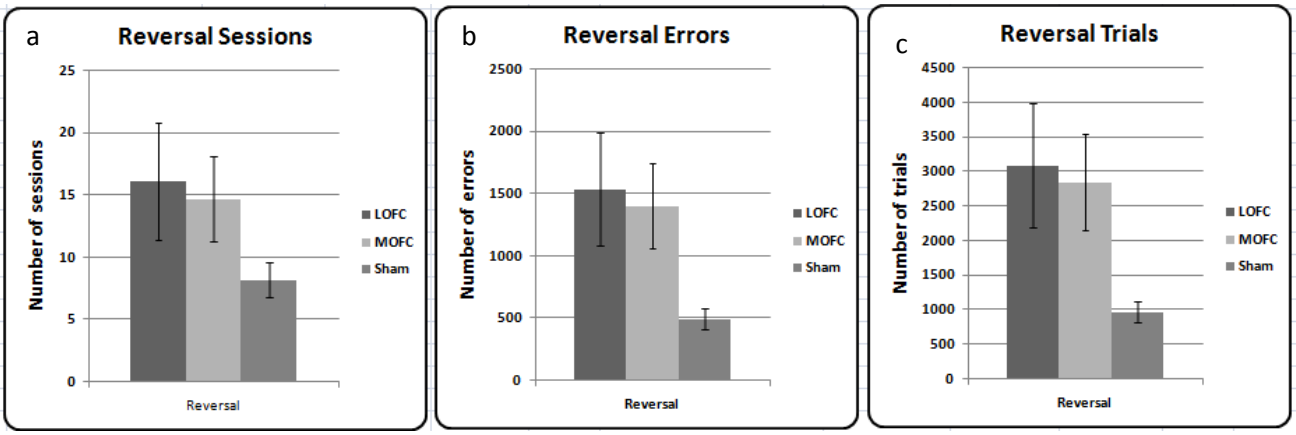


**Figure 23: Amount of sessions (a), errors (b) and trials (c) to criterion for the reversal stage.**

Intra-dimensional discrimination stage 4 comparison to reversal learning

In the result section above it is shown that there is no difference between any of the lesion groups within ID4 or reversal learning. However, it was expected that a strong effect was present between the two stages.

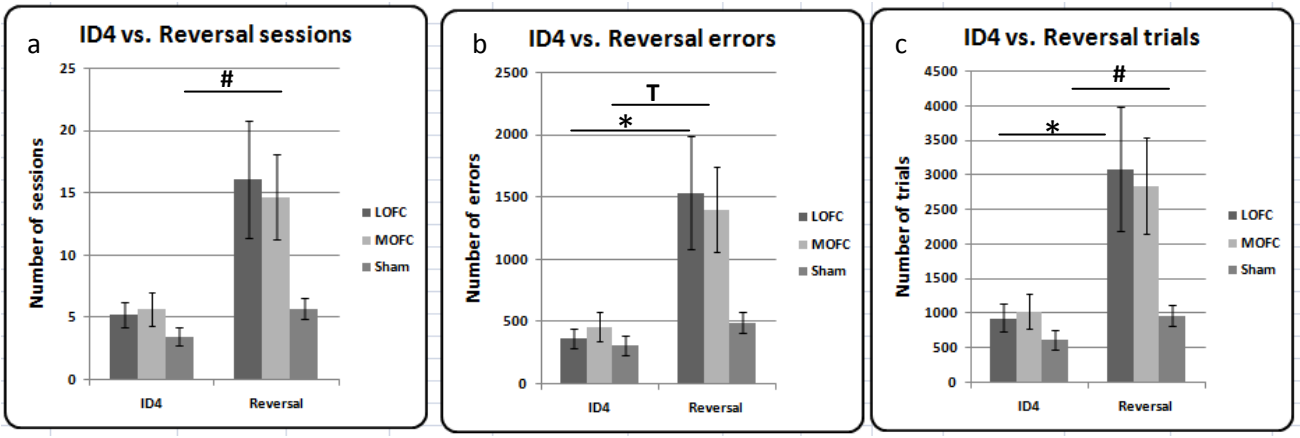


**Figure 24: Amount of sessions (a), errors (b) and trials (c) to criterion for ID4 and Reversal stage. The (*) sign shows a difference in lOFC. mOFC differences (T) and sham differences (#) are shows as statistical effect as well.**

The Friedman two-way ANOVA showed that this difference is very strong overall for the sessions, errors and trials to criterion($X^2$=9.800,P = 0.002; $X^2$=8.895,P=0.003; $X^2$=9.800,P=0.002; see figure 24). Individual independent group wise tests have revealed that from the lOFC ID4 vs. reversal, mOFC ID4 vs. reversal and sham ID4 vs. reversal several measurements reached significance or a trend towards it was shown (lOFC ID4 vs. reversal sessions: U=13.5,P=0.051; errors: U=7.0,P=0.015; trials: U=12.0,P=0.036; see *; mOFC ID4 vs. reversal sessions: U=12.5, P=0.123; errors: U=9.0,P=0.048; trials: U=11.0,P=0.085; see T; sham ID4 vs. reversal sessions: U=3.5,P=0.021; errors: U=6.0,P=0.062; trials: U=4.0,P=0.028; see #).

# Discussion

The present study provides the first demonstration of the role of the OFC in rats using newly developed touchscreen tasks. Blocking the receptors with selective SB242084 antagonist of 5-$HT_{2c}$ during the serial reversal learning paradigm revealed the specific role of the 5-$HT_{2c}$ receptors on the number and type of errors and total trials in rats: the 1.0 µg/ml dose enhanced the performance of the number of errors and trials during the perseverative phase of reversal learning. The intra-dimensional/extra-dimensional (IDED) set shifting paradigm, performed as the second experiment, investigated the role of the specific lateral and medial OFC subregions in dimensional set formation and reversal learning during several stages. These stages of the IDED task showed that all animals are able to perform intra-dimensional set shifting stages and therefore were able to form a dimensional set. These set shifting results also yielded differences between the lateral and medial OFC lesioned animals. The reversal results show, on the other hand, no clear differences between any of the groups. The results of both experiments might help explain the contradictive findings of previous research and will be discussed in more detail below.

## Reversal learning and OFC

In the first experiment, the serial reversal task, the animals where trained on discrimination of two stimuli, which revealed no pre-surgery performance differences. This means that the animals were assigned into equivalent groups in this experiment. Also the post-surgery re-baseline data showed that there were no problems in performing the task when the pair of discriminative stimuli used was the same as before the surgery. Therefore, the surgery did not affect the animals directly when no infusions were given. Only with the intra-OFC infusions of SB242084, as 5-$HT_{2c}$ antagonist, the difference appeared. When receiving the low dose of the antagonist of 5-HT2C receptor (1.0 µg/ml), the animals made fewer mistakes during the perseverative phase of reversal learning and a strong trend towards significance is found for the number of trials necessary to reach criterion was observed too. This is in agreement with previous research performed by Fraiser-Grinberg et al. (2008) and Boulougouris and Robbins (2010), and supports the hypothesis of a specific involvement of 5-$HT_{2c}$ receptors within the OFC in certain aspects of cognitive flexibility and response inhibition (Boulougouris et al. 2008; Boulougouris and Robbins 2010; Flaisher-Grinberg et al. 2008). The most appealing explanation is that an altered reversal learning performance may be caused by changes in the ability to overcome prior associations of either or both positive and negative valence (Nilsson et al. 2012) while the different postsynaptic 5-HT receptor subtypes make it more or less likely that alternative responses are promoted over previously reinforced responses in the reversal task (Lapiz-Bluhm et al. 2009; Roberts 2011). More specifically, it suggests that when the 5-$HT_{2c}$ receptor has a reduced activity, because of the infusion with the antagonist, the OFC infused rats tend to learn faster to inhibit responding to the previously rewarded stimulus and therefore enhances reversal learning by decreasing the influence of previously non-

rewarded associations (Boulougouris et al. 2008; Boulougouris and Robbins 2010; Brigman et al. 2010; Lapiz-Bluhm et al. 2009; Nilsson et al. 2012).

Another explanation might be that the enhancement effect in the perseverative phase is present because of another, indirect effect of the antagonist used. Roberts et al. (2011) mentioned that the neuronal activity of the dorsal part of the raphe nucleus (DRN) is enhanced when the 5-$HT_{2c}$ receptor antagonist is used(Roberts 2011), revealing a postsynaptic 5-HT feedback mechanism. It is therefore possible that SB242084, indirectly, is blocking the inhibitory effects of 5-HT stimulation. However, only if this regulation results from descending influences of the mPFC, just like in dopaminergic projections, a modulation like this could imply an indirect effect of 5-$HT_{2c}$ receptors in the OFC enhancing the activity of the DRN (Roberts 2011). This theory would also help to explain why there is an inconsistency in results between our findings and the studies of Roberts and colleagues in marmosets on the effects of orbitofrontal 5-HT depletion on the flexible control of behaviour (Clarke et al. 2004; Clarke et al. 2005; Clarke et al. 2007; Walker et al. 2006). These studies showed that orbitofrontal 5-HT depletion resulted in perseverative responding in both a de-tour-reaching and a discrimination reversal task(Clarke et al. 2004; Clarke et al. 2005; Clarke et al. 2007; Walker et al. 2006). This perseverative responding was suggested to be due to a failure to inhibit a conditioned stimulus response and to be related to compulsive responding. In contrast, the current study, in line with Boulougouris and collegues and Flaisher-Grinberg, found that SB242084 decreased compulsive responding, as the number of perseverative errors decreased (Boulougouris et al. 2008; Boulougouris and Robbins 2010; Flaisher-Grinberg et al. 2008). This difference may be attributed to the fact that the effects blockade of a specific 5-HT receptor type within the OFC might be different from the effects of 5-HT depletion from this cortical area.

But how about the higher dose, that did not show the enhancement effect? Although it seems that the 3.0 µg/ml dose has no effects on reversal learning, it is highly possible that it did affect the performance of the OFC during reversal. The functioning of the brain areas in the cortico-striatal loops, including the OFC, are influenced by a number of ascending neurotransmitter systems that are characterized by an inverted U-shaped curve (Di Giovanni et al. 2008). This means that for serotonin, as one of these monoaminergic neurotransmitters, it is also possible that dose-response curve can be made that looks like an inverted U-shape. In the current study it seems that the local blockade of the 5-$HT_{2c}$ receptor in the OFC is indeed dose-dependent. The low dose of 1.0 µg/ml SB242084 is increasing the level of serotonin towards, or on top of, the U-shape, reaching optimal performances and therefore enhancing the functioning of the OFC. The dose of 3.0 µg/ml, on the other hand, is possibly increasing the level of serotonin too much, and exceeding the optimal level of serotonin, not reaching significant enhancements in the functioning of the OFC. Boulougouris et al. (2008,2010) also found that the effect of different doses yield different, but dependent effects when infusing SB242084 into the OFC, with their highest dose of 1.0 µg/ml showing the most im-

provement, while with 0.3 and 0.1 µg/ml the improvement in reversal learning decreased by dose (Boulougouris and Robbins 2010).

The findings of the current study on the 5-$HT_{2c}$ receptor subtype antagonism, which are in accordance with previous findings in visual and spatial reversal learning, have received attention as a possible therapeutic target in psychiatric conditions such as OCD, psychoses, addiction, anxiety and Schizophrenia (Flaisher-Grinberg et al. 2008; Roth et al. 2004). Besides the given explanations, it is known that 5-HT and DA systems are interacting, which can also affect the performance of this task. The 5-$HT_{2C}$ receptor is found in a variety of forebrain structures, including cortical OFC, amygdala, hippocampal, striatal/ accumbens regions, as well as monoaminergic cell body areas such as the locus coeruleus, substantia nigra, and ventral tegmental area (VTA) (Fletcher et al. 2002). Previous research has shown evidence that suggests that activation of this receptor by serotonin inhibit dopamine (DA) release in certain areas of the brain including striatum, PFC, nucleus accumbens, hippocampus, hypothalamus and amygdala (Boulougouris et al. 2008; Di Giovanni et al. 2008; Fletcher et al. 2002; Nilsson et al. 2013; Roberts 2011). By using antagonists to block the 5-$HT_{2c}$ receptors, the mesolimbic dopamine release will be increased in the presence and absence of stimuli. In relation to the current study, this increased level of dopamine seems to enhance the perseverative phase during the performed visual serial reversal task. This interaction is, however, more complex and it might be dependent on other mentioned explanations. Therefore future studies should focus on the connections with other brain areas, like mPFC and DRN. Also the functional interaction between the serotonergic and dopaminergic systems should be investigated further to explain the mechanism of the 5-HT2 antagonists that potentially can be used in treatments for psychological disorders such as schizophrenia and depression.

## Intra-dimensional/extra-dimensional set shifting and OFC

In the second experiment, the IDED paradigm was started with two simple discrimination (SD) stages. In each of these the animals receive two stimuli in either the shape or line dimension. Both SD1 and SD2 results showed that none of the animals had problems with learning to discriminate between them. There were also no differences found between the later created groups, meaning that again the animals were assigned into equivalent groups in the experiment. The comparison between the first and second discrimination showed, although not significantly, that the animals improved in sessions, errors and trials to criterion on SD2 relative to SD1. This means that they show a general transfer of learning, as was expected, while the animals got more experienced with the task. By performing the task more often, the animals got more used to the testing environment and were able to find a strategy to learn to discriminate between the presented stimuli, as has been mentioned in previous research as well (Chase et al. 2012; Dias et al. 1996, 1997).

Following surgery on the visual discrimination tasks, the simple discrimination re-baseline was performed to show that no deficit was caused by the surgery. The results did not show any effect on the number of sessions, errors or trials to criterion for any of the three groups. This means that the animals still remembered the stimuli they had before the surgery and that they all had formed a dimensional set. The com-

pound discrimination stage, in which the second dimension was added, did not show any difference either. The distraction of the new dimension did therefore not prevent the animals for performing the same as during the re-baseline. Again this proves that the animals all seem to have learned to form a dimensional set in the shape or line dimension and were able to ignore the irrelevant dimension that was added in this stage.

The hypothesis that different areas within the orbitofrontal cortex subsume different aspect in the IDED task can partly be answered by using the results of the intra-dimensional acquisition stages. Just like in the study by Chase et al. (2012), the task had replaced the first reversal component, which usually followed the CD stage, of the set-shifting task with multiple intra-dimensional acquisitions. It was assumed that the medial OFC is implicated in monitoring associations between stimuli, responses and outcomes under changing circumstances, while the more lateral region is involved in overriding of behavioural choices based on the previous reward values of stimuli and responses (Elliott et al. 2000). In this study, the possibility for different functions can be found when looking at the performance during the first intra-dimensional stage. The ID1 stage showed that there were differences between the two OFC lesion groups. The animals with a lesion in medial OFC required a reduced number of sessions, errors and trials to criterion compared to the lateral OFC group, while the lateral OFC group performed almost equally to the sham group. The same pattern arose in the second and third intra-dimensional set shifting stage. The medial OFC lesioned animals kept performing better than the lateral OFC and the sham groups, but without reaching significance. Taking the cumulative scores into account, to show a more reflective learning curve and including scores for twenty-four lesioned animals, the difference was more clear. The medial OFC lesioned animals performed better in all four cumulative scores and were therefore overall significant better compared to the lateral group. Together with the fact that the mOFC lesioned animals showed to be unsignificantly more distracted by the addition of the irrelevant dimension in the CD stage, this finding indicate functional differences between the two subregions of the OFC.

As for the specific functions of the subregions, a comparison should be made between the OFC lesion groups and the sham animals. No differences were seen between the lateral OFC group and the sham lesioned during any of the intra-dimensional set shifting stages. Therefore no effect of lesion on the number of sessions, errors or trials compared to the sham group was found. When combining the intra-dimensional stages by calculating the cumulative score for sessions, errors and trials to criterion, the lateral OFC and sham group still did not differ from each other and none of them showed an improvement over the time course of the intra-dimensional set shifting stages. Both the lOFC and the sham lesioned animals have an attenuated performance, meaning that the subjects had a predisposition to attend to one stimulus dimension over another(Chase et al. 2012). This suggests that, like shown in previous research, OFC-lesioned rats could form a dimensional set and that the lateral part of the OFC does not play a role in forming this set (Chase et al. 2012; Hampshire et al. 2012; McAlonan and Brown 2003). As the animals already seem to have

formed a set in the compound discrimination stage, experience in performing intra-dimensional set shifting stages did not result in an improvement anymore. The new stimuli take, of course, longer to learn than the ones that the animals were trained on during the CD stage (which were the same as SD2 and the re-baseline). The constant performance over time during the first three stages, however, revealed that the dimensional was very stable during the intra-dimensional set shifting. The only exception to this observation is the fourth intra-dimensional (ID4) set shifting stage. In this stage new stimuli were introduced and all three groups showed increased numbers of all measurements. It might therefore be that these newly introduced stimuli are harder to discriminate between. Another reason for the higher number of sessions, errors and trials to criterion can be the order in which the stimuli were presented to the animals, although counterbalanced in each stage. A third and final reason for the problems occurring in this ID4 stage is the amount of training the animals had on the stage before. Quite some animals finished the third intra-dimensional (ID3) stage without moving on to ID4 directly. They still received the same stimuli in which they became very proficient. Therefore, it is possible that this set of stimuli was acquired much stronger and interfered while learning the stimuli in ID4. Garner et al. also showed this effect in mice that had problems learning an extra-dimensional set after overtraining on the stage occurring before the shift (Garner et al. 2006).

While the sham scores are almost identical to the lOFC group, this part of the OFC does not affect the intra-dimensional set shifting, however, the mOFC scores seem to suggest to play a role in set shifting.
This region was expected to be affected within the dimensional set shifting in the IDED paradigm by showing slower learning over the course of the intra-dimensional set shifting as this region is involved in making stimulus-reward associations and with the reinforcement of behaviour. Conversely, the results showed that mOFC lesioned animals had an enhanced performance on sessions, errors and trials to reach criterion.

Finding the specific function for the mOFC seems to be complex as less research is performed on this subregion. By having a significantly lower number of sessions, errors and trials to criterion compared to the lateral OFC lesioned animals, it is possible to conclude that the medial OFC seems to be more focussed during the intra-dimensional stage and learn the first new dimensional set faster. Over time their performance also seem to improve, in contrast to the stable performance over time of the other two groups, making the difference between the groups bigger. The lower scores on all measurements indicate an enhanced intra-dimensional set shifting in these medial lesioned animals. It is possible that this might be a direct or indirect effect of the connections between the medial OFC and brain regions it is connected with. As mentioned in the introduction, the strongest connections can be found with the hippocampus and associated areas of the cingulate, retrosplenial and entorhinal cortices, anterior thalamus and septal diagonal band (Elliott et al. 2000). Therefore it was thought that this subregion functions in responding to, monitoring, or adjusting the incentive value of stimuli. In the lesioned animals this function should be impaired. Therefore the question

rises how a deficit in this area could lead to an enhancement in performance during the intra-dimensional stages.

One explanation is that the medial subregion of the OFC function in comparing the value of different options in order to make choices among them. It does so by reducing contrasting representations of value to a single dimension in order to compare alternative choices(Rudebeck and Murray 2011b). This way comparisons between stimuli are no longer independent of alternative choices(Noonan et al. 2010). Lesions of this brain area might therefore keep an enhanced contrast between stimuli which makes the decision between the two stimuli in the relevant dimension easier. On top of that, the dimensional set that has been formed, will reduce the impact of the stimuli from the irrelevant dimension. These enhancements together are able to account for the enhancement shown in the mOFC lesioned animals.

Another possibility, that does not have to be completely based on a different mechanism as the first, is that the lesion in mOFC plays a role in decreasing impulsive choice relative to both the lateral OFC and sham lesioned animals. This is mentioned in previous research by Fuchs et al. (2004) and Mar et al. (2011). The study of Fuchs et al. (2004) on cocaine-seeking behaviour showed that the lOFC lesions augmented cocaine-primed reinstatement in a perseverative manner, whereas mOFC lesions attenuated cocaine-primed reinstatement. They mentioned that these results indicate a role of the mOFC in impairing perseverative, impulsive behaviour. Mar et al. (2011), who performed a delay-discounting task, also showed that the mOFC lesioned animals had a reduction in immediate reward choices, meaning the lesion reduces delay-discounting preferentially through enhanced sensitivity to relative reward magnitudes. As a possible explanation, Mar et al (2011) mentioned that the mOFC might be part of a circuitry that encodes associations between stimuli and/or responses that lead to feedback of reward and bias stimulus-driven, behavioural responses towards immediate goals (Elliott et al. 2000; Mar et al. 2011). Therefore the results of the present study might show that the animals are better in inhibiting responding to a CS- when they were biased towards it. This would be in line with the enhancements found in these multiple intra-dimensional stages of the IDED task. However, one would argue that if the disruption of the bias is general, also when the animals have a bias towards the CS+, the lesion in the mOFC might reduce this bias. Therefore, the disruption of the mOFC could play a role in diminishing the bias towards any of the stimuli and the animal would choose more objectively between the stimuli options. That animals with mOFC lesion still choose the rewarded stimulus may be because the animals with a lesion in the mOFC use a simpler, choice-bias strategy that does not use the integrative value comparison (Mar et al. 2011). On top of that, the lesion does not affect the whole valuation and decision-making system of which the mOFC is a component, and it leaves intact other independent value systems that exist in the brain (Noonan et al. 2010). It is also possible that the information provided from the lOFC has to play a more crucial role in the decision-making. This lateral part of the OFC is, as mentioned in the introduction, related to stimulus-outcome associations like with punishment-reward stimuli and the evaluation of behaviour when it needs to be changed (Elliott et al. 2000; Kringelbach and

Rolls 2004). Without the normal functioning of the mOFC, the lOFCand the other decision-making brain regions can therefore take over part of the process to value the different options, without the mOFC providing any bias, and adjust behaviour towards the rewarded stimulus.

Although this decrease in impulsivity seems like a possible explanation for the reduction in sessions, errors and trials to criterion when animals have a lesion in the medial OFC, there are also studies presenting the opposite results. Iversen and Mishkin (1970) showed for example, that animals with lesions in mOFC had moderate difficulty withholding response between trials on an auditory differentiation task (Iversen and Mishkin 1970) and also during a 5-CSRT increases of premature responding are found (Chudasama and Robbins 2003; Winstanley et al. 2005). Therefore, it seems that damage to the orbitofrontal cortex can have an increasing effect on premature responding and the similar lesion in mOFC can also decrease impulsive decision-making, as been shown in a delay-discounting procedure (Chudasama and Robbins 2003; Mar et al. 2011; Winstanley et al. 2005). This suggests that depending on the task, the mOFC might use different mechanisms that decrease or increase impulsive choices. As this study is not designed to answer which mechanism is really influenced by the lesion of the medial part of the OFC, it is important that further research is done to confirm if and which different mechanisms are activated in certain tasks to provide a more detailed and solid answer on the decreasing or increasing impulsivity caused by lesions in the mOFC.

For the reversal stage, which followed the intra-dimensional set shifting stages, none of the groups showed any significant results. Only a weak trend appeared when comparing lOFC with sham errors. Neither the absence of an overall effect, nor the lack of effect in the mOFC lesioned animals came as a surprise. That there was no overall effect was expected and also no effect in the mOFC lesioned animals did not come as a surprise. However, these results do not correspond to the expected outcome for the lateral OFC lesion group. The expectation was, after all, that the lOFC lesioned animals should have problems during the reversal stage, as shown in multiple studies before (G. B. Bissonette and Powell 2012; Chase et al. 2012; Chudasama and Robbins 2003; Dias et al. 1996, 1997; Fellows and Farah 2003; Hampshire et al. 2012; McAlonan and Brown 2003). On the other hand, there are also studies performed that did not show the reversal deficit within the medial or lateral OFC when this area is lesioned (Rudebeck and Murray 2011a). They studied rhesus monkeys (*Macaca mulatta*) with restricted excitotoxic lesions targeting either the lateral OFC (corresponding to Walker's areas 11/13) or medial OFC (corresponding to Walker's area 14). The performance of these two groups was compared to that of a group of unoperated controls and neither lesion affected monkeys performance on object reversal learning (Rudebeck and Murray 2011a). In accordance, also the findings of Kazama and Bachevalier (2009) reported that monkeys with lesions of areas 11 and 13 were unimpaired on object reversal learning, are in line with the current findings. In addition, Kazama and Bachevalier (2009) showed that area 14 is not essential for this task (Kazama and Bachevalier 2009).

There are a couple explanations for the lack of reversal effect. The first is that combined damage to the lateral and medial OFC is required to yield a deficit in this task. Each subregion of OFC can compensate for the other in reversal learning, so damaging either lOFC or mOFC alone is not sufficient to produce a deficit in reversal learning. Another possibility is that the method used to induce the lesion has an effect on the results. This explanation is consistent with the available data on the effects of either excitotoxic or aspiration lesions of subregions of PFC, mentioned in the article of Rudebeck and Murray (2011b). Following this, a third possibility is that complete damage to fibers of passage, either alone or together with damage to PFC, is responsible for the deficit. OFC itself might in this case not be important for reversal learning, but axons that pass through it are. Previous work used aspiration lesions which would have destroyed these axons. Determining the effects on object reversal learning of these fiber-sparing, excitotoxic lesions of the entire PFC, will be required to test this possibility (Rudebeck and Murray 2011a). Since, the animals did not complete the whole paradigm so far, the brains are not subjected to histology yet. This is however important to be fully able to use these explanations for the current study.

The final explanation that causes the lack of reversal effect might not lie in the OFC, but resides from the paradigm itself. As mentioned before, in the fourth intra-dimensional stage, stimulus pairs were newly introduced. The animals needed more sessions, errors and trials to criterion than in the previous three intra-dimensional stages. While each pair was pre-tested before the experiment and the animals seem to have learned to discriminate between the stimuli, it is still possible that the animals had an inherent bias towards any of the two relevant stimuli. The identification of the stimuli could also be more poorly performed by the animals. When this occurs, it could induce a general failure in identifying relevant stimulus features of compound stimuli. During the reversal stage, e.g. perseveration, as one of the factors, might be influenced by the poor stimulus identification. Which could explain- why the expected results did not come through during reversal learning.

When comparing the scores of the fourth intra-dimensional (ID4) and the reversal stage, there are supposed to be differences within each group. The reversal stage is expected to have increased scores compared to ID4, which are called reversal-costs. The lOFC and the sham lesioned animals did, as expected, show these reversal-costs on all three measurements, although some scores are only reaching towards significance (lOFC ID4 vs. reversal sessions: $U=13.5, P=0.051$; sham ID4 vs. reversal errors: $U=6.0, P=0.062$). The animals that had a mOFC lesion did, nevertheless, not show these reversal-costs overall, which seems to be odd. One explanation might come from Stopper et al. (2014). They suggested that the mOFC plays a selective role in decisions involving reward uncertainty, mitigating the impact that rewards exert on subsequent choice behaviour. This function may promote the exploration of novel options when reward contingencies change (Stopper et al. 2014). Therefore, the mOFC lesioned rats in the current study might not be inhibited to respond to the previously non-rewarded stimuli either when the reversal starts. If this is the case, another part

of the hypothesis that different areas within the orbitofrontal cortex subsume different aspect in the IDED task can be verified. However, a more plausible reason could be that, as mentioned in the section of the reversal learning results, the design of the current IDED paradigm needs revision to e.g. optimize stimuli.

## Validation of the present touchscreen study

The functional homology between rodent and primate OFC was concluded partly on the basis that lesions of these regions produced similar behavioural deficits across species(Chase et al. 2012). This study seems to show the same conclusive results as in non-human and primate monkeys when looking at the function of the OFC with reversal learning (Chudasama and Robbins 2003; Roberts 2011). Which would suggest that the homology is valid to use in this brain area when performing the serial reversal task. Furthermore, both serial reversal and set shifting are valid and reliable indexes of behavioural flexibility that can be measured in many species (G.B. Bissonette et al. 2013; Boulougouris and Robbins 2010; Dias et al. 1997; Owen et al. 1993). Although the touchscreen method may not appear particularly naturalistic, it exploits the rats innate tendency to investigate novel items in its environment, which it does primarily by looking, using its vibrissae and sniffing (McAllister 2012). When the rat investigates a rewarded stimuli this way, it leads to reward delivery. Following such reinforcement, the animal will approach the stimulus with increasing frequency, due to natural mechanisms of conditioning (McAllister 2012). Therefore, the used tasks have direct external construct validity to the human task, unlike most high-throughput tasks which lack human equivalents (Elliott et al. 2000; Garner et al. 2006). On top of that, the touchscreen method used in this study can easily be manipulated and the experimenter knows when the stimuli are on and off (McAllister 2012). Finally, it shows that the rats are able to form a dimensional set in a touchscreen setting using two-dimensional visual stimuli and therefore the newly developed paradigm also contains face validity. These advantages for translational research will lead to a recommendation that tasks of these types can be used in the development of new therapies for psychological disorders and the use of reversal and set-shifting tasks in drug development might increase (Chase et al. 2012; Lapiz and Morilak 2006).

However, although the method seems to provide a great step into increasing translational research, it should be noted that the current experiments have their limitations. They were performed to validate the use of the touchscreen task as they are the first that are performed in the modality that corresponds to human and primate research. The serial reversal learning task seems to show a perfect validation as the results are in coherence with previous results. A minor remark should still be made about this experiment. From the twenty animals that started in this task, only eight animals completed the reversals. As reversal data can be inconclusive without results of the full cohort, the results could be confirmed by repeating the experiment.

Also for the newly developed IDED task some remarks can be made on the task, while the results are evidently not completely as expected. First, as mentioned in several sections above, the current design might demonstrate that it did not optimize stimuli for use in a complex task such as this, which is always

challenging. Also the order in which the animals receive certain stimuli might have led to the results shown above. Counterbalancing the stimuli might, therefore, not be enough to prevent a bias towards a certain stimulus. Another remark that should be made concerns the automated testing method in general. The automation of testing allows for large numbers of rats to be tested simultaneously, in an automated, objective manner(McAllister 2012). It is therefore also possible that problems arise in the program during performance. When this happens the data cannot be used and it might affect learning in the animal. Finally, the current IDED study was not completed at the time this report was written. This means that the attentional set shifting is not incorporated. Also, the results of the final stages shown in this thesis (ID4, reversal learning and ID5), do not include all animals. Therefore the results can be inconclusive and they might change when more animals are included in the analysis.

## Conclusion

This study assessed the specific role of the OFC in flexible behaviour by using touchscreen tasks in which subjects must change an established behavioural response in order to adapt to new contingencies. The serial reversal learning task showed that the 5-$HT_{2c}$ receptor antagonist SB242084 affects reversal learning in the OFC in a phase- specific manner. The intra-dimensional/ extra-dimensional (IDED) set shifting task examined the role of OFC, using more selective lesions of the lateral and medial subregions, on the formation of a dimensional and attentional set, and reversal learning in which the specific subregions seem to perform distinct heterogenic functions. An important challenge for future studies will be to specify the nature of these results in both the tasks and experimental manipulations. This will have particular relevance in preclinical tests used to characterise novel pharmacological treatments of human psychopathology.

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# List of figures

# Supplementary Material

**Table 5: The Quartile lower and upper values for the lesion groups for sessions, errors and trials per stage. Including the rat number that is defined as outlier and the number of animals included in the data analysis.**

| Stage | Lower Quartile | | | Upper Quartile | | | Outliers numbers | | | Included per group | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SD1 | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham |
| Sessions | -3 | -2 | -6 | 12 | 19 | 20 | x | x | x | 6 | 7 | 9 |
| Errors | -471 | -75 | -545 | 994 | 1027 | 1432 | x | 3 | x | 6 | 6 | 9 |
| Trials | -846 | -351 | -1366 | 1953 | 2471 | 3355 | x | 3 | x | 6 | 6 | 9 |
| SD2 | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham |
| Sessions | 1 | -1 | 2 | 5 | 8 | 6 | 2 | 3 | 10,14,21 | 5 | 6 | 6 |
| Errors | -172 | -83 | 39 | 488 | 535 | 407 | 2 | 3 | 10,14,21 | 5 | 6 | 6 |
| Trials | -171 | -59 | 168 | 899 | 1034 | 887 | 2 | 3 | 10,14,21 | 5 | 6 | 6 |
| SD2 re-baseline | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham |
| Sessions | 1 | 1 | 1 | 1 | 1 | 1 | 19 | x | 14,28 | 5 | 7 | 7 |
| Errors | -57 | -22 | -40 | 116 | 83 | 120 | x | x | x | 6 | 7 | 9 |
| Trials | -92 | -19 | -114 | 261 | 203 | 334 | x | x | x | 6 | 7 | 9 |
| CD | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham |
| Sessions | 1 | 0 | 1 | 1 | 2 | 1 | 24 | x | x | 6 | 7 | 8 |
| Errors | -61 | -8 | -30 | 129 | 108 | 87 | 24 | 3 | x | 6 | 6 | 8 |
| Trials | -123 | -27 | -25 | 323 | 314 | 184 | 24 | x | x | 6 | 7 | 8 |
| ID1 | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham |
| Sessions | 1 | -3 | -14 | 14 | 9 | 26 | x | 3 | 18 | 8 | 6 | 8 |
| Errors | 0 | -327 | -1424 | 1170 | 781 | 2485 | x | 3 | 18 | 8 | 6 | 8 |
| Trials | 95 | -654 | -2925 | 2488 | 1660 | 5160 | x | 3 | 18 | 8 | 6 | 8 |
| ID2 | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham |
| Sessions | -3 | -1 | -2 | 8 | 5 | 11 | x | 25 | 14 | 8 | 6 | 8 |
| Errors | -308 | -48 | -166 | 593 | 326 | 922 | x | 25 | 14 | 8 | 6 | 8 |
| Trials | -651 | -127 | -311 | 1320 | 863 | 1889 | x | 25 | 14 | 8 | 6 | 8 |
| ID3 | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham |
| Sessions | -3 | -1 | -11 | 10 | 4 | 22 | 2 | 32 | x | 7 | 6 | 8 |
| Errors | -222 | -173 | -1008 | 723 | 340 | 1819 | 2 | x | x | 7 | 7 | 8 |
| Trials | -441 | -396 | -2176 | 157 | 3830 | 3993 | 2 | x | x | 6 | 6 | 9 |
| ID4 | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham |
| Sessions | 0 | -6 | -3 | 11 | 18 | 10 | x | x | 4 | 8 | 7 | 7 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Errors | -56 | -545 | -419 | 1958 | 1479 | 1047 | 2 | x | 4 | 7 | 7 | 7 |
| Trials | -846 | -1125 | -613 | 1953 | 3211 | 1842 | x | x | 4 | 8 | 7 | 7 |
| Reversal | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham |
| Sessions | -3 | -2 | -6 | 12 | 19 | 20 | x | 3 | x | 6 | 6 | 9 |
| Errors | -471 | -75 | -545 | 994 | 1027 | 1432 | x | 3 | x | 6 | 6 | 9 |
| Trials | -846 | -351 | -1366 | 1953 | 2471 | 3355 | x | 3 | x | 6 | 6 | 9 |
| ID5 | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham | lOFC | mOFC | Sham |
| Sessions | -2 | -2 | -4 | 6 | 7 | 9 | 16 | 20 | x | 4 | 5 | 5 |
| Errors | -176 | -292 | -493 | 445 | 547 | 843 | 16 | 20 | x | 4 | 5 | 5 |
| Trials | -525 | -588 | -926 | 1227 | 1159 | 1650 | 16 | 20 | x | 4 | 5 | 5 |

**Example boxplots**

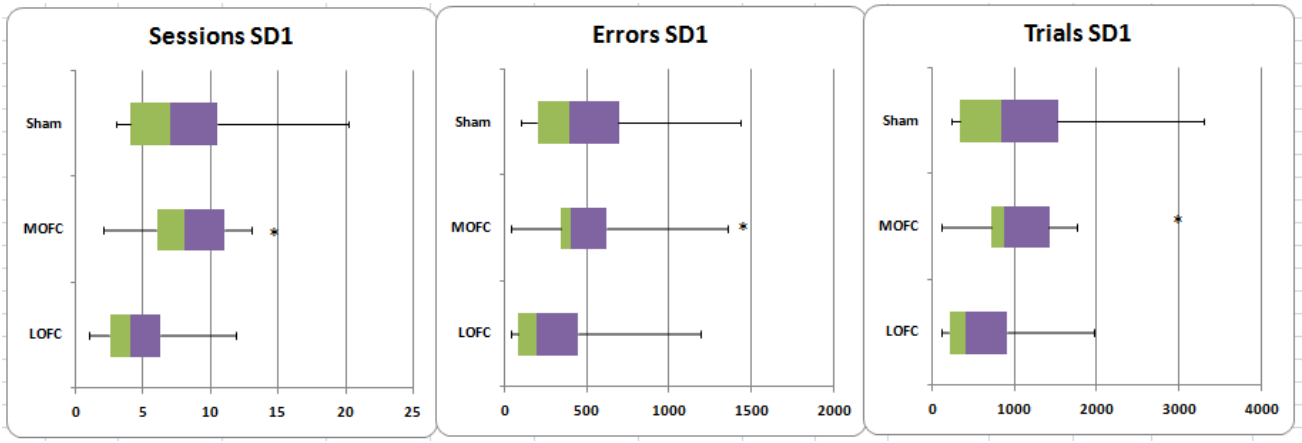


**Figure 25: Boxplots for the sessions (a), errors (b) and trials (c) for the first simple discrimination stage, including outliers (*).**