Effects of orbitofrontal, infralimbic and prelimbic cortical lesions on serial spatial reversal learning in the rat

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Abstract

Background: Recent evidence suggests that the neural correlates of reversal learning are localised to the orbitofrontal cortex whereas studies on the contribution of the medial prefrontal cortex to this capacity have produced equivocal results. This study examines the behavioural effects of selective lesions centred on orbitofrontal, infralimbic and prelimbic cortex on serial spatial reversal learning in the rat.

Methods: Rats were trained on a novel instrumental two-lever spatial discrimination and reversal learning task, measuring both ‘cognitive flexibility’ and constituent processes including response inhibition. Both levers were presented, only one of which was reinforced. The rat was required to respond on the reinforced lever under a fixed ratio 3 schedule of reinforcement. Following attainment of criterion, a series of reversals was presented.

Results: Bilateral excitotoxic lesions of the orbitofrontal cortex did not affect retention of a preoperatively acquired spatial discrimination but did impair reversal learning. This deficit manifested as increased perseverative responding on the previously correct lever. Although impairments were evident during reversal 1, OFC-lesioned animals performed significantly better than controls on reversal 2. There were no significant effects of infralimbic and prelimbic lesions on the retention of a spatial discrimination or reversal learning.

Conclusions: These results indicate that the orbitofrontal cortex is critical for flexible responding in serial spatial reversal learning. The present findings may be relevant to deficits in reversal learning and response inhibition in such neuropsychiatric disorders as obsessive-compulsive disorder.

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1. Introduction

Accumulating evidence suggests that different forms of behavioural flexibility are mediated by distinct regions of the prefrontal cortex (PFC) in rats [7,13,14,32,38,49] and non-human primates [15,17,29,49]. Studies of reversal learning in the human, marmoset and rat have localised some of the neural substrates of this capacity to the orbitofrontal cortex (OFC) [11,14,17,27,29,32,34,39,45] and the ventrolateral sector of caudate nucleus [20]. Lesions to the OFC have been suggested to impair animals’ capacity to adapt their responding following changes in stimulus reward contingencies. For example, OFC lesioned rats exhibit deficits after reversal in odour and visual discrimination tasks [8,14,32,42–44], while other studies using an olfactory-guided go/no-go discrimination task, show that rats with OFC lesions are impaired during the second, but not the first, reversal stage [22].

In contrast to the OFC, temporary inactivation, or targeted pharmacological manipulations centred on the rat mPFC have produced somewhat equivocal effects on reversal learning. During reversal of a spatial [30] or an olfactory [22] discrimination task, mPFC-lesioned rats made perseverative errors, as defined by repetitive responding to the previously correct stimulus. On the contrary, during a visual discrimination task, mPFC-lesioned rats only made more errors during reversal learning when the stimuli were difficult to discriminate [10]. Impairments were also observed in tasks which include shifting from a delayed non-matching-to-sample to a delayed matching-to-sample rule in an operant chamber [28,36,37], from a stimulus-matching to a stimulus-non-matching rule [31], from a place to a cue recognition rule in water maze [16] and from one perceptual dimension to another in attentional set shifting [7]. In all of these tasks...
contingencies were reversed so that inhibition of previously learned responses is required for optimal performance.

To our knowledge, although great emphasis has been placed on the mPFC and reversal learning, only two studies have addressed the effects of selective lesions to its subregions, namely the infralimbic (ILC) and prelimbic (PLC) cortex, on visual discrimination and reversal learning [13,14], showing that ILC damage causes deficits in the ‘learning’ stage of a reversal. The effects of OFC and ILC/PLC lesions on instrumental spatial reversal learning have not been thoroughly examined. The present study was therefore designed to determine the comparative contributions of OFC, ILC and PLC to performance on a novel instrumental two-lever spatial discrimination and serial reversal learning task, requiring flexible control and inhibition of responding. Specifically this investigation examines the effects of selective neurotoxic lesions of the OFC, PLC, ILC on (a) discriminative guidance of instrumental behaviour according to preoperatively acquired stimulus-reward contingencies, and (b) behavioural adaptation to a series of postoperative reversals of the spatial discrimination.

2. Methods

2.1. Subjects

Forty-one male Lister Hooded rats (Charles River, UK) weighing 280–320 g at the start of experiment, were pair-housed under a reversed light cycle (lights on from 19:00 to 07:00h). Prior to the beginning of training, rats were handled for ~5 min daily for 5 days and were put on a food-restriction schedule (15–18 g of Purina lab chow per day). Water was available ad libitum and testing took place between 13:00 and 16:00h 6–7 days per week. The work was carried out under UK Home Office licence in accordance with the UK Animals (Scientific Procedures) Act 1986.

2.2. Apparatus

The behavioural apparatus consisted of seven operant conditioning chambers (30 cm × 24 cm × 30 cm; Med Associates, Georgia, VT), each enclosed within a sound-attenuating wooden box fitted with a fan for ventilation and masking of extraneous noise. Each chamber was fitted with two retractable levers located on either side of a centrally positioned food magazine, into which an external pellet dispenser could deliver 45 mg sucrose pellets (Noyes dustless pellets; Sandown Scientific, Middlesex, UK), a light emitting diode (LED), which was positioned centrally above each lever, a magazine light, and a houselight. Magazine entry was detected by an infrared photocell beam located horizontally across the entrance. The apparatus was controlled by Whisker control software (www.whiskercontrol.com) and the task was programmed in Visual C++ (v.6).

2.3. Surgery

Subjects were divided into six groups, matched for their performance during the discrimination phase prior to surgery. Animals were anaesthetized using 10 ml/kg ketamine [10g of 2,2,2-tribromoethanol (Sigma, Poole, UK)] in 5 g of tertiary amyl alcohol, diluted in a solution of 40 ml of ethylol and 450 ml of PBS) and secured in a stereotaxic frame fitted with atraumatic earbars. For the PLC lesions it was set at 2.6, DV, −3.3 mm relative to the interaural line for a flat skull position, whereas for the PLC lesions it was set at 3.3 mm relative to the interaural line for a flat skull position, whereas for the PLC lesions it was set at 4.4, 0.2 mm. Bilateral excitotoxic lesions were made using either 0.09 M (OF lesions) or 0.06 M (ILC or PLC lesions) quisqualic acid, dissolved in 0.1 M phosphate buffer; the pH was adjusted with 0.1 M NaOH to between 6.5 and 7.0. Infusions (0.1 µl/min) were made according to the following coordinates [35]–OF lesions: site 1 AP, +4.0, L, ±0.8; DV, −3.4, 0.2 µl; site 2 AP, +3.7, L, ±2.0, DV, −3.6, 0.3 µl; site 3 AP, +3.2, L, ±2.6, DV, −4.4, 0.2 µl. PLC lesions: site 1 AP, +3.0, L, ±0.7, DV, −4.5, 0.2 µl; site 2 AP, +2.5, L, ±0.7, DV, −4.5, 0.2 µl. ILC lesions: site 1 AP, +4.0, L, ±0.8, DV, −3.3, 0.32 µl; site 2 AP, +2.7, L, ±0.8, DV, −3.8, 0.32 µl. Infusions were made 1 min after lowering the injector into the target region. The injector was left for a further 3–4 min after each infusion to allow for diffusion. Sham-operated animals received the same surgical procedure as the lesioned groups, except that they were infused with phosphate buffer 0.01 M. After surgery, animals were allowed for 7–10 days to recover prior to behavioural re-testing, during which time subjects were returned to their home cages.

2.4. Histology

After the completion of behavioural testing, animals were given a lethal dose of sodium pentobarbitone (1.5 ml/rat; Euthatal, 200 mg/ml; Genus Express, UK) and perfused transcardially with 0.01 M PBS followed by 4% paraformaldehyde. The brains were removed, postfixed in 4% paraformaldehyde for 24 h, and dehydrated in 20% sucrose in 0.01 M PBS overnight. Coronal sections of 40 µm were cut on a freezing microtome and processed for immunohistochemistry for the neuron-specific nuclear protein NeuN (Chemicon, Temecula, CA, USA). Specifically, after rinsing in 0.01 M PBS, free-floating sections were incubated overnight at room temperature with a primary mouse anti-NeuN antibody (1:10000) in a solution containing 0.4% Triton X-100 in 0.01 M PBS. After rinsing, they were incubated for 2 h at room temperature with a secondary biotinylated horse anti-mouse antibody (1:200; Dakopatts, Copenhagen, Denmark) followed by another rinse. The bound antibodies were then visualized by an avidin–biotin–peroxidase complex system (Vectastain ABC Elite Kit, Vector Labs, Burlingame, CA, USA) using 3,3-diaminobenzidine as the chromogen. All sections were mounted onto double-subbed glass slides and covered slipped with DePex mounting medium (BDH). The sections were then used to verify lesion placement and to assess the extent of the lesion-induced neuronal loss. 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 [35].

2.5. Behavioural procedure

2.5.1. Pretreatment

Subjects were initially given one 30 min exposure session to habituate to the test apparatus. During this time the houselight was on and the food magazine was loaded with pellets. After this habituation phase animals were trained to nosepoke in the central magazine in order to trigger presentation of the retractable levers and to respond on them for food delivery. This training took place on each lever separately, initially under a fixed ratio 1 (FR1) schedule to a criterion of 50 presses in 15 min, then under fixed ratio 3 (FR3) to a criterion of 150 presses in 15 min for each lever for two consecutive days. The subject was required to make a nosepoke response within 20 s to trigger presentation of a single lever. Responding on a lever within 10 s led to delivery of a single food pellet followed by retraction of the lever and the initiation of a 5 s intertrial interval. Every 10 s, a trial began with illumination of the houselight. The FR-3 schedule was used to preclude the possibility of reinforcing single, accidental presses on the correct lever. The order of left and right lever presentation was counterbalanced across subjects.

2.5.2. Acquisition of spatial discrimination

Training continued with the acquisition of a two-lever discrimination task. Now both levers were presented at trial onset and the rat had to learn that three lever presses on only one of these levers would result in reward. Each session lasted 15 min and consisted of a maximum of five 10-trial blocks. Each trial began with the presentation of both levers and a visual stimulus (a lit LED). The lit LED was used as a distractor and its location (left/right) varied from trial to trial according to a pseudo-random schedule so that the light was presented an equal number of times on each side for the session. This element was included to allow for the possible future addition of an extra-dimensional shift in our procedure (shift to the visual stimulus modality). Thus, the only stimulus with informational value for the discrimination was the spatial position of the retractable levers. Throughout the session, three lever presses on one lever (lever A) would produce a single pellet reward and the retraction of both levers, whereas three responses on lever B would result in lever retraction without
reward delivery. The position of the reinforced lever (left or right) was kept constant for each rat but was counterbalanced between subjects.

Each trial began with the switching on of the houselight. As in pretraining, there was a limited hold period of 20 s within which the rat had to nosepoke in the magazine to trigger presentation of the two-levers. Lever presentation initiated a 10-s response interval. Failure to respond in either the 20-s limited hold period, or the 10-s response interval resulted in the return to the inter-trial interval (ITI) state until the next trial was scheduled to begin, while the trial was recorded as an omission. Once the rat had responded on one of the levers, both levers were retracted and the houselight was turned off. Each rat had one training session per day and was trained to a criterion of 9 correct responses in one block of 10 trials (binomial distribution \( p < 0.01 \), likelihood of attaining criterion in a 10-trial block). Once this criterion was reached, this discrimination phase was considered complete, and the animal was returned to the home cage. If the criterion was not achieved this phase was repeated the next day till criterion achievement (Fig. 1).

### 2.5.3. Within session reversal learning task

In the next training session, reversal learning was introduced. By definition, reversal learning presupposes retention of a previously acquired discrimination. In serial reversals, in the first instance this would involve recall of the initially acquired discrimination described above. In subsequent reversals it would involve retention of the preceding reversal phase.

Accordingly, in the reversal session, animals were again exposed to the initial discrimination task described above (with the same lever rewarded as before: Discrimination retention in the first instance, latest reversal retention in subsequent runs). Once the criterion of 9 correct responses in a 10-trial block...
was achieved, the position of the reinforced lever was reversed (reversal phase). The reversal phase also consisted of a maximum of five 10-trial blocks. The learning criterion was the same as in the initial phase (9 correct responses in a 10-trial block).

A series of four reversal phases was given. Between successive reversals, animals were always given a single intervening day session where they were required to show retention of the previous reversal phase by reaching the 9/10 correct criterion (retention phase: same procedure as acquisition of spatial discrimination described above, Fig. 1).

Our procedure is within-session reversal since each daily session begins with a retention phase of the latest complete spatial discrimination acquired (criterion achievement). This minimizes the likelihood of contaminating results with memory effects while keeping the impact of contextual cues relatively constant. Another reason for utilising within-session reversal is because our ultimate aim was to develop a paradigm, which can be used for intra-cerebral infusions of drugs. It should be noted that Idris et al. [25] followed a similar procedure for testing the effects of drugs administered systemically but the time-consuming design of their task did not allow its use for intra-cerebral infusions.

2.6. Statistical analysis

The main measures of the animals' ability to learn the discriminations were: (i) the number of incorrect responses to criterion, and (ii) the number of trials to criterion. Additional measures recorded for each trial were (iii) the choice latency, (iv) the latency to collect the reward and (v) the number of omissions. Data for each variable were subjected to a repeated measures ANOVA. A square root (SQRT) transformation of data was used to ensure homogeneity of variance. Where significant interactions were found, they were further explored through separate ANOVAs or planned comparisons (contrast testing) to establish simple effects. The between-subject factor was group (four levels: sham, OFC, ILC and PLC lesions) and the within-subject factors were either retention phase (five levels: acquisition of pre-operative spatial discrimination, post-operative retention of spatial discrimination, retention of reversals 1–3) or reversal phases (four levels: reversals 1–4). Moreover, the number of incorrect responses during preoperative discrimination was added as a covariate in the analysis of the behavioural data.

3. Results

3.1. Histological results

The cytoarchitectonic borders and nomenclature are taken from the atlas by Paxinos and Watson [35]. The largest and smallest of the lesions for each group are depicted in Fig. 2A–C and photomicrographs are presented in Fig. 3A–C. Immunohistochemical analysis revealed that one animal from the OFC-lesioned presented with an incomplete, unilateral lesion and was thus discarded from the behavioural analyses. In all
other cases the area of the lesion was centred on the appropriate target region for that lesion group. Therefore, the final numbers in each group for subsequent behavioural analyses were as follows: sham-OFC = 4; sham-ILC = 4; sham-PLC = 4; OFC = 9; ILC = 11; PLC = 8.

All the remaining OFC-lesioned animals showed bilateral damage to the entire extent of the orbitofrontal region. The lesion started at bregma +4.7 and included the most ventral orbital (VO) and in some cases the most medial (MO) regions. At this most rostral extent, the lesion encroached into the prelimbic cortex (PLC). The lesion then continued to include the ventral and lateral orbital (LO) cortex (at bregma +3.2), where the most lateral extent of the ILC was also damaged although for the most part, the ILC was entirely spared, as was the dorsal peduncular (DP) and the PLC. At its most caudal extent (bregma +2.7), the lesion included the VO and LO and the most ventral agranular insular (AIV) cortex (Fig. 2A). ILC lesioned animals exhibited evidence of bilateral ILC damage, with almost complete neuronal loss. The lesion extended from approximately AP +3.7 mm to AP +2.2 mm from bregma (Fig. 2B). All animals showed slight sparing of the ILC at the most lateral and caudal limits of this region. The lesion in two animals was found to encroach ventrally into the PLC and dorsally into the dorsal peduncular cortex. Finally, the lesions in the PLC-lesioned group displayed extensive bilateral cell damage that began at the frontal pole and continued caudally to the level of the genu of the corpus callosum (Fig. 2C). In all PLC-lesioned animals, bilateral cell loss was evident in the prelimbic area with only the most caudal regions being spared.

3.2. Behavioural results

Because preliminary analysis of the sham-OFC, sham-ILC and sham-PLC groups’ data using ANOVA revealed no significant differences for any behavioural measure ($F$'s $< 1.0$, ns), we treated these animals as a single sham group during subsequent analyses.

3.2.1. Preoperative performance

3.2.1.1. Acquisition of the spatial discrimination. Preoperatively, the groups did not differ in the number of incorrect responses to reach the performance criterion on the acquisition of spatial discrimination ($F_{3,36} = 1.815$, $p = 0.494$; Fig. 4: AcqSD).

3.2.2. Postoperative performance

3.2.2.1. Retention phase: analysis of incorrect responses to criterion. The retention phases are shown in Fig. 4. There were no significant differences between the groups in their

![Fig. 3. Photomicrographs of coronal sections. (A) OFC in a representative OFC-lesioned (left) and sham (right) rat. (B) ILC in a representative ILC-lesioned (left) and sham (right) rat. (C) PLC in a representative PLC-lesioned (left) and sham (right) rat.](image)

![Fig. 4. Acquisition and retention phases. Data are means ± S.E.M. of incorrect responses to criterion (SQRT transformed values) in acquisition and retention phases. AcqSD: acquisition of pre-operative spatial discrimination. RetSD: post-operative retention test of pre-operatively acquired spatial discrimination. RetRev1, RetRev2 and RetRev3: postoperative retention test of reversal 1, 2 and 3.](image)
ability to retain the preoperatively acquired spatial discrimination (group: $F_{3,36} = 0.087$, $p = 0.966$; retention phase: $F_{1,36} = 0.319$, $p = 0.576$; group $\times$ retention phase: $F_{3,36} = 1.260$, $p = 0.303$) or to retain previously acquired reversals (group: $F_{3,36} = 0.144$, $p = 0.933$; retention phase: $F_{3,108} = 18.523$, $p < 0.001$; group $\times$ retention phase: $F_{9,108} = 0.460$, $p = 0.899$).

### 3.2.2.2. Serial reversal phase: analysis of number of incorrect responses to criterion.

Performance on serial reversals is shown in Fig. 5. Repeated measures ANOVA of the incorrect responses across series of reversals using the factors group (sham versus OFC versus ILC versus PLC) and phase (reversals 1–4) showed no effect for the covariate ($F_{3,105} < 1.0$). The covariate was thus left out and the analysis yielded a highly significant main effect of reversal phase ($F_{3,108} = 150.70$, $p < 0.001$) and reversal phase $\times$ group interaction ($F_{9,108} = 2.390$, $p = 0.016$). Planned comparisons demonstrated that OFC-lesioned rats made significantly more incorrect responses than controls in reversal 1 phase (reversal 1: sham versus OFC contrast: $F_{1,36} = 5.445$, $p = 0.025$) while no significant differences were noted between ILC or PLC groups and controls.

Whilst all groups showed a decline in the number of incorrect responses to reach criterion from the first to the fourth reversal, it is noteworthy that they did not improve across successive reversals in the same way. Planned comparisons showed that, although the performance of the shams and PLC-lesioned in reversal 2 phase rats were significantly better than that in reversal 1 phase (shams: reversal 1 versus reversal 2 contrast: $F_{1,36} = 9.549$, $p = 0.004$; PLC: reversal 1 versus reversal 2 contrast: $F_{1,36} = 7.656$, $p = 0.009$), they yielded a relatively less rapid rate of learning compared with the OFC- and ILC-lesioned groups (reversal 2: sham versus OFC contrast: $F_{1,36} = 4.378$, $p = 0.044$; sham versus ILC contrast: $F_{1,36} = 3.765$, $p = 0.06$; PLC versus OFC contrast: $F_{1,36} = 4.011$, $p = 0.053$; PLC versus ILC contrast: $F_{1,36} = 3.430$, $p = 0.072$).

### 3.2.2.3. Serial reversal phase: analysis of perseverative and learning errors.

Data were further analyzed according to the method of Dias et al. [17] and Bussey et al. [10], modified from Jones and Mishkin [29]. In this analysis, errors during reversal learning were broken down into two learning stages: errors committed before the attainment of chance level performance (39% correct) and errors committed between 39% and 85% correct trials. Jones and Mishkin regarded errors made during the first stage of learning as being indicative of perseverative responses to the previously reinforced stimulus. Thus, stage 1 errors are termed “perseverative errors” whereas stage 2 errors are termed “learning errors”.

The number of perseverative errors is shown in Fig. 6A. A repeated measures ANOVA revealed that there was no main effect of group ($F_{3,36} = 3.77$, $p = 0.77$), but there was a significant main effect of Reversal phase ($F_{3,108} = 151.12$, $p < 0.001$), and a significant group $\times$ reversal phase interaction ($F_{9,108} = 3.399$, $p = 0.001$). Planned comparisons demonstrated that, OFC-lesioned rats made significantly more perseverative errors than controls in reversal 1 phase (reversal 1: sham versus OFC contrast: $F_{1,36} = 7.751$, $p = 0.008$), while no significant differences were noted between ILC or PLC groups and controls. On the contrary, OFC lesions committed fewer perseverative errors than sham controls in reversal 2 phase (reversal 2: sham versus OFC contrast: $F_{1,36} = 5.674$, $p = 0.023$).
Values ± similar pattern (number of trials to criterion in the reversal phase revealed a significant main effect of reversal phase interaction \( F_{9,108} = 0.631, p = 0.769 \)).

3.2.2.4. Analysis of number of trials to criterion.

3.2.2.4.1. Retention phase. A repeated measures analysis of the number of trials to criterion in the retention phase revealed no significant main effect of group \( F_{3,36} = 0.917, p = 0.442 \), a significant main effect of reversal phase \( F_{3,108} = 20.213, p < 0.001 \), and no significant group × reversal phase interaction \( F_{9,108} = 0.631, p = 0.769 \).

3.2.2.4.2. Serial reversal phase. A similar analysis of the number of trials to criterion in the reversal phase revealed a similar pattern \( F_{3,36} = 0.84, p = 0.481; F_{3,108} = 85.09, p < 0.001; F_{9,108} = 0.936, p = 0.497 \). Data are presented as mean values ± S.E.M.s in Table 1.

3.2.2.4.3. Latencies and omissions. Lesioned animals did not omit more trials compared with sham-operated controls (retention phases: \( F_{12,144} < 1 \); reversal phases: \( F_{9,108} = 1.012, p = 0.435 \), and did not differ in their latencies to make correct or incorrect choice at any stage of the experiment pre- or post-operatively \( (F's < 1.0, ns) \). The latencies to collect the reward following correct trials were also similar across all groups \( (F's < 1.0, ns) \).

4. Discussion

This study has demonstrated differential effects of selective damage to the rodent OFC, ILC and PLC on serial reversal learning of an instrumental two-lever spatial discrimination task. Lesions to the rodent OFC impaired initial reversal learning, whereas lesions centred on ILC and PLC did not. This impairment was perseverative in nature, and occurred in the absence of significant effects on the ability to retain the previous stimulus-reward contingencies or to perform a spatial discrimination acquired preoperatively. Furthermore, the lesion did not affect the latency to respond to either the lever or the reward. These data suggest that the OFC may play a role in adjusting behaviour in response to changing relationships between cues and outcomes.

Despite the initial impairment in reversal learning, the performance of OFC-lesioned group improved across reversals. Specifically, they committed significantly fewer perseverative errors during reversal phase 2 compared to controls. This practice effect may suggest parallel processing of other structures, which are assumed to subsume prefrontal functions with practice [33].

4.1. OFC lesions and reversal learning

Although OFC lesions did not disrupt either rats’ ability to perform a spatial discrimination learned preoperatively or the late phases (i.e. “learning” phases) of reversal learning, there was a significant deficit in the early stage of reversal phase 1. This was characterized by an inability to inhibit previously reinforced responses, which led to perseveration, a finding reminiscent of previous studies in humans [39], monkeys [17,27,29] and rats [8,14,32,42–44]. For example, Dias et al. [17] reported that marmosets given neurotoxic lesions of OFC before training were impaired when the response contingencies of a previously acquired discrimination were subsequently reversed. Notably, these animals became substantially better at these reversals with additional training and were no longer impaired relative to controls, as in the present study and others [43]. These results suggest that a basic mechanism to inhibit responding remains intact after OFC lesions.

Furthermore, the present finding delineates a dissociation of the perseverative deficit from one of new learning in the context of instrumental discrimination: importantly, OFC-lesioned animals were not impaired in learning the new stimulus-response contingencies once the perseverative tendency had been overridden. Thus, this finding implies a selective ‘executive’ impairment of response control.

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**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Retention of spatial discrimination</th>
<th>Retention of reversal 1</th>
<th>Retention of reversal 2</th>
<th>Retention of reversal 3</th>
</tr>
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<tbody>
<tr>
<td>Shams</td>
<td>12</td>
<td>87.25 ± 14.49</td>
<td>65.58 ± 8.12</td>
<td>48.83 ± 6.78</td>
<td>39.17 ± 3.78</td>
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<tr>
<td>OFC</td>
<td>9</td>
<td>125.22 ± 16.73</td>
<td>51.11 ± 9.37</td>
<td>36.89 ± 7.83</td>
<td>43.0 ± 4.36</td>
</tr>
<tr>
<td>ILC</td>
<td>11</td>
<td>104.27 ± 15.13</td>
<td>46.09 ± 8.48</td>
<td>45.36 ± 7.08</td>
<td>43.36 ± 3.94</td>
</tr>
<tr>
<td>PLC</td>
<td>8</td>
<td>116.13 ± 17.74</td>
<td>65.75 ± 9.94</td>
<td>37.5 ± 8.3</td>
<td>38.75 ± 4.62</td>
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</tbody>
</table>

Data are presented as mean values ± S.E.M.

**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Reversal 1</th>
<th>Reversal 2</th>
<th>Reversal 3</th>
<th>Reversal 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shams</td>
<td>12</td>
<td>124.42 ± 12.39</td>
<td>81.67 ± 9.63</td>
<td>47.5 ± 5.73</td>
<td>34.0 ± 2.25</td>
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<tr>
<td>OFC</td>
<td>9</td>
<td>139.33 ± 14.31</td>
<td>51.67 ± 11.12</td>
<td>39.89 ± 6.61</td>
<td>29.56 ± 2.6</td>
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<tr>
<td>ILC</td>
<td>11</td>
<td>128.09 ± 12.94</td>
<td>53.82 ± 10.05</td>
<td>40.0 ± 5.98</td>
<td>32.45 ± 2.35</td>
</tr>
<tr>
<td>PLC</td>
<td>8</td>
<td>126.5 ± 15.17</td>
<td>77.88 ± 11.79</td>
<td>42.25 ± 7.01</td>
<td>32.13 ± 2.75</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± S.E.M.

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...
Despite the initial impairment on reversal, performance of OFC-lesioned rats improved with practice. Indeed, they performed significantly better than controls on reversal phase 2. A possible explanation of this paradoxical effect may be that these animals showed an acquired side bias resulting from the initial training. Since the contingencies in reversal phase 2 are the same as the ones acquired initially (on which they showed the perseverative tendency during reversal phase 1), OFC rats possibly reversed more readily than controls, since they were now required to respond to their ‘preferred lever’.

Overall, one may conclude that the deficit of the OFC-lesioned rats reflected a failure to inhibit a prepotent instrumental response. This failure in inhibition was possibly exacerbated by the effects of proactive interference from the previously established association, leading to an enhanced expression of a stimulus-response habit that is impervious to changes in the value of reinforcement [4,19,34]. This is consistent with prior literature where monkeys with frontal (ventral and orbital) lesions were impaired in the acquisition of new visuomotor associations, particularly when having to choose between three equally reinforced responses [9].

4.2 Lack of effect of mPFC lesions on serial spatial reversal learning

Recent reports have shown deficits in reversal learning after lesions of medial frontal cortex [22,30] but others [10] reported that reversal learning was impaired only when stimuli were difficult to discriminate. The latter authors argue that this reversal deficit might be caused by an inability to attend to relevant stimulus features. Moreover, Birrell and Brown [7] showed that ibotenic acid lesions in medial frontal cortex affected neither acquisition nor reversal learning of odour/texture discriminations. Here we investigated the effects of two distinct regions of the rodent mPFC, namely the ILC and PLC, in reversal learning of an instrumental two-lever spatial discrimination task. There was no effect of these lesions on acquisition, retention and any stage of serial spatial reversal learning.

The profound deficit in visual reversal learning reported by Bussey et al. [10] was observed during the later stage of reversal learning (learning errors), following lesions of the mPFC that included the PLC and overlying cingulate cortex rostral to the genu of the corpus callosum. In contrast, in the present study, rats with selective lesions to PLC were unimpaired during reversal learning, showing a similar pattern to that of rats with selective lesions to PLC during visual reversal learning [13]. Thus, all these findings possibly implicate involvement of pre- or perigenual anterior cingulate cortex, rather than of PLC in this type of learning. This hypothesis remains to be tested directly.

The effects of selective ILC lesions on reversal learning were assessed previously using a visual discrimination and reversal learning paradigm [14]. The authors report that the ILC lesioned rats group showed a specific impairment in the number of sessions required to reach criterion during reversal learning. Close examination of the type of errors in this stage revealed that these animals tended to make more ‘learning’ than ‘perseverative’ errors to reach criterion. This implies a deficit in new stimulus-reward learning rather than in the inhibitory control of previously reinforced responses [29]. In the present study, ILC-lesioned animals were not impaired at any reversal stage. This inconsistency may be due to the different modalities used (spatial versus visual discriminations) or the simplicity of our task which may have facilitated the learning of new stimulus-reward associations. Alternatively, the difference may be due to slight difference in the precise location of the ILC lesion. For example, the present ILC lesions did not include the PLC or the dorsal peduncular (DP) as in Chudasama and Robbins [14].

Given that rats with mPFC lesions are reported to exhibit difficulties in altering their behaviour when reinforcement contingencies are changed (e.g. [1,4,16,18,36,37]), the present finding that ILC and PLC lesions were without effect in reversal learning may seem surprising. However, the dissociation between OFC and PLC/ILC lesions reported here is consistent with previous observations that mPFC and OFC subserve different types of inhibitory control or behavioural flexibility [7,17,18,32]. Most relevant to the present study are data suggesting that the OFC plays a role in behavioural flexibility at the level of stimulus-reinforcement associations, while mPFC is implicated in switching of general rules, strategies or attentional sets [7,8,17,18,28,32,36,37].

4.3 Obsessive-compulsive disorder (OCD) and reversal learning

The results presented here may be relevant to the pathophysiology of OCD. Recent neuropsychological studies suggest that prominent component of the cognitive impairments characterizing OCD is a deficit in response inhibition, which is extensively interconnected with the PFC [2,3,24]. Indeed, OCD sufferers often show impairments on laboratory tests of frontal lobe function involving response shifting and inhibitory processing that correlate positively with the severity of their symptoms. Specifically, they fail to inhibit inappropriate responses at the behavioural and cognitive levels [5,23,40], and extensive evidence now implicates overactivity in the lateral OFC and associated circuitry [6,26,41,46,47]. Moreover, there is some evidence that OFC volume is reduced in OCD patients [12,48]. Thus, one hypothesis is that the compulsive behaviours and obsessive rumination present in OCD are partially due to the inability of the PFC – particularly the lateral OFC, but not the lateral PFC – to inhibit compulsions. These findings of OFC dysfunction in a disorder in which there are deficits in response inhibition are therefore consistent with previous literature identifying the lateral OFC as a key mediator in the ability to inhibit a prepotent response [11,21,27]. Thus, although the perseverative deficits on reversal learning, observed in the present study following lesions to the rodent OFC and not to ILC or PLC, are unlikely to resemble the aetiology of OCD, it may represent a model of compulsive responding characteristic of OCD.

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