‘Compulsive’ lever pressing in rats is enhanced following lesions to the orbital cortex, but not to the basolateral nucleus of the amygdala or to the dorsal medial prefrontal cortex

Daphna Joel, Julia Doljansky and Daniela Schiller
Department of Psychology, Tel Aviv University, Ramat-Aviv, Tel Aviv 69978, Israel

Keywords: animal model, conditioned reinforcement, obsessive–compulsive disorder (OCD), orbitofrontal cortex, reinforcer devaluation, signal attenuation

Abstract
In a new rat model of obsessive–compulsive disorder (OCD), ‘compulsive’ behaviour is induced by attenuating a signal indicating that a lever-press response was effective in producing food. We have recently found that compulsive lever pressing is increased following lesions to the rat orbital cortex, in accordance with several lines of evidence implicating the orbitofrontal cortex in the pathophysiology of OCD. In view of the functional similarities between the orbital cortex, the basolateral nucleus of the amygdala and the medial prefrontal cortex, the present study compared the effects of lesions to these three regions. The present study replicated the finding that lesions to the rat orbital cortex enhance compulsive lever pressing. In contrast, lesions to the dorsal medial prefrontal cortex and to the basolateral amygdala did not affect compulsive lever pressing. A comparison of these findings to current knowledge regarding similarities and differences in the functioning of the three regions sheds light on the mechanism by which signal attenuation induces compulsive lever pressing and on the role played by the orbital cortex in compulsive behaviour.

Introduction
Obsessive–compulsive disorder (OCD) is a psychiatric affliction with a lifetime prevalence of 1–3% (Rasmussen & Eisen, 1992; Weissman et al., 1994). According to the American Psychiatric Association (1994), the essential features of OCD are recurrent obsessions or compulsions (e.g. doubting, checking, washing).

We (Joel & Avisar, 2001) have recently developed a new animal model of OCD, the signal attenuation model, on the basis of the theoretical proposition that compulsive behaviours result from a deficit in the feedback associated with the performance of normal goal-directed responses (e.g. Reed, 1977; Gray, 1982; Malloy, 1987; Pitman, 1987, 1991; Baxter, 1999; Szechtman & Woody, 2004; for review see Otto, 1990). In the model, the attenuation of an external feedback for lever-press responding leads, in a subsequent extinction test, to excessive lever pressing that is not accompanied by an attempt to collect a reward. This behaviour, which we have named ‘compulsive’ lever pressing because it may be analogous to the excessive and unreasonable behaviour seen in OCD, is abolished by the selective serotonin reuptake inhibitors fluoxetine, paroxetine and fluvoxamine, but not by the anxiolytic drug diazepam or the tricyclic antidepressant desipramine (Joel & Avisar, 2001; Joel et al., 2004), in accordance with the differential efficacy of these drugs in alleviating obsessions and compulsions in OCD patients (e.g. Zohar et al., 1992; Piccinelli et al., 1995; Dolberg et al., 1996).

We have recently found that ‘compulsive’ lever pressing is increased following lesion to the rat orbital cortex. This finding is in line with functional imaging data from patients with idiopathic OCD and evidence from patients with acquired OCD which implicate the orbitofrontal cortex (OFC) in the pathophysiology of this disorder (e.g. Baxter et al., 1987, 1988; Benkelfat et al., 1990; Insel, 1992; Swedo et al., 1992; McGuire et al., 1994; Rauch et al., 1994; Berthier et al., 1996; Breiter & Rauch, 1996; Breiter et al., 1996; Cottraux et al., 1996; Hugo et al., 1999; Saxena et al., 1999; Stein et al., 1999; for a review see Saxena et al., 1998). The aim of the present study was to compare the effects of orbital lesions to the effects of (i) lesions to the basolateral nucleus of the amygdala (BLA), which is anatomically connected (Uylings & van Eden, 1990; Hatfield et al., 1996; Rolls, 1996, 1999, 2000a,b; Gallagher & Schoenbaum, 1999; Gallagher et al., 1999; Holland & Gallagher, 1999; Schoenbaum & Setlow, 2001; Cardinal et al., 2002; Schoenbaum et al., 2002, 2003a; Setlow et al., 2002), and (ii) lesions to the medial prefrontal cortex (mPFC), another major subdivision of the rat prefrontal cortex (for a recent review see Uylings et al., 2003).

Materials and methods
Subjects
Male Wistar rats (Tel Aviv University, Sackler Faculty of Medicine, Israel) 4 months old and weighing 350–480 g, were housed four to
a cage under reversed-cycle lighting (lights on 19.00–07.00) with ad lib access to food and water except for the duration of the experiment, in which they were maintained on a 22-h food-restriction schedule (see below) with freely available water. They were weighed twice a week to ensure that their body weight was not reduced below 90%. All experimental protocols conformed to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University, Israel. All efforts were made to minimize the number of animals used.

Surgery
Rats received 0.6 mg/kg diazepam and 5 min later were anaesthetized with i.p. injection of Avertin (10 mL/kg). They were placed in a stereotaxic frame and an incision was made into the scalp to expose the skull. The vertical coordinates of bregma and lambda were measured in order to align them in the same (level head) plane. A small square of bone was removed over the spots where the cannula would later enter. Orbital lesions were made by bilateral infusion of 0.3 µL N-methyl-d-aspartate (0.12 m; Sigma Chemicals, Israel) dissolved in phosphate-buffered saline (PBS; pH adjusted to 7.4 with 1 N NaOH) at the following coordinates (Paxinos & Watson, 1998): 3.2 mm anterior to bregma, 2.4 mm lateral to the midline and 5.5 mm ventral to the skull. BLA lesions were made by bilateral infusions of 0.4 µL quinolinic acid (QA; 0.12 m; Sigma Chemicals, Israel) dissolved in PBS, at the following coordinates: 2.8 mm posterior to bregma, 5.0 mm lateral to the midline and 8.6 mm ventral to the skull. mPFC lesions were made by bilateral infusions of 0.5 µL QA dissolved in PBS, at the following coordinates: anterior, −3.7 mm anterior to bregma, 0.7 mm lateral to midline and 2.2 mm ventral to the skull; posterior, −2.7 mm anterior to bregma, 0.7 mm lateral to midline and 2.2 mm ventral to the skull. For BLA and orbital lesions, infusions were made through the tip of a Hamilton syringe (26-gauge cannula) using a manually driven pump (Kopf, microinjection unit, model 5000) mounted onto the stereotaxic frame. For mPFC lesions, infusions were made through a Hamilton syringe mounted onto an electrically driven pump (model CMA/100, Medecin AB, Solona, Sweden) and connected to the injection cannula (31-gauge) with polyethylene tubing. All infusions were at a flow rate of 0.1 µL/min. Following injections the cannulae were left at the injection site for a period of 5 min to allow complete diffusion of the neurotoxin. Sham-operated controls underwent the same surgical procedures but received injections of a corresponding dose of PBS alone. At the end of surgery, the hole in the bone was covered with sterispon and the scalp incisions were sutured with Michel clips. Following surgery rats were returned to their home cages and were monitored on a daily basis.

Apparatus and behavioural procedure
At least 14 days after recovery from operation, rats were tested for latent inhibition (retarded conditioning to a stimulus after it had been repeatedly presented without consequences) in a conditioned emotional response procedure. The emotional response was conditioned by exposing each rat to two or five pairings of a tone (10 s, 80 dB, 2.8 kHz) and a shock (0.5 mA and 1 s duration). The results are reported elsewhere (Schiller & Weiner, 2004).

Training in the post-training signal attenuation procedure began 1–2 weeks after the conclusion of the latent inhibition procedure. The task was conducted in four operant chambers (Campden Instruments, Loughborough, UK), housed in sound-attenuated boxes and equipped with a 3-W house light and two retractable levers on either side of a food magazine (fitted with a 3-W magazine light), into which 45 mg Noyes precision food pellets (Noyes, Sandown Chemical Limited, Hampton, UK) could be delivered. Access to the food magazine was through a hinged panel, the opening of which activated a microswitch. Equipment programming and data recording were computer-controlled.

Prior to the beginning of training, rats were handled for ≈2 min daily for 5 days. A 22-h food restriction schedule began simultaneously with handling and continued throughout behavioural testing. Food was provided in the home cages between 14.00 and 16.00 h, at least half an hour after the end of the session. On the last 2 days, after handling, 20–30 food pellets used as reinforcement for operant training were introduced into the home cages on a tray. The tray was removed from the cage after each rat was observed to consume at least two pellets.

Post-training signal attenuation
The post-training signal attenuation procedure included four stages.

Stage 1: magazine training
On days 1–3, rats were trained to collect food pellets from the food magazine in the operant chamber, with the levers retracted. On each trial, a single food pellet was dropped into the food magazine, simultaneously with the onset of the magazine light (the stimulus). The stimulus was turned off after the rat’s head entered the food magazine or after 15 s had elapsed, and a 30-s intertrial interval began (for more details see Fig. 1). On each day, each rat was trained until it completed 30 trials in which it inserted its head into the food magazine during stimulus presentation (collected trials), or until a total of 40 trials was reached. The number of collected trials and the total number of trials were recorded.

Stage 2: lever-press training
On days 4–6, rats were trained to lever-press in a discrete-trial procedure. On each trial, both levers were inserted into the chamber. Responding on one of the levers (reinforced lever; RL) resulted in the delivery of a single food pellet into the magazine, accompanied by the presentation of the magazine light stimulus. The levers were retracted and the stimulus was turned off, after the rat’s head entered the food magazine or after 15 s from the rat’s first lever-press had elapsed (see Fig. 1). Further lever presses on the RL as well as responding on the other lever (nonreinforced lever; NRL) had no programmed consequences. The lever designated RL was counterbalanced over subjects and remained the same for each rat over the entire experimental procedure. Each trial was followed by a 30-s intertrial interval. On day 4, each rat was trained until it completed 24 trials, that is, pressed the lever and inserted its head into the food magazine during stimulus presentation, or for a total of 60 trials. Rats that failed to attain at least 20 completed trials were returned to the test chamber at the end of the day for an additional session. Those that did not attain at least 20 completed trials in the second session were excluded from the experiment. On days 5 and 6, all rats were trained as on day 4 except that the stimulus was turned off after 10 s instead of after 15 s and training ended when the rat had attained 40 completed trials or a total of 60 trials.

In order to assess acquisition of the lever-press response, the number of trials on which the rat did not press the RL (unpressed trials) and the number of trials on which the rat pressed the RL without inserting its head into the food magazine (uncompleted trials) were recorded in addition to the number of completed trials (see Fig. 1). In order to assess rats’ tendency for excessive lever pressing, the number of lever presses on the NRL, and the number of lever presses on the RL after the first response (extra lever presses; ELP) were recorded.
The latter measure was further subdivided into ELP in uncompleted trials (that is, ELP not followed by insertion of the head into the food magazine; ELP-U), and ELP in completed trials (ELP-C).

### Stage 3: signal attenuation

On days 7–9, with the levers retracted, rats were exposed to the presentation of the stimulus as on days 1–3 but no food was delivered to the food magazine (see Fig. 1). Rats received 40 such trials on day 7 and 30 trials on days 8 and 9. The number of collected trials was recorded. Rats that had > 15 collected trials on day 9 were returned to the test chamber at the end of the day for an additional session.

### Stage 4: test

On day 10, rats were trained as in the lever-press training stage except that no food was delivered to the food magazine, that is, pressing the lever resulted in the presentation of the stimulus only (see Fig. 1). The session lasted for 50 trials. The behavioural measures recorded were the same as in the lever-press training stage. Compulsive lever pressing is operationally defined as the number of extra lever presses that are not followed by magazine entry (ELP-U) in the test stage of the post-training signal attenuation procedure.

### Histology

After the completion of behavioural testing, lesion and sham rats were overdosed with sodium pentobarbital (60 mg/kg, i.p.) and perfused intracardially with a solution of 0.9% NaCl at room temperature for 2 min (flow rate 35 mL/min), followed by 10% buffered formalin for 15 min. The brains were removed from their skulls and placed in 10% buffered formalin for at least 24 h. The brains were sectioned in the coronal plane using a freezing microtome at 50 μm thickness and stained with Cresyl Violet. The atlas of Paxinos & Watson (1998) was used to verify the placement and the extent of the lesions.

### Results

#### Experiment 1. Effects of orbital lesion in post-training signal attenuation

#### Anatomical

Figure 2A presents a photomicrograph of coronal sections taken from a representative orbital lesioned (left) and sham (right) rat. Figure 3A presents a schematic reconstruction of individual cases sustaining the largest (shaded) and smallest (solid) lesions to the orbital cortex. In most animals, the lesion extended from 4.7 to 2.2 mm anterior to Bregma (AP), from 0.6 to 5.1 mm lateral to the midline (ML) and from 3.8 to 6.8 mm ventral to the skull (DV). Neuronal loss and accumulation of glia cells was evident in the ventral and lateral orbital cortex and occasionally extended to the dorsolateral orbital and agranular insular cortex, but this was typically a minor and unilateral damage. Examination of the histological materials from vehicle-injected rats confirmed that there was no discernable damage in any of the sham-lesioned control rats. The only visible damage was the cannulae tracks toward the target areas.

#### Behavioural

Eighteen rats were randomly assigned to two groups (orbital lesion, \( n = 9 \), and sham operation, \( n = 9 \)). One sham rat did not attain the criterion of 20 completed trials in the second session and was excluded from the experiment. On the last day of lever-press training, all rats achieved 40 completed trials with no uncompleted trials and therefore with no ELP-U; there were no significant differences between the groups on the number of unpressed trials or the number of ELP-C \([\text{ANOVA with a main factor of Group (sham, lesion) yielded } F \text{ values } < 1]\). At the signal attenuation stage, there were no differences between the groups in the extinction of the compound stimulus, as reflected in the number of collected trials, in either the rate of extinction or in the performance level at the end of this stage (Table 1). From the second day of lever-press training rats rarely
Fig. 2. Photomicrographs of coronal sections. (A) Orbital cortex in a representative orbital-lesioned (left) and sham (right) rat. Arrows denote lesion borders. (B) mPFC in a representative mPFC-lesioned (left) and sham (right) rat. Arrows denote lesion borders. (C) Amygdala in a representative sham (top) and BLA-lesioned (bottom) rat. Arrows denote lesion borders and injection cannula track. BLA, basolateral nucleus of the amygdala; CE, central nucleus of the amygdala.

Table 1. Number of collected trials on the signal attenuation stage

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Mixed ANOVA</th>
</tr>
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<tr>
<td></td>
<td>Factor</td>
<td>F-value</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td>Experiment 1, orbital lesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>22.0 ± 2.23</td>
<td>6.1 ± 1.73</td>
<td>2.7 ± 0.94</td>
<td>Group: $F_{1,15} = 1.58$, 0.23</td>
</tr>
<tr>
<td>Lesion</td>
<td>23.7 ± 2.01</td>
<td>11.0 ± 2.57</td>
<td>4.6 ± 1.52</td>
<td>Day: $F_{2,30} = 101.7$, &lt; 0.0001</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>$G \times D$: $F_{2,30} = 0.88$, 0.43</td>
</tr>
<tr>
<td>Experiment 2, mPFC lesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>20.6 ± 2.12</td>
<td>5.1 ± 1.43</td>
<td>3.9 ± 0.23</td>
<td>Group: $F_{1,14} = 0.012$, 0.91</td>
</tr>
<tr>
<td>Lesion</td>
<td>21.9 ± 2.65</td>
<td>3.3 ± 0.41</td>
<td>5.0 ± 1.05</td>
<td>Day: $F_{2,28} = 96.0$, &lt; 0.0001</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>$G \times D$: $F_{2,28} = 0.79$, 0.47</td>
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<tr>
<td>Experiment 3, BLA lesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>22.3 ± 1.99</td>
<td>11.3 ± 1.19</td>
<td>5.4 ± 1.13</td>
<td>Group: $F_{1,16} = 0.006$, 0.94</td>
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<tr>
<td>Lesion</td>
<td>21.9 ± 2.45</td>
<td>10.3 ± 1.35</td>
<td>6.5 ± 1.92</td>
<td>Day: $F_{2,32} = 54.7$, &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$G \times D$: $F_{2,32} = 0.244$, 0.79</td>
</tr>
</tbody>
</table>

Data are presented as mean values ± SEM.

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Fig. 3. Reconstruction of individual cases sustaining the largest (shaded) and smallest (solid) lesions to the orbital cortex (A), mPFC (B) and BLA (C). Coordinates of the coronal sections are indicated with reference to bregma according to the stereotaxic atlas of Paxinos & Watson (1998).
Table 2 Number of completed, uncompleted and unpressed trials on the test

<table>
<thead>
<tr>
<th></th>
<th>Completed trials</th>
<th>Uncompleted trials</th>
<th>Unpressed trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1, orbital lesion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>9.4 ± 2.13</td>
<td>3.6 ± 1.4</td>
<td>37.0 ± 3.00</td>
</tr>
<tr>
<td>Lesion</td>
<td>13.6 ± 2.51</td>
<td>3.8 ± 0.76</td>
<td>32.7 ± 2.87</td>
</tr>
<tr>
<td>$F$-value</td>
<td>$F_{1,15} = 1.57$</td>
<td>$F_{1,15} = 0.01$</td>
<td>$F_{1,15} = 1.09$</td>
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<tr>
<td>$P$-value</td>
<td>0.23</td>
<td>0.92</td>
<td>0.31</td>
</tr>
<tr>
<td>Experiment 2, mPFC lesion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>11.0 ± 1.40</td>
<td>6.3 ± 1.10</td>
<td>32.8 ± 1.72</td>
</tr>
<tr>
<td>Lesion</td>
<td>10.5 ± 0.98</td>
<td>6.7 ± 1.21</td>
<td>32.8 ± 1.72</td>
</tr>
<tr>
<td>$F$-value</td>
<td>$F_{1,14} = 0.09$</td>
<td>$F_{1,14} = 0.09$</td>
<td>$F_{1,14} = 0.00$</td>
</tr>
<tr>
<td>$P$-value</td>
<td>0.77</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Experiment 3, BLA lesion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>10.7 ± 1.42</td>
<td>4.4 ± 1.11</td>
<td>35.0 ± 2.08</td>
</tr>
<tr>
<td>Lesion</td>
<td>13.0 ± 2.52</td>
<td>4.0 ± 1.77</td>
<td>34.0 ± 2.48</td>
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<tr>
<td>$F$-value</td>
<td>$F_{1,16} = 0.70$</td>
<td>$F_{1,16} = 0.04$</td>
<td>$F_{1,16} = 0.10$</td>
</tr>
<tr>
<td>$P$-value</td>
<td>0.42</td>
<td>0.84</td>
<td>0.76</td>
</tr>
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</table>

Data are presented as mean values ± SEM.

pressed the NRL. In both groups there was no increase in lever presses on the NRL in the test stage.

Figure 4 presents the number of ELP-U (Fig. 4A) and ELP-C (Fig. 4B) in five 10-trial blocks of the sham and orbital groups in the test. As can be seen, orbital rats exhibited a higher number of ELP-U than did sham-operated rats (main effect of Group, $F_{1,15} = 5.63$, $P < 0.05$), but the number of ELP-C was similar in the two groups (main effect of Group, $F_{1,15} = 1.82$, $P = 0.20$; Group × Block interaction, $F_{4,60} = 0.55$, $P = 0.70$). There were also no significant differences between the two groups in the number of completed, uncompleted and unpressed trials (Table 2).

Experiment 2. Effects of mPFC lesion in post-training signal attenuation

Anatomical

Figure 2B presents a photomicrograph of coronal sections taken from a representative mPFC-lesioned (left) and sham (right) rat. Figure 3B presents a schematic reconstruction of individual cases sustaining the largest (shaded) and smallest (solid) lesions to the mPFC. In most animals, the lesion extended from 4.7 to 2.2 mm anterior to bregma (AP), 1.4 mm lateral to the midline (ML) and 4.1 mm ventral to the skull (DV). Neuronal loss and accumulation of glia cells was evident in the cingulate cortex and the dorsal part of the prelimbic cortex, which comprise the dorsal medial prefrontal cortex (Heidbreder & Groenewegen, 2003), and in the most medial aspect of the secondary motor cortex (medial agranular cortex). Examination of the histological materials from vehicle-injected rats confirmed that there was no discernable damage in any of the sham-lesioned control rats. The only visible damage was the cannulae tracks toward the target areas.

Behavioural

Sixteen rats were randomly assigned to two groups (mPFC lesion, $n = 8$, and sham operation, $n = 8$). On the last day of lever-press training, all rats achieved 40 completed trials with no uncompleted trials and therefore with no ELP-U; there were no differences between the groups on the number of unpressed trials or the number of ELP-C (all $F < 1$). At the signal attenuation stage, there were no significant differences between the groups in the extinction of the compound stimulus, as reflected in the number of collected trials, in either the rate of extinction or in the performance level at the end of this stage (Table 1). From the second day of lever-press training rats rarely pressed the NRL. In both groups there was no increase in lever presses on the NRL in the test stage.

Figure 5 presents the number of ELP-U (Fig. 5A) and ELP-C (Fig. 5B) in five 10-trial blocks of the sham and mPFC groups in the test. As can be seen, mPFC rats exhibited a similar number of ELP-U and of ELP-C to that of sham operated rats (main effect of Group and Group × Block interaction were not significant; see Fig. 5 for the results of the statistical analysis). There were also no significant differences between the two groups in the number of completed, uncompleted and unpressed trials (Table 2).
The effect of Group, pattern of change in ELP-U over the five blocks tended to be different in the two groups (Group × Blocks interaction, F_{4,64} = 2.30, P = 0.068). There were no significant differences between the two groups in the number of completed, uncompleted and unpressed trials (Table 2).

**Discussion**

As shown by us previously (Joel et al., 2005), rats sustaining lesions to the orbital cortex exhibited a profound increase in compulsive lever pressing (i.e. ELP-U) in an extinction of lever-press responding that was preceded by signal attenuation. Importantly, as in our previous study, increased compulsivity in orbital rats could not be attributed to the effects of the lesion in the early stages of the task (i.e. magazine training, lever-press training and signal attenuation), because orbital-lesioned rats performed similarly to their sham-operated controls in these stages. In contrast to the compulsivity-enhancing effect of the orbital lesions, neither mPFC nor BLA lesions had any effect on compulsive lever pressing.

The post-training signal attenuation procedure bears some similarities to two procedures which have been used to study the effects of lesions to the orbital cortex and BLA, namely, procedures in which the value of the primary reinforcer is devalued and procedures in which a conditioned reinforcer is omitted. In reinforcer-devaluation procedures, after animals acquire a response, the value of the reinforcer is reduced (by sickness-induced conditioned aversion to the reinforcer or by a motivational shift from a deprived to a nondeprived state), and this is followed by assessment of the response under extinction conditions.

**Fig. 6**

The mean and SEM number of (A) extra lever presses that were not followed by an attempt to collect a reward (ELP-U) and (B) extra lever presses that were followed by an attempt to collect a reward (ELP-C), in five 10-trial blocks in sham and mPFC-lesioned rats in the test. (A) ELP-U: main effect of Group, F_{1,16} = 0.18, P = 0.68; repeated-measurements factor of Block, F_{4,64} = 2.96, P < 0.005; Group × Block interaction, F_{4,64} = 2.30, P = 0.068. (B) ELP-C: main effect of Group, F_{1,16} = 0.07, P = 0.79; repeated-measurements factor of Block, F_{4,64} = 11.64, P < 0.0001; Group × Block interaction, F_{4,64} = 0.11, P = 0.98.
conditions. Thus, both reinforcer devaluation procedures and the present procedure intersperse between the training stage and the extinction test a stage in which response outcome is manipulated without the rat emitting the response. In studies assessing the effects of omission of a conditioned reinforcer, responding is first reinforced by a primary reinforcer accompanied with a neutral stimulus, thus establishing the latter as a conditioned reinforcer. Subsequently, the response requirement for the presentation of the primary reinforcement is increased and, after responding on this second-order schedule stabilizes, the effects of the omission of the conditioned reinforcer (but not of the primary reinforcer) are assessed. Thus, both this procedure and the post-training signal attenuation procedure include a stage in which the consequences of the operant response are altered, either by omitting the conditioned reinforcer altogether or by presenting a conditioned reinforcer that has undergone extinction (in the signal attenuation stage).

Lesions to the orbital cortex and BLA have been shown to render animals insensitive to the two types of manipulations. Thus, whereas control rats reduce responding following reinforcer devaluation, BLA- and orbital-lesioned rats respond to a devalued reinforcer similarly to lesioned and control rats that did not undergo reinforcer devaluation (Hatfield et al., 1996; Gallagher et al., 1999; Balleine et al., 2003; see also Baxter et al., 2000 and Malkova et al., 1997 for related studies in primates). Similarly, whereas control marmosets decrease their rate of responding when the conditioned reinforcer is omitted, BLA- and OFC-lesioned marmosets do not change their rate of responding when the conditioned reinforcer is omitted (Parkinson et al., 2001; Pears et al., 2003). It should, however, be noted that this conclusion should be taken with caution because Parkinson et al. (2001) and Pears et al. (2003) report only the mean rate of responding on the conditioned reinforcer omission session as a whole. It is therefore possible that the rate of responding initially increased and only later decreased, as happens in the post-training signal attenuation procedure. Moreover, a direct comparison of the behaviour of animals in the two procedures is also complicated by the fact that in the procedure used by Parkinson et al. (2001) and by Pears et al. (2003) a response leads to the disappearance of the stimulus to which responding is directed, so animals cannot perform excessive responses as they do in the post-training signal attenuation procedure.

In contrast to the effects of orbital and BLA lesions in reinforcer devaluation and omission of conditioned reinforcer procedures, in post-training signal attenuation procedure, they amplify, rather than abolish, the effects of signal attenuation. That is, orbital-lesioned rats exhibit more ELP-U than sham rats that underwent signal attenuation (which exhibit more ELP-U than sham (and orbital) rats that did not undergo signal attenuation; Joel et al., 2004; Joel et al., 2005).

The reduced sensitivity of orbital- and BLA-lesioned rats to reinforcer devaluation and to the omission of a conditioned reinforcer, together with findings obtained in related paradigms, have been taken to suggest that these two regions form a functional system which enables stimuli to take on reinforcing properties and/or to access the current motivational and/or affective value of the associated outcome (Cador et al., 1989; Everitt et al., 1989; Everitt & Robbins, 1992; Hatfield et al., 1996; Rolls, 1996, 1999, 2000a,b; Whitelaw et al., 1996; Gallagher & Schoenbaum, 1999; Gallagher et al., 1999; Holland & Gallagher, 1999; Baxter et al., 2000; Parkinson et al., 2001; Schoenbaum & Setlow, 2001; Cardinal et al., 2002; Schoenbaum et al., 2002, 2003a; Setlow et al., 2002; Coussens & Otto, 2003; Pears et al., 2003). The present findings that orbital lesions affect performance in the post-training signal attenuation procedure, whereas BLA lesions are without an effect, point to dissociable contributions of these two regions to such processes. This is consistent with electrophysiological (Rolls, 1996; Thorpe et al., 1983; Schoenbaum et al., 1998, 1999, 2000, 2003b) and recent lesion studies (Coussens & Otto, 2003; Parkinson et al., 2001; Setlow et al., 2002; Pears et al., 2003; Pickens et al., 2003), suggesting that the orbital cortex and BLA play distinct roles in mediating the effects of motivationally significant stimuli on behaviour. Specifically, it has been suggested that the BLA is primarily involved in the acquisition of the motivational significance of stimuli whereas the orbital cortex is particularly critical for flexible adjustment of responding when reinforcement contingencies change (Pickens et al., 2003; Rolls, 1996, 1999, 2000a,b; Schoenbaum & Setlow, 2001). The lack of effect of BLA lesions in the present study may thus be taken to suggest that the critical factor in inducing compulsive lever pressing is not the reduction in the motivational significance of the stimulus in the signal attenuation stage but rather the reduction in its ability to provide a feedback that the lever-press response has had an impact on the environment (e.g. Williams, 1994). This latter function has been shown not to depend on the BLA (Parkinson et al., 2001). The observation that orbital-lesioned rats extinguish responding similarly to control rats (Joel et al., 2005), but exhibit elevated levels of responding when the extinction session is preceded by signal attenuation (present findings and Joel et al., 2005), suggests that, in the intact brain, the orbital cortex is crucial for suppressing behaviour on the basis of the information acquired at the signal attenuation stage (i.e. that the stimulus no longer signals food). This suggestion is in line with current views of OFC function which emphasize its involvement in suppressing behaviour in the context of changed task contingencies (e.g. Dias et al., 1996, 1997; Gallagher et al., 1999; Nobre et al., 1999; Schoenbaum et al., 1999, 2000; Rolls, 2000b; Zald & Kim, 2001).

Given that rats sustaining mPFC lesions are also reported to exhibit difficulties in altering their behaviour when reinforcement contingencies change (e.g. Agglet et al., 1995; Kolb, 1984; de Bruin et al., 1994; Dias et al., 1997; Joel et al., 1997; Balleine & Dickinson, 1998; Ragozzino et al., 1999a,b), the present finding that mPFC lesions were without an effect in the post-training signal attenuation procedure may seem surprising. However, the present dissociation is consistent with previous demonstrations that the mPFC and the orbital cortex subserve different types of inhibitory control or behavioural flexibility (Birrell & Brown, 2000; Dias et al., 1996, 1997; McAlonan & Brown, 2003). Most relevant in the present context are findings suggesting that the orbital cortex plays a role in behavioural flexibility at the level of stimulus–reinforcement associations (e.g. as in reversal of a spatial discrimination) whereas the mPFC is involved in switching of general rules, strategies or attentional sets (Dias et al., 1996, 1997; Joel et al., 1997; Ragozzino et al., 1999a,b; Birrell & Brown, 2000; Kesner, 2000; Brown & Bowman, 2002; McAlonan & Brown, 2003). Because the critical behavioural manipulation in the post-training signal attenuation procedure is a change in stimulus–reinforcement association, i.e. the extinction of the stimulus–food association in the signal attenuation stage, this manipulation would be expected to be sensitive to orbital but not to mPFC lesion. It should be noted, however, that the lack of effect of the mPFC lesion in the present study may alternatively be attributed to the fact that the mPFC lesions spared the ventral parts of the mPFC (i.e. the ventral prelimbic cortex and the infralimbic cortex), because this region has been recently shown to play a critical role in mediating the effects of a prior extinction learning on subsequent behaviour (Milad & Quirk, 2002; Quirk et al., 2000).

Specifically, although rats sustaining lesions to the ventral mPFC extinguished conditioned fear similarly to control rats, they exhibited a higher degree of spontaneous recovery on a subsequent extinction session (Quirk et al., 2000). It is thus possible that the ventral mPFC
also participates in the retention of the extinction of the stimulus–food contingency that takes place in the signal attenuation stage, and that its lesion would interfere with rats’ performance at the test stage of the post-training signal attenuation procedure. It is noteworthy, however, that such interference would be expected to reduce the effects of signal attenuation on rats’ behaviour in the test, rather than enhance it, as found here following orbital lesions.

We have previously proposed that the extinction of the classical contingency between the stimulus and food, in the signal attenuation stage, alters the ability of the stimulus to signal that the response was effective in producing food and that this leads, in the subsequent test stage, to repeated emission of the lever-press response (Joel & Avisar, 2001; Joel & Doljansky, 2003). We have further speculated that signal attenuation may simulate a deficient response feedback or a deficient signalling that the conditions have changed following the organism’s response which, it has been hypothesized, underlies obsessions and compulsions in patients (e.g. Reed, 1977; Gray, 1982; Malloy, 1987; Pitman, 1987, 1991; Baxter, 1999; Szechman & Woody, 2004, for review see Otto, 1990). The possibility that ELP-U induced by signal attenuation may provide an animal model of compulsive behaviour in OCD is further supported by (i) a similar pharmacological profile (i.e. alleviation by selective serotonin reuptake inhibitors but not by anxiolytic, antipsychotic and classical tricyclic antidepressant drugs, Joel & Avisar, 2001; Joel & Doljansky, 2003; Joel et al., 2004); (ii) behavioural similarity, that is, both ELP-U and compulsions are excessive and inappropriate, and (iii) similarities in the neurotransmitter systems involved, namely, the serotonergic and dopaminergic systems (Joel & Avisar, 2001; Joel et al., 2001, 2004; Joel & Doljansky, 2003).

Although there are significant differences in the details and in the complexity of the organization of the cortex of rats and primates, hodological, electrophysiological and behavioural data suggest that the rat orbital cortex may be analogous to the primate OFC whereas the rat mPFC may correspond to regions in the dorsal and lateral subdivisions of the primate prefrontal cortex (for recent reviews see Groenewegen & Uylings, 2000; Kesner, 2000; Ongur & Price, 2000; Schoenbaum & Setlow, 2001; Uylings et al., 2003). Given that, in humans, lesions to the OFC may result in compulsive behaviour which is similar to that of idiopathic OCD (Berthier et al., 1996; Hugo et al., 1999), the demonstration that compulsive lever pressing is enhanced following lesions to the orbital cortex supports our hypothesis that this behavioural pattern may serve to model compulsive behaviour in OCD. Moreover, the finding that compulsive lever pressing is enhanced following lesions to the orbital cortex but not to the mPFC or to the BLA is consistent with functional imaging findings in OCD. Moreover, the finding that compulsive lever pressing is enhanced following lesions to the orbital cortex supports our hypothesis that this finding that compulsive lever pressing is enhanced following lesions to the orbital cortex supports our hypothesis that this

References


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Abbreviations

BLA, basolateral nucleus of the amygdala; ELP, extra lever presses; ELP-C, extra lever presses in completed trials; ELP-U, extra lever presses in uncompleted trials; mPFC, medial prefrontal cortex; NRTL, nonreinforced lever; OCD, obsessive–compulsive disorder; OFC, orbitofrontal cortex; PBS, phosphate-buffered saline; RL, reinforced lever; QA, quinolinic acid.


