Evaluation of animal models of obsessive-compulsive disorder: correlation with phasic dopamine neuron activity

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Abstract

Obsessive compulsive disorder (OCD) is a psychiatric condition defined by intrusive thoughts (obsessions) associated with compensatory and repetitive behaviour (compulsions). However, advancement in our understanding of this disorder has been hampered by the absence of effective animal models and correspondingly analysis of the physiological changes that may be present in these models. To address this, we have evaluated two current rodent models of OCD; repeated injection of dopamine D\textsubscript{2} agonist quinpirole and repeated adolescent injection of the tricyclic agent clomipramine in combination with a behavioural paradigm designed to produce compulsive lever pressing. These results were then compared with their relative impact on the state of activity of the mesolimbic dopaminergic system using extracellular recoding of spontaneously active dopamine neurons in the ventral tegmental area (VTA). The clomipramine model failed to exacerbate compulsive lever pressing and VTA dopamine neurons in clomipramine-treated rats had mildly diminished bursting activity. In contrast, quinpirole-treated animals showed significant increases in compulsive lever pressing, which was concurrent with a substantial diminution of bursting activity of VTA dopamine neurons. Therefore, VTA dopamine activity correlated with the behavioural response in these models. Taken together, these data support the view that compulsive behaviours likely reflect, at least in part, a disruption of the dopaminergic system, more specifically by a decrease in baseline phasic dopamine signalling mediated by burst firing of dopamine neurons.

Key words: Animal models, compulsive lever pressing, obsessive compulsive disorder, phasic dopamine.

Introduction

Obsessive compulsive disorder (OCD) is characterized by obsessions (intrusive recurrent thoughts) and compulsions (repetitive aberrant behaviour). Current effective therapeutic intervention in patients suggests that dopaminergic and serotonergic systems are involved in controlling compulsive behaviour (Denys et al., 2004b; Koo et al., 2010). Thus, dopamine antagonists are often substituted or added to serotonin reuptake inhibitors to enhance therapeutic efficacy (Koo et al., 2010). Furthermore, imaging studies suggest the presence of a complex imbalance in the dopamine system in OCD patients (Kim et al., 2003; Denys et al., 2004a; van der Wee et al., 2004; Hesse et al., 2005; Moresco et al., 2007; Perani et al., 2008; Olver et al., 2009). Albeit debatable, the data suggest that OCD patients exhibit lower dopamine receptor availability primarily in the striatum secondary to increased dopamine drive.

Obsessions cannot be effectively approximated in animal models. Thus, research has focused on developing models for compulsivity (for review, see Albelda and Joel, 2011). Szechtman et al. (1998) proposed a pharmacological model produced by repeated injection of the dopamine D\textsubscript{2} agonist quinpirole that induces compulsive checking. This model is responsive to treatments used therapeutically for OCD such as clomipramine (Szechtman et al., 1998) and deep brain stimulation (Winter et al., 2008; Klavir et al., 2009; Mundt et al., 2009). Recently, Andersen et al. (2010) introduced an OCD model based on repeated injection of a drug during a sensitive developmental period. Adult rats showed increased anxiety level, perseveration and working memory deficits, which occurred in concert with increased levels of mRNA for serotonin 5-HT\textsubscript{2c} receptors and dopamine D\textsubscript{2} receptors in the orbitofrontal cortex and striatum.

In this paper, we challenge these two models in combination with post-training signal attenuation (PTSA) procedure, a behavioural model developed to assess compulsive lever pressing (Joel and Avisar, 2001). Additionally we examined elevated plus maze (EPM)
and spontaneous alternation behaviour (SAB) to compare with previously published data on these models. Finally, in order to further characterize the role of the dopamine system in compulsive behaviour, we evaluated the activity state of mesolimbic dopamine neurons to parallel the behavioural testing.

**Materials and method**

**Subjects**

Male Sprague–Dawley rats (Harlan Laboratories, USA) were housed pair-wise on a 12-h light/dark cycle. Litters for clomipramine treatment were acquired at post-natal day (PD) 4 and were initially housed with the dam in a normal light cycle. Weaning occurred at PD 24 and the mother and all the females were euthanized. An average litter provided between five and six males, which were moved to a 12 h/12 h light/dark reverse cycle (lights on 07:00 hours) room at PD 75, 10 d prior to any behavioural experiments. The quinpirole-treatment rats arrived weighing ~300 g and were placed into reverse light cycle housing. Animals that underwent electrophysiological recording remained under normal light cycle. All protocols were consistent with the guidelines outlined in the USPHS Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

**Procedures**

**Spontaneous alternation behaviour**

SAB is T-maze based. Subjects are expected to randomly alternate between right and left arm choices to optimize the area investigated during exploration. Arm choice was recorded for a total of nine trials. Failure to choose within 90 s resulted in trial cancellation. The maze was cleaned with 70% ethanol solution between each attempted trial; the subject waited in a holding cage.

**Elevated plus maze**

The EPM consisted of two open and two closed arms attached to a central platform (hub) and raised 50 cm above the floor. Each rat’s exploratory behaviour was examined over a 5-min period and included measures of time in each compartment (open arms, closed arm and hub) and total entries into the open and closed arms.

**Signal attenuation and regular extinction**

The signal attenuation (SA) model was developed (Joel and Avisar, 2001) based on the concept that compulsive behaviours result from a deficit in feedback associated with performance of normal goal-directed responses. Three days before experiments, rats were subjected to food deprivation that maintained ≥ 85% of free feeding body weight, monitored daily prior to testing.

During stage 1, days 1–3, rats were trained to collect single food pellets paired with a compound stimulus (CS: magazine light and tone). The CS was turned off after the rat visited the food magazine or after 15 s, followed 30 s inter-trial intervals. Daily training continued until 30 collected trials (e.g. when the rat visited the food magazine during CS) or until 40 total trials were reached.

During stage 2, day 4–6, rats were presented with two levers into the chamber. Responding on the reinforced lever (RL) resulted in the delivery of a single food pellet into the magazine contingent to CS presentation. The lever designated as RL was counter-balanced among subjects, but was constant for each rat across the procedure. Further lever presses on the RL or responses on the non-reinforced lever (NRL) had no programmed outcome, but were recorded. The levers were retracted and the CS was turned off following entry into the magazine panel or 15 s following the initial press on the RL. Each trial was followed by a 30 s inter-trial interval. A completed trial (CT) means that a RL press is followed by magazine entry during CS presentation. On day 4, the training stopped after 24 CTs or 60 total trials. Rats that failed to reach 20 CTs were returned to the test chamber at the end of the day for an additional session; rats that failed for the second time to reach 20 CT were excluded from the study. On days 5 and 6, training ended following 40 CT or 60 total trials.

We monitored the number of CT, the number of unpressed trials (UPT: no RL press) and uncompleted trials (UCT: rat pressed RL but did not visit magazine). In addition, the general behavioural output was assessed in terms of NRL press (NRLP) and extra lever presses after the first RL press (ELP). For the latter measure we also monitored whether ELP occurred during uncompleted trials (ELP-U) or during completed trials (ELP-C).

In stage 3, days 3–9, rats were subjected to regular extinction (RE; 3 d in home cage) or to signal attenuation post-training (PTSA). PTSA consisted of presentation of the CS without food delivery. No levers were presented. Daily sessions lasted 30 trials. The number of first visits to the magazine following CS (collected trial) was monitored.

In stage 4, day 10, rats were tested as in stage 2, but pressing the lever resulted in the presentation of the CS without food delivery. The session lasted for 50 trials. The behavioural measures monitored were identical to stage 2.

A pro-compulsive effect is evidenced by an increase in ELP that is not followed by magazine entry in rats that underwent SA but not RE (for review, see Joel, 2006). A correct extinction response is defined as a decrease in CT and increase in UPT. General behavioural output is estimated with ELPs and NRLP. A contingent change in UCT and ELP-U was interpreted as an increase (or decrease) in compulsive lever pressing.
Drug pretreatment and procedures

Clomipramine-treated rats

Injection schedule and drug administration. The injection schedule was based on the published OCD model (Andersen et al., 2010). Between PD 9 and PD 16, rats were weighed daily at 09:00 hours and given an i.p. injection of physiological saline vehicle or 15 mg/kg clomipramine hydrochloride dissolved in saline (0.9 mg/ml; Sigma-Aldrich, USA), followed 7 h later by a second injection. Pups and matched littersmates received a total of 16 injections. After 1 wk of handling the testing periods started at PD 85. As a general observation, during the injection schedule rats exposed to clomipramine tended to weigh less than controls (10–20%, data not shown), but this was no longer the case at testing.

Single exposure behavioural battery. For this behavioural series, four litters were divided into clomipramine exposure (Clo) and saline (control) groups. Rats were tested during the 2 wk following PD 85 (10 Clo and 12 controls). During the first week rats were subjected to SAB and the following week to EPM; testing occurred during the dark phase. For 3 d prior to each experiment the rats were acclimated to the transportation and the testing environment for 1 h/d. During EPM testing, two Clo and one control were removed from the analysis due to falling off the open arms.

Post-training signal attenuation procedure. Six litters of rats that were pretreated with either clomipramine or vehicle (saline) were subjected to PTSA procedure. Training started at PD 85. Two rats (one from each group) were excluded because they failed stage 1, leaving 16 rats in the Clo group (post-training procedure: 10 SA and 6 RE, respectively Clo-SA and Clo-RE) and 16 rats in the control group (post-training procedure: 10 SA and 6 RE, respectively Sal-SA and Sal-RE).

Ventral tegmental area activity recording. Recordings were performed on four litters, evenly divided between saline and clomipramine injection. One rat died during the surgery (Clo) and two rats were excluded due to incorrect electrode placements. The final analysis of the collected data is based on 10 rats subjected to clomipramine and 10 rats subjected to saline, yielding recordings from 70 and 62 dopamine neurons, respectively.

Quinpirole-treated rats

Injection schedule and drug administration. Quinpirole hydrochloride (Sigma-Aldrich, USA) was dissolved in physiological saline (0.5 mg/ml) and injected s.c. under the nape of the neck (Quin; 0.5 mg/kg) twice per wk at 3–4 d intervals for 7 wk, for a total of 14 injections (Szechtmian et al., 1994). Vehicle animals were injected in parallel with equal volume of vehicle (saline).

Single exposure behavioural battery. A total of 24 animals were subjected to the same order of testing as described for the clomipramine group. Additionally, the rats were tested for SAB and EPM 30–60 min following respectively the 12th and 14th injection of quinpirole or saline. Each group initially consisted of 12 rats; two Quin animals did not reach a total of nine choices after 50 attempted trials during the SAB test and were excluded from the SAB analysis. Additionally, five rats fell of the maze and were excluded from the EPM analysis, leaving nine Quin rats and eight control rats.

Post-training signal attenuation procedure. A total of 40 rats were injected with quinpirole (Quin) or saline (control) for 5 wk prior to starting the PTSA procedure. Day 1 of training occurred following the 11th injection while day 10 occurred at the last injection of the schedule (i.e. 14th).
One control rat failed stage 1 and three failed at stage 2 and were excluded from analysis. Therefore, 20 rats in the Quin group (10 SA and 10 RE; respectively Quin-SA and Quin-RE) and 19 rats in saline group (10 SA and 9 RE; respectively Sal-SA and Sal-RE) were analysed.

**Histology**

Following each electrophysiological experiment, the recording site was marked using electrophoretic ejection of Chicago Sky Blue dye (-20 μA constant current: 20–30 min). Rats were euthanized with an overdose of chloral hydrate (400 mg/kg i.v.), decapitated and their brains removed. Brains were submerged in 8% paraformaldehyde (in PBS) for fixation for a period of at least 48 h and then transferred to a 25% sucrose solution (in PBS) for cryoprotection before being frozen and sectioned on a cryostat in the coronal plane (thickness: 60 μm). Sections were placed on gelatin-chromalum-coated slides and stained using Cresyl Violet for histochemical verification of electrode placement with reference to a stereotaxic atlas (Paxinos and Watson, 1998).

**Statistics**

For electrophysiological recordings, Clo data were analysed using the Student’s *t* test for all parameters. The three control Quin treatments were compared using one-way analysis of variance (ANOVA). Because no differences were observed (all *F* <0.36; n.s.), the control data were combined. The three treatment groups and the controls were compared using one-way ANOVA for all parameters and if significant, Dunnett’s *t* (two-sided) *post hoc* test was performed (using control group as reference).

The behavioural data for stage 2 and 3 was compared between treated animal (Clo and Quin) and their respective control groups using Student’s *t* test for all parameters. Stage 2 and stage 4 were compared using a two-way ANOVA for repeated measures with post-training period (day 6 and day 10) and treatment (treated vs. controls) as independent factors. This analysis was done separately if rats underwent RE or PTSA. Stage 2 has 40 trials while stage 4 has 50 trials in total. We computed a ratio of type of trials (CT, UPT and UCT) to allow for a comparison. At day 6 none of the animals expressed UCT so no ELP-U could be measured.

Finally, the data collected at stage 4 for RE and PTSA groups were compared using a two-way ANOVA with extinction type (RE vs. SA) and treatment (treated vs. controls). The least significant difference method was used for *post hoc* analysis.

For all tests, a two-tailed *p* <0.05 was considered to be significantly different between the tested groups.

**Results**

**Clomipramine model**

All Clo-series behavioural measures are summarized in Table 1 (EPM and SAB) and Table 2 (PTSA).

**Spontaneous alternation, elevated plus-maze and post-training signal attenuation behaviour**

There was no difference between the Clo rats and the saline controls in alternations at PD 85 (*t*<sub>na</sub> = 0.90, n.s.).
Table 1. Summary of the behavioural measurements collected during spontaneous alternation behaviour and elevated plus maze tests of animals exposed to clomipramine or quinpirole chronically and their respective controls

<table>
<thead>
<tr>
<th>Behavioural measures</th>
<th>Controls</th>
<th>Clomipramine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated with clomipramine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous alternation behaviour</td>
<td></td>
<td></td>
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<tr>
<td>Number of alternations</td>
<td>4.8 ± 0.4</td>
<td>4.3 ± 0.4</td>
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<tr>
<td>Elevated plus maze</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time spent in open arms (%)</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Treated with quinpirole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous alternation behaviour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of alternations</td>
<td>4.3 ± 0.4</td>
<td>2.7 ± 0.6*</td>
</tr>
<tr>
<td>Elevated plus maze</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time spent in open arms (%)</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Time spent in open arms (%)</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
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</tbody>
</table>

* Indicates significant effect compared to respective controls (p<0.05).

Results are reported as mean ± S.E.M.

Furthermore, no difference between groups was found during the EPM test (time in closed arms: t₁₀ = 0.25, n.s.; time in open arm: t₁₀ = 0.32, n.s.) or in CT at stage 2 (day 6) or in general behavioural output and type of trials composition at stage 3 (day 9; t<1 for all, n.s.).

Effect of juvenile exposure to clomipramine on regular extinction. Both saline and Clo rats at stage 4 were affected by the RE procedure (all F₁,₁₉ > 18.72, p<0.001 for all). Post hoc analysis showed that following RE the ratio of CT decreased, while the UPT and UCT increased. This is indicative of an extinction process in reaction to the absence of expected reward.

Both behavioural output measures increased after RE, but only NRLP was sensitive to clomipramine treatment. Rats produce more ELP-C following RE (post-training period: t₁₉ = 42.82, p<0.001), without treatment effect or interaction (F<1.32 for all, p>0.05). However, the number of NRLP was affected by both clomipramine exposure (treatment: F₁,₁₉ = 5.17, p = 0.046) and RE (post-training period: F₁,₁₉ = 25.04, p = 0.001) and their interaction (F₁,₁₉ = 5.16, p = 0.046). Animals exposed to clomipramine showed a higher increase of NRLP than controls after RE. The increase of lever presses following RE matches previous observations and was referred to as extinction burst (Joel and Avisar, 2001).

Effect of juvenile exposure to clomipramine on post-training signal attenuation. The ratio of CT decreased in saline and clomipramine rats at day 10, while UPT and UCT increased (post-training period: all F>73.21, p<0.001 for all) indicating an extinction process.

NRLP were sensitive to SA (post-training period: t₁₉ = 42.82, p<0.001), but not to clomipramine treatment or their interaction (all F<1.83, n.s.); NRLP were greater at day 10. ELP-C remained unaffected by clomipramine (F₁,₁₉ = 2.14; n.s.) or SA (F₁,₁₉ = 3.51, n.s.). Overall, clomipramine treatment did not affect the general behavioural output following SA.

Comparison between regular extinction and signal attenuation at day 10. The number of UCT was higher in rats that underwent SA than RE (Fig. 1b; extinction type: F₁,₁₉ = 9.83, p = 0.004). No treatment effect or interaction was found (all F<2.5, n.s.). Similarly, rats produced more ELP-U following SA (extinction type: F₁,₁₉ = 6.46, p = 0.017) without treatment or interaction effects (all F<2.2, n.s.; Fig. 1d). Clomipramine exposure did not potentiate compulsive behaviour induced by PTSA.

Rats that underwent RE exhibited more CT than the groups trained with SA (extinction type: F₁,₁₉ = 24.55, p<0.001 Fig. 1a). Conversely, rats exposed to SA produced more UPT than rats exposed to RE (extinction type: F₁,₁₉ = 14.85, p = 0.001). No treatment effect or interaction was found (all F<2.6, n.s.). SA increased the sensitivity to reward extinction.

Animals exposed to RE produced more ELP-C (extinction type: F₁,₁₉ = 8.71, p = 0.006). No treatment or interaction effects were observed (all F<2.3, n.s.; Fig. 1c). Despite no extinction type or treatment effects (all F<0.67, n.s.), NRLP analysis showed an interaction effect (F₁,₁₉ = 6.60, p = 0.016): Clo-RE animals expressed more NRLP than any other group. Clomipramine exposure did not alter the general behavioural output in this paradigm.

VTA recordings

No differences were found between controls and Clo group in number of active dopamine neurons (firing t₈₄ = 0.09, n.s.; Fig. 2a, Table 3), their average firing rate (t₈₄ = 1.62, n.s.; Fig. 2b) or the ratio of bursting dopamine neurons (t₈₄ = 1.50, n.s.; Fig. 2c). Furthermore, there were no differences in activity across the anterior–posterior axis of the VTA. However, dopamine neurons in Clo animals exhibited fewer spikes occurring in bursts (t₈₄ = 2.97, p = 0.04; Fig. 2d) and burst frequency (t₈₄ = 3.51, p = 0.001), plus a higher interburst interval (t₈₄ = 3.35, p = 0.001). No effect was found in the average number of spikes per burst (t₈₄ = 1.699, n.s.). Therefore, clomipramine exposure led to a decrease in bursting activity in the VTA by altering burst frequency but not burst structure.

Quinpirole model

All behavioural measures are summarized in Table 1 (EPM and SAB) and Table 2 (PTSA).
Table 2. Summary of the behavioural measurements collected during post-training signal attenuation procedure of animals exposed to clomipramine or quinpirole chronically and their respective controls

<table>
<thead>
<tr>
<th>Behavioural measures</th>
<th>Controls (RE)</th>
<th>Clomipramine (RE)</th>
<th>Controls (SA)</th>
<th>Clomipramine (SA)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lever-press training</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Completed trials</td>
<td>40.0 ± 0.0</td>
<td>40.0 ± 0.0</td>
<td>40.0 ± 0.0</td>
<td>40.0 ± 0.0</td>
</tr>
<tr>
<td>Unpressed trials</td>
<td>0.3 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.6 ± 0.2</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Uncompleted trial</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Extra-lever press – C</td>
<td>10.7 ± 1.4</td>
<td>10.8 ± 2.6</td>
<td>19.3 ± 6.3</td>
<td>12.7 ± 3.2</td>
</tr>
<tr>
<td>Non rewarded-lever press</td>
<td>0.7 ± 0.5</td>
<td>1.0 ± 0.3</td>
<td>1.1 ± 0.5</td>
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<td><strong>Signal attenuation</strong></td>
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</tr>
<tr>
<td>Completed trials</td>
<td>10.0 ± 1.0</td>
<td>8.6 ± 1.1</td>
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<td></td>
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<tr>
<td>Uncompleted trial</td>
<td>20.0 ± 1.0</td>
<td>21.4 ± 1.1</td>
<td></td>
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<tr>
<td><strong>Test</strong></td>
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<td></td>
</tr>
<tr>
<td>Completed trials</td>
<td>24.2 ± 4.0</td>
<td>27.0 ± 2.3</td>
<td>12.4 ± 1.7a</td>
<td>13.0 ± 2.4a</td>
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<td>Unpressed trials</td>
<td>20.7 ± 3.1</td>
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<td>27.2 ± 1.4a</td>
<td>29.4 ± 1.8a</td>
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<td>5.2 ± 1.8</td>
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<tr>
<td>Extra-lever press – C</td>
<td>34.5 ± 6.8</td>
<td>42.2 ± 4.4</td>
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<td>21.7 ± 4.3a</td>
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<td>Extra-lever press – U</td>
<td>29.2 ± 10.1</td>
<td>22.5 ± 5.2</td>
<td>61.8 ± 10.1a</td>
<td>43.4 ± 10.3a</td>
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<td>Non rewarded-lever press</td>
<td>7.7 ± 1.6</td>
<td>19.7 ± 5.0b</td>
<td>18.2 ± 3.5</td>
<td>12.0 ± 2.6</td>
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<td><strong>Lever-press training</strong></td>
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<tr>
<td>Completed trials</td>
<td>40.0 ± 0.0</td>
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<tr>
<td>Unpressed trials</td>
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<td>0.6 ± 0.4</td>
<td>0.1 ± 0.1</td>
<td>0.3 ± 0.2</td>
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<tr>
<td>Uncompleted trial</td>
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<td>23.7 ± 6.6</td>
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<td>Non rewarded-lever press</td>
<td>3.7 ± 2.0</td>
<td>3.7 ± 1.6</td>
<td>3.5 ± 1.9</td>
<td>2.0 ± 0.9</td>
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<tr>
<td><strong>Signal attenuation</strong></td>
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</tr>
<tr>
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<td>7.7 ± 1.4</td>
<td>11.2 ± 2.0</td>
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<tr>
<td>Uncompleted trial</td>
<td>22.3 ± 1.4</td>
<td>18.8 ± 2.0</td>
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<tr>
<td><strong>Test</strong></td>
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<tr>
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<td>23.1 ± 2.5</td>
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<td>12.5 ± 1.9a</td>
<td>16.6 ± 3.2a,b</td>
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<tr>
<td>Unpressed trials</td>
<td>17.9 ± 1.7</td>
<td>7.9 ± 2.6b</td>
<td>26.6 ± 1.9a</td>
<td>14.2 ± 3.5a,b</td>
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<tr>
<td>Uncompleted trial</td>
<td>9.0 ± 1.5</td>
<td>5.7 ± 1.7</td>
<td>10.9 ± 1.2a</td>
<td>19.2 ± 3.2a,c</td>
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<td>12.4 ± 4.7b</td>
</tr>
<tr>
<td>Extra-lever press – U</td>
<td>37.7 ± 4.3</td>
<td>9.0 ± 3.7</td>
<td>45.6 ± 4.7b</td>
<td>43.3 ± 16.2a</td>
</tr>
<tr>
<td>Non rewarded-lever press</td>
<td>11.7 ± 2.2</td>
<td>2.0 ± 0.8b</td>
<td>15.9 ± 3.5</td>
<td>2.9 ± 0.9b</td>
</tr>
</tbody>
</table>

RE, Regular extinction; SA, signal attenuation; C, completed trials; U, uncompleted trials.

abc Indicate significant effect respectively for extinction type, drug treatment and extinction type × drug treatment interaction (p < 0.05).

Results are reported as mean ± S.E.M.

Spontaneous alternation, elevated plus maze and post-training signal attenuation behaviour

Animals exposed to quinpirole performed significantly less alternations than controls (t18 = 2.225, p = 0.038). There was no difference between the Quin rats and controls when exposed to the EPM (time in closed arms: t18 = 0.33, n.s.; time in open arms: t18 = 0.68, n.s.) or in CT at stage 2 (day 6) or in general behavioural output and type of trials composition at stage 3 (day 9; all t < 1.1, n.s.).

Effect of chronic exposure to quinpirole on regular extinction. At day 10, CT and UPT occurrences were affected by RE (post-training period: CT F1,15 = 92.73, p < 0.001; UPT F1,15 = 72.37, p < 0.001), quinpirole exposure (treatment: CT F1,15 = 9.07, p = 0.008; UPT F1,15 = 9.60, p = 0.007) and their interaction (CT F1,17 = 10.55, p = 0.005; UPT F1,17 = 11.80, p = 0.003). Quin rats expressed more CT and less UPT than controls (Sal-RE) after RE, which could indicate an impaired extinction process.

The ratio of UCT was sensitive to RE (post-training period: F1,17 = 41.40, p < 0.001) with no effect of quinpirole injections and no interaction (all F < 0.20, n.s.). UCT were non-existent at stage 2 (day 6) but represented 11% of the trials at stage 4 (day 10).

ELP-C were unaffected by RE or quinpirole injections (all F < 1.2, n.s.). RE procedure promoted NRP (post-training period: F1,17 = 5.38, p = 0.033), but only in controls (interaction: F1,17 = 12.54, p = 0.003). Quin rats expressed less NRP than controls (treatment: F1,17 = 5.28, p = 0.035). Unlike clomipramine rats, Quin animals did not express extinction burst.
Fig. 1. Clomipramine exposure effect on post-training signal attenuation (PTSA). Juvenile exposure to clomipramine does not further affect compulsive lever pressing following PTSA or performance after regular extinction. The upper panels show the number (mean ± S.E.M.) of (a) completed (CT) and (b) uncompleted trials (UCT) in rats exposed to clomipramine (black bars) and their controls (white bars). The mean number of CT decreases when exposed to signal attenuation in both groups and the number of UCT increased in both groups as well. The lower panels show the number (mean ± S.E.M.) of extra lever presses (ELPs) that were followed by an attempt to collect a reward (c), ELP-CT and that were not followed by an attempt to collect a reward (d), ELP-UCT. The mean number of ELP-CT decreases when trained with signal attenuation while the number of ELP-UCT increases in both controls and treated animals. * Indicates significant effect (p < 0.005).

Fig. 2. Clomipramine exposure effect on ventral tegmental area (VTA) activity. Juvenile exposure to clomipramine decreases the bursting activity of dopamine neurons in the VTA at the same age as that of behavioural testing. Measures are presented as mean ± S.E.M.; controls are shown as white bars and clomipramine-exposed animals (Clo) as black bars. The upper panels show the average number of active dopamine neurons per electrode track (a) and their average firing rate (b). The lower panels present the proportion of bursting neurons in the recorded population (c) and the percentage of spikes that occurred in bursts for the spontaneously active dopamine neurons (d). Only the percentage of spikes in bursts was affected by the clomipramine pre-treatment and showed a 41% decrease compared to controls. *Indicates significant effect (p < 0.005).
Table 3. Summary of the extracellular recording data from dopamine neurons in the ventral tegmental area (VTA) of animals exposed to clomipramine, quinpirole chronically and then withdrawn (Quin-Wth), chronically (Quin-Chc), acutely (Quin-Acu) and their respective controls

<table>
<thead>
<tr>
<th>VTA recording measures</th>
<th>Controls</th>
<th>Quin-Wth</th>
<th>Quin-Chc</th>
<th>Quin-Acu</th>
<th>Statistical significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bursting neuron ratio</td>
<td>0.66 ± 0.05</td>
<td>0.63 ± 0.05</td>
<td>0.27 ± 0.10*</td>
<td>0.43 ± 0.15</td>
<td>&lt;0.009</td>
</tr>
<tr>
<td>Cells per track</td>
<td>1.01 ± 0.05</td>
<td>1.49 ± 0.99*</td>
<td>0.60 ± 0.09*</td>
<td>0.43 ± 0.08*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Firing rate (Hz)</td>
<td>3.41 ± 0.18</td>
<td>4.07 ± 0.25</td>
<td>2.48 ± 0.33</td>
<td>2.24 ± 0.35*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spikes in bursts (%)</td>
<td>28.03 ± 2.33</td>
<td>27.35 ± 3.38</td>
<td>8.75 ± 3.42*</td>
<td>13.48 ± 4.48*</td>
<td>0.001</td>
</tr>
<tr>
<td>Burst frequency (Hz)</td>
<td>0.52 ± 0.04</td>
<td>0.66 ± 0.06</td>
<td>0.25 ± 0.07</td>
<td>0.37 ± 0.10</td>
<td>n.s.</td>
</tr>
<tr>
<td>Spikes per burst</td>
<td>3.29 ± 0.19</td>
<td>3.21 ± 0.16</td>
<td>3.35 ± 0.45</td>
<td>2.84 ± 0.15</td>
<td>n.s.</td>
</tr>
<tr>
<td>Interburst interval (s)</td>
<td>3.35 ± 0.38</td>
<td>2.54 ± 0.42</td>
<td>5.15 ± 1.67</td>
<td>5.33 ± 2.05</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VTA recording measures</th>
<th>Control</th>
<th>Clomipramine</th>
<th>Statistical significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bursting neuron ratio</td>
<td>0.72 ± 0.07</td>
<td>0.56 ± 0.08</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cells per track</td>
<td>0.99 ± 0.06</td>
<td>0.98 ± 0.10</td>
<td>n.s.</td>
</tr>
<tr>
<td>Firing rate (Hz)</td>
<td>3.69 ± 0.21</td>
<td>3.24 ± 0.18</td>
<td>n.s.</td>
</tr>
<tr>
<td>Spikes in bursts (%)</td>
<td>24.83 ± 2.93</td>
<td>14.68 ± 1.89</td>
<td>0.004</td>
</tr>
<tr>
<td>Burst frequency (Hz)</td>
<td>0.53 ± 0.06</td>
<td>0.29 ± 0.04</td>
<td>0.001</td>
</tr>
<tr>
<td>Spikes per burst</td>
<td>2.85 ± 0.11</td>
<td>2.62 ± 0.08</td>
<td>n.s.</td>
</tr>
<tr>
<td>Interburst interval (s)</td>
<td>2.92 ± 0.42</td>
<td>5.94 ± 0.80</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Indicates significant difference from controls (p < 0.005). Results are reported as mean ± S.E.M.

Effect of chronic exposure to quinpirole on post-training signal attenuation at day 10. The ratio of UCT was affected by SA (post-training period: $F_{1,35} = 78.07$, p < 0.001), quinpirole injections (treatment: $F_{1,35} = 5.94$, p = 0.025) and their interaction ($F_{1,35} = 5.94$, p = 0.025). This indicates that the PTSAs triggered more non-goal-directed behaviour in Quin animals (Quin-SA) than in controls (Sal-SA).

SA affected both the ratio of CT (post-training period: $F_{1,35} = 327.57$, p < 0.001) and UPT (post-training period: $F_{1,35} = 99.65$, p < 0.001), respectively decreasing and increasing these measures following SA. Only UPT was also sensitive to quinpirole exposure (treatment: $F_{1,35} = 9.77$, p = 0.006) and their interaction ($F_{1,35} = 9.79$, p = 0.006). The Sal-SA group expressed more UPT than Quin-SA. This effect could result from the great increase in UCT which would have had to occur at the expense of another type of response and did not necessarily preclude any extinction process deficiency.

Similar to that in RE groups, no differences were found with ELP during CT (ELP-C: all F < 2.80, n.s.). In addition, NRLP was sensitive to SA (post-training period: $F_{1,35} = 13.92$, p = 0.002), quinpirole exposure (treatment: $F_{1,35} = 9.20$, p = 0.007) and their interaction ($F_{1,35} = 10.41$, p = 0.005). The number of NRLP increased following stage 3 only in controls, which together shows that the general behavioural output is lower in Quin animals.

Comparison between regular extinction and signal attenuation post-training. The number of UCT was higher when animals underwent SA (extinction type: $F_{1,35} = 13.86$, p = 0.001; Fig. 3b). There was a treatment × extinction type factors interaction ($F_{1,35} = 7.88$, p = 0.008), but no treatment effect ($F_{1,35} = 1.44$, n.s.). Quin-SA UCT occurrences were significantly higher than in controls. Moreover, the number of ELP-U was increased by SA (extinction type: $F_{1,35} = 5.37$, p = 0.026; Fig. 3d). No other effect was found (all F < 2.89, n.s.). The compulsive behaviour (UCT and ELP-U) was consistent with previously published work on PTSA schedule (Joel and Dolmansky, 2003); furthermore, chronic exposure to quinpirole increased the number of non-goal-directed responses following SA and ELP-U apparently was not affected by the general decrease in behavioural output. Overall these data suggest a potentiation of the effect of the procedure on compulsive behaviour following quinpirole injections.

The number of CT was higher following RE (Fig. 3d), while the number of UPT was higher in groups that underwent PTSA (extinction type: CT $F_{1,35} = 29.42$, p < 0.001; UPT $F_{1,35} = 8.95$, p = 0.005). Moreover, Quin animals performed more CT and less UPT than their respective controls, suggesting an alteration of extinction processes induced by the drug (treatment: CT $F_{1,35} = 9.66$, p = 0.004; UPT $F_{1,35} = 19.95$, p < 0.001). No interaction was found (all F < 2.71; n.s.). SA again promoted the extinction process, while quinpirole injections tended to act against it.

The general behavioural output (ELP-C and NRLP) was affected in the same way by chronic quinpirole injection (treatment: respectively, $F_{1,35} = 4.37$, p = 0.044;
Both types of lever presses were decreased in Quin animals regardless of the type of post-training to which they were exposed (post-training and interactions: all $F < 2.98$, n.s.).

**VTA recordings**

All control groups were merged and compared to rats: withdrawn after chronic exposure (Quin-Wth), chronically exposed (Quin-Chc) and acutely exposed (Quin-Acu) to quinpirole. All measurements are reported in Table 3.

The number of spontaneously active dopamine neurons was altered by the various quinpirole injections regimen ($F_{3,32} = 34.18$, $p < 0.001$): Quin-Chc and Quin-Acu rats had significantly fewer active dopamine neurons per track; Quin-Wth had more active dopamine neurons, compared to controls (Fig. 4a). Furthermore, there were no differences in activity across the anterior–posterior axis of the VTA. Quin-Chc animals had fewer bursting dopamine neuron than controls ($F_{3,32} = 4.52$, $p = 0.009$; Fig. 4c). This change was associated with a lower %SIB ($F_{3,223} = 5.55$, $p = 0.001$; Fig. 4d). Quin-Acu resulted in a lower average firing rate compared to controls ($F_{3,223} = 7.00$, $p < 0.001$; Fig. 4b) and this was associated with a lower %SIB ($F_{3,223} = 5.57$, $p = 0.001$).

The burst structure was unaltered when comparing between groups. ANOVAs revealed a significant effect on burst frequency ($F_{3,223} = 3.18$, $p = 0.026$), but post hoc tests did not reveal any differences between treated and control groups. The other parameters were not affected (all $F < 2.20$, n.s.). Therefore, acute and chronic exposure to quinpirole decreased VTA activity both in terms of active cells and bursting activity; withdrawal in contrast increased overall VTA output.

**Discussion**

In comparing two models of OCD, we found that the clomipramine model, in our hands, did not robustly replicate the behavioural features of OCD adequately to use as a screen. The clomipramine model did not further affect indices of behaviour using EPM, SAB or PTSA paradigms. In contrast, the quinpirole model showed several alterations consistent with an augmentation of compulsive behaviour but without affecting anxiety level. This included the highest number of UCT and a 300% increase in ELP during these trials compared to the performance prior to the SA stage. We also found that dopamine neuron activity corresponded to behavioural outcomes. Exposure to clomipramine reduced VTA output.

$F_{3,32} = 26.08, p < 0.001$; ELP-C: Fig. 3c). Both types of lever presses were decreased in Quin animals regardless of the type of post-training to which they were exposed (post-training and interactions: all $F < 2.98$, n.s.).
dopamine neuron bursting activity, whereas repeated injection of quinpirole reduced both the number of bursting dopamine neurons and the %Sib. Given that this was a decrease in baseline burst firing, stimulus-evoked bursting should therefore have a greater relative impact in the Quin rats.

Compulsive behavioural model

Rats treated with clomipramine did not show differences in EPM or SAB performance, which differs from a previous report (Andersen et al., 2010). Treated animals also did not exhibit potentiation in the PTSA paradigm. Behavioural outcomes for both RE and SA groups were comparable to controls. The Clo-RE group expressed a specific increase in NRLP. No other lever presses were affected; therefore, an increase in general behavioural output resulting from RE is unlikely. Alternatively, it can indicate an enhanced sensitivity to reward extinction which translates to a greater extinction burst specific to the unrewarded lever.

Rats treated with quinpirole showed increased preservative behaviour (decreased alternation in SAB) with no effect on the level of anxiety (EPM). A decrease in alternation has been reported previously for the quinpirole model (Einat and Szechtman, 1995). To our knowledge, no data were reported previously on the effects of repeated injection of quinpirole on EPM exploration. However, it was reported that local infusion of high doses of quinpirole into the basal lateral amygdala resulted in less time spent in open arms without altering the time spent in closed arms (Bananej et al., 2012). A single local high dose may not compare to chronic systemic injection. Repeated injection of quinpirole potentiated the compulsive lever pressing (combined effect on UCT and ELP-U). The increase in UCT is substantial, while the effect on ELP-U is dampened by the general decrease in behavioural output. The increase in ELP-U following SA is specific because all other lever pressing parameters were decreased in Quin groups regardless of the post-training (i.e. NRLP and ELP-C). Additionally, rats treated with quinpirole showed impaired extinction: the number of CT in the non-rewarded condition increased in all groups exposed to quinpirole injections at the expense of the UPT (correct extinguished response) similar to that reported previously (Kurylo and Tanguay, 2003; Kurylo, 2004; Dubrovina and Zinov’eva, 2010).
effect of the treatment on UCT is specific to Quin-SA (treatment × procedure factors interaction effect). The fact that the Quin-SA group expresses more UCT than any other group can explain the weaker drug effect on its CT numbers.

Mesolimbic dopaminergic activity and compulsive lever pressing

Clomipramine-exposed rats exhibited a decrease in VTA dopamine neurons without exhibiting behavioural alterations.

Acute injection of quinpirole decreased the number of active dopamine neurons in the VTA and decreased the activity of the spontaneously firing dopamine neurons (lower firing rate and fewer spikes/burst). To our knowledge, no behavioural data are available for compulsive behaviour at that stage.

Repeated injection of quinpirole is known to induce long-term changes in dopaminergic receptors by down-regulating both the number of receptors available and their mRNA levels (Chen et al., 1993). If dopamine D$_2$ receptors are down-regulated, we expected the VTA activity in the Quin-Chc group to be less affected by the final exposure prior to recording. However, chronic exposure to quinpirole induced stronger changes in VTA activity: it reduced the number of active neurons as well as producing a marked shift in dopamine neuron activity from bursting to non-bursting, with the remaining bursting neurons showing fewer spikes in bursts and less frequent burst episodes. Therefore, at baseline the amount of bursts reaching the VTA projection sites would be significantly diminished. Coincidentally, we observed the strongest effect on compulsive behaviour following chronic treatment. The compulsive lever pressing observed in the PTSA schedule was hypothesized to reflect a decrease in phasic dopamine mediated by dopamine D$_1$ receptors in the striatum (Joel and Doljansky, 2003). Both low-affinity D$_1$ receptors (Gonon, 1997; Lodge and Grace, 2006a; Dreyer et al., 2010) and intra-synaptic D$_2$ receptors (Floresco et al., 2003) can be stimulated by phasic dopamine release secondary to burst firing of dopamine neurons (Floresco et al., 2003; Goto and Grace, 2005). Therefore, phasic burst firing and the number of dopamine neurons that can be recruited to burst fire (Lodge and Grace, 2006a) could provide an effective correlate of dopamine disruption in OCD. Given that the baseline, non-stimulated burst firing is less following quinpirole, this would suggest that stimulus-driven bursting should have a greater impact on behaviour, which is consistent with increased dopamine responsivity believed to contribute to this pathology. It has been reported that repeated daily injection of quinpirole induced more compulsive responses in the withdrawn state (Joel et al., 2001). Our results suggest that a withdrawn state induces an overactive VTA by increasing the number of active neurons. In this early work on the PTSA model, the effect on compulsive lever pressing was due to a general increase of ELP (regardless of the type of trials during which it was produced), in which the majority of them were generated during CT. The number of UCT previously reported in the Quin rats (Joel et al., 2001) was on average four against one–two in controls. Animals tested in our experiment generated on average 19.2 ± 3.2 UCT when exposed to quinpirole and 10.9 ± 1.2 in controls. In both cases response almost doubles.

Conclusion

In our hands neonatal injection of clomipramine failed to produce any sort of compulsive behaviour. This model has also been used as an animal model for endogenous depression (Vogel et al., 1988, 1990a, b, c, d; Andersen et al., 2002; Cassano et al., 2006; Bhagya et al., 2008). More investigation is needed to clarify which model juvenile exposure to clomipramine better approximates. In contrast, Quin animals demonstrated clear increases in compulsive behaviour in the PTSA test and a reliable perseveration in SAB test, which further strengthens the face validity of this model. Furthermore, we found that the degree of alteration in bursting activity of the VTA is predictive of the amount of uncompleted type of trials observed following SA and the associated amount of repetitive lever pressing.

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Statement of Interest

A. A. Grace has received consulting honoraria from Johnson & Johnson, Lundbeck, Pfizer, GSK, Puretech Ventures, Merck, Takeda, Dainippon Sumitomo, Otsuka, Eli Lilly and Roche.

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