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ARTICLE

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The role of 5-HT_{2A} and 5-HT_{2C} receptors in the signal attenuation rat model of obsessive–compulsive disorder

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Abstract

Serotonin 5-HT_{2A} and 5-HT_{2C} receptors have been implicated in the pathophysiology of obsessive–compulsive disorder (OCD) and in the mechanism mediating the anti-compulsive effects of serotonin reuptake inhibitors. Yet it is currently unclear whether activation or blockade of these receptors would have an anti-compulsive effect. The present study tested the effects of 5-HT_{2A} and 5-HT_{2C} activation and blockade in the signal attenuation rat model of OCD. In this model, 'compulsive' behaviour is induced by attenuating a signal indicating that a lever-press response was effective in producing food. Experiments 1–4 revealed that systemic administration of the 5-HT_{2C} antagonist RS 102221 (2 mg/kg) selectively decreases compulsive lever-pressing, whereas systemic administration of the 5-HT_{2A} antagonist MDL 11,939 (0.2–5 mg/kg) or of the 5-$HT_{2A/2C}$ agonist DOI (0.05–5 mg/kg) did not have a selective effect on this behaviour. Experiments 5 and 6 found that systemic co-administration of DOI (0.5 mg/kg) with MDL 11,939 (1 mg/kg) or with RS 102221 (2 mg/kg) had a non-selective effect on lever-press responding, with the former manipulation increasing and the latter manipulation decreasing lever-pressing. Finally, experiment 7 demonstrated that administration of RS 102221 directly into the orbitofrontal cortex also exerts an anti-compulsive effect. The results of these experiments suggest that blockade of 5-HT_{2C} receptors may have an anti-compulsive effect in OCD patients, and that this effect may be mediated by 5-HT_{2C} receptors within the orbitofrontal cortex.

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Introduction

Obsessive–compulsive disorder (OCD) is a psychiatric affliction with a lifetime prevalence of 2–3% (Sasson and Zohar, 1996). According to DSM-IV criteria (APA, 1994), the essential features of OCD are recurrent, intrusive and unwanted thoughts (obsessions) and/or repetitive ritualistic behaviours (compulsions). To date, the recommended pharmacotherapy for OCD is treatment with serotonin re-uptake inhibitors (SRIs; Dougherty et al., 2004). However, about 40–50% of patients exhibit no or only partial response to SRI therapy (Albert et al., 2002). Although some of these patients benefit from pharmacological augmentation treatment (for review see Albert et al., 2002) and cognitive-behavioural therapy (Miguel et al., 2003), there is clearly a need for improved psychotherapeutic drugs for OCD (Moreno et al., 2006). Because administration of SRIs leads to changes in serotonergic neurotransmission, one strategy for the development of new anti-compulsive drugs is to target directly specific 5-HT receptors.

Two of the receptors which have been implicated in the pathophysiology of OCD and in the mechanism mediating the therapeutic effect of SRIs in this disorder are the 5-HT_{2A} and 5-HT_{2C} receptors. However, whether activation or blockade of these receptors would have an anti-compulsive effect is currently unclear. For example, while intoxication with psychedelic drugs possessing potent 5-$HT_{2A/2C}$ agonist activity has been reported to alleviate symptoms in OCD patients (see Moreno et al., 2006 and references within), there is evidence that activation of 5-HT_{2C} receptors (typically by administration of m-chlorophenylpiperazine; mCPP) can exacerbate obsessive

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compulsive symptoms (see Gross-Isseroff et al., 2004 and references within; although other studies have failed to obtain this effect, see Khanna et al., 2001 and references within).

A similar inconsistency regarding the role of 5-HT_{2A} and 5-HT_{2C} receptors in compulsive behaviours exists in the animal literature. For example, activation of 5-HT_{2C} receptors has been shown to induce compulsive behaviour in some animal models of OCD (e.g. grooming and persistent alternation; Graf, 2006; Tsaltas et al., 2005), but to decrease it in other models (e.g. marble-burying and schedule-induced polydipsia; Bös et al., 1997; Martin et al., 2002).

Studies aiming to elucidate the mechanism of action of selective serotonin reuptake inhibitors (SSRIs) have also yielded inconsistent conclusions with regard to the role of 5-HT_{2A} and 5-HT_{2C} receptors. Blier and colleagues assessed in rodents the effects of chronic administration of SSRIs on 5-HT release and on 5-HT receptors. On the basis of their findings these authors have suggested that the therapeutic action of SSRIs in OCD is mediated by enhanced 5-HT release in the orbitofrontal cortex that activates normosensitive post-synaptic 5-HT_2 receptors (for review see El Mansari and Blier, 2006). Hence, these and other authors have suggested that compounds possessing strong 5-HT_{2A} (for review see Carlsson, 2001) or 5-HT_{2C} (Bös et al., 1997; Martin et al., 2002; Moreno et al., 2006) agonism would have a therapeutic effect in OCD. On the other hand, several lines of evidence suggest that chronic treatment with SSRIs leads to desensitization or down-regulation of 5-HT_{2A} and 5-HT_{2C} receptors in OCD patients and in animals (for review see Van Oekelen et al., 2003). These findings have led to the hypothesis that 5-HT_{2A} and/or 5-HT_{2C} receptor antagonists may offer a potential therapeutic advance in OCD (Graeff et al., 1996).

The present study tested the role of 5-HT_{2A} and 5-HT_{2C} receptors in compulsive behaviour using the signal attenuation rat model of OCD (for a recent review of the model see Joel, 2006). In this model, attenuation of a signal indicating that a lever-press response was effective in producing food, 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 SSRIs fluoxetine, paroxetine and fluvoxamine, but not by the anxiolytic drug, diazepam, or the tricyclic antidepressant, desipramine (Joel and Avisar, 2001; Joel et al., 2004), in accordance with the differential efficacy of these drugs in alleviating obsessions and compulsions in OCD patients (Zohar et al., 1992). Compulsive lever-pressing is also sensitive to manipulations of the orbitofrontal cortex (Joel et al., 2005a,b; Joel and Klavir, 2006), in line with different lines of evidence implicating this cortical region in the pathophysiology of OCD (for a recent review see Friedlander and Desrocher, 2006).

Experiments 1–3 tested the effects of systemic administration of the 5-HT_{2C} antagonist RS 102221 and of the 5-HT_{2A} antagonist MDL 11,939 on compulsive lever-pressing. RS 102221 is a selective 5-HT_{2C} antagonist, i.e. it is 100–200 times more selective for the 5-HT_{2C} receptor compared to other 5-HT_2 receptors and binding sites, in both rats and primates (Acuna-Castillo et al., 2002; Barnes and Sharp, 1999; Bonhaus et al., 1997, 1998; Knight et al., 2004). MDL 11,939 is a selective 5-HT_{2A} antagonist, showing greater selectivity for the 5-HT_{2A} vs. the 5-HT_{2C} and 5-HT_{1A} receptor subtypes and low or no affinity for non-5-HT_2 receptors (Pehek et al., 2006; Sramek et al., 1995). Because there are currently no well characterized highly selective agonists for 5-HT_{2A} and 5-HT_{2C} receptors (Baxter et al., 1995; Higgins and Fletcher, 2003; Knight et al., 2004), expts 4–6 tested the effects of systemic administration of the 5-$HT_{2A/2C}$ agonist DOI as well as of combined administration of DOI with either RS 102221 or MDL 11,939 on compulsive lever-pressing. Because the results of expts 1–6 have shown that only the 5-HT_{2C} antagonist RS 102221 had a selective effect on rats' compulsive lever-pressing, and in view of data implicating the orbitofrontal cortex in the pathophysiology of OCD (for a recent review see Friedlander and Desrocher, 2006) and in the mediation of the therapeutic effects of SSRIs (El Mansari and Blier, 2006), expt 7 tested the effects of intra-orbitofrontal injection of RS 102221 on compulsive lever-pressing.

Methods

Subjects

Sprague–Dawley rats (Harlan; Jerusalem, Israel) approximately 3–5 months old, were housed 2–4 to a cage under a reversed 12-h light–dark cycle (lights on 19:00 hours). Rats were maintained on a 22-h food restriction schedule (see below), with water freely available. They were weighed twice a week to ensure that their body weight was not reduced to below 90%. All experimental protocols were carried out according to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University.

Apparatus and behavioural procedure

Behavioural testing was conducted in four operant chambers (Campden Instruments, Loughborough, UK), housed in sound-attenuated boxes and equipped with a 3 W house light, a Sonalert module (Model SC 628) that could produce a 80 dB 2.8 kHz tone, 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 micro-switch. Equipment programming and data recording were computer controlled.

Prior to the beginning of the experiment, rats were handled for about 2 min daily for 5 d. A 22-h food restriction schedule began simultaneously with handling and continued throughout behavioural testing. Food was provided in the home cage at least half an hour after the end of the session. On the last 3 d, 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 (PTSA)

The PTSA procedure included four stages (in expt 7, surgery for cannulae implantation was conducted within the second stage).

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, simultaneous with the onset of a compound stimulus consisting of the magazine light and the tone. The compound stimulus was turned off after the rat's head entered the food magazine or after 15 s had elapsed, and a 30 s inter-trial interval began. 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 day 4, rats received a session of pre-training using a free-operant schedule. The house light was on and one lever was present in the operant box throughout the entire session. Responding on this lever (reinforced lever, RL) resulted in the delivery of a single food pellet into the magazine, accompanied by the presentation of the compound stimulus (magazine light and tone). 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. The lever designated as RL was counterbalanced over subjects and remained the same for each rat over the entire experimental procedure. Each rat was trained until it completed 30 trials, i.e. pressed the lever and inserted its head into the food magazine during stimulus presentation. Rats that failed to attain 30 completed trials within 30 min, were returned to the test chamber at the end of the day for an additional session. On days 5–7 (days 5–6 in expt 7), rats were trained to lever-press in a discrete-trial procedure. On each trial, both levers were inserted into the chamber. Responding on the RL resulted in the delivery of a single food pellet into the magazine, accompanied by the presentation of the compound stimulus. The levers were retracted and the compound stimulus was turned off, after the rat's head entered the food magazine or after 10 s from the rat's first lever-press had elapsed. Further lever-presses on the RL as well as responding on the other lever (non-reinforced lever, NRL) had no programmed consequences. Each trial was followed by a 30 s inter-trial interval. Each rat was trained until it completed 40 trials, i.e. pressed the lever and inserted its head into the food magazine during stimulus presentation, or for a total of 60 trials. Rats were randomly assigned to the different experimental groups at the end of this stage.

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. In order to assess the 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).

In expt 7, following the two sessions of lever-press training (on days 5 and 6), rats underwent surgery for cannulae implantation (see below). Following at least 7 recovery days with food and water available ad libitum, rats were returned to the 22-h food restriction

schedule, and 3 d later were given two additional sessions of lever-press training (one session per day), identical to the sessions given pre-surgery.

Stage 3: Signal attenuation

On the following 3 d, with the levers retracted, rats were exposed to the presentation of the compound stimulus as on days 1–3, but no food was delivered to the food magazine. Rats received 30 such trials on each day, and the number of collected trials was recorded. Rats that had more than 13 collected trials on the last day of signal attenuation were returned to the test chamber at the end of the day for an additional session.

Stage 4: Test

On the following day, rats were trained as in the lever-press training stage, except that no food was delivered to the food magazine, i.e. pressing the lever resulted in the presentation of the compound stimulus only. 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 ELP-U in the test stage of the PTSA procedure.

Regular extinction

Rats were run exactly as in the PTSA procedure, with the exception that they did not undergo the signal attenuation stage. Instead, on these days, rats were brought to the laboratory and left in their home cages for a period equivalent to the average duration of the signal attenuation stage.

Systemic drug administration

In order to systematically assess the effects of RS 102221 hydrochloride (8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulphonamido) phenyl-5-oxo-pentyl]-1,3,8-triazaspiro[4,5]decane-2,4-dione hydrochloride); MDL 11,939 [α-phenyl-1-(2-phenylethyl)-4-piperidinemethanol] and DOI hydrochloride [(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride] on compulsive lever-pressing, the effects of each drug in the PTSA procedure were assessed using several doses, ranging from low doses that had no effect on behaviour, to high doses that almost abolished lever-press responding. Doses were selected on the basis of previous studies that tested the behavioural effects of these drugs (e.g. RS 102221: Conductier et al., 2005; Popova and Amstislavskaya, 2002; MDL 11,939: Goudreau et al., 1993; Mechan et al., 2002; Schmidt et al., 1991; DOI: Hawkins et al., 2002, Koskinen et al., 2000). Because in the PTSA procedure the effects of signal attenuation are assessed under extinction conditions, when a drug was found to exert an anti-compulsive effect in the PTSA procedure, a subsequent experiment tested the effects of this drug also in a control procedure ('regular extinction') that is identical to the PTSA procedure but does not include a signal attenuation stage (this experimental design enables a differentiation between the drug's effects on the behavioural response to signal attenuation and on extinction per se, for a detailed discussion of the use of such a design see Joel, 2006).

Drugs were systemically administered i.p. in a volume of 1 ml/kg (DOI) or 2 ml/kg (RS 102221, MDL 11,939), 15 min (DOI) or 30 min (RS 102221, MDL 11,939) before the beginning of the test stage. DOI (Sigma, Rehovot, Israel) was dissolved in saline with a few drops of Tween-80 to a dose of 0.05, 0.1, 0.2, 0.5, 1.5 and 5.0 mg/kg. RS 102221 (Tocris, St Louis, MO, USA) was suspended in 90% distilled water with 10% Tween-80 to a dose of 0.5, 2.0 and 4.0 mg/kg. MDL 11,939 (Tocris) was dissolved in 5% acetic acid (1 M) and adjusted to pH 6.4–6.7 using NaOH and saline to a dose of 0.2, 1.0 and 5.0 mg/kg. No-drug controls received an equivalent volume of the corresponding vehicle.

Surgery (*expt 7*)

Rats received 3 mg diazepam, and 20 min later were anaesthetized with i.p. injection of avertin (10 ml/kg). *Cannulae implantation*: bilateral 26-gauge, stainless-steel, guide cannulae (Bilaney, Düsseldorf, Germany), were implanted at the following coordinates (Paxinos and Watson, 1998): 3.7 mm anterior to bregma, 2.4 mm lateral to the midline, and 3.3 mm ventral to dura. Removable stylets were placed in the guide cannulae and held in place with a screw-on dust cap.

Microinjection (*expt 7*)

Thirty minutes before the test, intracerebral microinjections were made bilaterally using a dual-syringe infusion pump (CMA/100 microinjection pump; Medecin AB, Solna Sweden). Rats were lightly sedated with halothane, the stylets were removed, and the injection needles (30-gauge) were inserted into the guide cannulae to protrude 1 mm below their tips. RS 102221 (0.5 μl) (dissolved in HCl, pH adjusted to 6–7 using NaOH and saline, to a concentration of 0.3 μg/μl) were slowly delivered at a constant rate over 60 s. One minute following the injection, the needles were slowly removed and replaced by the stylet. Control

rats received an equivalent volume of vehicle at pH 6–7. The volume and concentration of RS 102221 were selected on the basis of reports in the literature (Ramos et al., 2005).

Histology (*expt 7*)

One to three weeks after the completion of behavioural testing, all rats were overdosed with avertin (30 ml/kg, i.p.) and perfused intracardially with phosphate-buffered saline followed by 10% buffered formalin. The brains were removed and placed in 10% buffered formalin for at least 24 h, followed by 20% sucrose solution. The brains were sectioned in the coronal plane at 50 μm thickness and stained with Thionin Blue.

Statistical analysis

The number of ELP-C and ELP-U of rats undergoing the test stage was analysed using analyses of variance (ANOVAs) with the following factors: in experiments testing the effects of several drug doses in the PTSA procedure (expts 1, 3 and 4) – a main factor of dose. In experiments testing the effects of a single drug dose in the PTSA and regular extinction procedures (expts 2 and 7) – main factors of procedure (PTSA/regular extinction) and drug (RS 102221/vehicle). In experiments testing the effects of two different drugs in the PTSA procedure (expts 5 and 6) – main factors of drug A (e.g. DOI/vehicle) and drug B (e.g. RS 102221/vehicle). Significant main effects and/or interactions were followed by post-hoc least significant difference (LSD) comparisons.

Although drugs were administered only prior to the test stage, the rats' performance on the lever-press training and signal attenuation stages was also analysed, to ensure that differences in performance at the test stage were not a result of an earlier difference. For the former, the number of ELP-C on the last day of lever-press training was analysed (the variability of the other variables was too low to enable statistical analysis, as all rats achieved 40 completed trials with almost no uncompleted and unpressed trials). Performance on the signal attenuation stage was analysed using a mixed ANOVA performed on the number of completed trials on the three sessions of the signal attenuation stage.

Results

Table 1 presents the number of rats allocated to each experiment, the number of rats that were excluded from each experiment, the doses used (where relevant), and the final number of rats in each group.

Expt 1: The effects of systemic administration of 0.5, 2.0 and 4.0 mg/kg of the 5-HT$_{2C}$ antagonist RS 102221 in the PTSA procedure

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown). Figure 1(a, b) presents the mean number of extra lever-presses that were followed by magazine entry (ELP-C) and that were not followed by magazine entry (ELP-U), respectively, in RS 102221 and vehicle-treated rats undergoing the test stage of the PTSA procedure. As can be seen, RS 102221 did not affect the number of ELP-C, although the highest dose tested (4 mg/kg) tended to decrease this measure [Figure 1a, ANOVA: dose, $F(3,55)=1.26$, $p=0.296$], whereas both 2 and 4 mg/kg RS 102221 decreased the number of ELP-U [Figure 1b, ANOVA: dose, $F(3,55)=4.76$, $p<0.01$; see the Figure for the results of post-hoc LSD comparisons].

Expt 2: The effects of systemic administration of 2.0 mg/kg of the 5-HT$_{2C}$ antagonist RS 102221 in the PTSA and regular extinction procedures

Of the three doses of RS 102221 that were tested in expt 1, only the intermediate dose (2 mg/kg) exerted a selective anti-compulsive effect (i.e. a reduction in the number of ELP-U, but not in the number of ELP-C), whereas the lowest dose (0.5 mg/kg) had no effect, and the highest dose (4.0 mg/kg) decreased compulsive lever-pressing and in addition tended to decrease the number of ELP-C. Expt 2 therefore assessed the effects of 2 mg/kg RS 102221 in the PTSA and regular extinction procedures.

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown). Figure (1c, d) presents the mean number of ELP-C and ELP-U, respectively, in RS 102221 and vehicle-treated rats undergoing the test stage of the PTSA or regular extinction procedures. As has previously been reported (Joel, 2006) rats undergoing regular extinction exhibited a higher number of ELP-C compared with rats undergoing PTSA. In both procedures, however, RS 102221 had no effect on the number of ELP-C [Figure 1c, ANOVA: procedure, $F(1,34)=13.28$, $p<0.001$; drug, $F(1,34)=0.29$, $p=0.592$; procedure × drug interaction, $F(1,34)=2.49$, $p=0.124$]. In contrast, RS 102221 significantly decreased the number of ELP-U in the PTSA procedure, without affecting the number of ELP-U in

Table 1. Summary of experiments

Expt	Drug	Injection	Procedure	No. of rats in expt	No. of rats excluded	Dose (mg/kg)	Final no. per group
1	RS 102221	Systemic	SA	74	4, statistical	Vehicle	24
					5, acquisition failure	0.5	9
					4, computer failure	2.0	17
					2, illness	4.0	9
2	RS 102221	Systemic	SA × RE	48	2, statistical	SA, Vehicle	10
					3, acquisition failure	SA, RS 102221	9
					4, computer failure	RE, Vehicle	10
					1, illness	RE, RS 102221	9
3	MDL 11,939	Systemic	SA	54	3, statistical	Vehicle	12
					3, acquisition failure	0.2	10
					1, computer failure	1.0	12
						5.0	13
4	DOI	Systemic	SA	99	7, statistical	Vehicle	21
					1, acquisition failure	0.05	13
					6, computer failure	0.1	15
					1, injection failure	0.2	9
					1, illness	0.5	12
						1.5	7
						5.0	6
5	DOI *and* RS 102221	Systemic	SA	92	4, statistical	DOI, RS 102221	21
					1, acquisition failure	DOI, RS 102221 vehicle,	22
					3, computer failure	DOI vehicle, RS 102221	19
					1, injection failure	DOI vehicle, RS 102221 vehicle	19
					2, illness		
6	DOI *and* MDL 11,939	Systemic	SA	89	6, statistical	DOI, 0.2 mg/kg MDL 11,939	11
					5, acquisition failure	DOI, 1.0 mg/kg MDL 11,939	8
					5, computer failure	DOI, MDL 11,939 vehicle	12
					2, injection failurex	DOI vehicle, 0.2 mg/kg MDL 11,939	13
					3, illness	DOI vehicle, 1.0 mg/kg MDL 11,939	13
						DOI vehicle, MDL 11,939 vehicle	11
7	RS 102221	Intra-orbital	SA × RE	71	4, statistical	SA, Vehicle	13
					6, computer failure	SA, RS 102221	11
						RE, Vehicle	11
						RE, RS 102221	11

SA, Post-training signal attenuation; RE, regular extinction.
Acquisition failure: rats were excluded if they did not acquire lever-press responding.
Statistical: rats were excluded if their score on at least one variable was more than 4 S.D. above their group mean.

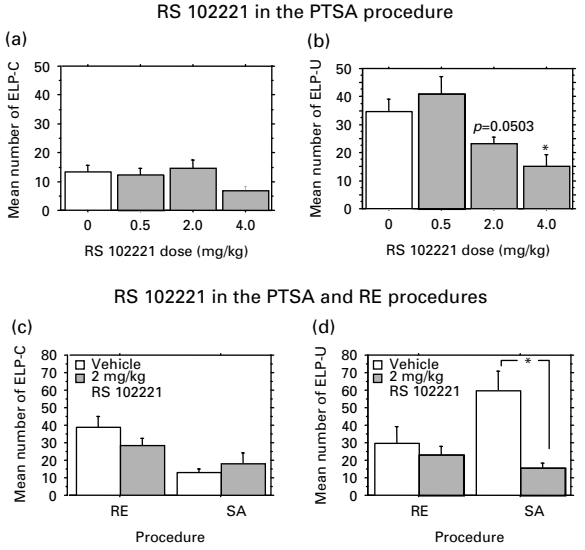

Figure 1. Effects of systemic administration of RS 102221 in the post-training signal attenuation (PTSA) and regular extinction (RE) procedures. Mean and standard error of the mean number of extra lever-presses (a, c) that were followed by magazine entry (ELP-C) and (b, d) that were *not* followed by magazine entry (ELP-U) of (a, b) rats treated with vehicle, 0.5, 2 or 4 mg/kg RS 102221 on the test day of the PTSA procedure (expt 1), and (c, d) of rats treated with 2 mg/kg RS 102221 on the test day of the PTSA and the RE procedures (expt 2). * Significantly different from the vehicle group.

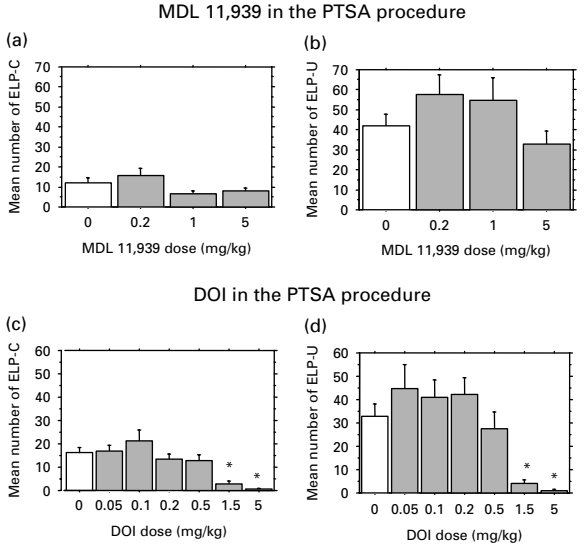

Figure 2. Effects of systemic administration of MDL 11,939 and DOI in the post-training signal attenuation (PTSA) procedure. Mean and standard error of the mean number of extra lever-presses (a, c) that were followed by magazine entry (ELP-C) and (b, d) that were *not* followed by magazine entry (ELP-U) of (a, b) rats treated with vehicle, 0.2, 1.0 or 5.0 mg/kg MDL 11,939 on the test day of the PTSA procedures (expt 3), and (c, d) of rats treated with vehicle, 0.05, 0.1, 0.2, 0.5, 1.5 or 5.0 mg/kg DOI on the test day of the PTSA procedure (expt 4). * Significantly different from the vehicle group.

regular extinction [Figure 1d, ANOVA: procedure, $F(1,34)=1.81$, $p=0.187$; drug, $F(1,34)=9.46$, $p<0.005$; procedure × drug interaction, $F(1,34)=5.24$, $p<0.05$; see the Figure for the results of post-hoc LSD comparisons].

Expt 3: The effects of 0.2, 1.0 and 5.0 mg/kg of the 5-HT$_{2A}$ antagonist MDL 11,939 in the PTSA procedure

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown). Figure 2(a, b) presents the mean number of ELP-C and ELP-U, respectively, in MDL 11,939 and vehicle treated rats undergoing the test stage of the PTSA procedure. As can be seen, at the highest doses tested, MDL 11,939 tended to reduce the number of ELP-C [Figure 2a; dose, $F(3,43)=3.35$, $p<0.05$, none of the post-hoc LSD comparisons revealed a significant difference from the vehicle group]. MDL 11,939 had no effect on the number of ELP-U [Figure 2b; dose, $F(3,43)=1.81$, $p=1.60$].

The finding that MDL 11,939 tended to decrease the number of ELP-C at doses that did not affect the number of ELP-U, suggests that this drug does not exert an anti-compulsive effect in the signal attenuation model. The effects of MDL 11,939 in regular extinction were therefore not assessed.

Expt 4: The effects of 0.05, 0.1, 0.2, 0.5, 1.5 and 5.0 mg/kg of the 5-HT$_{2A/2C}$ agonist DOI in the PTSA procedure

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown). Figure 2(c, d) presents the mean number of ELP-C and ELP-U, respectively, in DOI and vehicle treated rats undergoing the test stage of the PTSA procedure. As can be seen, only the highest DOI doses tested decreased the number of ELP-C [Figure 2c; dose, $F(6,76)=4.19$, $p<0.01$, see the figure for the results of post-hoc LSD comparisons] and of ELP-U [Figure 2d; dose, $F(6,76)=3.86$, $p<0.01$, see the figure for the results of post-hoc LSD comparisons].

Because of the DOI doses that did not completely abolish lever-press responding, none exerted a selective decrease in the number of ELP-U, no further

evaluation of DOI in the regular extinction procedure was performed.

Expt 5: The effects of co-administration of the 5-HT$_{2A/2C}$ agonist DOI and the 5-HT$_{2A}$ antagonist MDL 11,939 in the PTSA procedure

In order to test the effects of activation of 5-HT$_{2C}$ receptors in the absence of 5-HT$_{2A}$ receptor activation, expt 5 tested the effects of co-administration of the 5-HT$_{2A/2C}$ agonist DOI and the 5-HT$_{2A}$ antagonist MDL 11,939 in the PTSA procedure. The DOI dose used in this experiment was chosen based on the following considerations – DOI has been shown to possess high affinity at both 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in rodents and humans (Aloyo et al., 2001; Baxter et al., 1995; Glennon et al., 1992; Porter et al., 1999; Rojas-Corrales et al., 2007), and was found to induce both 5-HT$_{2A}$- and 5-HT$_{2C}$-mediated behaviours (Dave et al., 2002; Ouagazzal et al., 2001; Rojas-Corrales et al., 2007 and references within). However, several studies suggest that DOI holds higher selectivity at 5-HT$_{2A}$ receptors (see Ripoll et al., 2006 and references within, but see Acuna-Castillo et al., 2002, for the opposite finding), and therefore that higher doses of DOI are needed to induce 5-HT$_{2C}$-mediated behaviours compared to 5-HT$_{2A}$-mediated behaviours (Dave et al., 2002; Nic Dhonnchadha et al., 2003a; Ripoll et al., 2006). We therefore chose to use the highest DOI dose tested that did not exert a general decrease in lever-press responding (i.e. 0.5 mg/kg). The MDL 11,939 doses used in this experiment were 0.2 and 1.0 mg/kg. Thus, expt 5 used a complete factorial design with main factors of DOI (vehicle, 0.5 mg/kg DOI) and MDL 11,939 (vehicle, 0.2, 1.0 mg/kg MDL 11,939).

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown). Figure 3(a, b) presents the mean number of ELP-C and ELP-U, respectively, in the six groups on the test stage of the PTSA procedure. As found in expts 3 and 4, administration of MDL 11,939 and of DOI did not significantly affect either ELP-C or ELP-U. Co-administration of 0.5 mg/kg DOI and 0.2 mg/kg MDL 11,939 was also without an effect on these measures. However, co-administration of 0.5 mg/kg DOI and 1.0 mg/kg MDL 11,939 increased the number of both ELP-C and ELP-U [ELP-C: Figure 3a, ANOVA: DOI, $F(1,62)=4.71$, $p<0.05$; MDL 11,939, $F(2,62)=1.84$, $p=0.16$; DOI × MDL 11,939 interaction, $F(2,62)=5.09$, $p<0.01$. ELP-U: Figure 3b, ANOVA: DOI, $F(1,62)=0.69$, $p=0.40$; MDL 11,939, $F(2,62)=0.83$, $p=0.44$; DOI × MDL 11,939 interaction, $F(2,62)=4.12$, $p<0.05$; see the figure for results of post-hoc LSD comparisons].

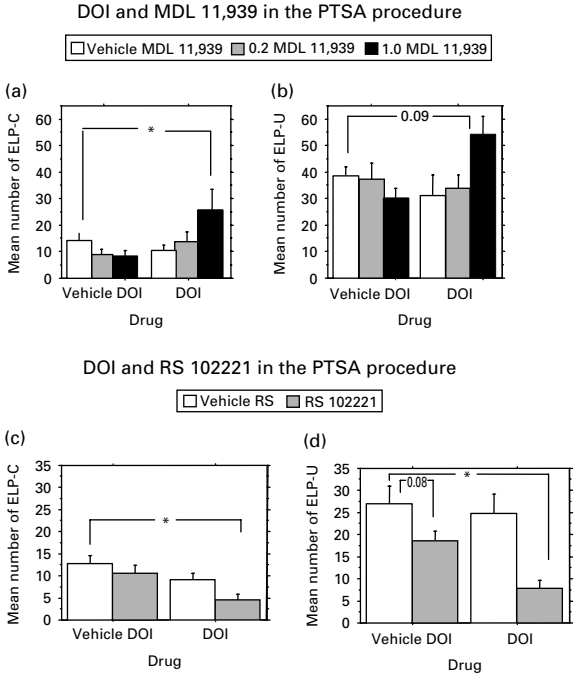

Figure 3. Effects of systemic co-administration of DOI with either MDL 11,939 or RS 102221 in the post-training signal attenuation (PTSA) procedure. Mean and standard error of the mean number of extra lever-presses (a, c) that were followed by magazine entry (ELP-C) and (b, d) that were *not* followed by magazine entry (ELP-U) of (a, b) rats treated with DOI + VEH$_{MDL}$, VEH$_{DOI}$ + 0.2 mg/kg MDL 11,939, VEH$_{DOI}$ + 1.0 mg/kg MDL 11,939, DOI + 0.2 mg/kg MDL 11,939, DOI + 1.0 mg/kg MDL 11,939 and VEH$_{DOI}$ + VEH$_{MDL}$ on the test day of the PTSA procedure (expt 5), and (c, d) of rats treated with DOI + VEH$_{RS}$, VEH$_{DOI}$ + RS 102221, DOI + RS 102221 or VEH$_{DOI}$ + VEH$_{RS}$ on the test day of the PTSA procedure (expt 6). * Significantly different from the vehicle group.

Expt 6: The effects of co-administration of the 5-HT$_{2A/2C}$ agonist DOI and the 5-HT$_{2A}$ antagonist RS 102221 in the PTSA procedure

In order to test the effects of 5-HT$_{2A}$ receptor activation in the absence of 5-HT$_{2C}$ receptor activation, expt 6 tested the effects of co-administration of the 5-HT$_{2A/2C}$ agonist DOI and the 5-HT$_{2C}$ antagonist RS 102221 in the PTSA procedure. The same DOI dose (0.5 mg/kg) used in expt 5 was chosen for the present experiment. On the basis of the results of expts 1 and 2, the RS 102221 dose chosen was the dose (2.0 mg/kg) that was found to exert an anti-compulsive effect. Thus, expt 6 used a complete factorial design with main

factors of DOI (vehicle, 0.5 mg/kg DOI) and RS 102221 (vehicle, 2.0 mg/kg RS 102221).

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown). Figure 3(c, d) presents the mean number of ELP-C and ELP-U, respectively, in the four groups on the test stage of the PTSA procedure. Similarly to the results obtained in expts 1 and 4, DOI did not affect the rats' behaviour whereas RS 102221 decreased the number of ELP-U (although this effect failed to reach statistical significance) but not of ELP-C. Interestingly, co-administration of DOI and RS 102221 significantly decreased the number of both measures [ELP-C: Figure 3c, DOI, $F(1,77)=9.62$, $p<0.01$; RS 102221, $F(1,77)=4.73$, $p<0.05$; DOI × RS 102221 interaction, $F(1,77)=0.69$, $p=0.41$. ELP-U: Figure 3d, DOI, $F(1,77)=3.69$, $p=0.06$; RS 102221, $F(1,77)=14.5$, $p<0.001$; DOI × RS 102221 interaction, $F(1,77)=1.64$, $p=0.20$; see figure for the results of post-hoc LSD comparisons].

Expt 7: The effects of intra-orbitofrontal infusion of the 5-HT$_{2C}$ antagonist RS 102221 in the PTSA and regular extinction procedures

On the basis of the results of expts 1–6, showing that only the 5-HT$_{2C}$ antagonist RS 102221 had a selective effect on the rats' compulsive lever-pressing, and in view of data implicating the orbitofrontal cortex in the pathophysiology of OCD (for a recent review see Friedlander and Desrocher, 2006) and in the mediation of the therapeutic effects of SSRIs (El Mansari and Blier, 2006), expt 7 tested the effects of intra-orbitofrontal injection of RS 102221 to rats undergoing the PTSA or regular extinction procedures.

Anatomical

Figure 4a presents a photomicrograph of a coronal section taken from a representative rat. The only visible damage seen in this rat was the cannulae tracks towards the target areas. Figure 4b presents a schematic reconstruction of cannulae placement in the orbitofrontal cortex of all rats. In all animals, cannulae tips were located within the lateral and dorsolateral orbitofrontal cortex.

Behavioural

There were no differences between the groups at the lever-press training and signal attenuation stages (data not shown). Figure 4(c, d) presents the mean number of ELP-C and ELP-U, respectively, in vehicle- and RS 102221-infused rats undergoing the test stage of the PTSA or regular extinction procedures. As found in expt 2, rats undergoing regular extinction exhibited a higher number of ELP-C compared with rats undergoing PTSA, and RS 102221 failed to affect this type of excessive lever-pressing [Figure 4c, ANOVA: procedure, $F(1,42)=5.78$, $p<0.05$; drug, $F(1,42)=1.31$, $p=0.259$; procedure × drug interaction, $F(1,42)=0.14$, $p=0.711$]. In contrast, RS 102221 significantly decreased the number of ELP-U in the PTSA procedure, and tended to increase the number of ELP-U in regular extinction [Figure 4d, ANOVA: procedure, $F(1,42)=0.16$, $p=0.688$; drug, $F(1,42)=0.54$, $p=0.465$; procedure × drug interaction, $F(1,42)=6.69$, $p<0.05$, see figure for LSD post-hoc comparison].

Discussion

The present study tested the role of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in compulsive behaviour, as assessed in the signal attenuation rat model of OCD. Systemic administration of the 5-HT$_{2C}$ antagonist RS 102221 (0.5, 2 and 4 mg/kg) prior to the test stage of the PTSA procedure, resulted in dose-dependent effects on rats' lever-press responding. Specifically, the lowest dose tested (0.5 mg/kg) had no effect on lever-press responding, the intermediate dose (2 mg/kg) decreased the number of excessive lever-presses that were *not* followed by magazine entry (i.e. ELP-U) while having no effect on the number of excessive lever-presses that were followed by magazine entry (i.e. ELP-C), and the highest dose (4 mg/kg) decreased both types of excessive lever-presses (expt 1). The finding that in the PTSA procedure, 2 mg/kg RS 102221 decreased ELP-U without affecting ELP-C was replicated in expt 2. Expt 2 further revealed that when given prior to an extinction test that was not preceded by signal attenuation (i.e. in regular extinction), 2 mg/kg RS 102221 did not affect either ELP-C or ELP-U.

Taken together, these results show that at 2 mg/kg, RS 102221 selectively decreases compulsive lever-pressing (i.e. signal attenuation-induced ELP-U). Although 5-HT$_{2C}$ receptors have been implicated in the control of locomotion (e.g. Grottick et al., 2000; Takahashi et al., 2001) and feeding (for review see Bickerdike, 2003; Giorgetti and Tecott, 2004), the anti-compulsive effect of RS 102221 seen here cannot be attributed to non-specific effects on lever-press responding because at 2 mg/kg the drug did not affect either ELP-U in regular extinction or ELP-C in both procedures. Moreover, the anti-compulsive effect of RS 102221 cannot be attributed to a non-selective effect on the rats' tendency to enter the magazine, because the drug did not affect the number of nose-pokes in

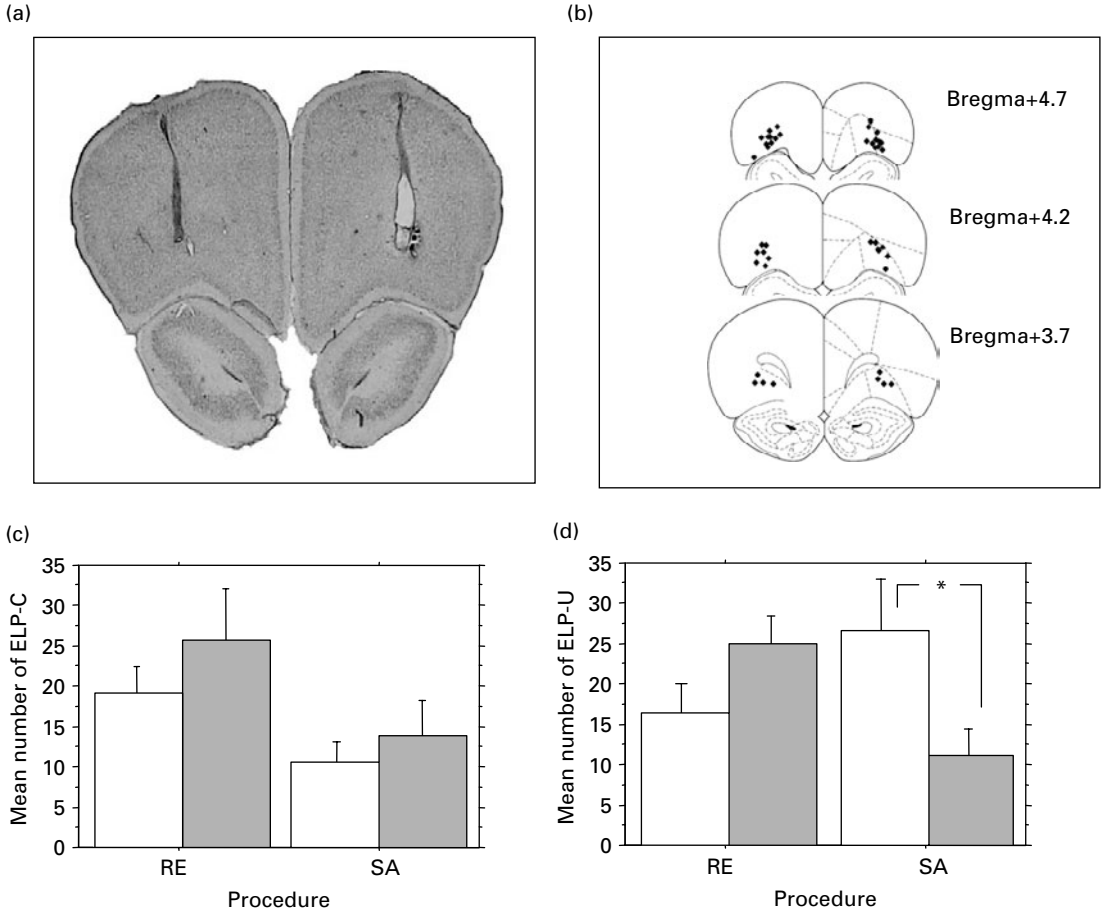

Figure 4. Effects of intra-orbital administration of RS 102221 in the post-training signal attenuation (PTSA) and regular extinction (RE) procedures. (a) A photomicrograph of a coronal section taken from a representative rat that sustained intra-orbitofrontal injection of RS 102221. (b) A schematic reconstruction of cannulae placement in the orbitofrontal cortex of rats treated with RS 102221. (c, d) Mean and standard error of the mean number of extra lever-presses (c) that were followed by magazine entry (ELP-C) and (d) that were *not* followed by magazine entry (ELP-U) of rats that received an intra-orbitofrontal injection of RS 102221 (■) or vehicle (□) on the test day of the PTSA and the RE procedures (expt 7). * Significantly different from the vehicle group.

either the PTSA or regular extinction procedures (data not shown, p values >0.45). Finally, because RS 102221 did not affect the rats' responding on the regular extinction procedure it is unlikely that the drug disrupted the conditioned reinforcing properties of the compound stimulus, because one of the hallmarks of a conditioned reinforcer is its ability to support responding in extinction (Mackintosh, 1974).

RS 102221 exerted a selective anti-compulsive effect also when administered into the orbitofrontal cortex (expt 7). Specifically, intra-orbitofrontal RS 102221 decreased ELP-U in rats undergoing PTSA, while having no effect on the number of ELP-C in these rats or on the number of ELP-C and ELP-U in rats undergoing regular extinction. The selectivity of the effect suggests that also in the case of intra-orbitofrontal administration, the anti-compulsive effect of RS 102221 cannot be attributed to non-specific effects on lever-press responding or on nose-poking, or to a disruption of the conditioned reinforcing properties of the compound stimulus.

There are relatively few studies which examined the behavioural effects of serotonergic manipulations of the orbitofrontal cortex. Of these, the ones which seem most relevant in the present context are the studies of Roberts and colleagues in marmosets on the effects of orbitofrontal 5-HT depletion on the flexible control of behaviour (Clarke et al., 2004, 2005, 2007; Walker et al., 2006). These studies found that orbitofrontal 5-HT depletion resulted in perseverative

responding in both a detour-reaching and a discrimination reversal task (Clarke et al., 2004, 2005, 2007; Walker et al., 2006). This perseverative responding was suggested to be due to a failure to inhibit a conditioned stimulus (CS)-elicited Pavlovian approach response (Clarke et al., 2007; Walker et al., 2006) and to be related to compulsive responding (Clarke et al., 2007; Walker et al., 2006). In contrast, the present study found that RS 102221 decreased compulsive responding, as defined in the signal attenuation model. Furthermore, RS 102221 had no effect on the number of nose-pokes (the CS-elicited Pavlovian approach response) in both the PTSA and regular extinction procedures (data not shown, $p = 0.80$), suggesting that a failure to inhibit a CS-elicited Pavlovian approach response cannot account for decreased compulsivity following blockade of orbitofrontal 5-HT$_{2C}$ receptors. These differences may be attributed to the fact that the effects of blockade of a specific 5-HT receptor type within the orbitofrontal cortex may be different from the effects of 5-HT depletion from this cortical area.

In contrast to the anti-compulsive effect of RS 102221, systemic administration of the 5-HT$_{2A}$ antagonist MDL 11,939 and of the 5-HT$_{2A/2C}$ agonist DOI prior to the test stage of the PTSA procedure did not affect compulsive lever-pressing. More specifically, MDL 11,939 tended to decrease the number of ELP-C at doses that did not affect the number of ELP-U (expt 3), and DOI either did not effect (at low doses) or almost completely suppressed (at high doses) the two types of excessive lever-presses (expt 4). The present findings are in line with previous reports that MDL 11,939 and other 5-HT$_{2A}$ antagonists suppress rats' motor activity in several behavioural procedures (Kehne et al., 1991; Nic Dhonnchadha et al., 2003a,b; but see Herin et al., 2005; Higgins et al., 2003, who did not find this effect), and that DOI decreases motor activity (Dave et al., 2002; Nic Dhonnchadha et al., 2003a; Ripoll et al., 2006), including lever-press responding (Engleman et al., 1992; Liao and Chang, 2001).

Co-administration of DOI and MDL 11,939 prior to the test stage of the PTSA procedure resulted in an increase in both ELP-C and ELP-U (expt 5), suggesting that activation of 5-HT$_{2C}$ receptors concomitant with blockade of 5-HT$_{2A}$ receptors non-selectively increases lever-press responding. The opposite effect was obtained by co-administration of DOI and RS 102221 (expt 6). The finding that primary 5-HT$_{2A}$ and 5-HT$_{2C}$ activation induced opposite effects in the PTSA procedure are in agreement with previous findings showing that activation of these two receptors exerts opposite effects on locomotion (Higgins et al., 2001; Nic Dhonnchadha et al., 2003b; Ouagazzal et al., 2001), drug-related consummatory behaviours and stimulant effects of several drugs of abuse (for review see Higgins and Fletcher, 2003; Muller and Huston, 2006).

In summary, of the different pharmacological manipulations tested in the present study, only blockade of 5-HT$_{2C}$ receptors had a selective effect on compulsive lever-pressing. To the best of our knowledge, there is only one study that assessed the effects of systemic administration of a 5-HT$_{2C}$ antagonist in an animal model of OCD and found increased compulsive drinking in the schedule-induced polydipsia model (Martin et al., 2002), in contrast to the present finding that systemic administration of RS 102221 decreased compulsive lever-pressing. This contradiction is paralleled by reports of increased and decreased compulsive responding following activation of 5-HT$_{2C}$ receptors in different animal models of OCD (see Introduction). In agreement with the present results are Boulougouris and colleagues' findings that in a reversal of a two-lever spatial discrimination, blockade of 5-HT$_{2C}$, but not 5-HT$_{2A}$, receptors decreased perseverative responding, which has been suggested to be related to compulsive responding (Boulougouris et al., in press).

It is of special interest to note that 5-HT$_{2C}$ blockade has been found to have a pro-addictive effect (for review see Higgins and Fletcher, 2003). Because compulsivity and addiction are closely related conceptually (e.g. addiction is defined as compulsive drug use in DSM-IV), and they seem to share underlying neural substrates (e.g. the orbitofrontal cortex and the dopaminergic system, for review see Adinoff, 2004; Jentsch and Taylor, 1999; Kalivas and Volkow, 2005; Stein, 2002), it could have been expected that 5-HT$_{2C}$ blockade would also have a pro-compulsive effect. The present finding that 5-HT$_{2C}$ blockade has an anti-compulsive effect may be taken to suggest that distinct subpopulations of 5-HT$_{2C}$ receptors are involved in compulsivity and in drug addiction. This suggestion receives support from the present demonstration that RS 102221 decreased compulsive lever-pressing when administered directly into the orbitofrontal cortex, because the pro-addictive effects of 5-HT$_{2C}$ antagonists have been attributed to their indirect facilitatory effect on dopamine neurons in the ventral tegmental area (for review see Higgins and Fletcher, 2003).

The present findings that systemic and intra-orbitofrontal administration of RS 102221 selectively decreased compulsive lever-pressing suggest that blockade of 5-HT$_{2C}$ receptors may have an anti-compulsive effect in OCD patients, and that this effect

may be mediated by 5-HT_{2C} receptors within the orbitofrontal cortex. The suggestion that 5-HT_{2C} antagonists may alleviate symptoms in patients is consistent with the finding that in OCD patients activation of 5-HT_{2C} receptors exacerbates symptoms (see Introduction). This suggestion is also in line with the hypotheses, derived from challenge studies in OCD patients, that 5-HT_{2C} receptors are hyper-sensitive in OCD patients (Graf et al., 2003; Yamauchi et al., 2004) and that SSRI-induced de-sensitization of these receptors may contribute to the therapeutic effects of SSRIs (Greenberg et al., 1998; Kennett et al., 1994; Rojas-Corrales et al., 2007; Stahl, 2000; Yamauchi et al., 2004). However, this latter hypothesis is inconsistent with data derived by Blier and colleagues from a rodent model of SSRIs' action. Specifically, studies assessing the effects of chronic SSRI administration in rodents have suggested that the anti-compulsive effect of SSRIs is mediated by enhanced 5-HT release in the orbitofrontal cortex that activates normosensitive post-synaptic 5-HT_2 receptors (El Mansari and Blier, 2006).

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Statement of Interest

None.

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