5-HT$_{2A}$ receptors in the orbitofrontal cortex facilitate reversal learning and contribute to the beneficial cognitive effects of chronic citalopram treatment in rats

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Abstract

Chronic stress is a risk factor for depression, and chronic stress can induce cognitive impairments associated with prefrontal cortical dysfunction, which are also major components of depression. We have previously shown that 5 wk chronic intermittent cold (CIC) stress induced a reversal-learning deficit in rats, associated with reduced serotonergic transmission in the orbitofrontal cortex (OFC) that was restored by chronic treatment with a selective serotonin reuptake inhibitor (SSRI). However, the mechanisms underlying the beneficial cognitive effects of chronic SSRI treatment are currently unknown. Thus, the purpose of this study was to investigate the potential modulatory influence specifically of 5-HT$_{2A}$ receptors (5-HT$_{2A}$ARs) in the OFC on reversal learning, and their potential contribution to the beneficial cognitive effects of chronic SSRI treatment. Bilateral microinjections of the selective 5-HT$_{2A}$AR antagonist, MDL 100,907 into OFC (0.02–2.0 nmol) had a dose-dependent detrimental effect on a reversal-learning task, suggesting a facilitatory influence of 5-HT$_{2A}$ARs in the OFC. In the next experiment, rats were exposed to 5 wk CIC stress, which compromised reversal learning, and treated chronically with the SSRI, citalopram (20 mg/kg.d) during the final 3 wk of chronic stress. Chronic citalopram treatment improved reversal learning in the CIC-stressed rats, and bilateral microinjection of MDL 100,907 (0.20 nmol, the optimal dose from the preceding experiment) into OFC once again had a detrimental effect on reversal learning, opposing the beneficial effect of citalopram. We conclude that 5-HT$_{2A}$ARs in the OFC facilitate reversal learning, and potentially contribute to the beneficial cognitive effects of chronic SSRI treatment.

Introduction

Chronic stress is a risk factor in depression (Anisman & Zacharko, 1982; Caspi et al. 2003; Kendler et al. 1999; Kessler, 1997). Moreover, altered regulation of serotonergic neurotransmission interacts with stress to increase the risk for depression (Caspi et al. 2003). However, little is known about the mechanisms underlying this interaction, or how long-term regulatory changes in serotonergic function induced by chronic stress might contribute to the symptoms of depression. Similarly, although selective serotonin reuptake inhibitors (SSRIs) are the most widely used class of antidepressant drugs, little is known about how long-term drug-induced alterations in serotonergic function might contribute to the beneficial effects of SSRIs on mood and cognition in depression.

The prefrontal cortex (PFC) is involved in executive functions that are disrupted in depression, including cognitive flexibility, the ability to modify previously learned associations and behavioural patterns in response to a changing environment (see Kehagia et al. 2003).
2010). Cognitive dysfunction, specifically deficits of cognitive flexibility, may contribute to the perseverative emotional biases that are important in the development and maintenance of depression (Beck, 1976; Coles & Heimberg, 2002; Mathews & Mackintosh, 1998). Depressed patients exhibit cognitive biases for emotionally salient material, particularly related to stressful life events (Beck, 1976), abnormal responses to performance feedback, a narrowing of attentional focus to depression-relevant thoughts, and difficulty shifting attentional set from one affective dimension to another, consistent with perseverative attention to themes of loss and worthlessness, and the persistent ruminations prevalent in depression (Austin et al. 2001; Fossati et al. 1999; Merriam et al. 1999; Murphy et al. 1999). In addition to cognitive impairments, there is evidence of decreased cortical volume and functional dysregulation of the PFC in depression, including regions of both hypo- and hyper-metabolic activity (Mayberg, 2003; Rogers et al. 2004; Sheline, 2003; see Price & Drevets, 2010).

In previous studies, we have used a behavioural assay for cognitive flexibility, the attentional set-shifting test (AST; Birrell & Brown, 2000), to address mechanisms by which monoaminergic neurotransmission in PFC of rats can modulate cognitive flexibility, and how chronic stress can induce deficits of cognitive flexibility that are sensitive to antidepressant drug treatment (Bondi et al. 2008, 2010; Lapiz et al. 2007; Lapiz-Bluhm & Morilak, 2010). We have shown that 2 wk of chronic intermittent cold (CIC) stress produced a selective reversal-learning deficit (Lapiz-Bluhm et al. 2009), that was subsequently reversed by 3 wk of chronic SSRI treatment, administered while the stress continued (Lapiz-Bluhm & Morilak, 2010). Similarly, selective reversal-learning deficits have been observed following lesions or 5-HT depletion specifically in the orbitofrontal cortex (OFC) (Boulougouris et al. 2007; Clarke et al. 2007; McAlonan & Brown, 2003), and the reversal-learning deficit in rats exposed to CIC stress was accompanied by reduced 5-HT release in OFC during the AST (Lapiz-Bluhm et al. 2009).

Thus, 5-HT has been implicated in reversal learning and cognitive flexibility in the OFC, but the mechanisms by which these effects are mediated remain unknown. Systemic administration of a 5-HT AR antagonist increased perseverative errors in an operant two-choice serial reversal task, whereas systemic blockade of 5-HTCs Rs had the opposite effect, improving performance (Boulougouris et al. 2008), as did direct administration of a 5-HTCR antagonist into OFC (Boulougouris & Robbins, 2010). Thus, 5-HTCs Rs may compromise, and 5-HTAs Rs facilitate reversal learning, but it is unknown if either of these has a role in the beneficial effects of SSRI treatment.

We showed recently that the beneficial effects of selective norepinephrine reuptake blockade on cognitive set-shifting, another form of cognitive flexibility, are mediated by a1-adrenergic receptors in the medial PFC (Bondi et al. 2010; Lapiz & Morilak, 2006). 5-HTAs Rs and a1-receptors have similar distributions in PFC, and exert similar effects on the electrical activity of PFC pyramidal cells (Marek & Aghajanian, 1999). Thus, in the present study, we hypothesized that 5-HTAs Rs in the OFC may similarly facilitate reversal learning, and contribute to the beneficial effects of chronic SSRI treatment in restoring cognitive flexibility compromised by prior exposure to CIC stress.

First, bilateral microinjections of the selective 5-HTAs R antagonist, MDL 100,907, were made directly into OFC prior to a reversal-learning task. Then, the role of 5-HTAs Rs in the beneficial effect of chronic SSRI treatment was investigated by microinjecting MDL 100,907 into the OFC of rats whose reversal-learning capability had been compromised by prior exposure to CIC stress and restored by chronic SSRI treatment. Portions of this work have been presented in abstract form (Furr et al. 2010).

Methods

Animals

A total of 72 adult male Sprague–Dawley rats were used in these experiments. Rats weighed 220–240 g upon arrival, and were individually housed under a 12-h light/dark cycle (lights on 07:00 hours) with food and water available ad libitum. Experiments were conducted during the light phase of the cycle. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio, and were consistent with NIH guidelines for the care and use of laboratory animals. All efforts were made to minimize pain, distress, and the number of animals used.

CIC stress

CIC stress was applied as described previously (Lapiz-Bluhm & Morilak, 2010). During the light phase of the cycle, rats in the CIC stress condition were weighed and transported in their home cages, with food, water, and bedding, into a cold room maintained at 4 °C for 6 h, then returned to the housing room. This was
sawdust-filled pots. Consecutive trials to retrieve the reward from both transferred to the testing arena and given three retrieved the reward from the pots each time, it was completely full). Once the rat had successfully third full, three with the pots half-full and three (three trials with no sawdust, three with the pots one-

Cheerio covered with increasing amounts of sawdust in the home cage and re-baited every 5 min, with the

Cereals, USA) was buried 2 cm below the surface of the texture of the digging medium with which it was defined as vigorous displacement of the medium making choices by smelling the reward. Digging was that the rat learned the discrimination and was not

was placed in each section. Each pot was defined by a pair of cues along two stimulus dimensions; the

One-fourth of a Honey Nut Cheerio (General Mills Cereals, USA) was buried 2 cm below the surface of the digging medium in the ‘positive’ pot. In all discrimination trials, a small quantity of powdered Cheerio was sprinkled onto the medium in both pots to ensure that the rat learned the discrimination and was not making choices by smelling the reward. Digging was defined as vigorous displacement of the medium to retrieve the reward buried in the pot. Simply investigating the rim of the pot or the surface of the digging medium with paws or snout without displacing material was not scored as a ‘dig’. Therefore, the rats were able to access visual, tactile and olfactory cues associated with the pots to make their choices. The behavioural procedure was conducted over 3 d.

Day 1: Habitation. Two unscented pots were placed in the home cage and re-baited every 5 min, with the Cheerio covered with increasing amounts of sawdust (three trials with no sawdust, three with the pots one-third full, three with the pots half-full and three completely full). Once the rat had successfully retrieved the reward from the pots each time, it was transferred to the testing arena and given three consecutive trials to retrieve the reward from both sawdust-filled pots.

Day 2: Training. On the following day rats were trained to complete two simple discriminations, with six consecutive correct responses required to reach criterion in each. In the first discrimination, both pots were filled with the same medium (sawdust) and scented with different odours (lemon vs. rosewood), with only one odour associated with reward. After reaching criterion, two new unscented pots were introduced, each filled with a different medium (shredded paper vs. felt strips). All rats were trained using the same stimulus exemplars in the same order. The positive and negative cues for each rat were randomly determined and equally represented. These training stimuli were not used again during testing.

Day 3: Testing. On the following day, the rats were tested on a series of discriminations. To proceed to the next stage, they had to reach a criterion of six consecutive correct trials. The first task was a simple discrimination, similar to the training trials, involving only one stimulus dimension. Half the rats were required to discriminate between two odours, only one of which was associated with reward, with both pots filled with sawdust. For the other half, this first discrimination involved the digging media, and both pots were unscented (for clarity, the remainder of this description will refer only to the example with odour discrimination). The second stage was then a compound discrimination, in which the same discrimination was required (e.g. odour), but the second, irrelevant stimulus was introduced. Only one odour was associated with reward, and the two different digging media were paired randomly with the odours over successive trials. Once the rats reached criterion, the last stage (R1) was then a reversal of the previous compound discrimination, in which the same odours and media were used, and odour was still the relevant dimension, but the negative odour from the previous stage was now positive (i.e. associated with the reward), and the positive odour from the previous stage was now negative. The same was true for rats tested with medium as the discriminative dimension. The dependent measure on this test was trials to criterion (TTC), the number of trials required to reach the criterion of six consecutive correct responses on the R1 reversal task.

Drugs

MDL 100,907 [(R)-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol], is a selective 5-HT_{2A}R antagonist (Axon Medchem, The
Netherlands). Due to the low solubility of MDL 100,907 in aqueous solution, it was dissolved with sonication in a vehicle comprised of 20% 2-hydroxypropyl-β-cyclodextrin (HBC) in filtered saline. For chronic delivery by osmotic minipump, the SSRI, citalopram HBr (Cit; Shanco International, USA), was dissolved in a vehicle of 10% ethanol in filtered saline to a final concentration of approximately 150 mg/ml, calculated to deliver 20 mg/kg.d free-base.

**Expt 1: testing the suitability of 20% HBC as a vehicle for microinjections into OFC**

Because MDL 100,907 had to be dissolved in 20% HBC, it was first necessary to conduct a control experiment to determine if microinjections of the HBC vehicle into the OFC would have any effect on reversal learning in the R1 task compared to microinjections of saline vehicle, which we have demonstrated previously to have no effect (Lapiz-Bluhm & Morilak, 2010).

Thirteen rats were given 1 wk to acclimatize to the animal facility after arrival. They were anaesthetized (43 mg/ml ketamine, 1.4 mg/ml acepromazine, 8.6 mg/ml xylazine given in 1.0 ml/kg i.m.) and placed in a stereotaxic apparatus for bilateral implantation of microinjection guide cannulae (22-gauge stainless-steel, 11 mm length), aimed so that their tips were located 1 mm above the OFC (coordinates from bregma: AP +2.9 mm, ML ±2.6 mm, DV −4.2 mm, corresponding to plate 9 in Paxinos & Watson, 1998). After surgery, rats were given antibiotic (Penicillin G, 300 000 IU/ml/kg), and individually housed for 14 d. Beginning 7 d prior to testing, food was restricted to 14 g/d, with water freely available. Rats then underwent habituation, training and behavioural testing on the AST over 3 d, as described above. On both the habituation and training days, they were also acclimated to handling and removal of the obdurators. On the test day, all rats were taken through the simple and compound discrimination stages. After completion of the compound discrimination, the obdurators were removed and replaced with microinjectors (30-gauge stainless-steel tubing) connected by a fluid-filled line to a Hamilton syringe mounted on a syringe pump (Instech Labs, USA). The injectors extended 1 mm beyond the tips of the guide cannulae, placing them in the OFC. The rats were then given acute bilateral microinjections into the OFC (0.50 μl/side) of either saline vehicle or 20% HBC vehicle, delivered over 2 min at a rate of 0.25 μl/min (n = 6–7/group). The microinjectors were left in place for an additional 2 min to allow for diffusion before withdrawing. After the completion of the microinjections, the rats were returned to their home cage for a 5-min recovery period before testing resumed with the R1 reversal task.

**Expt 2: effects of the selective 5-HT6R antagonist, MDL 100,907 microinjected into the OFC on reversal learning**

Having confirmed that the HBC vehicle alone had no effect on reversal learning when microinjected into OFC (see Results section), 68 rats were used in expt 2 to test the effects of direct microinjections of MDL 100,907 (n = 7–12/group) into the OFC. The rats were given at least 1 wk to acclimatize to the housing facility after arrival. They were then stereotaxically implanted with bilateral guide cannulae aimed at the OFC as above. Antibiotic was administered, and rats were individually housed for 14 d following surgery. Seven days prior to testing, food was restricted to 14 g/d. The rats were habituated, trained and tested over 3 d, as described above. After the habituation and training procedures, rats were briefly acclimatized on each day to handling, removal and replacement of the obdurators. On the test day, rats were taken through the simple and compound discrimination stages. After completion of the compound discrimination, bilateral microinjections were made into the OFC of either 20% HBC vehicle (0.50 μl/side) or MDL 100,907 (0.02, 0.10, 0.20, 1.00, or 2.00 nmol/0.50 μl per side) at a rate of 0.25 μl/min over a 2-min period. The microinjectors were left in place for an additional 2 min to allow for diffusion before withdrawal. Rats were returned to their home cages for 5-min post-injection before testing resumed with the R1 reversal stage of the AST.

To test for anatomical specificity, two groups of rats (n = 8/group) subsequently received bilateral microinjections of vehicle or an effective dose of MDL 100,907 (0.20 nmol) using the same approach, but 2.2 mm dorsal to the OFC target, in the Fr1 region of frontal cortex.

**Expt 3: role of 5-HT6Rs in the OFC in the beneficial effects of chronic SSRI treatment on reversal learning that has been compromised by chronic stress**

Seventy-five rats were randomly assigned to eight groups defined by the chronic stress treatment (control or CIC), chronic drug treatment (vehicle or Cit) and the drug microinjected acutely into OFC prior to R1 reversal testing (HBC-vehicle or MDL 100,907). Rats underwent 14 d of control or CIC stress treatment. On day 15, the rats were stereotaxically implanted with bilateral microinjection guide cannulae aimed at the
An osmotic minipump was also implanted intraperitoneally (Alzet model 2ML4, USA), pre-filled with either vehicle (10% ethanol in saline) or Cit at a concentration calculated to deliver 20 mg/kg.d of the free base. This dose was determined in pilot studies to achieve steady-state plasma drug levels within the target therapeutic range (Gould, Frazer & Morilak, unpublished data). After antibiotic administration and 3 d of post-surgical recovery, CIC stress treatment or control conditions were resumed for an additional 3 wk. As above, food was restricted to 14 g/d, 7 d prior to test day. Testing occurred 3 d after the termination of CIC or control treatments.

On the test day, all rats were taken through the simple and compound discriminations, after which they received acute bilateral microinjections into the OFC of either HBC vehicle (0.50 µl/side) or MDL 100,907 (0.20 nmol/0.50 µl per side), given at a rate of 0.25 µl/min over a 2-min period, as described above. The microinjectors remained in place for an additional 2 min to allow for drug diffusion before withdrawal. Rats were returned to their home cages for 5 min post-injection before testing resumed with the R1 reversal stage of the AST.

Statistical analyses
In all experiments, investigators conducting the behavioural test were blind to the experimental treatment condition of the rat being tested. Mean TTC on the simple discrimination task on the training day were first compared by ANOVA, to ensure that acquisition and general performance capability were comparable between experimental groups. A two-way MANOVA was also performed on the tasks preceding the R1 reversal stage on the testing day, to ensure that the groups were comparable prior to the acute microinjections.

In all experiments, the dependent measure of interest was the number of trials required to reach criterion (TTC) on the R1 reversal stage. In expt 1, the effect of microinjecting 20% HBC vehicle compared to saline vehicle microinjections into the OFC. There were no significant differences on the number of TTC (t = 0.297, p = 0.77, n = 6–7/group), indicating that 20% HBC is a suitable vehicle for microinjections into the OFC prior to testing on the reversal learning task.

Results
Figure 1 shows a representative example of the histological localization of bilateral guide cannulae tracks and microinjection sites in the OFC.

Expt 1: suitability of 20% HBC as a vehicle for microinjections into OFC
Performance on the reversal task was comparable after 20% HBC and saline vehicle microinjections into OFC. There were no significant differences on the number of TTC (t = 0.297, p = 0.77, n = 6–7/group), indicating that 20% HBC is a suitable vehicle for microinjections into the OFC prior to testing on the reversal learning task.
Fig. 2. Acute bilateral microinjections of the selective 5-HT2A R antagonist, MDL 100,907, into the orbitofrontal cortex immediately prior to testing on the reversal stage of the attentional set-shifting test impaired reversal learning compared to vehicle microinjections in control rats. Significant reversal deficits were observed in rats given MDL 100,907 at 0.10, 0.20, 1.00 and 2.00 nmol/0.50 μl per side (\(^* p < 0.05\) compared to the vehicle-injected control group by Newman–Keuls post-hoc comparisons; mean ± s.e.m., \(n = 7–12/group\)).

**Expt 2: effects of 5-HT2A R blockade in OFC on reversal learning**

Analysis of TTC on the two test stages prior to drug microinjections revealed no pre-test differences between the groups (\(F_{5,46} = 1.449, p > 0.20\), data not shown). Bilateral microinjections of MDL 100,907 had a significant, dose-related detrimental effect on reversal learning compared to HBC-vehicle control injections (\(F_{5,46} = 5.086, p < 0.001, n = 7–12/group\); see Fig. 2). Post-hoc comparisons revealed a significant increase in TTC in rats that received MDL 100,907 at doses from 0.10 to 2.00 nmol compared to HBC-vehicle controls (\(p < 0.05\)). Based on this result, 0.20 nmol was selected as the optimal dose of MDL 100,907 to be used in expt 3. This same dose was also used to test for anatomical specificity of the observed effect, and to control for potential diffusion of drug up the cannulae tracks. Bilateral microinjection of MDL 100,907 (0.20 nmol/side) into a site 2.2 mm dorsal to the OFC target had no effect on reversal learning compared to vehicle (\(t_{14} = 0.531, p = 0.60\)).

**Expt 3: role of 5-HT2A Rs in the OFC in the beneficial effects of chronic SSRI treatment on reversal learning that has been compromised by CIC stress**

Control and CIC-stressed rats learned the simple discrimination during the training session comparably, indicating no pre-existing difference between treatment groups in the ability to learn the contingency and perform the required tasks, and there were no group differences in performance on the two test stages prior to the R1 reversal task (all \(p > 0.05\), data not shown).

Figure 3 shows the effect of acute bilateral microinjections of 0.20 nmol MDL 100,907 into the OFC on the beneficial effects of chronic Cit treatment in CIC-stressed rats. Three-way ANOVA indicated a significant main effect for Cit stress (\(F_{1,67} = 6.71, p < 0.01\)), replicating the detrimental effect on reversal learning reported previously (Lapiz-Bluhm et al. 2009). There were also significant main effects of chronic Cit treatment (\(F_{1,67} = 4.12, p < 0.05\)) and MDL 100,907 microinjection (\(F_{1,67} = 34.69, p < 0.001\), and a significant CIC stress × Cit interaction (\(F_{1,67} = 4.69, p < 0.05\). Post-hoc comparisons indicated that Cit stress significantly increased TTC in vehicle-treated animals, and that Cit treatment significantly reduced TTC in CIC-stressed animals, also in replication of our previous results (Lapiz-Bluhm & Morilak, 2010). There was no interaction involving MDL 100,907, suggesting that it exerted similar effects in all treatment conditions. This was confirmed by subsequent post-hoc two-way ANOVAs, indicating that chronic Cit treatment had no significant effect in control rats, but exerted a
beneficial effect in CIC-stressed rats ($F_{1,31} = 17.16$, $p < 0.001$), and that a significant main effect of MDL 100,907 was observed in both control ($F_{1,34} = 13.52$, $p < 0.001$) and CIC-stressed ($F_{1,31} = 32.38$, $p < 0.001$) rats. A significant $\text{Cit} \times \text{MDL}$ interaction existed only in the CIC-stressed group ($F_{1,31} = 7.57$, $p < 0.01$), with post-hoc comparison by the Newman–Keuls test confirming that MDL 100,907 exerted a detrimental effect on reversal learning in all treatment conditions, and reversed the cognitive improvement seen in CIC-stressed rats treated chronically with Cit (Fig. 3).

Discussion
This study addressed the potential role of 5-HT$_{2A}$Rs in modulation of reversal learning in OFC, and in the beneficial cognitive effects of chronic treatment with the antidepressant SSRI, Cit. Reversal learning is a form of cognitive flexibility that enables behavioural adaptation to changing internal states and environmental circumstances (see Rygula et al. 2010). The neural substrate of reversal learning has been shown to include the OFC in rodents (Boulougouris et al. 2007; Clarke et al. 2007; McAlonan & Brown, 2003), as well as in humans (Cools et al. 2002; Fellows & Farah, 2003; Hampshire & Owen, 2006). Further, 5-HT has been implicated in the modulation of reversal learning in the OFC (Clarke et al. 2007; Lapiz-Bluhm et al. 2009). We have shown previously that systemic treatment with the SSRI, Cit, reversed the selective impairment in reversal learning induced in rats following CIC stress treatment, and this improvement was associated with an increase in 5-HT release in the OFC (Lapiz-Bluhm & Morilak, 2010). However, the mechanism by which 5-HT might exert its beneficial effects on reversal learning in the OFC is unknown.

The primary observation in the present study is that local administration of the selective 5-HT$_{2A}$R antagonist, MDL 100,907 into the OFC prior to testing on the R1 task of the AST attenuated reversal learning. This is consistent with a previous study in which systemic administration of MDL 100,907 compromised serial reversal learning on a spatial two-choice operant task (Boulougouris et al. 2008). In contrast with the present results, however, local infusion of MDL 100,907 into OFC by the same group had no effect on reversal learning, whereas the 5-HT$_{2C}$R antagonist, SB 242084, enhanced reversal learning similarly after either systemic or local administration into OFC (Boulougouris et al. 2008; Boulougouris & Robbins, 2010). One possible explanation for the discrepant findings after local microinjections of MDL 100,907 into OFC may be in the nature of the learning involved in the two experiments. Lesion studies have demonstrated that the OFC is essential to reversal learning on the AST (McAlonan & Brown, 2003), which involves associating reward with a specific cue in one of two different sensory modalities (e.g. odour vs. texture), then switching contingencies between the cues within one of those modalities. By contrast, the two-choice operant task in the studies cited above (Boulougouris et al. 2008; Boulougouris & Robbins, 2010) is a spatial learning task, wherein the lever associated with reward is defined by its spatial location, and reversal involves switching the spatial location associated with reward, rather than switching the valence of distinct sensory cues. This difference may render the OFC less essential in the spatial task, as other regions involved in both reversal learning and spatial learning (e.g. hippocampus or striatum) may maintain reversal learning capability in the face of compromised OFC function, and may also be sites at which facilitation by 5-HT$_{2A}$Rs can be maintained despite blockade of these receptors in the OFC.

Second, we examined the potential contribution of 5-HT$_{2A}$Rs in OFC to the beneficial cognitive effects of chronic treatment with the SSRI antidepressant drug, Cit. Replicating our previous result (Lapiz-Bluhm & Morilak, 2010), chronic treatment with Cit improved reversal learning that had been impaired by prior exposure to CIC stress, but had no effect on cognitive capability in unstressed controls. This is consistent with the literature suggesting that beneficial effects of antidepressant drug treatment are evident only in affected subjects, both in human clinical studies (e.g. Gelfin et al. 1998), and in animal studies comparing antidepressant responses of controls to subjects that have been compromised prior to treatment, for instance by genetic predisposition or chronic stress, using both behavioural and molecular-cellular measures (e.g. see Balu et al. 2009; David et al. 2009; El Khoury et al. 2006; Murray et al. 2008; Piras et al. 2010; Willner, 1997). In the present study, bilateral microinjections of MDL 100,907 into the OFC reversed the Cit-induced improvement in reversal learning after 5 wk of CIC stress treatment, indicating that the beneficial effects of Cit may be due, at least in part, to activation of 5-HT$_{2A}$Rs in OFC. However, a definitive conclusion regarding the degree to which the beneficial effects of SSRI treatment may require 5-HT$_{2A}$R activation cannot be determined from these data, because local blockade of 5-HT$_{2A}$Rs in OFC had a detrimental effect on reversal learning in control as well as chronically stressed rats, treated either with Cit or vehicle. Thus, it is possible that the effects of MDL 100,907 may be additive with those of Cit, rather than
strictly antagonistic. Nonetheless, the fact that 5-HT$_2A$Rs activation remains capable of facilitating reversal learning in animals compromised by chronic stress suggests that, even if these receptors are not primarily responsible for the beneficial cognitive effects of SSRI treatment, it may be possible to enhance these effects by promoting or maintaining 5-HT$_2A$R activity in the OFC during chronic antidepressant treatment.

The detrimental effect on reversal learning resulting from blockade of 5-HT$_2A$Rs in the OFC is reminiscent of the facilitatory influence exerted by $\alpha_1$-adrenergic receptors in the medial PFC on cognitive set-shifting (Lapiz & Morilak, 2006), and the role of $\alpha_1$-adrenergic receptors in the beneficial cognitive effects of antidepressant drugs that block norepinephrine reuptake (Bondi et al. 2010). The cellular actions of 5-HT$_2A$Rs and $\alpha_1$-adrenergic receptors are mediated by similar signal transduction pathways, involving activation of phospholipase C by $G_{q/11}$ (Hannon & Hoyer, 2008; Wu et al. 1992). Moreover, 5-HT$_2A$Rs and $\alpha_1$-adrenergic receptors both facilitate glutamate release in PFC, and both enhance the excitatory responses of pyramidal cells to glutamate (Marek & Aghajanian, 1999). It is unlikely that the effects of MDL 100,907 were attributable to non-selective blockade of $\alpha_1$ receptors, as MDL 100,907 has >100- to 500-fold greater affinity and potency at 5-HT$_2A$Rs compared to $\alpha_1$ receptors (Kehne et al. 1996). In addition, we have shown that the selective reversal-learning deficit induced by CIC stress is sensitive to serotonergic modulation and chronic treatment with a SSRI, but not with the selective norepinephrine reuptake blocker, desipramine (Lapiz-Bluhm & Morilak, 2010; Lapiz-Bluhm et al. 2009). Thus, similarities in the modulatory influence of 5-HT$_2A$Rs and $\alpha_1$-adrenergic receptors in PFC may underlie similar but specific facilitatory roles in the beneficial cognitive effects of antidepressant drugs that block the reuptake of 5-HT and norepinephrine, respectively.

Reversal learning is a complex process, requiring error detection, behavioural inhibition (i.e. suppression of the previously learned pre-eminent response), and overcoming the learned avoidance of a previously negative stimulus that is now positive (see Clarke et al. 2007). Depletion of 5-HT in the OFC specifically impaired the inhibition of pre-eminent responses, resulting in perseverative behaviour (Clarke et al. 2007). A subsequent study using a modified probabilistic reversal-learning task showed that increasing serotonergic tone with SSRI treatment enhanced cognitive flexibility and decreased perseverative behaviour by facilitating reward sensitivity and reducing negative feedback sensitivity, thus increasing the successful completion of reversal learning, whereas 5-HT depletion had the opposite effect (Bari et al. 2010). This is perhaps consistent with the negative emotional biases and enhanced sensitivity to negative feedback seen in depressed patients (Beats et al. 1996; Murphy et al. 2003).

However, the role of 5-HT, and specifically of 5-HT$_2A$Rs in different aspects of cognitive flexibility is complex and sometimes contradictory. A lack of behavioural inhibition can also manifest as impulsivity, and 5-HT depletion has been shown to increase impulsivity as measured by premature responding on the five-choice serial reaction time test (Winstanley et al. 2004). However, by contrast with results indicating that blockade of 5-HT$_2A$Rs with MDL 100,907 is detrimental to reversal learning (Boulougouris et al. 2008, and the present study), MDL 100,907 improved performance and reduced impulsivity on the five-choice test (Winstanley et al. 2004). Interestingly, despite these disparate observations, the effects of 5-HT$_2C$Rs were found in both cases to be opposite to those of 5-HT$_2A$Rs.

Reduced 5-HT activity has been implicated in depression, and 5-HT is critical to the clinical efficacy of SSRI antidepressants (Delgado et al. 1999). However, elucidation of the involvement specifically of 5-HT$_2A$Rs either in depression or in the efficacy of antidepressant drug treatment has remained elusive and controversial (see Carr & Lucki, 2011; Celada et al. 2004). Consistent with a beneficial effect of 5-HT$_2A$R antagonism on impulsivity, MDL 100,907 has been shown to exhibit antidepressant-like effects alone, and to enhance the effects of a low dose of fluoxetine on a test in which impulsivity is a component of the behavioural measure, namely, the differential reinforcement of a low rate 72-s schedule of reinforcement (DRL 72-s, Marek et al. 2005). Such observations are further consistent with both clinical and preclinical studies suggesting that blockade of 5-HT$_2A$Rs contributes to or enhances antidepressant efficacy (e.g. see Pandey et al. 1995).

Effects of chronic stress on 5-HT$_2A$R expression are similarly complex. Differential changes in 5-HT$_2A$R expression have been reported in hippocampus, hypothalamus and frontal cortex following acute or repeated footshock associated with learned helplessness. Whereas reduced 5-HT$_2A$R expression in hippocampus was associated with learned helplessness behaviour after both acute and repeated shock, it was suggested that the elevated expression of 5-HT$_2A$Rs in frontal cortex seen only after repeated shock may have been an adaptive response to reduced serotonergic transmission (Dwivedi et al. 2005). Chronic
unpredictable stress also elevated cortical 5-HT\textsubscript{2A}R-binding density in rats, and this was reversed by antidepressant treatment (Ossowska et al. 2002). By contrast, repeated restraint stress had no effect on cortical 5-HT\textsubscript{2A}R binding (Watanabe et al. 1993). Thus, in at least some contexts, chronic stress may up-regulate 5-HT\textsubscript{2A}R expression, as in depression, and in both cases this may represent a compensatory response to altered serotonergic tone (e.g. see Shelton et al. 2009). In such contexts, the restoration of serotonergic activity at 5-HT\textsubscript{2A}Rs in OFC by chronic SSRI treatment may improve cognitive flexibility. However, the apparently opposing effects of 5-HT\textsubscript{2A}R blockade on cognitive flexibility on the one hand, and on depressed mood and other indices of antidepressant efficacy on the other, may account for the disturbingly high incidence of partial antidepressant response, persistence of residual symptoms, loss of clinical efficacy over time, or even treatment resistance.

In sum, the primary observation in this study is that blockade of 5-HT\textsubscript{2A}Rs in the OFC compromised reversal-learning capability on the AST. Second, activation of this receptor subtype may contribute to the beneficial cognitive effects of chronic SSRI treatment in alleviating a selective deficit in reversal learning induced by exposing rats to chronic cold stress. These results add to an accumulating body of both clinical and preclinical evidence suggesting that deficits of cognitive flexibility may be central to the aetiology and symptomatology of psychiatric disorders associated with chronic stress, in particular major depressive disorder. Such observations suggest that a more fine-grained assessment of the cognitive deficits exhibited by depressed patients might identify specific aetiological substrates, and may eventually indicate more specific, or even individualized therapeutic approaches. More generally, elucidating the mechanisms by which antidepressant drugs can exert their therapeutic benefits may lead to the development of more effective or specific antidepressant strategies.

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**Statement of Interest**

None.

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