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5-HT₂ receptors modulate the expression of antipsychotic-induced dopamine supersensitivity



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Abstract

Antipsychotic treatment can produce supersensitivity to dopamine receptor stimulation. This compromises the efficacy of ongoing treatment and increases the risk of relapse to psychosis upon treatment cessation. Serotonin 5-HT₂ receptors modulate dopamine function and thereby influence dopamine-dependent responses. Here we evaluated the hypothesis that 5-HT₂ receptors modulate the behavioural expression of antipsychotic-induced dopamine supersensitivity. To this end, we first treated rats with the antipsychotic haloperidol using a clinically relevant treatment regimen. We then assessed the effects of a 5-HT₂ receptor antagonist (ritanserin; 0.01 and 0.1 mg/kg) and of a 5-HT_{2A} receptor antagonist (MDL100,907; 0.025–0.1 mg/kg) on amphetamine-induced psychomotor activity. Antipsychotic-treated rats showed increased amphetamine-induced locomotion relative to antipsychotic-naïve rats, indicating a dopamine supersensitive state. At the highest dose tested (0.1 mg/kg for both antagonists), both ritanserin and MDL100,907 suppressed amphetamine-induced locomotion in antipsychotic-treated rats, while having no effect on this behaviour in control rats. In parallel, antipsychotic treatment decreased 5-HT_{2A} receptor density in the prefrontal cortex and nucleus accumbens core and increased 5-HT_{2A} receptor density in the caudate-putamen. Thus, activation of either 5-HT₂ receptors or of 5-HT_{2A} receptors selectively is required for the full expression of antipsychotic-induced dopamine supersensitivity. In addition, antipsychotic-induced dopamine supersensitivity enhances the ability of 5-HT₂/5-HT_{2A} receptors to modulate dopamine-dependent behaviours. These effects are potentially linked to changes in 5-HT_{2A} receptor

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density in the prefrontal cortex and the striatum. These observations raise the possibility that blockade of 5-HT_{2A} receptors might overcome some of the behavioural manifestations of antipsychotic-induced dopamine supersensitivity.

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1. Introduction

Antipsychotic drugs suppress the symptoms of psychosis by attenuating dopamine-mediated neurotransmission at striatal dopamine D2/D3 receptors (Farde et al., 1988; Kapur and Remington, 2001; Seeman and Lee, 1975). Paradoxically, long-term treatment with antipsychotic medications can evoke a state of dopaminergic supersensitivity. Dopamine supersensitivity is seen in approximately 13–39% of patients chronically exposed to either typical or atypical antipsychotics, compared to 0–2% of antipsychotic-naïve psychiatric patients (Fallon et al., 2012; Woerner et al., 1991). Studies conducted in patients or laboratory animals show that antipsychotic-induced dopamine supersensitivity is linked to an elevated incidence of psychosis (Chouinard et al., 1978), compromised antipsychotic efficacy (Fallon et al., 2012; Gill et al., 2014; Samaha et al., 2008, 2007; Suzuki et al., 2015), an increased psychomotor response to dopaminergic drugs (Asper et al., 1973; Sayers et al., 1975), and augmented amphetamine-induced operant responding for cues that predict reward (Bedard et al., 2011, 2013).

Antipsychotic-induced dopamine supersensitivity has significant clinical implications, yet little is known about its underlying neurobiological mechanisms. On the presynaptic side, studies suggest no obvious changes in extracellular dopamine levels in response to dopamine-releasing drugs such as amphetamine (Ichikawa and Meltzer, 1992; Samaha et al., 2007) or in cortical or subcortical dopamine transporter densities (Ase et al., 1999). On the postsynaptic side, some studies report augmented numbers of striatal D2 receptors (Muller and Seeman, 1977) and D2 receptors that are in a high-affinity state for dopamine (Samaha et al., 2008, 2007). However, behavioural supersensitivity to dopamine agonists and changes in striatal D2 receptor number are dissociable (Pierce et al., 1991; Samaha et al., 2007). Preliminary data also suggest no change in D2 high-affinity states in antipsychotic-treated schizophrenia patients (Graff-Guerrero et al., 2009).

Beyond changes at dopamine receptors, serotonin receptors can modulate dopamine function and thereby influence behaviour. Of particular relevance here, dopamine supersensitivity outside of the context of antipsychotics enhances the ability of serotonin 5-HT₂ receptors to mediate dopamine-dependent responses. For instance, in dopamine-depleted or cocaine-sensitised animals, the ability of 5-HT_{2A} receptor activity to mediate striatal gene expression (Brown and Gerfen, 2006), extracellular dopamine levels in the nucleus accumbens (Yan et al., 2000) and psychomotor activity (Bishop et al., 2004) is augmented. Dopamine-denervated animals also have increased striatal levels of 5-HT_{2A} receptors (Basura and Walker, 1999), and blockade of these receptors normalises the exaggerated psychomotor response to dopamine agonists seen in these animals (Bishop et al., 2005).

Similarly, in cocaine sensitised animals, microinjection of either a 5-HT_{2A} or a 5-HT_{2C} receptor antagonist into the nucleus accumbens suppresses the expression of locomotor sensitization to cocaine, and blockade of 5-HT_{2A} receptors in this region also disrupts the expression of augmented cocaine-evoked dopamine release (Zayara et al., 2011).

One way 5-HT₂ receptors influence dopamine-mediated behaviours is by regulating dopamine release. Within the nucleus accumbens, activation of 5-HT_{2A} receptors increases dopamine release, while activation of 5-HT_{2B/C} receptors has the opposite effect (De Deurwaerdere and Spampinato, 1999; Lucas et al., 2000). Similarly, activation of 5-HT_{2A} receptors within the medial prefrontal cortex increases the activity of dopamine cells in the ventral tegmental area, and the release of dopamine in both the ventral tegmental area and the medial prefrontal cortex (Bortolozzi et al., 2005).

In light of this literature, we asked the following question, do 5-HT₂ receptors mediate the expression of dopamine supersensitivity evoked by antipsychotic treatment? To address this question, we exposed rats to the typical antipsychotic haloperidol. While treatment with either typical or atypical antipsychotic medications can evoke dopamine supersensitivity (Chouinard et al., 1978; Fallon et al., 2012; Woerner et al., 1991), typical agents more readily induce this response (Bedard et al., 2013; Samaha et al., 2007). We used a treatment regimen that is clinically pertinent and that also evokes dopamine supersensitivity, as indicated by sensitization to both the psychomotor activating and the conditioned reward-enhancing effects of the indirect dopamine agonist amphetamine (Bedard et al., 2013; Samaha et al., 2007). Following haloperidol treatment, we assessed the psychomotor response to acute amphetamine co-administered with either ritanserin, a 5-HT₂ receptor antagonist, or MDL100,907, a potent and selective 5-HT_{2A} receptor antagonist with comparatively little affinity for 5-HT_{2B/C} receptors (Palfreyman et al., 1993). At the neurobiological level, we also quantified the density of 5-HT_{2A} receptors in cortical and striatal regions.

2. Experimental procedures

2.1. Subjects and housing

Male Sprague-Dawley rats (Charles River, Montréal, Canada) weighing 200–225 g upon arrival were housed two per cage on a 12-h reverse light/dark cycle (lights off at 8 a.m.) with *ad libitum* access to food and water. All behavioural testing was conducted during the dark phase of the animals' circadian cycle, in a testing room with lights off save for a desk lamp containing a red-tinted, 13-watt light bulb. Different cohorts of rats were used in each experiment. All reasonable efforts were made to minimise animal suffering and the number of animals used. The Université de Montréal's animal care committee approved all experimental procedures.

2.2. Drugs

Haloperidol (Sandoz, Boucherville, Canada) was dissolved in a 0.5% glacial acetic acid/water solution (pH adjusted to ~5 with sodium hydroxide [1 M NaOH]) and was administered at a dose of 0.5 mg/kg/day via subcutaneous minipump (Alzet model 2ML2, 15-17 days of drug delivery depending on the batch and according to manufacturer's specifications; Durect, Cupertino, CA, USA). D-amphetamine sulphate (Sigma-Aldrich, Dorset, UK) and MDL100,907 [(+)- α -(2,3-Dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidine-methanol Hydrochloride; Toronto Research Chemicals, Toronto, Canada] were each dissolved in 0.9% saline. Ritanserin (Sigma-Aldrich, Oakville, Canada) was dissolved in a 0.9% saline solution containing 15 μ l/ml of glacial acetic acid (pH adjusted to ~6-7 with sodium hydroxide [1 M NaOH]). Doses of MDL100,907 refer to the weight of the base. Amphetamine (1.5 mg/kg) was injected subcutaneously. MDL100,907 (0.025, 0.05 and 0.1 mg/kg) and ritanserin (0.01 and 0.1 mg/kg) were administered via intraperitoneal injection. All injections were given in a volume of 1 ml/kg.

2.3. Antipsychotic treatment

To assess the role of 5-HT₂ receptors in the expression of antipsychotic-induced dopamine supersensitivity, we used a clinically relevant dose and mode of haloperidol treatment. When typical antipsychotics are used, therapeutic efficacy and a reduced risk of motor side effects are observed with 65-75% striatal D₂ receptor occupancy (Farde et al., 1992; Kapur et al., 2000). Pre-clinical studies also show that typical antipsychotics have antipsychotic-like efficacy with a reduced risk of motor side effects at doses that occupy 65-80% of striatal D₂ receptors (Wadenberg et al., 2000). Thus, we used a dose of 0.5 mg/kg/day haloperidol because this achieves clinically relevant levels of D₂ occupancy [(73% \pm 14 SD; unpublished observations. See also Samaha et al. (2007)]. As regards the mode of treatment, we administered haloperidol via subcutaneous osmotic minipump because this models the kinetics of standard antipsychotic treatment in patients. In patients, the typical antipsychotic treatment regimen involves daily oral administration. However, in humans, a single dose of antipsychotic can maintain high striatal D₂ receptor occupancy levels for several days (Farde et al., 1989; Tauscher et al., 2002). In rats, striatal D₂ occupancy declines significantly 24 h following a single subcutaneous injection, but is continuous if antipsychotics are given via subcutaneous osmotic minipump (Kapur et al., 2003). This is because the half-life of haloperidol is 1.5 h in rats (Cheng and Paalzow, 1992) and 14-36 h in humans (Kudo and Ishizaki, 1999). Thus, to achieve the relatively continuous D₂ receptor occupancy that is produced by clinical antipsychotic treatment regimens, we administered haloperidol via minipump.

For minipump implantation, rats were anaesthetised with isoflurane (5% for induction and 1.5% for maintenance during the surgery; CDMV, Ste-Hyacinthe, Canada) and implanted with subcutaneous minipumps filled with haloperidol and set to lie in between the scapulae, as described in (Samaha et al., 2007). Amphetamine-induced locomotion is similar in control rats that had been implanted with a saline-containing minipump (Samaha et al., 2007) compared to control rats that had received a sham surgery without minipump implantation (Samaha et al., 2008). Thus, in the present experiments, control rats received a sham surgery consisting of an incision and sutures. Seventeen to 19 days following implantation, minipumps were removed from the haloperidol rats, thus terminating antipsychotic treatment. Control rats received a second sham surgery.

All behavioural tests were conducted between the 2nd and 10th days after antipsychotic treatment cessation. In animals, the persistence of antipsychotic-induced dopamine supersensitivity is proportional to the length of the antipsychotic treatment (Muller and Seeman, 1977). The haloperidol treatment regimen used here produces dopamine

supersensitivity that persists for at least 10-11 days after antipsychotic treatment cessation (Samaha et al., 2007; Bedard et al., 2011). Importantly, we would not expect haloperidol to be present in the system 2 days after antipsychotic treatment cessation. In rats, 24 h after the last haloperidol administration, striatal D₂ receptor occupancy by the antipsychotic is close to zero (e.g., 24 h after a s.c. injection of 0.25 mg/kg haloperidol, there is 5% occupancy \pm 2 S.D.; (Kapur et al., 2003).

2.3.1. Experiment 1: the effects of 5-HT₂ receptor blockade on amphetamine-induced locomotion

Here we tested the hypothesis that 5-HT₂ receptor blockade disrupts the expression of antipsychotic-induced dopamine supersensitivity, as measured by the locomotor response to the indirect dopamine agonist, amphetamine. Thus, we assessed the effects of ritanserin on amphetamine-induced locomotion in rats withdrawn from chronic haloperidol treatment and in control rats. Figure 1 shows the sequence of experimental events. Starting two days after minipump removal, amphetamine-induced locomotor activity was assessed in Plexiglas cages equipped with infrared photocells. A computer recorded the number of full-cage crossings, and this was used as a measure of locomotor activity. Rearing behaviour or vertical activity, as well as stereotyped behaviours represent other aspects of the psychomotor response to amphetamine. However, our locomotor activity cages are not equipped to measure rearing behaviour, and the experimenters did not score stereotyped behaviours. Rats were injected with ritanserin (0, 0.01 or 0.1 mg/kg) and placed in the test cages for a 30-min habituation. Rats were then injected with saline or amphetamine and locomotor activity was assessed for another 90 min. This yielded six possible treatment combinations. Each rat received four out of these six combinations, in a counterbalanced order, administered every other day. We used a total of 32 rats for this experiment, 8 rats for each treatment combination.

2.3.2. Experiment 2: the effects of 5-HT_{2A} receptor blockade on amphetamine-induced locomotion

Experiment 1 revealed that 5-HT₂ receptor blockade suppresses the expression of antipsychotic-induced dopamine supersensitivity, as indicated by attenuation of the exaggerated locomotor response to

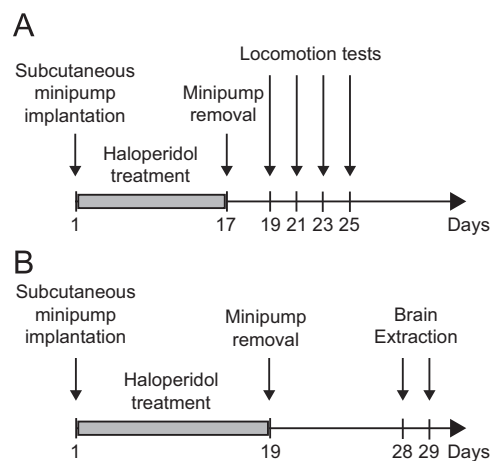


Figure 1 Timeline of experimental manipulations. (A) Illustrates the procedures used in Experiments 1 and 2, where we assessed the effects of either the 5HT_{2A/B/C} receptor antagonist, ritanserin or the 5HT_{2A} receptor antagonist MDL100,907 on amphetamine-induced locomotion in rats previously exposed to haloperidol. (B) Illustrates the procedures used in Experiment 3, where we assessed changes in 5-HT_{2A} receptor binding in rats previously exposed to haloperidol.

amphetamine observed in antipsychotic-treated rats. Here we tested the hypothesis that 5-HT_{2A} receptors mediate this effect. Procedures were similar to those described in Experiment 1 except that the rats were injected with MDL100,907 instead of ritanserin. In addition, we did not include amphetamine-naïve rats in Experiment 2 because testing the hypothesis does not require doing so, and this also keeps the number of animals manageable. We used 42 rats for this experiment, 10-21 rats for each treatment combination.

2.3.3. Experiment 3: ex-vivo 5-HT_{2A} receptor binding

Haloperidol-exposed rats show an exaggerated locomotor response to amphetamine, and Experiment 2 revealed that 5-HT_{2A} receptors modulate the expression of this response. Here we determined whether haloperidol treatment alone or combined with an acute amphetamine injection alters 5-HT_{2A} density in corticolimbic structures.

2.4. Brain preparation

Nine or 10 days following haloperidol treatment cessation, rats were injected with either saline or amphetamine one hour prior to decapitation. Our prior work shows that at this time point, haloperidol-treated rats still show robust dopamine supersensitivity, as indicated by an augmented locomotor response to amphetamine (Bedard et al., 2013). Brains were immediately frozen in isopentane (−40 °C), stored at −80 °C and later sectioned into 12 μm coronal slices using a cryostat.

2.5. [³H]Ketanserin binding

Procedures were adapted from Riahi et al. (2011). Briefly, 5-HT_{2A} receptors were labelled with [Ethylene-³H]-Ketanserin hydrochloride (47.3 Ci/mmol; Perkin Elmer, Woodbridge, ON, Canada). Brain slices were pre-incubated in 170 mM Tris-HCl buffer (pH 7.4) for 15 min at room temperature. Brain slices were then incubated in the previous buffer containing 5 nM [³H]Ketanserin and 50 μM prazosin (to block alpha-1 adrenergic receptor binding sites) for 2 h at room temperature. At a dose of 5 nM, [³H]Ketanserin saturates 5-HT_{2A} receptors in rat brain (Leysen et al., 1982). 1 μM methysergide was added in binding buffer to assess non-specific [³H]Ketanserin binding. Slides were washed in 170 mM Tris-HCl buffer (pH 7.4 at room temperature) twice for 10 min at 4 °C and dipped in distilled water for 10 s at 4 °C. Brain slices were dried and exposed to a BiomaxMR film (Kodak, New Haven, CT, USA) for 7 weeks.

2.6. Quantification

Sections were quantified using Image J software (NIH, Bethesda, MD, USA) by an experimenter blind to experimental condition. The Paxinos and Watson (1986) atlas was used to identify the caudate-putamen (divided into quadrants), the nucleus accumbens core and shell, cingulate cortex areas 1 and 3 (Cg1/Cg3), infralimbic and orbitofrontal (lateral and ventrolateral) cortices. In rat cortex, 5-HT_{2A} receptors show a laminar distribution, with the highest density found in layer V_a, a layer which is located mid-way through the depth of the cortex (Blue et al., 1988). In accordance with this, visual inspection of our autoradiograms revealed that in cortical areas, 5-HT_{2A} density was notable in a discrete layer also located mid-way through the depth of the cortex, and 5-HT_{2A} density was faint in the remaining layers. Thus, we quantified receptor binding in this densely labelled layer. Optical densities were translated in nCi/mg with a ³H standard curve (ART 0123A; American Radiolabeled Chemicals, St-Louis, MI, USA). A background value taken in the rhinal fissure (for regions analysed between +3.8 and +2.6 mm from Bregma) or the corpus callosum (+2.0-0.0 mm) was subtracted from values in each slice. Nonspecific binding was

determined in each region from 2 rats per group and the mean value was subtracted from values obtained in the corresponding region of each experimental subject. We used 6-14 rats per treatment condition, for a total of 25-27 rats per brain region quantified.

3. Statistical analysis

Mixed-model ANOVA was used to analyse locomotor activity (Treatment condition × Time, with 'Treatment condition' as a between-subjects variable and 'Time' as a within-subjects variable). Mixed-model ANOVA was also used to analyse 5-HT_{2A} receptor density (Treatment condition × Rostro-caudal position, with 'Treatment condition' as a between-subjects variable and 'Rostro-caudal position' as a within-subjects variable). When treatment condition effects were significant, within group comparisons were analysed using the Bonferroni test. Values in all figures represent mean ± SEM.

4. Results

4.1. Experiment 1: the effects of 5-HT₂ receptor blockade on amphetamine-induced locomotion

In both control rats and haloperidol-exposed rats, there was a significant main effect of Treatment condition (minutes 35-120; Figure 2A; $F(3,28)=16.33$; Figure 2B; $F(3,28)=21.03$; Figure 2C; $F(3,28)=44.37$; Figure 2D; $F(3,28)=30.99$; all $P's < 0.0001$). In both groups, regardless of whether the rats were pre-treated with ritanserin or not, amphetamine increased locomotor activity relative to saline (main effect of Treatment condition on minutes 35-120: VEH/AMPH vs. VEH/SAL; Figure 2A; $F(1,14)=31.72$; Figure 2B; $F(1,14)=30.01$; Figure 2C; $F(1,14)=83.79$; Figure 2D; $F(1,14)=38.72$; RIT/SAL vs. RIT/AMPH; Figure 2A; $F(1,14)=18.53$; Figure 2B; $F(1,14)=32.90$; Figure 2C; $F(1,14)=57.30$; Figure 2D; $F(1,14)=60.70$; all $P's < 0.001$). In addition, amphetamine-induced locomotion was greater in haloperidol-exposed rats compared to control rats (two-way ANOVA comparing VEH/AMPH between the two groups: Figure 2A vs. 2B; Treatment condition × Time interaction effect on minutes 35-120; $F(17,238)=3.20$, and main effect of Group on minutes 40-70; $F(1,14)=7.65$; Figure 2C vs. 2D; Treatment condition × Time interaction effect on minutes 35-120 $F(17,238)=1.81$, and main effect of Group on minutes 35-120; $F(1,14)=9.94$; All $P's < 0.03$). Thus, haloperidol treatment evoked dopamine supersensitivity.

A low dose of ritanserin (0.01 mg/kg) had no effect on either spontaneous or amphetamine-induced locomotion, in either experimental group (Figure 2A and B; all $P's > 0.05$). At a higher dose (0.1 mg/kg), ritanserin had no effect on spontaneous-induced locomotion, in either group (Figure 2C and D; all $P's > 0.05$). However, this dose of ritanserin attenuated amphetamine-induced locomotion only in haloperidol-exposed rats (Figure 2D; minutes 30-120, Treatment condition × Time interaction effect; $F(18,252)=1.678$; $p=0.043$; Figure 2C; all $P's > 0.05$). Furthermore, ritanserin (0.1 mg/kg) attenuated amphetamine-induced locomotion in haloperidol-exposed rats to levels similar to those seen in control rats not pre-treated with ritanserin [RIT/AMPH in

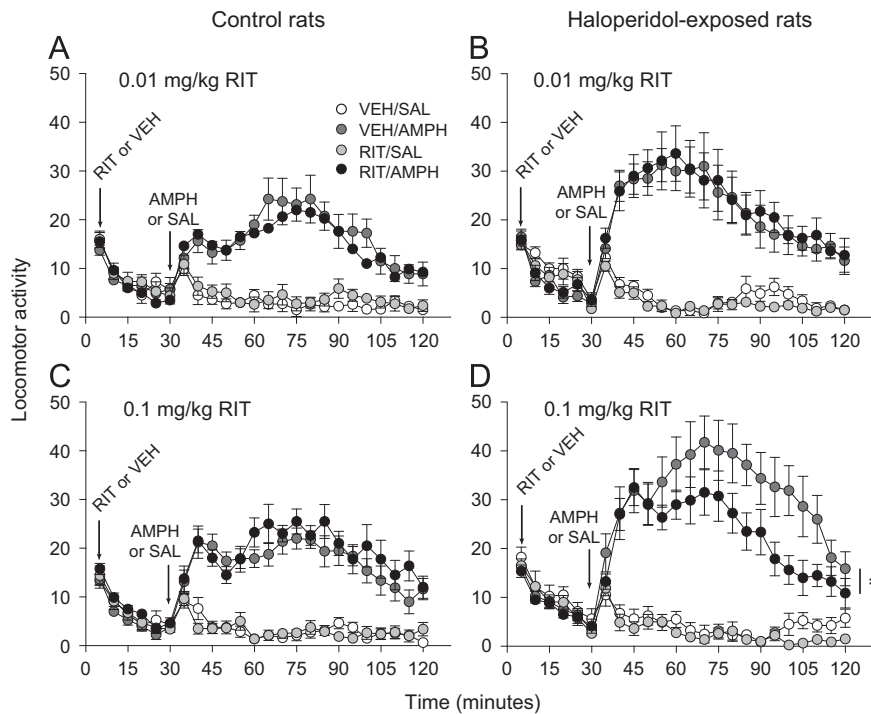


Figure 2 Blockade of 5-HT₂ receptors with ritanserin (RIT) attenuates amphetamine-induced locomotion in haloperidol-exposed rats but not in control rats. * $p < 0.05$, minutes 30-120. SAL, saline. VEH, Vehicle. AMPH, amphetamine. $N_s = 8$ /treatment condition.

haloperidol-exposed rats (Figure 2D) vs. VEH/AMPH in control rats (Figure 2C); main effect of Group on minutes 35-120; $F(1,14) = 3.553$; $p = 0.08$. Thus, haloperidol-exposed rats showed exaggerated amphetamine-induced locomotion, and 5-HT₂ receptor blockade normalised this response, while having no effect in antipsychotic-naïve rats.

4.2. Experiment 2: the effects of 5-HT_{2A} receptor blockade on amphetamine-induced locomotion

Amphetamine-induced locomotion was greater in haloperidol-exposed rats compared to control rats [SAL/AMPH in haloperidol-exposed rats (Figure 3A) versus in control rats (Figure 3B); Treatment condition \times Time interaction effect on minutes 35-120; $F(17, 306) = 2.32$, and main effect of Group on minutes 40-70; $F(1,18) = 4.547$; all P 's < 0.05]. Thus, as in Experiment 1, the haloperidol-exposed rats developed dopamine supersensitivity.

In control rats, MDL100,907 (0.025-0.1 mg/kg) had no effect on amphetamine-induced locomotion (Figure 3A, C and E; all P 's > 0.05), but MDL100,907 attenuated spontaneous locomotion at all doses tested (main effect of treatment on minutes 0-30; Figure 3A; $F(1,29) = 5.027$; $p = 0.033$; Figure 3C; $F(1,24) = 8.580$; $p = 0.007$; Figure 3E; $F(1,24) = 12.27$; $p = 0.002$). In haloperidol-exposed rats, the highest dose of MDL100,907 (0.1 mg/kg) attenuated amphetamine-induced locomotion (0.1 mg/kg; Figure 3F; main effect of Treatment condition on minutes 60-120; $F(1,24) = 4.579$; $p = 0.04$), as well as spontaneous locomotion (Figure 2F; main effect of treatment on minutes 0-30; $F(1,24) = 6.919$; $p = 0.015$). No other comparisons were significant. Thus, haloperidol-exposed rats showed exaggerated amphetamine-induced locomotion, and 5-HT_{2A} receptor blockade normalised this response, while having no

effect on amphetamine-induced locomotion in antipsychotic-naïve rats. In parallel, a higher dose of MDL100,907 was required to alter spontaneous locomotion in haloperidol-exposed rats as compared to control animals.

4.3. Experiment 3: ex-vivo 5-HT_{2A} receptor binding

In Experiment 3, we determined whether haloperidol treatment combined with an acute injection of saline or amphetamine alters 5-HT_{2A} density in corticolimbic structures. In the caudate-putamen, nucleus accumbens core and all cortical regions analysed, there was no effect of amphetamine on 5-HT_{2A} density, in either haloperidol-exposed rats or control rats (data not shown; all P 's > 0.05). Thus, in these regions, rats that had received saline or amphetamine were pooled within each experimental group. In contrast, as will be detailed below, amphetamine had a small but significant effect on 5-HT_{2A} density in the nucleus accumbens shell. Thus, data from animals that received amphetamine or saline prior to sacrifice are presented separately for this brain region.

There was no effect of haloperidol exposure on 5-HT_{2A} receptor density in the infralimbic or orbitofrontal cortices (Figure 4B-D; all P 's > 0.05). However, haloperidol decreased 5-HT_{2A} receptor density in the cingulate cortex (Figure 4A; $F(1,24) = 5.583$, $p = 0.03$), and increased 5-HT_{2A} density in the caudate-putamen (Figure 5; between +1.6 and 0.0 mm relative to Bregma in the dorsolateral quadrant; $F(1,23) = 4.981$, $p = 0.04$; between +0.8 and 0.0 mm in the remaining quadrants, dorsomedial; $F(1,24) = 12.36$, $p = 0.002$; ventromedial; $F(1,24) = 7.892$, $p = 0.01$; ventrolateral; $F(1,24) = 9.461$, $p = 0.005$). In the nucleus accumbens

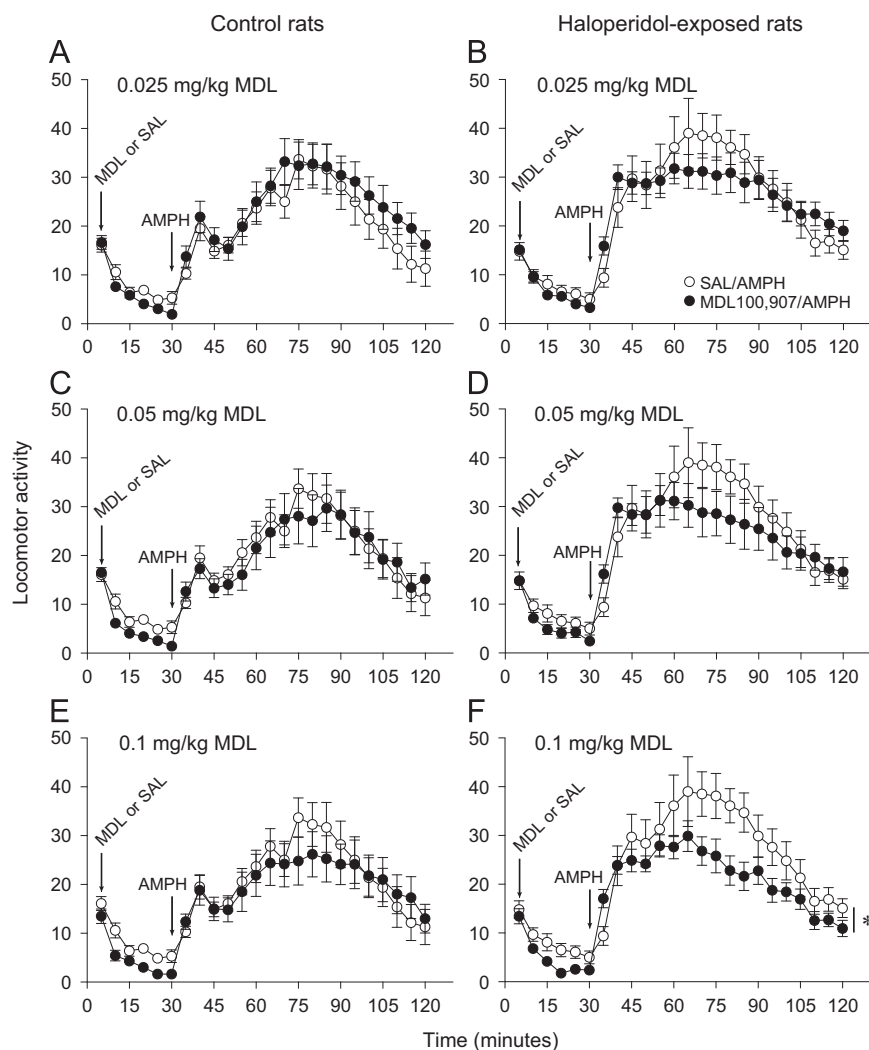


Figure 3 Blockade of 5-HT_{2A} receptors with MDL100,907 attenuates amphetamine-induced locomotion in haloperidol-exposed rats but not in control rats. * $p < 0.05$, minutes 60-120. SAL, saline. AMPH, amphetamine. MDL, MDL100,907. N's=10-21/treatment condition.

core, there was a significant Treatment condition \times Rostro-caudal position interaction effect (Figure 6A; $F(3,69) = 6.561$, $p = 0.0006$). Post-hoc investigation of this interaction effect revealed that haloperidol decreased 5-HT_{2A} density at +1.2 mm relative to Bregma ($p = 0.003$). In the nucleus accumbens shell, haloperidol-exposed rats had slightly greater 5-HT_{2A} density following amphetamine versus saline (Figure 6B; main effect of Treatment condition; $F(1,10) = 5.728$, $p = 0.04$), but there were no differences between haloperidol-exposed rats and control rats (all P 's > 0.05). No other comparisons were significant. Thus, prior haloperidol exposure decreased the density of 5-HT_{2A} receptors in the cingulate cortex and in a restricted region of the nucleus accumbens core (at +1.2 mm relative to Bregma), and haloperidol increased the density of 5-HT_{2A} receptors in the caudate-putamen.

5. Discussion

Chronic antipsychotic treatment evokes compensatory neurobiological changes that result in a state of supersensitivity

to dopamine stimulation. Here we investigated the contributions of 5-HT₂ receptors to the behavioural expression of antipsychotic-induced dopamine supersensitivity. Consistent with our prior findings (Bedard et al., 2011, 2013; El Hage et al., 2015; Samaha et al., 2008, 2007), withdrawal from a clinically pertinent regimen of haloperidol revealed a state of dopamine supersensitivity, as indicated by an augmented locomotor response to systemic amphetamine. Pharmacological blockade of either 5-HT₂ receptors or 5-HT_{2A} receptors selectively attenuated the locomotor response to amphetamine in dopamine supersensitive rats, while having no effect on this behaviour in antipsychotic-naïve rats. In parallel, antipsychotic treatment evoked persistent changes in 5-HT_{2A} receptor density in the prefrontal cortex and the striatum. These results support two conclusions. First, activation of 5-HT₂ receptors is required for the full expression of antipsychotic-induced dopamine supersensitivity. Second, dopamine supersensitivity enhances the ability of 5-HT₂ receptors to modulate dopamine-dependent behaviours.

In humans, the symptoms of antipsychotic-induced dopamine supersensitivity can emerge several weeks following treatment cessation. Here, rats were treated with

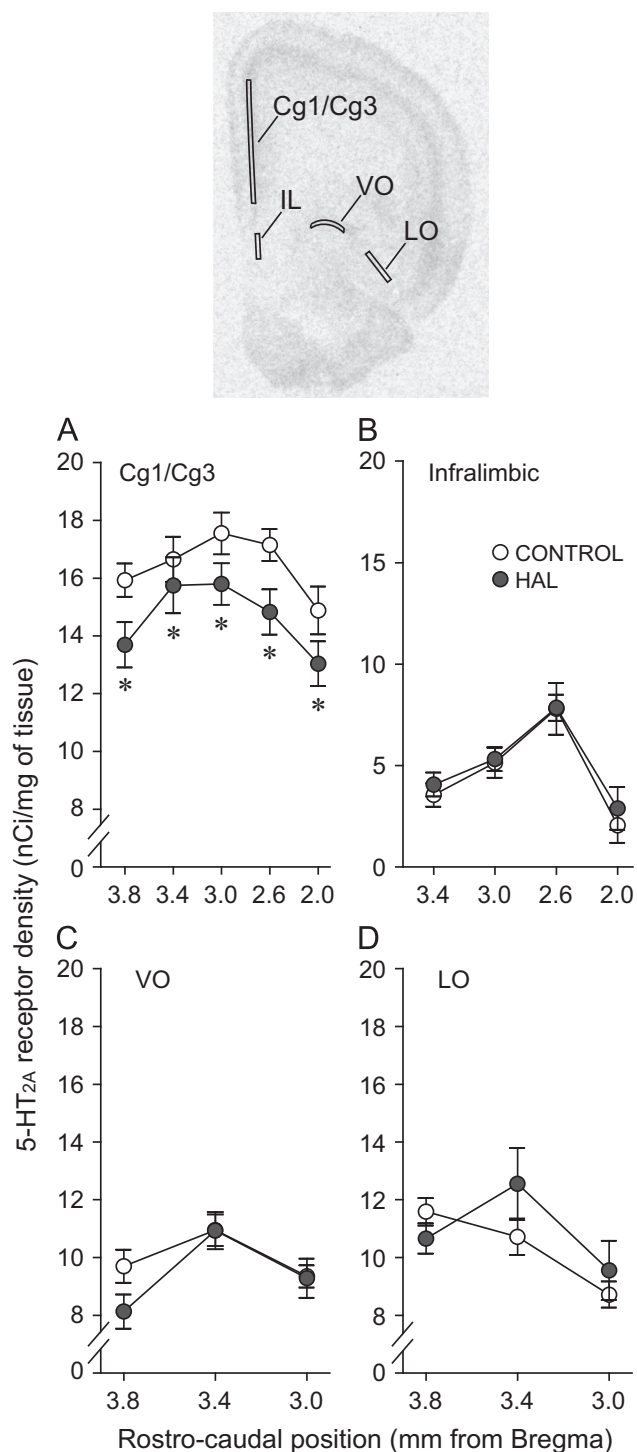


Figure 4 Haloperidol (HAL) treatment decreases 5-HT_{2A} density in the cingulate cortex (Cg1/Cg3). IL, infralimbic. VO, ventral orbitofrontal cortex. LO, lateral orbitofrontal cortex. **p* < 0.05, HAL-exposed rats compared to control rats. *N*s = 12-14/treatment condition.

haloperidol for 15-17 days, and behavioural and receptor density measurements were taken 8-10 days after treatment cessation. We did not measure dopamine supersensitivity at longer time points. However, in animals treated with antipsychotics, the persistence of dopamine

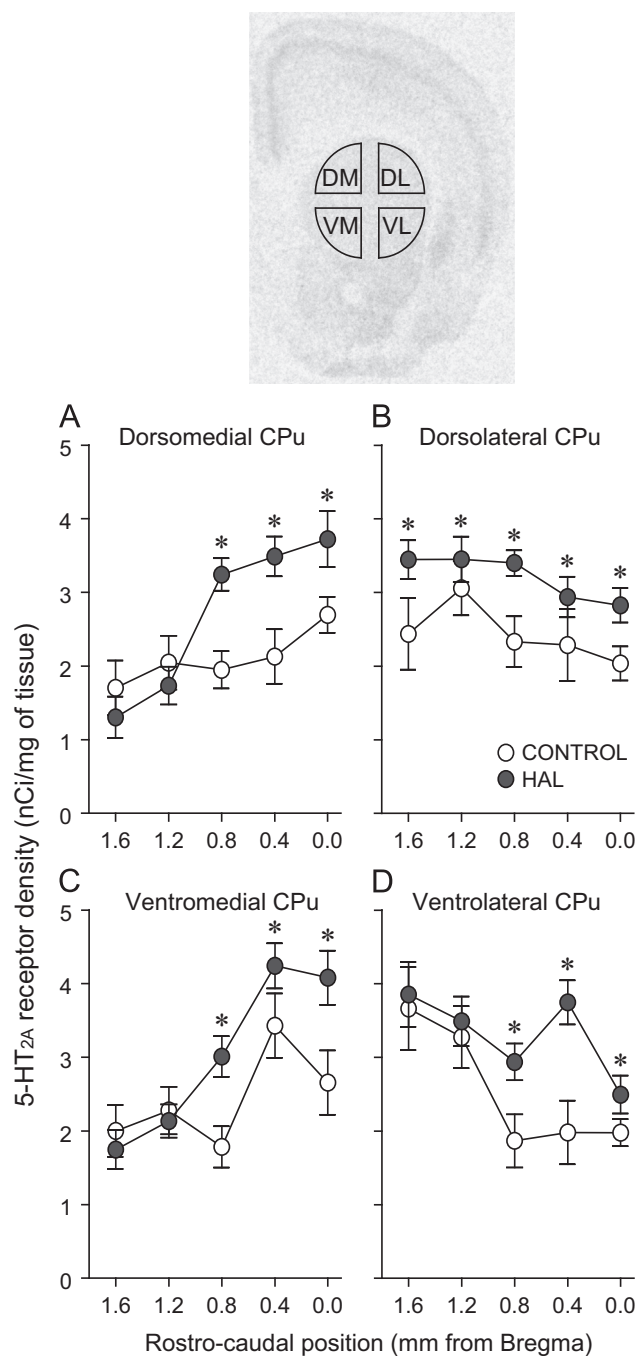


Figure 5 Haloperidol (HAL) treatment increases 5-HT_{2A} density in the caudate-putamen. **p* < 0.05, HAL-exposed rats compared to control rats at the same rostrocaudal position. CPU (caudate putamen). *N*s = 12-13/group.

supersensitivity is proportional to the length of the antipsychotic treatment (Muller and Seeman, 1977). As such, we would expect that dopamine supersensitivity in the present animal model would begin to wane at longer time periods following antipsychotic treatment cessation. Within this context, an interesting question concerns what a 10-day washout period in the rat might be equivalent to in human years. The answer is not simple, but based on developmental milestones, anatomy and physiology, it is estimated that in adult rats, 12 days are equivalent to 1 human year

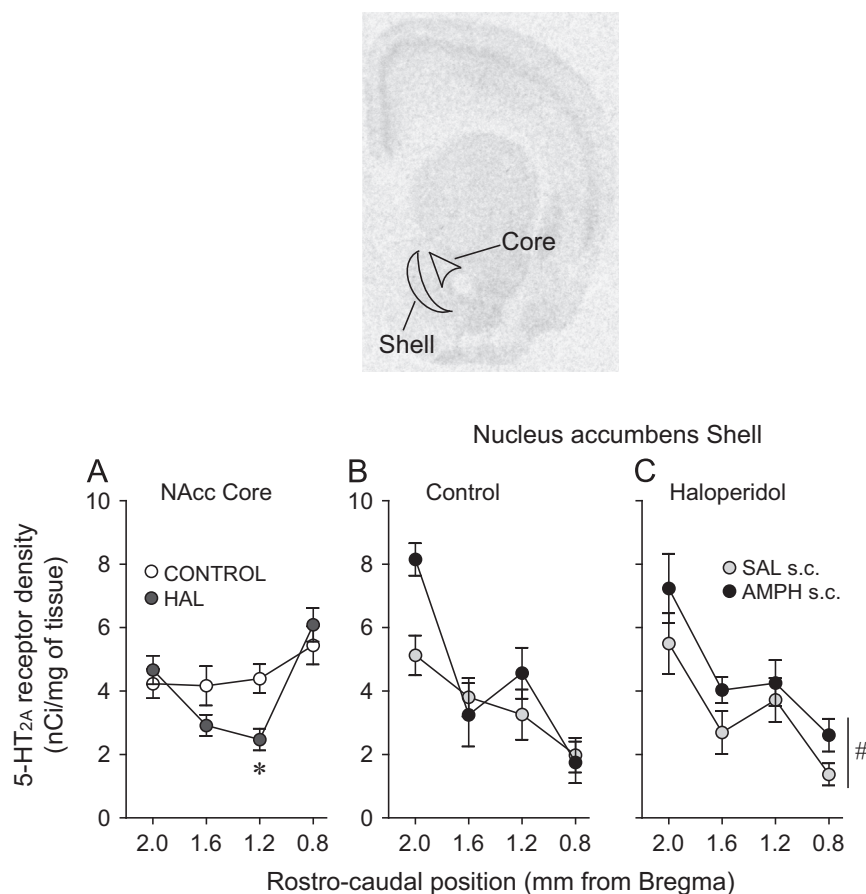


Figure 6 Haloperidol treatment (HAL) alters 5-HT_{2A} density in the nucleus accumbens. * $p < 0.05$, haloperidol-exposed rats compared to control rats at the same rostrocaudal position. $p < 0.05$. s.c., Subcutaneous injection. N 's=12-13/group.

(Sengupta, 2013). As such, an 8-10 day washout period is a significant amount of time in a rat's lifespan.

5.1. The relative contributions of 5-HT_{2A} and 5-HT_{2B/C} receptors

Both a 5-HT₂ receptor antagonist (ritanserin) and a selective 5-HT_{2A} receptor antagonist (MDL100,907) suppressed amphetamine-induced locomotion in dopamine supersensitive rats, at doses that were ineffective in control animals. The highest doses of ritanserin and MDL100,907 we tested did not disrupt amphetamine-induced locomotion in control animals, and this is in accordance with previous reports (Millan et al., 1999; Moser et al., 1996). We did not assess the effects of selective 5-HT_{2B} or 5-HT_{2C} receptor blockade. However, the ability of ritanserin to disrupt amphetamine-induced locomotion in antipsychotic-treated animals is likely driven by blockade of 5-HT_{2A} rather than 5-HT_{2B/C} receptors. Both ritanserin and MDL100,907 have high affinity for 5-HT_{2A} receptors, and both antagonists suppressed amphetamine-induced locomotion in haloperidol-exposed rats with similar efficacy. Ritanserin also binds to 5-HT_{2B/C} receptors. Its 5-HT_{2A} versus 5-HT_{2B/C} receptor selectivity ratio is less than 10:1, compared to greater than 300:1 for MDL100,907 (Palfreyman et al., 1993). However, in both control animals (Ball and Rebec, 2005) and in dopamine

supersensitive animals outside of the context of antipsychotics (Bishop et al., 2005; Filip et al., 2004), blockade of either 5-HT_{2B} or 5-HT_{2C} receptors generally does not affect the locomotor response to dopamine agonists [c.f., (Zayara et al., 2011)].

Blockade of 5-HT₂ receptors with ritanserin had no effect on spontaneous locomotion, in either experimental group. In contrast, blockade of 5-HT_{2A} receptors with MDL100,907 had dissociable effects on spontaneous versus amphetamine-evoked locomotor activity in control animals. In these animals, all doses of MDL100,907 suppressed spontaneous locomotor behaviour, but none altered amphetamine-induced locomotion. In contrast, in dopamine supersensitive rats, only the highest dose of MDL100,907 altered locomotor behaviour, and it suppressed both spontaneous and amphetamine-induced locomotion. This suggests that the neural mechanisms that regulate spontaneous versus amphetamine-induced locomotion are at least partially distinct in control animals. The mesolimbic system tightly regulates both spontaneous and amphetamine-induced locomotion, making it unlikely that the differential effects of MDL100,907 observed in control rats are due to the fact that different brain regions mediate the two behaviours. However, it is possible that the mesolimbic networks mediating spontaneous and amphetamine-evoked locomotion are neurochemically dissociable, in that they are differentially modulated by 5-HT_{2A} receptors. The

findings also suggest that dopamine supersensitive rats do not simply have a general and diffuse increase in 5-HT_{2A} receptor function. In these animals, 5-HT_{2A} receptors have an increased influence on amphetamine-induced locomotion but a reduced influence on spontaneous locomotion. While the mechanisms underlying these effects are not known, the findings indicate that dopamine supersensitivity produces enduring changes in 5-HT_{2A} receptor function.

5.2. How might antipsychotic treatment enhance the ability of 5-HT_{2A} receptors to modulate amphetamine-induced locomotion?

Blockade of 5-HT_{2A} receptors with MDL100,907 suppressed the locomotor response to amphetamine in antipsychotic-treated rats, while having no effect on this behaviour in control animals. Blockade of 5-HT_{2A} receptors with MDL100,907 can suppress amphetamine-induced locomotion while leaving other dopamine-dependent behavioural effects of amphetamine unchanged (Moser et al., 1996). This rules out the possibilities that MDL100,907 suppresses the behavioural response to amphetamine by preventing it from entering the brain and augmenting dopamine overflow or by directly inhibiting the actions of extracellular dopamine. Instead, blockade of 5-HT_{2A} receptors might attenuate the expression of dopamine supersensitivity by i) reducing dopamine neurotransmission through an indirect process, or ii) through a dopamine-independent mechanism.

The psychomotor activating effects of amphetamine are mediated by increased dopamine transmission in the striatum. Activation of 5-HT_{2A} receptors increases dopamine release in the nucleus accumbens (De Deurwaerdere and Spampinato, 1999; Lucas et al., 2000). In addition, in a dopamine supersensitivity model outside of the context of antipsychotics, the ability of 5-HT_{2A} receptors to promote the activity of D1-receptor bearing striatonigral cells is augmented (Basura and Walker, 2001). These observations suggest the hypothesis that antipsychotic treatment could potentiate the ability of 5-HT_{2A} receptors to promote dopamine release and/or D1-mediated signalling in the striatum, thereby enhancing the psychomotor response to drugs like amphetamine. Two predictions of this hypothesis would be that dopamine supersensitive animals have augmented amphetamine-induced dopamine overflow in the nucleus accumbens and that injecting amphetamine directly into the nucleus accumbens produces an enhanced psychomotor response in these rats. However, both baseline and amphetamine-evoked extracellular levels of dopamine in the nucleus accumbens are normal in rats showing antipsychotic-induced dopamine supersensitivity (Samaha et al., 2007). In addition, in these same rats, injecting amphetamine into the nucleus accumbens does not produce an exaggerated psychomotor response, suggesting that the actions of amphetamine in the nucleus accumbens are not sufficient to evoke the expression of dopamine supersensitivity (El Hage et al., 2015). Taken together, these results suggest that the ability of 5-HT_{2A} receptor antagonists to suppress the expression of dopamine supersensitivity involves blockade of 5-HT_{2A} receptors outside of the nucleus accumbens. One candidate region is the medial prefrontal cortex. Activation of 5-HT_{2A} receptors in the medial

prefrontal cortex increases the activity of dopamine cells in the ventral tegmental area, and the release of dopamine both in the ventral tegmental area and the medial prefrontal cortex (Bortolozzi et al., 2005).

Non-dopamine mechanisms could also contribute to the enhanced ability of 5-HT_{2A} receptors to modulate amphetamine-induced psychomotor activity in dopamine supersensitive rats. There is evidence that—outside of the context of antipsychotics—dopamine supersensitive animals have sensitised serotonin neurons, as indicated by enhanced serotonin overflow in the prefrontal cortex in response to application of a serotonin releaser, and that the development of this sensitised response depends upon the activation of 5-HT₂ receptors (Lanteri et al., 2008). Amphetamine releases serotonin and one possibility is that antipsychotic treatment potentiates this effect of amphetamine, leading to augmented signalling at 5-HT_{2A} receptors. However, this potential mechanism would have to involve additional neurotransmitter systems. First, at doses similar to the one used here, acute exposure to amphetamine increases extracellular levels of dopamine, but not 5-HT, in the nucleus accumbens (Kankaanpaa et al., 1998). Second, at least in otherwise naïve rats, selectively increasing 5-HT release does not enhance locomotor behaviour (Ziance et al., 1972).

5.3. Antipsychotic-treated rats have altered 5-HT_{2A} receptor density in corticostriatal regions

Antipsychotic-treated animals had pronounced changes in 5-HT_{2A} receptor density in the prelimbic subdivision of the medial prefrontal cortex (Cg1/Cg3). Consistent with findings in control animals (Blue et al., 1988), visual inspection of autoradiograms from both control and antipsychotic-treated rats revealed that cortical 5-HT_{2A} density was most abundant in a discrete layer located mid-way through the depth of the cortex. Within this middle layer, antipsychotic treatment decreased receptor density across the rostrocaudal axis of the prelimbic cortex. This is in accord with post-mortem studies showing attenuated 5-HT_{2A} receptor levels in the prefrontal cortex of antipsychotic-treated schizophrenia patients (Burnet et al., 1996). Within the middle layers of the rat prelimbic cortex, 5-HT_{2A} receptors are primarily postsynaptic and are found both on pyramidal cells and local circuit neurons (Miner et al., 2003). The minority of 5-HT_{2A} receptors located presynaptically are found mainly on putative dopamine fibres (Miner et al., 2003). Activation of 5-HT_{2A} receptors in the prefrontal cortex promotes the local release of glutamate and serotonin (Aghajanian and Marek, 1997), as well as dopamine overflow in the prefrontal cortex and the ventral tegmental area (Bortolozzi et al., 2005). Thus, if there is a causal link between downregulation of 5-HT_{2A} receptors in the prelimbic cortex and the expression of antipsychotic-induced dopamine supersensitivity, this could involve changes in the balance of serotonin, glutamate and/or dopamine neurotransmission within mesocortical networks, leading to an exaggerated behavioural response to dopamine agonist stimulation.

Antipsychotic treatment also evoked changes in 5-HT_{2A} receptor density within the striatum. Compared to control animals, antipsychotic-treated animals had a small decrease

in 5-HT_{2A} receptor density at a restricted rostrocaudal level of the nucleus accumbens core. It is unlikely that such a small change substantially contributes to the behavioural effects we report. In contrast, antipsychotic-treated rats showed significant upregulation of 5-HT_{2A} receptors in all quadrants of the caudate putamen, and across several rostrocaudal levels. 5-HT_{2A} receptors are abundant in the caudate putamen. They are equally distributed on medium spiny projection neurons forming the direct and indirect basal ganglia pathways, and are also expressed on GABA and cholinergic interneurons, and on putative cortical afferents (Navailles and De Deurwaerdere, 2011). Unlike what is observed in the nucleus accumbens, where activation of 5-HT_{2A} receptors promotes dopamine release, activation of 5-HT_{2A} receptors within the caudate putamen decreases stimulated dopamine release [with no effect on basal levels of extracellular dopamine (Lucas et al., 2000)]. Activation of striatal 5-HT_{2A} receptors also markedly increases the firing rate of cholinergic interneurons (Blomeley and Bracci, 2005). Note however that Blomeley and Bracci (2005) did not indicate whether measurements were made in the nucleus accumbens or caudate-putamen. Finally, activation of 5-HT_{2A} receptors within the caudate putamen positively regulates basal levels of extracellular glutamate (Ferguson et al., 2014). If antipsychotic-induced upregulation of 5-HT_{2A} receptors in the caudate putamen contributes to the behavioural expression of dopamine supersensitivity, this could occur via changes in the ability of 5-HT_{2A} receptors to regulate dopamine, acetylcholine and/or glutamate-mediated neurotransmission in caudate putamen-dependent networks.

Although the caudate putamen might contribute to the behavioural expression of dopamine supersensitivity, it is unlikely to play a central role. In prior work we have investigated the behavioural effects of injecting amphetamine into the caudate putamen of dopamine supersensitive rats (El Hage et al., 2015). These behaviours included amphetamine-induced locomotion and amphetamine-induced potentiation of operant responding for conditioned reward. Injecting amphetamine into the caudate putamen had no influence on these behaviours, in either control rats or dopamine supersensitive rats. However, in addition to locomotor activity, the psychomotor response to drugs like amphetamine also includes vertical activity and stereotyped behaviours. Future studies can determine whether the caudate putamen—and in particular 5-HT_{2A} receptors in this region—mediates these amphetamine-induced behaviours in dopamine supersensitive rats.

5.4. Limitations

We have evaluated the contributions of 5-HT₂ receptors to the expression of antipsychotic-induced dopamine supersensitivity in neurologically intact animals. This has allowed us to establish cause-and-effect relationships between chronic antipsychotic treatment and changes in 5-HT₂ receptor function in an otherwise unaltered brain. An important next step is to extend the present observations to animal models of schizophrenia-like symptoms. We have assessed the effects of 5-HT₂ receptor blockade following antipsychotic treatment cessation. Antipsychotic-induced dopamine supersensitivity can also occur during ongoing

treatment and undermine antipsychotic efficacy (Samaha et al., 2008, 2007). We do not know how 5-HT₂ receptors might be altered during ongoing antipsychotic treatment. However, our findings are clinically relevant because regular periods of medication cessation appear to be the norm rather than the exception in patients with schizophrenia (Masand et al., 2009). We did not measure amphetamine-induced locomotion in the animals used to assess the effects of antipsychotic treatment on 5-HT_{2A} receptor density. However, the antipsychotic treatment regimen used here evokes dopamine supersensitivity that is robust, reliable and highly reproducible (Bedard et al., 2011, 2013; El Hage et al., 2015; Samaha et al., 2008, 2007; Tadokoro et al., 2011).

The behaviour we used here to assess the expression of antipsychotic-induced dopamine supersensitivity is an empirical one. In humans treated with antipsychotic drugs, dopamine supersensitivity can manifest as tolerance to antipsychotic treatment (Fallon et al., 2012; Suzuki et al., 2015) and an increased likelihood of relapse to psychosis upon treatment withdrawal (Chouinard et al., 1978). The relationship of amphetamine-induced locomotion to these symptoms is unclear. However, amphetamine-induced locomotion permits an accessible and rapid assessment of dopamine function following antipsychotic treatment. In parallel, other studies have shown that antipsychotic-induced dopamine supersensitivity is also characterized by an enhanced ability of the direct dopamine receptor agonist apomorphine to stimulate both horizontal locomotor activity and stereotyped behaviour (Asper et al., 1973; Clow et al., 1979; Gianutsos et al., 1974; Montanaro et al., 1982; Sayers et al., 1975; Smith and Davis, 1975, 1976). Finally, a question raised by our findings is whether treatment with an atypical antipsychotic would induce similar alterations in 5-HT₂ receptor density and function. We would predict that any antipsychotic treatment protocol that produces dopamine supersensitivity would also enhance the ability of 5-HT₂ receptors to modulate dopamine-dependent behaviours. On this note, treatment with the atypical antipsychotic, olanzapine, using the same treatment regimen as used here with haloperidol, does not produce a dopamine supersensitive state (Bedard et al., 2013).

6. Conclusions

Within the context of our findings, it is noteworthy that compared to typical antipsychotic drugs such as haloperidol, atypical antipsychotics have higher affinities at several serotonin receptor types (Meltzer et al., 1989). This pharmacological difference might contribute to the lower incidence of dopamine supersensitivity following treatment with atypical versus typical antipsychotic drugs (Bedard et al., 2013; Chouinard et al., 1978; Fallon et al., 2012; Samaha et al., 2007; Woerner et al., 1991). In parallel, atypical antipsychotic medications have their own profile of negative side effects, some of which can be life-threatening, such as antipsychotic-induced metabolic syndrome. In light of these observations, it appears that improving existing treatments or developing new ones with fewer side effects is long overdue for patients who suffer from schizophrenia.

The findings presented here provide evidence that i) activation of either 5-HT₂ receptors or of 5-HT_{2A} receptors selectively is required for the full expression of antipsychotic-induced dopamine supersensitivity, ii) dopamine supersensitivity enhances the ability of 5-HT₂ receptors to modulate dopamine-dependent behaviours (as modelled by amphetamine-induced locomotion), and iii) these effects are potentially linked to changes in 5-HT_{2A} receptor density in the prefrontal cortex and the striatum. These observations raise the possibility that once antipsychotic-induced dopamine supersensitivity has developed, its behavioural manifestations might be overcome by blockade of 5-HT_{2A} receptors with a systemic antagonist.

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Author contributions

Anne-Noël Samaha designed all studies. Alexandra Charron and Cynthia El Hage conducted all behavioural studies. Alice Servonnet measured and quantified 5-HT_{2A} receptor density in corticostriatal regions. Cynthia El Hage and Alice Servonnet statistically analysed all data presented in the manuscript. Cynthia El Hage, Alice Servonnet and Anne-Noël Samaha wrote the manuscript. All authors have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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