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5-HT_6 receptor antagonist reversal of emotional learning and prepulse inhibition deficits induced by apomorphine or scopolamine

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Abstract

5-HT_6 receptors have been implicated in consolidation of visuospatial and reward-based learning tasks. Since 5-HT_6 receptors may be important in modulation of sensory gating which is often affected in schizophrenic patients, we tested whether Ro 4368554, a 5-HT_6 selective antagonist at a dose of 10 mg/kg, could reverse the loss of prepulse inhibition from apomorphine or scopolamine. In addition, we also tested whether Ro 4368554 altered fear conditioning using fear potentiated startle, a model for emotional learning. Prepulse inhibition of startle was disrupted by apomorphine (0.5 mg/kg) when prepulse emissions were 5 dB above background but not above 15 dB, while scopolamine (0.5 mg/kg) caused disruption at both prepulse levels. Scopolamine-mediated disruption was not reversed by Ro 4368854 but apomorphine-mediated disruption was significantly ameliorated by 5-HT_6 inhibition. For fear potentiated startle, scopolamine and/or Ro 4368554 were administered before two daily fear conditioning sessions; rats were tested on the following day. Rats that received scopolamine displayed no fear potentiated startle but Ro 4368554 reversed this scopolamine deficit. Additionally, we mapped Fos induction in rats treated with scopolamine and/or Ro 4368554; scopolamine increased Fos expression in the central nucleus of the amygdala and this was attenuated by Ro 4368554. In summary, we have demonstrated the efficacy of 5-HT_6 antagonists in modulating sensory gating and fear conditioning, and thus may be of therapeutic use for schizophrenia-related disorders.

Keywords: Serotonin receptor; Fos; Scopolamine; Amygdala; Apomorphine; Schizophrenia; Fear potentiated startle

1. Introduction

Schizophrenia is a neurodegenerative psychiatric disease with the hallmarks of disordered thought, auditory and visual hallucinations, emotional dysregulation, and cognitive impairment (Thomas and Woods, 2006). Cognitive symptoms impact attention, working memory and other aspects of memory consolidation, emotion discrimination and predict functional outcome (Milev et al., 2005). Newer antipsychotics may offer some advantages in treating cognitive symptoms (Keefe et al., 2004), but the pharmacological basis for improvement is not known and does not correlate strongly with improvement in positive symptoms. Some of these atypical antipsychotics have potent 5-HT_6 antagonist properties which may contribute to their efficacy (Mitchell and Neumaier, 2005; Roth et al., 2004). A number of studies have shown that 5-HT_6 antagonists can improve memory consolidation using several animal models (Mitchell et al., 2006, 2007); however, the contribution of 5-HT_6 receptors to emotional learning has not been described. This study investigated the potential use of a 5-HT_6 antagonist in prepulse inhibition of startle, an index of sensory motor gating that is relevant to attentional processing, and in fear potentiated startle, a model of emotional learning.

The 5-HT_6 receptor is a G-protein-linked receptor which activates the production of cAMP, and is expressed primarily in the striatum, nucleus accumbens, cortex and to a lesser degree in the hippocampus and thalamus (Gerard et al., 1997; Kohen et al., 2001; Monsma et al., 1993; Ruat et al., 1993). Antagonists of 5-HT_6 receptors have been shown to enhance memory consolidation in novel object recognition, social discrimination, and in Morris water maze. However, the greatest enhancement has been seen in memory deficit models, i.e. after scopolamine administration or in aged animals (Foley et al., 2004; Meneses,

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2001; Mitchell et al., 2006; Sleight et al., 1998). To date, there has been one study investigating the effects of 5-HT_6 antagonists on prepulse inhibition disrupted by amphetamine and PCP with negative results, although the compound used has limited brain penetrance (Pouzet et al., 2002). Ro 4368554 is a high affinity antagonist (pKi of 9.4) with >50-fold selectivity for 5-HT_6 receptors over other receptors (Bonhaus et al., 2002) and acceptable brain penetrance (brain/plasma ratio 0.8–1.1) (Schreiber et al., 2007). Ro 4368554 has been shown to improve memory in autoshaping, and reverse the effects of scopolamine in passive avoidance, social recognition and objection recognition, though had no effect on Morris water maze performance (Schreiber et al., 2007). In the present study, Ro 4368554 reversed the disrupting effects of apomorphine at lower prepulse noise levels, and also attenuated the amnesic effects of scopolamine in fear potentiated startle.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (240–260 g) were purchased from Charles River Laboratories and pair-housed for at least a week before behavioral testing. All animals were kept on a 12 h light/dark schedule and fed ad-lib water and chow. The rats were handled daily for several days before testing. Four groups of 8–10 rats each were given either scopolamine and/or Ro 4368554 for fear potentiated startle testing. For prepulse inhibition testing, 4 groups of 8–10 rats each were used; for the Fos mapping study, 6–8 rats were used per group. All animal procedures were approved by the Institutional Animal Care and Use Committee.

2.2. Drugs

Apomorphine was purchase from Sigma (Rockford, IL) and dissolved in saline and injected intraperitoneally as a 0.5 mg/mL solution. Scopolamine was purchased from American Pharmaceutical Partners (Schaumberg, IL) and injected intraperitoneally as a 0.4 mg/mL solution. We thank Roche for their kindness in providing Ro 4368554. Ro 4368554 was dissolved in 1% acetic acid in phosphate buffer and sonicated, then heated to 50 °C. Ro 4368554 was administered intraperitoneally as a 10 mg/mL solution.

2.3. Apparatus

All rats were tested in one of three SR-LAB startle units (San Diego Instruments, San Diego, CA) which had identical shock generators and stereo speakers. Each unit was equipped with a clear acrylic cylinder (8 cm diameter) in which a gridded shock floor was inserted. Each cylinder had sliding plastic panel doors and was mounted on a platform attached to a piezoelectric accelerometer. Fans ventilated the cabinet and speakers provided a background noise level of 70 dB; lighting was provided by a 15 W halogen bulb affixed to the ceiling of the chamber. The software used to run the boxes was SR-LAB program (San Diego Instruments), on a PC-compatible computer.

3. Procedures

3.1. Prepulse inhibition

Rats were assessed for individual gating, which refers to the capacity of the brain to "gate" or filter out irrelevant stimuli. The rats were tested for baseline startle by exposure to 10 trials of a pulse noise (110 dB) and also 3 each of background, low prepulse noise only (75 dB) and high prepulse noise only (85 dB). Based on their performance in the baseline session, the rats were separated into groups of similar average startle amplitudes. Three days after individual baseline startles were determined, rats were tested for prepulse inhibition. The rats were brought to the behavior room an hour before testing for acclimatization; the holding cylinder and shock floor were thoroughly cleaned with a disinfectant before each trial. Rats were injected with scopolamine (0.4 mg/kg i.p.) or apomorphine (0.5 mg/kg i.p.) 30 min before each conditioning session; Ro 4368554 (10 mg/kg i.p.) or saline was given 10 min before each conditioning session. There were separate vehicle only groups for both apomorphine and scopolamine treatment groups. A noise generator produced background noise of 70 dB throughout the session. The session began with 5 min of acclimatization before onset of the first trial, in which a noise prepulse burst (40 ms in length) was followed by a test pulse burst (60 ms in length), and the amplitude of startle was recorded for 120 ms. The interstimulus interval (ISI) was 100 ms. Each session consisted of 52 trials including 12 trials of test pulse only (110 dB), 2 trials of low prepulse tone only (75 dB), 2 trials of high prepulse tone only (85 dB), 14 trials of low prepulse plus pulse burst and 16 trials of high prepulse plus pulse burst. The interval time between trials was randomly varied between 15 and 20 s. Six trials of startle to background were also recorded. Data are expressed as a PPI percentage calculated as: $100-[(\text{mean startle amplitude for prepulse plus pulse trials}/\text{mean startle amplitude for pulse only trials})\times 100]$.

3.2. Fear potentiated startle

Fear potentiated startle was tested using the same apparatus described above and a previously described method (Clark et al., 2004). Briefly, rats were brought to the testing room 1 h beforehand. Each chamber was cleaned with NPD and dried before individual testing. A gridded shock floor was inserted in the testing chamber for both conditioning and testing days. Rats were injected with either Ro 4368554 (10 mg/kg i.p.) or saline 10 min before conditioning. Scopolamine (0.4 mg/kg) or saline was injected 5 min before conditioning. The rats acclimatized to the startle chamber for 5 min before the trials began. Each conditioning session, run by SR-LAB software, consisted of 15 trials of shock/light pairing, lasting one half hour. The interval between each trial was 1–3 min. During each conditioning session trial, a 3.7 s light stimulus was presented; 3.2 s after initiation of the light stimulus, a 500 ms

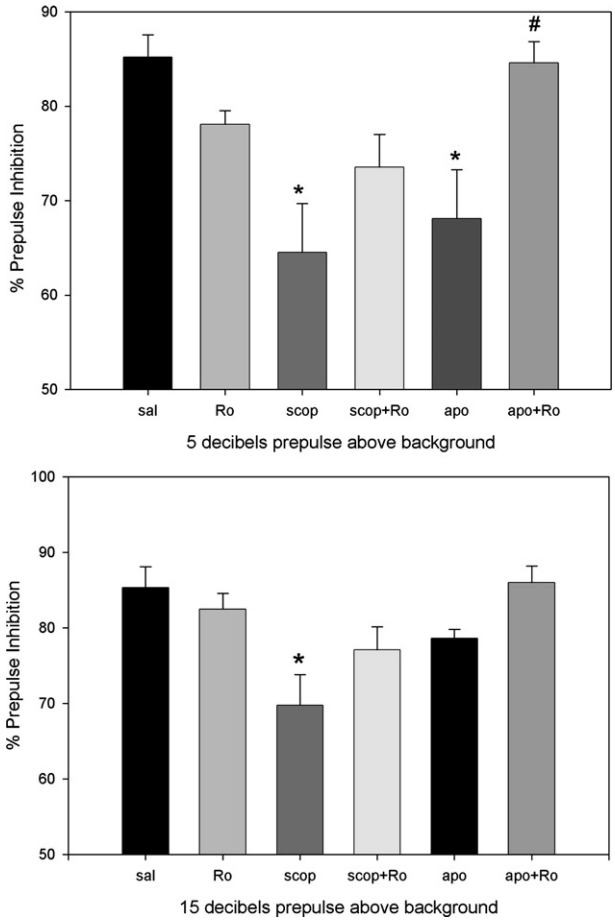

Fig. 1. Loss of prepulse inhibition from apomorphine is reversed by Ro 4368854. Means are shown with standard errors. sal: saline; Ro: Ro 4368554; scop: scopolamine; scop+Ro: scopolamine and Ro 4368554; apo: apomorphine; apo+Ro: apomorphine and Ro 4368554. Prepulse inhibition with prepulse 5 dB or 15 dB above background. Ro 4368554 (10 mg/kg i.p.) or saline was pretreated 30 min before an i.p. injection of scopolamine (0.4 mg/kg), or apomorphine (0.5 mg/kg,). Values are means±S.E.M. with group $n=8–12$. $^{*}p<0.05$, when compared to saline, $^{\#}p<0.05$, when compared to the apomorphine.

0.8 mA shock was delivered via a shock grid. Conditioning sessions were run daily for two days, during morning hours. The startle testing session was given one day after the last conditioning session. This testing session entailed partial habituation with 10 trials each of 95 and 105 dB noise bursts followed by 15 presentations of startle stimulus alone (dark startle) and 15 presentations of startle stimulus plus light (light–startle pairings), given in random order. For startle–light pairings, animals were presented with a 3.7 s light stimulus; 3.2 s into the light stimulus, a 50 ms 110 dB SPL white noise burst was initiated. Startle response was measured by the accelerometer at 1 ms intervals for 200 ms after the startle-inducing acoustic stimulus (the 50 ms white noise pulse at 110 dB). In order to normalize individual differences in startle response between animals, the startle amplitudes are normalized by expressing the ratio of averaged light/dark startle amplitudes. In order to observe rate of extinction, the trial averages are collapsed into three sets of trials, where each set is comprised of 5 trials.

3.3. Fos immunohistochemistry

Rats were injected with saline, Ro 4368554 (10 mg/kg i.p.), scopolamine (0.4 mg/kg i.p.) or a combination of scopolamine and Ro 4368554 where Ro 4368554 was injected 15 min before scopolamine. Two hours later, rats were perfused transcardially with phosphate-buffered saline and then 4% paraformaldehyde. Brains sections of 40 μm were cut with a freezing microtome.

As previously described (Mitchell et al., 2006), the sections were placed in wells and briefly washed in PBS, then incubated in 0.2% Triton X-100/PBS. Following incubation in 5% normal goat serum (NGS)/0.2% Triton/PBS for 1 h, the sections were set in a solution of 2.5% NGS/0.1% Triton/PBS and the primary antibody to Fos (rabbit, 1:5000, Chemicon) and agitated gently for two nights at 4 °C. The sections were then washed in PBS and incubated for 1 h with a secondary antibody (goat anti-rabbit or horse anti-mouse, Vector) in a 1:200 solution made in 2.5% NGS/0.1% Triton/PBS. After further rinsing, sections were incubated in an avidin–biotin peroxidase (ABC) solution, prepared according to the manufacturer directions (Vector Laboratories), for 90 min. Diaminobenzidine (0.05%) with

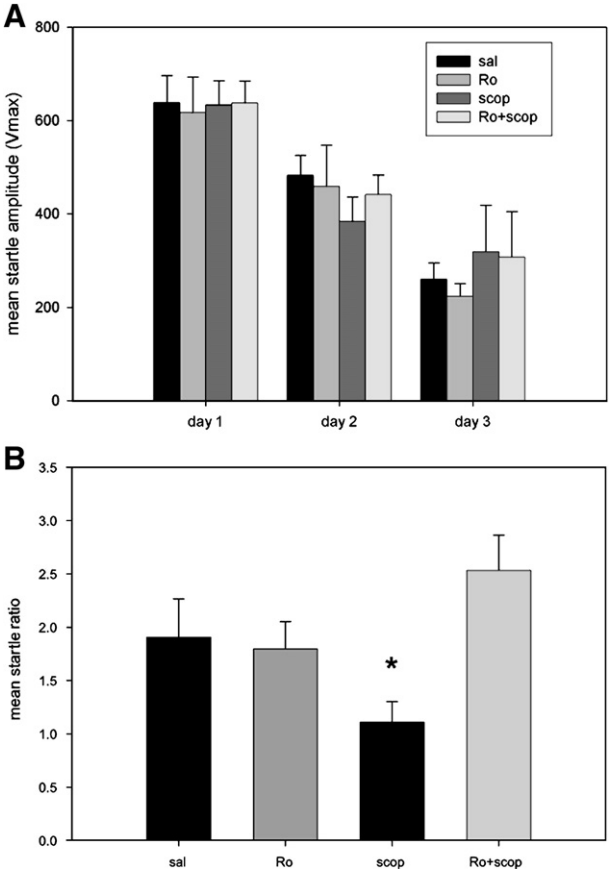

Fig. 2. A. No effect of scopolamine or Ro 4368554 during the 105 dB pulse: conditioning sessions (day 1 and day 2) and the testing session (day 3). Shown are the mean Vmax startle amplitudes with standard error. B. Ro 4368554 reverses scopolamine deficit in fear potentiated startle. Shown are the mean startle ratios (light vs. dark startle) during the third day testing session, with standard error bars: saline, Ro 4368554, scopolamine, Ro 4368554+scopolamine. $n=8–12$. *Significantly different from saline group ($p<0.05$).

nickel ammonium sulfate (0.25%) and H_2O_2 (0.0015%) was used to stain the sections. After mounting on slides coated with gelatin and dried, the sections underwent submersions in 70%, 90%, 100% ethanol, (2 min each) and xylene (5 min). The slides were coverslipped with Permount.

Brain sections were analyzed with ImageJ (distributed by NIH). Only darkly-stained cells that exceeded a threshold set to remove all background staining were counted. One section was used to count immunoreactive cells from a specific brain region, and counts from each side were averaged together. Adjacent sections, which were also processed immunohistochemically, were examined to verify the location of specific brain regions on a chosen section, in order to obtain consistency in counting. The following regions were demarcated according to anatomical designations detailed in Paxinos and Watson (Paxinos and Watson, 1986): the centromedial amygdala (CeM), centrolateral amygdala (CeL), CA1 and CA3 region of the hippocampus. A 200 × 400 μm region of the medial striatum (StrM) and lateral striatum (StrL) was quantified from each brain. For smaller regions (the amygdala nuclei and CA1 and CA3 regions of the hippocampus), the region was demarcated using the outline tool in NIH Image, and the number of cells within that entire area was counted.

3.4. Data analysis

Data was analyzed for significance using a 2 × 2 analysis of variance (ANOVA) with significance set at $p < 0.05$. All bar graphs show the means of each group and error bars represent the standard error of the means.

4. Results

4.1. Prepulse inhibition

Prepulse inhibition of startle is a measure of sensorimotor gating, in which a moderate-level noise stimulus preceding a larger one is sufficient to inhibit the acoustic startle reflex. Two

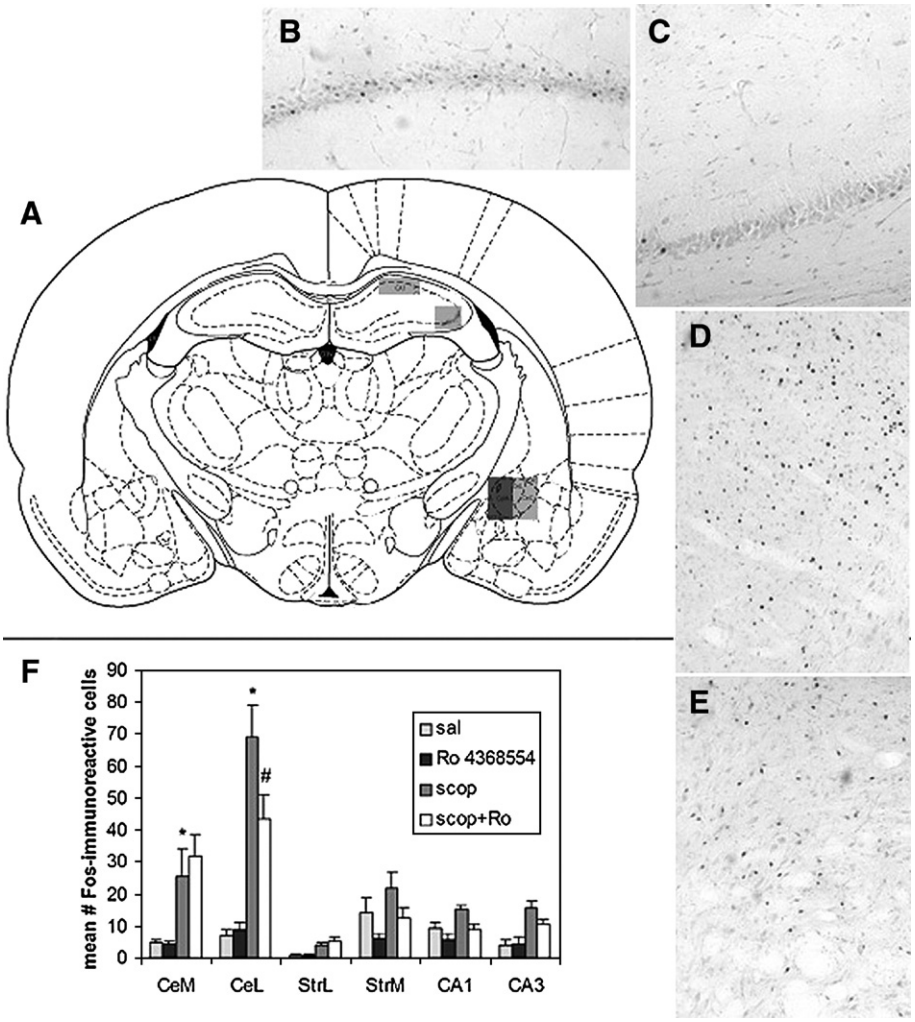

Fig. 3. Fos induction after treatment with scopolamine is increased in the central amygdala. A. Schematic drawing depicting typical regions of analysis for the hippocampus and central amygdala B. Fos expression in the CA1 region of the hippocampus. C. Fos expression in the CA3 region of the hippocampus. D. Fos expression in the centromedial amygdala. E. Fos expression in the centrolateral amygdala. F. Mean number of Fos-immunoreactive cells in the centromedial (CeM) and centrolateral amygdala (CeL), and lateral (StrL) and medial striatum (StrM), CA1 and CA3 regions of the hippocampus. SAL, saline; RO, Ro 4368554; SCOP, scopolamine; RO + SCOP, Ro 4368554 + scopolamine. Significantly different from saline group ($p < 0.05$). $^{\#}$Significantly different from scopolamine group ($p < 0.05$).

prepulse intensities, 5 and 15 dB above background, were used to test low and high sensorimotor gating. Either apomorphine, a dopamine agonist, or scopolamine, a muscarinic receptor antagonist, was used to disrupt such gating and the selective 5-HT_6 antagonist, Ro 4368554, was tested for reversal potential.

Ro 4368554 (10 mg/kg i.p.) given alone before the testing session had no effect on startle amplitude (243+42) as compared to two vehicle groups (241+45 and 250+32) or prepulse inhibition (Fig. 1) as compared to vehicle injection. Scopolamine (0.4 mg/kg i.p.) had no effect on startle amplitude (304+43) but disrupted prepulse inhibition for both prepulse levels (5 dB above background and 15 dB above background, ($F(1,33)=2.42$, $p<0.039$) (Fig. 1). Apomorphine (0.5 mg/kg i.p.), which had no effect on baseline startle (221+36), disrupted prepulse inhibition for prepulse emissions 5 dB above background; the apomorphine group displayed significantly lower mean prepulse inhibition than saline controls ($F(1,27)=6.04$, $p<0.018$) (Fig. 1). However, for the prepulse trials above 15 dB, apomorphine had no effect on prepulse inhibition as compared to controls ($F(1,27)=0.9$, $p<0.37$).

Ro 4368554 significantly normalized prepulse inhibition in apomorphine-treated animals at the 5 dB prepulse condition (apomorphine X Ro 4368554: $F(1,27)=5.3$, $p<0.029$) (Fig. 1). The effect of Ro 4368554 on apomorphine was not significant for the 15 dB prepulse condition (apomorphine X Ro 4368554: $F(1,27)=1.46$, $p<0.237$). Ro 4368554 given in conjunction with scopolamine did not significantly alter the scopolamine-mediated disruption of prepulse inhibition although there was a nonsignificant trend for scopolamine reversal for the 5 dB prepulse trial (scopolamine X Ro 4368554: $F(1,33)=3.03$, $p<0.08$).

4.2. Fear potentiated startle

Fear potentiated startle measures Pavlovian conditioning of a fear response to a light stimulus that has been previously paired with a footshock; startle responses to a sound stimulus given in conjunction with the light stimulus (but without shock) or in the dark are measured subsequently in a drug free session. Scopolamine had no effect on baseline startle on the testing day (Fig. 2A), but prevented the potentiation of startle associated with the conditioned stimulus (light) as demonstrated by the decrease in startle–light/dark ratio ($F(1,29)=4.8$, $p<0.035$) (Fig. 2B), presumably by interfering with the learned association (Yap et al., 2005). Ro 4368554 (10 mg/kg i.p.) given prior to the training sessions reversed the scopolamine-mediated decrease in mean startle ratios (scopolamine X Ro 4368554: $F(1,29)=3.9$, $p<0.047$) (Fig. 2B). There was no effect of Ro 4368554 alone on extinction of fear potentiated startle mean ratios during the testing session as compared to vehicle (data not shown).

4.3. Fos immunohistochemistry

Naïve animals were injected with saline, scopolamine, Ro 4368554 or a combination of both scopolamine and Ro 4368554 and their brains were analyzed for Fos expression 2 h later. No changes were seen in any brain region in animals given Ro 4368554 as compared to saline controls (Fig. 3F). Scopolamine-treated rats displayed significantly increased Fos expression in the centrolateral and centromedial amygdala regions ($F(1,24)=10.5$, $p<0.003$, $F(1,24)=5.2$, $p<0.02$). Ro 4368554 alone did not affect Fos expression as compared to saline-treated animals, but it decreased Fos expression induced by scopolamine in the centrolateral amygdala ($F(1,24)=10.7$, $p<0.003$) but not in the centromedial amygdala.

5. Discussion

5.1. Prepulse inhibition

Prepulse inhibition occurs when the motoric startle response to an intense stimulus (such as a loud noise) is reduced by prior exposure to a less intense stimulus, and as such is commonly referred to as a form of sensory gating. Sensory gating is impaired in schizophrenics and the animal correlate may be a useful probe into the neurocognitive pathophysiology of schizophrenia (Roth et al., 2004). Prepulse inhibition is disrupted by agents that act on several neurotransmitter signaling pathways, including dopamine, glutamate and serotonin (Geyer et al., 2001).

Recently anticholinergic drugs have been shown to disrupt prepulse inhibition (Jones et al., 2005; Sipos et al., 2001; Ukai et al., 2004). This is relevant to the pathophysiology of schizophrenia but is also important since these drugs are frequently used in treating the side effects induced by antipsychotic drugs that produce extrapyramidal side effects. Additionally, anticholinergic drugs have been shown to disrupt prepulse inhibition in humans and may intensify symptoms in schizophrenics(Kumari et al., 2001). Previously, Jones and co-workers demonstrated that scopolamine-mediated disruption of prepulse inhibition is reversed by D2 but not D1 antagonists (Jones et al., 2005). They proposed that the dopaminergic system modulates the rate of decay of the gating of the startle stimulus by the prepulse stimulus, while the muscarinic cholinergic system modulates the signal-to-noise ratio during detection of the prepulse stimulus. Thus they projected that muscarinic antagonists would be less effective at higher noise levels of prepulse. In our hands, scopolamine disrupted prepulse inhibition at the highest prepulse level while apomorphine did not. However, since we used a slightly higher dose of scopolamine (0.4 mg/kg as compared to the 0.3 mg/kg dose of the Jones and Shannon study), this concentration may have masked the cholinergic sensitive signal-to-noise detection at lower prepulse tone intensities. The mechanism of such a proposed action of scopolamine-mediated disruption requires further investigation. In any case, 5-HT_6 receptor blockade did not reverse scopolamine-mediated disruption of prepulse inhibition. This is an important observation, given that 5-HT_6 antagonists have been effective in reversing scopolamine-mediated impairment of memory consolidation in other behavioral models (Mitchell and Neumaier, 2005). It may be that scopolamine disruption of prepulse inhibition occurs by a mechanism that is insensitive to

modulation of 5-HT$_6$ receptor activity, unlike other cognitive processes that show interactions between 5-HT$_6$ and cholinergic receptors.

While we cannot rule out the possibility that a higher dose of Ro 4368554 would achieve greater reversal of PPI deficit, higher doses have been found to cause nonspecific problems using an i.p. route of administration, such as irritation at the injection site which could contribute to stress in the experimental subject (personal communication, Rudy Schreiber). Moreover, in a recent paper by Schreiber et al. (2007), 30 mg/kg Ro 4368554 actually decreased performance in several cognitive tasks such as object recognition and autoshaped learning. It is a limitation of this study that multiple doses of Ro4368554 were not tested; however, higher doses were not feasible and lower doses are unlikely to have been more effective.

The disruptive effects of apomorphine on prepulse inhibition have been well documented and its mechanism of action partially uncovered (Auclair et al., 2006). However, this drug can be somewhat variable in eliciting prepulse disruption, perhaps depending in part on apomorphine dose (Auclair et al., 2006; Davis et al., 1990). Jones and co-workers have demonstrated that when high doses of apomorphine are used (>0.5 mg/kg), antipsychotic drugs with 5-HT$_6$ activity such as olanzapine are less effective at reversing prepulse inhibition deficits; this is most apparent at higher prepulse sound levels (Jones et al., 2005). Therefore we used a relatively low dose of apomorphine to allow greater sensitivity for a reversal effect from the 5-HT$_6$ antagonist. Thus, 0.5 mg/kg apomorphine disrupted prepulse inhibition at the lower prepulse tone level (5 dB above background) but not at a higher level of prepulse (15 dB above background). Ro 4368554 reversed apomorphine's effects at the lower prepulse tone level, which indicates that this drug may counteract the disrupting effects of increased dopamine stimulation. A useful follow-up study would involve testing a variety of 5-HT$_6$ antagonists at several doses against incremental doses of apomorphine, to fully evaluate the potential of 5-HT$_6$ antagonists in this particular model.

Dopamine has important effects on attention and signal to noise sensitivity (Winterer and Weinberger, 2004), as seen by dopamine agonists' disruption of prepulse inhibition, so it is possible that 5-HT$_6$ antagonists attenuate dopaminergic function, through an indirect pathway. 5-HT$_6$ expression is most abundant in the striatum, but there is limited information about how these receptors affect other neurotransmitters in basal ganglia circuits. Microdialysis studies have not supported a role for 5-HT$_6$ receptors in modulating dopamine transmission (Dawson et al., 2000, 2001), although one study did report elevated extracellular dopamine in the cortex during 5-HT$_6$ blockade (Lacroix et al., 2004). 5-HT$_6$ agonists increase phosphorylation of DARPP-32 in striatal neurons, suggesting a point of intersection with dopaminergic function (Svenningsson et al., 2002). Serotonin-6 blockade may be changing the rate of firing of dopamine neurons, since 5-HT$_6$ inhibition decreased spontaneous firing of dopamine neurons in the VTA (Minabe et al., 2004). More experiments are needed to further elucidate the role of 5-HT$_6$ receptors' influence over particular dopamine systems and receptor subtypes.

Previous studies found that 5-HT$_6$ antagonists such as Ro 04-6790 did not reverse disruption of prepulse inhibition by amphetamine, LSD or PCP (Leng et al., 2003; Ouagazzal et al., 2001). One possible explanation for these results are that some of the 5-HT$_6$ antagonists used in these studies have low brain penetrance and may not have achieved sufficient brain concentration to be effective. Another potential reason is that 5-HT$_6$ receptor antagonists have a modest effect that could not overcome the impact of the moderately high doses of drugs used in those studies. In this study we used the minimal doses of scopolamine and apomorphine necessary to significantly disrupt prepulse inhibition; this may have increased the sensitivity for detecting 5-HT$_6$ reversal of these drugs.

5.2. Fear potentiated startle

To our knowledge, this is the first report of a scopolamine-mediated inhibition of fear conditioning as measured by fear potentiated startle. Greba and co-workers found that direct infusion of methylscopolamine into the ventral tegmental area impaired the expression, but not the acquisition or consolidation, of fear potentiated startle (Greba et al., 2000). We can rule out the possibility that scopolamine was inhibiting startle in general, since scopolamine had no effect on startle amplitude during the light/shock pairings as compared to saline controls. Since scopolamine decreased fear potentiated startle on the testing day but had no effect on startle during the conditioning sessions; this suggests an amnesic, rather than anxiolytic, mechanism of scopolamine action under these experimental conditions.

Ro 4368554 alone did not alter fear potentiated startle, but did reverse the effects of scopolamine on fear conditioning learning. As stated above, a number of studies have demonstrated the ability of 5-HT$_6$ antagonists to alleviate memory deficits caused by scopolamine in nonrewarding learning tasks (Mitchell et al., 2006; Rogers and Hagan, 2001; Sleight et al., 1998). There is little data on the effect of 5-HT$_6$ inhibition in fear conditioning paradigms, but one study reported that the 5-HT$_6$ antagonist, SB-271046, reversed a scopolamine-mediated deficit in avoidance of a shock floor (Foley et al., 2004). However, the mechanism through which this reversal occurs is unknown. Administration of the 5-HT$_6$ antagonist SB 271046 elevates cholinergic overflow in several brain regions, including the hippocampus (Dawson et al., 2001), which has been shown to modulate the amygdala's response to arousing stimuli (Huff et al., 2006). While 5-HT$_6$ receptors are expressed in amygdala (Gerard et al., 1997), their functional role in this region has not previously been examined. Interestingly, a previous study has shown that picrotoxin injection into the amygdala increases 5-HT$_6$ receptor mRNA in the hippocampus (Benes et al., 2004). Therefore, it is possible that 5-HT$_6$ inhibition is mediating its pro-cognitive effect in fear potentiated startle via the amygdala, an area important for fear learning (Davis, 1992), although the present data does not address whether this might be a direct or indirect effect.

5.3. Fos expression

In the present study we used the immediate early gene Fos to identify populations of neurons activated by systemic Ro

4368854, scopolamine or a combination of both drugs. We focused on regions activated with memory processing during fear, most notably the hippocampus and amygdala, as well as a region of abundant 5-HT_6 receptor expression, the striatum. Surprisingly, we observed increased neuronal expression of Fos protein by scopolamine in the centromedial and centrolateral amygdala; increased Fos expression in the former region was blocked by Ro 4368854.

Previous studies have shown that scopolamine, a muscarinic antagonist, has minimal effects on Fos activation by itself and, indeed, systemic scopolamine decreases striatal Fos expression invoked by dopamine agonists (Wang and McGinty, 1996). However, a recent study found that ERK phosphorylation is increased by scopolamine in the central nucleus of the amygdala, an area which exhibits stress-induced Fos expression (Valjent et al., 2004). Additionally, Sugita et al. has reported that stimulation of m_1 cholinergic receptors decreases GABA release in amygdala interneurons and, conversely, blockade of m_1 receptors activates GABAergic interneurons and enhances Fos induction (Sugita et al., 1991). As stated above, there is a moderate level of 5-HT_6 receptor expression the amygdala, however, there has been no detailed study of expression in the central nucleus subregions. Thus it is difficult to resolve the mechanism by which 5-HT_6 antagonists attenuate scopolamine-mediated Fos in centrolateral, but not centromedial amygdala.

Systemic scopolamine-mediated deficits in fear conditioning may be due to posttraining block of long-term consolidation. Previously it has been shown that fear conditioned freezing was decreased with intraamygdala infusions of scopolamine (Rodgers and Cole, 1995), and that intraamygdala scopolamine inhibits LTP (Watanabe et al., 1995). This indicates a role of cholinergic receptors in amygdala in the consolidation of fear learning. However, scopolamine has been shown to have discrepant effects on contextual vs. tone-related fear conditioning, where scopolamine blocks contextual learning but not tone learning. It is interesting to note that scopolamine increased Fos expression in the central nucleus of the amygdala, which mediates contextual but not tone-mediated fear conditioning (Sullivan et al., 2004), but had no effect in the basolateral amygdala.

In summary, the present data support the possible utility of 5-HT_6 antagonists as precognitive agents to treat disorders that involve impaired attention and sensory gating, such as schizophrenia. In addition, the 5-HT_6 antagonist Ro 4368554 demonstrated efficacy at reversing scopolamine-mediated blockade of fear conditioning. Together, these findings advocate further examination of 5-HT_6 antagonists as novel medications for treating a broader spectrum of symptoms associated with schizophrenia.

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