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Effect of Dopamine D_3 Antagonists on PPI in DBA/2J Mice or PPI Deficit Induced by Neonatal Ventral Hippocampal Lesions in Rats

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Schizophrenic patients typically exhibit impairment of sensorimotor gating, which can be modeled in animal models such as the test of prepulse inhibition of startle response (PPI) in rodents. It has been found that antipsychotics enhanced PPI in DBA mice and reversed the PPI deficit induced by neonatal ventral hippocampal (NVH) lesions in rats. However, the relative involvement of D_3 and D_2 receptors in these effects is unknown since all antipsychotics are D_2/D_3 antagonists with limited binding preference at D_2 receptors. Therefore, in the current study, we investigated the influence of several dopamine antagonists with higher selectivity at D_3 *vs* D_2 receptors on PPI in DBA/2J mice and in NVH-lesioned rats. The PPI in DBA/2J mice was enhanced by the nonselective D_2/D_3 antagonists, haloperidol at 0.3–3 mg/kg, or risperidone at 0.3–1 mg/kg, while PPI-enhancing effects were observed after the administration of higher doses of the preferential D_3/D_2 antagonist, BP 897 at 8 mg/kg, and the selective D_3 antagonists, SB 277011 at 30 mg/kg and A-437203 at 30 mg/kg. No effect was observed following the treatment with the selective D_3 antagonist, AVE 5997 up to 30 mg/kg. The PPI deficits induced by NVH lesions were reversed by haloperidol but not by the more selective D_3 antagonists, A-437203 and AVE 5997. BP 897 enhanced PPI nonselectivity, that is, in both lesioned and nonlesioned rats. In summary, the present study indicates that PPI-enhancing effects induced by antipsychotics in DBA/2J mice and in NVH-lesioned rats are unlikely to be mediated by D_3 receptors.
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INTRODUCTION

The discovery of the existence of dopamine subtypes, D_2, D_3 and D_4 receptor in the D_2-like receptor family (Giros *et al,* 1990; Mills *et al,* 1993), has evoked great interest in developing new antipsychotic agents specifically targeting D_3 and D_4 receptor subtypes with the desire to limit the presumed D_2 receptor-mediated side effects. The clinical significance of D_4 antagonists has come into question after clinical trials showed the lack of antipsychotic activity with D_4 receptor antagonists (Corrigan *et al,* 2004; Kramer *et al,* 1997). The rationale behind the development of compounds interacting with D_3 receptor remains intriguing according to preclinical and clinical data. Firstly, all antipsychotics are D_2/D_3 antagonists with limited binding preferences at D_2 receptor (Leysen *et al,* 1993; Schwartz *et al,* 2000; Seeman and Tallerico, 1998). Secondly, D_3 receptors have been implicated in behavioral sensitization, which could be a potential contributor to the pathogenesis of schizophrenia (Glenthoj and Hemmingsen, 1997; Guillin *et al,* 2001; Lieberman *et al,* 1997; Pilla *et al,* 1999; Richtand *et al,* 2001). Indeed, schizophrenic patients showed exacerbated psychotic symptoms to the administration of psychostimulants at low doses, which did not produce psychotic symptoms in healthy subjects (Janowsky and Davis, 1976; Lieberman *et al,* 1987). Thirdly, increased expression of D_3 receptors was observed in the post-mortem brains of unmedicated schizophrenic patients and the expression was normal in patients treated with antipsychotics (Gurevich *et al,* 1997). Fourthly, the D_3 receptor is predominantly expressed in limbic areas such as shell of the nucleus accumbens, olfactory tubercle, amygdala, and cortical structures important for emotional, cognitive, and motivational processes, and is scarce in side effect-related regions such as striatum and pituitary (Diaz *et al,* 1995; Gurevich and Joyce, 1999). Indeed, accumulating evidence

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from combined use of mutant mice and pharmacological approaches supports the notion that it is probably D_2 receptor, but not D_3 receptor, that contributes to the induction of the secondary negative symptoms, adverse cognitive effects, extrapyramidal symptoms, and hyperprolactinemia (Ballard *et al,* 2003; Browman *et al,* 2003; Depoortere, 1999; Millan *et al,* 2004).

In preclinical studies of schizophrenia, animal models have been used to model certain aspects of dysfunctions associated with the disease. For example, schizophrenic patients showed impairments in sensorimotor gating (Braff *et al*, 1978, 2001, 1992), probably a reflection of the disrupted information filtering mechanism contributing to overloading irrelevant stimuli in the cortical regions and subsequently causing confusion, hallucination, and attentional/cognitive deficits in the patients (Carlsson *et al,* 1997; McGhie and Chapman, 1961). One way to assess impaired sensorimotor gating is to measure prepulse inhibition (PPI) of the startle reflex, an operational measure of sensorimotor gating in which the involuntary startle reflex is reduced when a startling stimulus is preceded by a weak acoustic stimulus. Consistent with DA-, glutamate- and serotonin-based hypotheses of schizophrenia, PPI in rats can be disrupted by direct or indirect dopamine agonists, NMDA receptor blockers and 5-HT_{2A} agonists (Mansbach and Geyer, 1989; Mansbach *et al,* 1988; Sipes and Geyer, 1994; Swerdlow and Geyer, 1993; Swerdlow *et al,* 1991; Varty and Higgins, 1998). The reversal of these pharmacologically induced PPI deficits has been considered to indicate antipsychotic potential. However, these pharmacological models are tied to a specific mechanism and may thus be limited for detecting novel mechanisms of action. Recently, it has been suggested that the increase of PPI responding in DBA/2J mice displaying naturally occurring low PPI responses compared to other mouse strains may represent a more attractive animal model for assessing novel antipsychotic mechanisms (Browman *et al,* 2004; Olivier *et al,* 2001).

Another recently developed nonpharmacological model, which also exhibits a fair degree of validity to several aspects of schizophrenia, is the 'neonatal ventral hippocampal (NVH) lesion-induced deficits' model proposed by Lipska and co-workers as offering a valid simulation of psychosis disorders (Lipska *et al,* 2002, 1993; Lipska and Weinberger, 2000). Indeed, NVH-lesioned rats showed behavioral deficits resembling several aspects of schizophrenia. For instance, NVH-lesioned rats showed hyperactivity, PPI deficits, disruption of social interaction, and cognitive deficits (Becker and Grecksch, 2000; Becker *et al,* 1999; Lipska *et al,* 2002, 1995; Sams-Dodd *et al,* 1997), resembling positive, negative, and cognitive symptoms of schizophrenia, respectively. The onset of the schizophrenic symptoms typically appears in early adulthood, and the NVH lesion-induced behavioral abnormality in rats also appeared only after puberty (Lipska *et al,* 1993, 1995). Furthermore, in line with the DA and glutamate hypothesis of schizophrenia, NVH-lesioned rats exhibited exacerbated responses to dopamine direct/indirect agonists and NMDA antagonists, suggesting intrinsic dopaminergic and glutamatergic dysfunctions in these rats (Al-Amin *et al,* 2000; Lipska *et al,* 1993, 1995). As schizophrenic patients whose symptoms could be precipitated by stressful life events (Howes *et al,* 2004), the lesioned rats also exhibited hypersensitivity to stress (Lipska *et al,* 1993). Finally, some of the behavioral deficits observed in NVH-lesioned rats, such as PPI deficits, were reversible by antipsychotics (Le Pen and Moreau, 2002; Rueter *et al,* 2004).

As mentioned above, antipsychotics have been shown to increase PPI in DBA/2J mice and in NVH-lesioned rats. Since these antipsychotics in general are nonselective at D_2 and D_3 receptors (as shown in Table 1), it was of interest to determine whether D_3 receptors contribute to the efficacy on normalizing PPI functions in these models. Therefore, in the current study we investigated several dopamine antagonists with higher selectivity at D_3 receptors *vs* D_2 receptors (see Table 1) in these two animal models: BP 897 (Pilla *et al,* 1999; Wicke and Garcia-Ladona, 2001; Wood *et al,* 2000), SB 277011 (Ballard *et al,* 2003; Reavill *et al,* 2000), A-437203 (Gross *et al,* 1997; Unger *et al,* 2002) and AVE 5997 (Kongsamut, 2003). Unlike SB 277011, A-437203 and AVE 5997, BP 897 is less selective at D_3 and its intrinsic activity has been controversial. It has been characterized as a partial D_3 agonist and a D_3 antagonist depending on the assay used. For instance, BP 897, as a partial agonist, was able to inhibit forskolin-induced cAMP accumulation and induce mitogenesis in NG-108-15 cells transfected with human dopamine D_3 receptors (Pilla *et al,* 1999; Pilon *et al,* 1994). However, it was unable to increase GTPγS binding in human (h) D_3/CHO cells and potently inhibited the increase induced by dopamine, a reflection of antagonistic properties (Wicke and Garcia-Ladona, 2001). In support of its intrinsic activity as a D_3 antagonist, BP 897 did not affect neuronal firing rate in substantia nigra after acute treatment, while in the same assay D_3/D_2 agonists such as PD128907 and quinpirole showed dose-dependent inhibitory effects, which could be blocked by BP 897 (Wicke and Garcia-Ladona, 2001). Furthermore, as potent D_3 antagonsits such as SB 277011 (Vorel *et al,* 2002), BP 897 has been shown to reduce cocaine-seeking behavior in rats (Garcia-Ladona and Cox, 2003; Pilla *et al,* 1999). The doses of D_3 antagonsits have been proven to be bioactive by published pharmacological studies. For instance, BP 897 at 1 mg/kg reduced cocaine-seeking behavior in rats (Garcia-Ladona and Cox, 2003); SB 277011 at the dose of 3 mg/kg significantly reversed PPI deficit induced by social isolation (Reavill *et al,* 2000); A-437203 significantly reversed

Table 1 Drug Affinities (nM) of Antagonists to Cloned Human D_2 and D_3 Receptors

Compounds	K_i D_2 (nM)	K_i D_3 (nM)	Selectivity D_3/D_2
Haloperidol[a]	0.3–0.6	3	≈0.1
Risperidone[a]	1–4	5–11	≈1
BP 897[b]	61	0.92	66.3
SB-277011[c]	1047	11.2	93.5
A-437203[d]	351.00	2.92	120.2
AVE 5997[e]	1470	5.1	288.2

[a]Joyce (2001).
[b]Pilla *et al* (1999).
[c]Reavill *et al* (2000).
[d]Unger *et al* (2002).
[e]Kongsamut (2003).

PD-128907-induced social interaction deficit in rats at the doses of 1–4.64 mg/kg (Drescher *et al,* 2002); AVE 5997 antagonized MK-801-induced hyperactivity with an ED_{50} of about 0.1 mg/kg (Kongsamut, 2003).

MATERIALS AND METHODS

Animals

For mouse PPI studies male 4-week-old DBA/2J mice were obtained from Jackson Laboratories (USA) and housed in climate-controlled animal facilities under 12-h light–dark cycle (lights on at 0600). The methodology of the study in NVH-lesioned rats was as described previously (Rueter *et al,* 2004). Pregnant female Sprague–Dawley rats (16 day) were purchased from Charles River (USA). Since birth, mother and litter size can influence the physical and behavioral development of pups, multiple measures were taken to control for these factors. Litters were culled to all male pups on postnatal day (PD) 4 with cross fostering in order to control for differences between birth mothers and to equate litter size, which allows each pup equal access to feeding. Surgery was performed on PD 7 with pups from each dam being assigned to the lesioned and nonlesioned groups. Pups were immediately returned to the dams following recovery, again employing cross fostering to equate litter size. Each dam subsequently raised a litter of lesioned or nonlesioned pups. At PD 28, pups were weaned and housed three per cage in a reversed light cycle (lights off 0800, lights on 2100). Animals had access to food and water ad libitum. Testing began after the rats were 8 weeks old. All experiments were conducted in accordance with Abbott Animal Care and Use Committee and National Institutes of Health Guide for Care and Use of Laboratory Animals guidelines in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Surgical Procedures

As previously described (Rueter *et al,* 2004), pups were anesthetized by hypothermia on ice for 10–15 min. Pups were placed in a stereotaxic device with modified ear bars that had rubber cups on the tips that gently held the head in place. An incision was made through the skin of the skull, and the skin was retracted in order to expose the skull. Bilateral injections were made using needle tips cut from 10 μl Hamilton syringes spaced 7 mm apart on the carrier. The stereotaxic coordinates from bregma were AP-3.0, L-3.5, V-5.0. Ibotenic acid (2.5 μg) was infused over 2 min and the cannulae were left in place for a subsequent 2 min. The skin was sutured using VetBond (Webster Veterinary Supply, Sterling, MA, USA) and pups were immediately placed in a heated recovery area. Pups were constantly monitored and periodically stimulated using gentle stroking until they fully recovered. Following recovery, pups were immediately returned to the dams.

PPI Assay

In all of the behavioral tests, the animals were brought to a holding room adjacent to the testing room at least 60 min prior to the drug administration.

DBA/2J mice were tested in their light phase in Hamilton Kinder PPI equipment (SM 100 version 4.1, Poway, CA, USA) and the rats were tested in their dark phase in San Diego Instruments equipment (SRLAB, San Diego Instruments, San Diego, CA, USA). The animals were given a 5-min acclimation period in the startle chambers during which a 65-decibel (dB) background noise was presented. This background noise remained throughout the entire test. Following the 5-min acclimation period, four successive trials of 40-ms noise bursts at 120 dB were presented. These trials were not included in data analysis. The rats were then exposed to five different types of acoustic stimuli: Pulse Alone (120-dB noise for 40 ms), No Stimulus (no stimulus was presented), and three Prepulse + Pulse with prepulse set at three sound levels of 70, 75 and 80 dB for 20 ms followed by 40-ms pulse at 120 dB. There were 100 ms from the initiation of prepulse to the onset of the pulse. A total of 12 trials under each acoustic stimulus condition were presented with 15-s variable intervals. Finally, the test ended with four trials of 40-ms 120-dB pulse, which were excluded from data analysis. The inclusion of four Pulse Alone trials in the beginning of the experiment were presented to help normalize the responses of the mice, as there is rapid habituation to the startle response seen within the first few trials (Dulawa and Geyer, 2000). The four Pulse Alone trials at the end of the test session are part of the standard lab PPI program and are included to provide a means to assess startle reflex habituation to acoustic stimuli when it is of interest in a study. As we have not seen a differential effect of antipsychotics on habituation within these models (Rueter *et al,* 2004), those eight trials were excluded from data analysis in the current experiments. Percent PPI was calculated as following: $(1-(\text{startle response to Prepulse + Pulse})/(\text{startle response to Pulse Alone})) \times 100$.

Compounds and Doses

Risperidone (ICN Biomedicals Inc., Irvine, CA, USA), ibotenic acid (Sigma Chemical Co., St Louis, MO, USA), and haloperidol (Sigma Chemical Co., St Louis, MO, USA) were purchased from commercial suppliers. SB 277011, BP 897, AVE 5997, and A-437203 were synthesized at Abbott Laboratories (Ludwigshafen, Germany). A-437203 was dissolved in 1 N NaOH and then titrated to a final pH 9–10 with 1 N HCL. The other compounds were dissolved in 1 N HCl or acetic acid and then titrated to a final pH 5–6 with 10 N NaOH. In mouse PPI studies, all solutions were administered intraperitoneally (i.p.) in a volume of 10 ml/kg. In rat PPI studies, the solutions were administered in a volume of 1 or 2 ml/kg dependent upon solubility of the compounds, and the solutions were given i.p. except SB 277011 and haloperidol, which were administered subcutaneously (s.c.). In both mouse and rat studies, risperidone, haloperidol and BP 897 were administered 30 min before the PPI tests, while the other compounds were given 60 min prior to testing. The administration route, pretreatment time and doses were chosen based upon our preliminary studies.

Histological Verification of Lesions

Following the final experiment, rats were deeply anesthetized with pentobarbital (Nembutal; Abbott, Abbott Park,

IL, USA) and transcardially perfused with saline and then 10% formalin. Following sectioning on a cryostat, brain slices were stained with cresyl violet and assessed for the degree of lesion. Figure 1 represents the largest (stripes) and smallest (gray) amount of damage found upon histological investigation with animals falling all along the continuum between the two.

Statistical Analysis

In mouse PPI studies, % PPI data were analyzed by a two-way ANOVA with prepulse intensity as repeated measures and treatment as between-subjects factor. If a significant treatment effect and interaction of treatment and prepulse were identified, the % PPI was then analyzed by Fisher's *post hoc* PLSD test to compare group means at each prepulse level. If a significant treatment effect was identified without the presence of significant interaction of treatment and prepulse, % PPI was collapsed across prepulse levels and analyzed by Fisher's PLSD to compare means of treatment groups. Startle responses to pulse alone in the PPI studies were analyzed using one-way ANOVA with treatment as an independent variable, followed by Fisher's PLSD *post hoc* comparison to compare means of treatment groups. Alpha was set at 0.05.

In rat, PPI studies involving NVH lesions, % PPI data were analyzed using a three-way ANOVA (lesion × drug treatment × prepulse intensity) with repeated measures on prepulse intensity, followed when appropriate by separate two-way ANOVA or by the Fisher's PLSD. Startle responses to pulse alone were analyzed by two-way ANOVA (lesion × treatment) followed by Fisher's PLSD. Alpha was set at 0.05.

RESULTS

Assessing Nonselective and Selective D_3 Antagonists on PPI in DBA/2J Mice

Haloperidol. Two-way ANOVA revealed a significant main effect of treatment on % PPI ($F(3, 35) = 4.014$, $p < 0.05$) and prepulse intensity ($F(2, 70) = 167.546$, $p < 0.01$). No significant interaction of treatment and prepulse intensity was observed. Follow-up Fisher's PLSD analysis of treatment effect indicated a significant enhancement of % PPI in mice treated with haloperidol at all of the doses tested ($p < 0.05$ or $p < 0.01$ *vs* vehicle-treated group, as shown in the left panel of Figure 2a). No significant main effect of treatment on startle was revealed by one-way ANOVA (right panel of Figure 2a).

Risperidone. Two-way ANOVA revealed a significant main effect of treatment on % PPI ($F(3, 32) = 5.039$, $p < 0.01$) and prepulse intensity ($F(2, 64) = 52.319$, $p < 0.01$). No significant interaction of treatment and prepulse intensity was observed. Fisher's PLSD analysis of treatment effect indicated a significant enhancement of % PPI in mice treated with risperidone at 0.3 mg/kg and 1 mg/kg ($p < 0.01$ *vs* vehicle-treated group, as shown in the left panel of Figure 2b). One-way ANOVA revealed a strong trend towards a main effect on startle ($F(3, 32) = 2.829$,

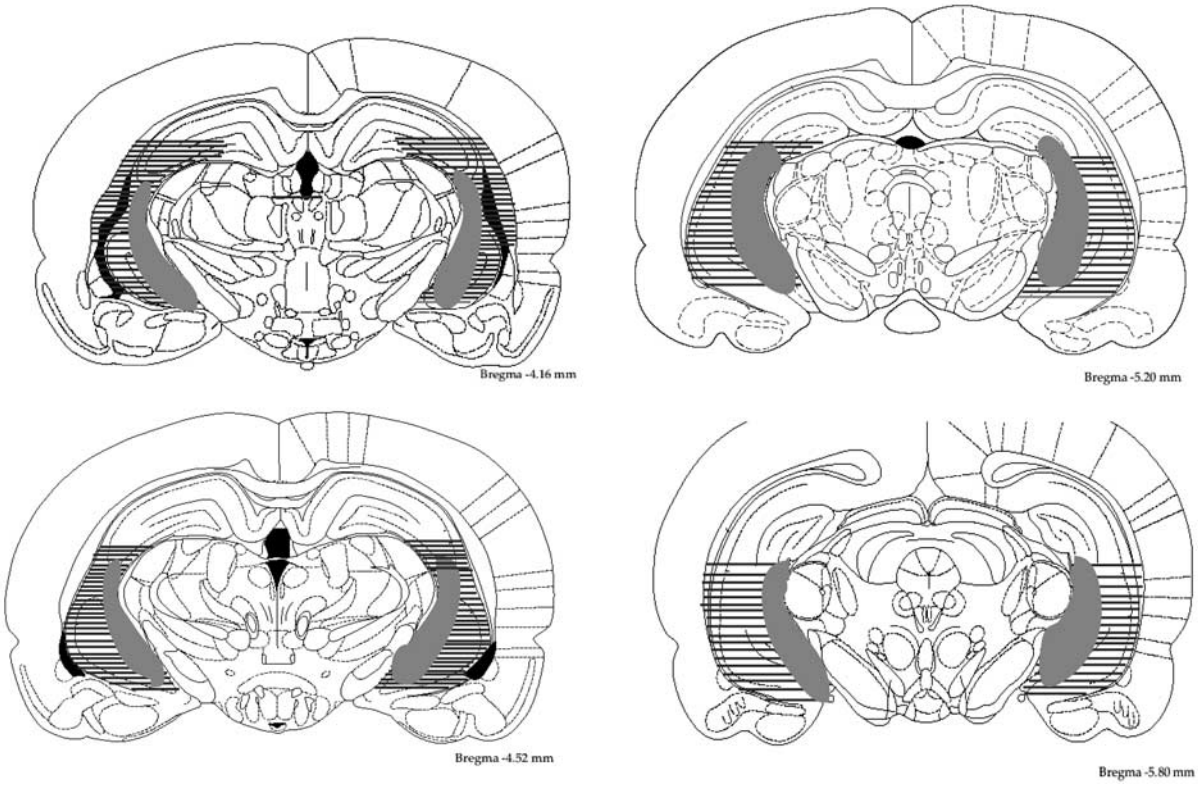

Figure 1 Neonatal hippocampal lesions: the amount of damage to the hippocampus found upon histological investigation of the lesioned animals is noted for the largest lesion found (strips) and the smallest lesion (gray shading). The distance from bregma is denoted along side each slice. The sections are redrawn from the stereotaxic atlas of Paxinos and Watson (1998).

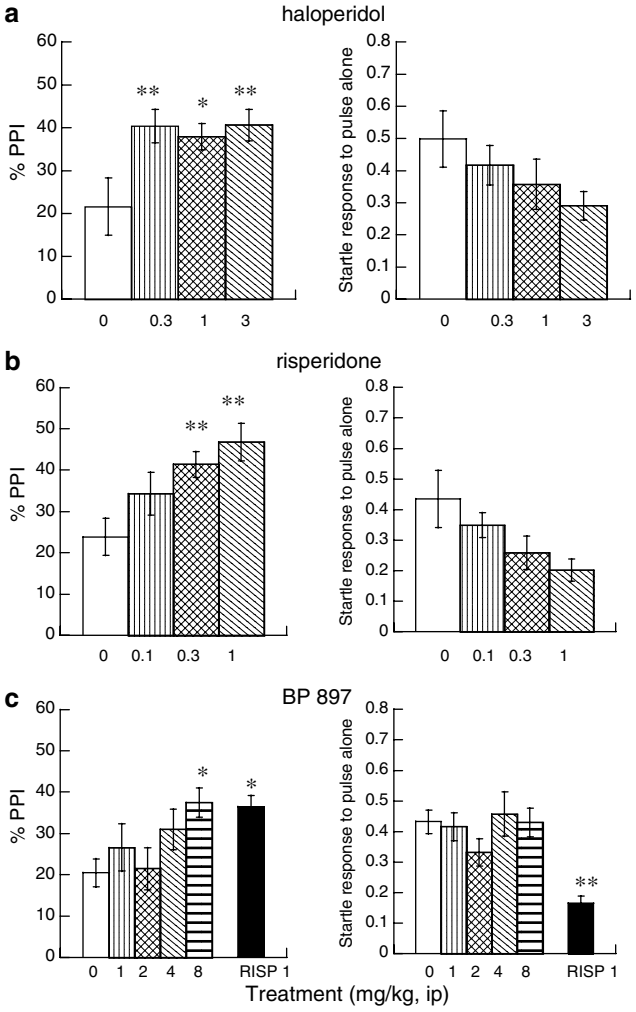

Figure 2 Effect of nonselective D_2/D_3 antagonists, haloperidol, and risperidone, and the preferential D_3 antagonist, BP 897, on PPI in DBA/2J mice. The effects on PPI and startle responses were presented on the left and the right side of the panels, respectively. Haloperidol (panel a, $N = 9$–10/group) significantly increased PPI at all of the doses tested while eliciting a nonsignificant reduction of startle response to pulse alone. Risperidone (panel b, $N = 9$/group) significantly increased PPI at 0.3 and 1 mg/kg. BP 897 (panel c, $N = 11$–13/group), significantly increased PPI at 8 mg/kg whose effect was comparable to its comparator, risperidone, although it did not reduce startle response as risperidone did in the same experiment. *$p < 0.05$ and **$p < 0.01$, compared to vehicle-treated alone group.

$p = 0.054$) (right panel of Figure 2b). Risperidone at 1 mg/kg was used as a comparator and a positive control in the following studies investigating more selective D_3 antagonists.

BP 897. Two-way ANOVA revealed a significant main effect of treatment on % PPI ($F(5, 66) = 2.936$, $p < 0.05$) and a significant main effect of prepulse intensity ($F(2, 132) = 103.263$, $p < 0.01$). No significant interaction of treatment and prepulse intensity was observed. Fisher's PLSD analysis of treatment effect demonstrated a significant enhancement of % PPI in mice treated with BP 897 at 8 mg/kg and with the comparator, risperidone, with regard to vehicle-treated mice, as shown in the left panel of Figure 2c ($p < 0.05$). One-way ANOVA revealed a significant main effect of treatment on startle responses to pulse alone ($F(5, 66) = 5.575$, $p < 0.01$) with follow-up Fisher's PLSD demonstrating that the comparator, risperidone, significantly reduced startle responses ($p < 0.01$, *vs* vehicle-treated group, as shown in the right panel of Figure 2c).

SB 277011. Two-way ANOVA revealed a significant main effect of treatment on % PPI ($F(6, 65) = 3.696$, $p < 0.01$) and a significant main effect of prepulse intensity ($F(2, 130) = 77.677$, $p < 0.01$). No significant interaction of treatment and prepulse intensity was observed. Fisher's PLSD analysis of treatment effect demonstrated a significant enhancement of % PPI in mice treated with SB 277011 at 30 mg/kg, as shown in the left panel of Figure 3a ($p < 0.05$, *vs* vehicle-treated group). No significant main effect of treatment on startle was revealed by one-way ANOVA, as shown in the right panel of Figure 3a.

A-437203. Two-way ANOVA revealed a significant main effect of treatment on % PPI ($F(4, 74) = 3.465$, $p < 0.05$) and a significant main effect of prepulse intensity ($F(2, 148) = 202.557$, $p < 0.01$). No significant interaction of treatment and prepulse intensity was observed. Fisher's PLSD analysis of treatment effect demonstrated a significant enhancement of % PPI in mice treated with A-437203 at 30 mg/kg and with the comparator, risperidone, as shown in the left panel of Figure 3b ($p < 0.05$ and < 0.01, respectively). One-way ANOVA revealed a significant main effect of treatment on startle responses to pulse alone ($F(4, 74) = 4.102$, $p < 0.01$) with Fisher's PLSD analysis of treatment effect demonstrating that risperidone significantly reduced startle responses ($p < 0.01$, *vs* vehicle-treated group), as shown in the right panel of Figure 3b.

AVE 5997. Two-way ANOVA revealed a significant main effect of treatment on % PPI ($F(5, 42) = 3.273$, $p < 0.05$) and prepulse intensity ($F(2, 84) = 134.672$, $p < 0.01$). No significant interaction of treatment and prepulse intensity was observed. Fisher's PLSD analysis of treatment effect showed a significant enhancement of % PPI only following the treatment of the comparator, risperidone ($p < 0.05$, *vs* vehicle-treated group), but not the treatment of AVE 5997 at any of the doses tested, as shown in the left panel of Figure 3c. A marginal significant main effect of treatment on startle was revealed by one-way ANOVA ($F(5, 42) = 4.402$, $p = 0.086$).

Assessing Nonselective and Selective D_3 Antagonists on PPI Deficits Induced by NVH Lesion

Haloperidol. For the measure of % PPI as shown in the left panel of Figure 4a, three-way ANOVA revealed a significant main effect of treatment ($F(1, 76) = 15.024$, $p < 0.01$), prepulse intensity ($F(2, 152) = 145.414$, $p < 0.01$), and lesion ($F(1, 76) = 31.327$, $p < 0.01$), as well as a significant lesion × treatment interaction ($F(1, 76) = 4.229$, $p < 0.05$) suggesting that the effect of haloperidol on PPI was different between lesioned and nonlesioned groups. Follow-up Fisher's PLSD indicated that NVH lesion-induced PPI reduction was reversed by haloperidol in lesioned ($p < 0.01$) but not in nonlesioned rats. For the measure of startle responses to

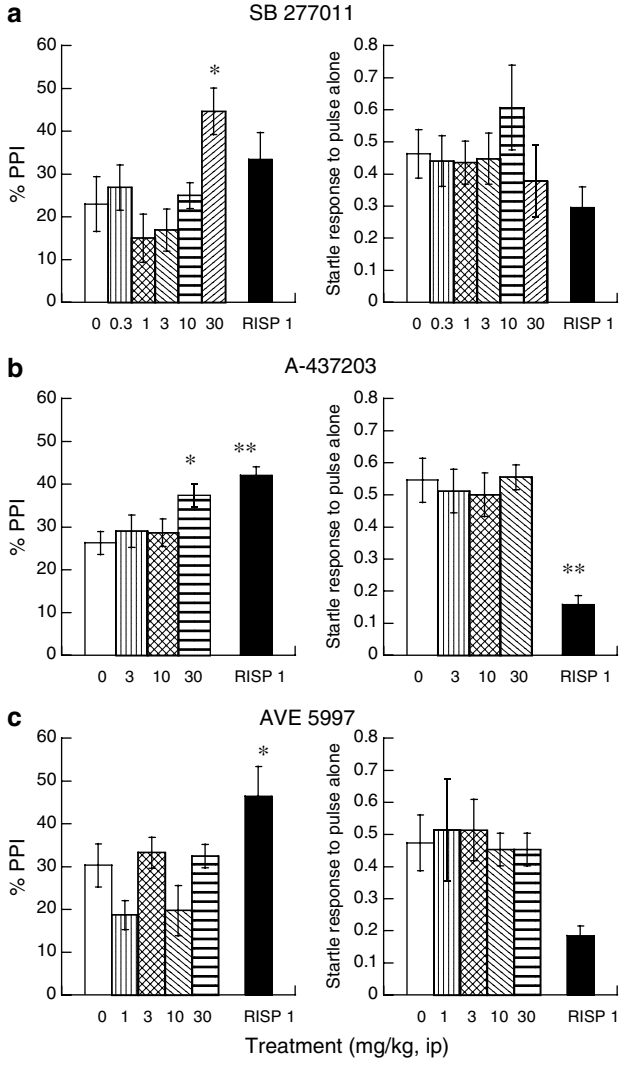

Figure 3 Effect of selective D_3 antagonists, SB 277011, A-437203, and AVE 5997 on PPI in DBA/2J mice. The effects on PPI and startle responses were presented on the left and the right side of the panels, respectively. SB 277011 (panel a, $N=10$–11) and A-437203 (panel b, $N=17$–18 for vehicle- and A-437203-treated groups; $n=8$ for risperidone-treated group) significantly increased PPI at 30 mg/kg, an effect at least comparable to risperidone without affecting startle responses as much as risperidone. However, AVE 5997 (panel c, $N=8$/group) did not produce a significant effect on PPI or startle response. $^{*}p<0.05$ and $^{**}p<0.01$, compared to vehicle-treated group.

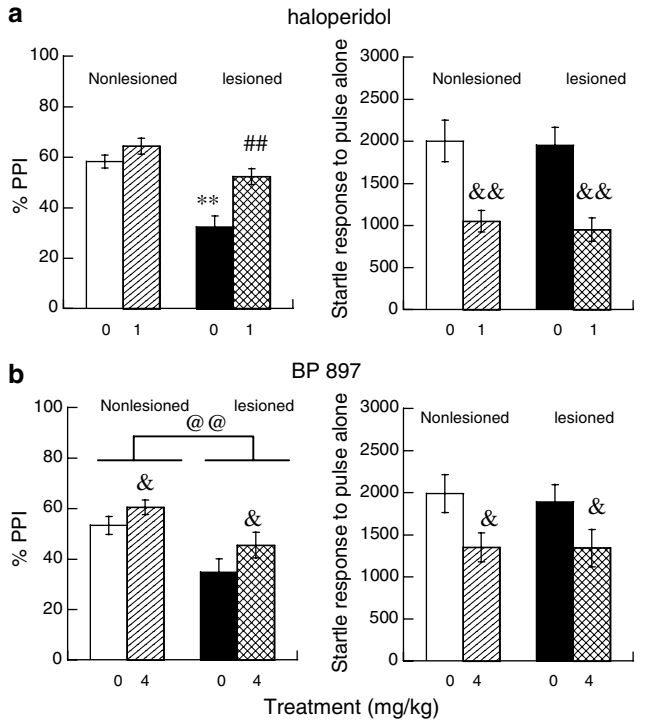

Figure 4 Effect of haloperidol and BP 897 on PPI deficits induced by NVH lesions. Neonatal lesion induced significant PPI deficits. Haloperidol (panel a, $N=20$) significantly enhanced PPI in NVH-lesioned group but not in nonlesioned group. BP 897 (panel b, $N=20$) only produced a significant main effect of treatment on PPI. No significant interaction of treatment and lesion was observed following BP 897 treatment. Both compounds significantly reduced startle response. $^{**}p<0.01$, compared to NVH-nonlesioned group receiving vehicle treatment; $^{\#\#}p<0.01$, compared to NVH-lesioned group receiving vehicle treatment; $^{\&}p<0.05$ and $^{\&\&}p<0.01$, significant main effect of treatment on startle response or PPI; $^{@@}p<0.01$, significant main effect of lesion on PPI.

pulse alone as shown in the right panel of Figure 4a, two-way analysis of ANOVA only revealed a significant main effect of treatment ($F(1,76)=26.441$, $p<0.01$), indicating that haloperidol reduced startle independent of surgery condition.

BP 897. For the measure of % PPI as shown in the left panel of Figure 4b, three-way ANOVA revealed a significant main effect of treatment ($F(1,76)=4.251$, $p<0.05$), prepulse intensity ($F(2,152)=180.434$, $p<0.01$), and lesion ($F(1,76)=14.991$, $p<0.01$). No significant interaction of treatment and lesion was found, suggesting that BP 897-induced PPI enhancing effect was not selective for the lesioned group. For the measure of startle responses to pulse alone as shown in the right panel of Figure 4b, two-way analysis of ANOVA only revealed a significant main effect of treatment ($F(1,76)=8.159$, $p<0.05$), indicating that the treatment of BP 897 significantly reduced startle.

A-437203. For the measure of % PPI as shown in the left panel of Figure 5a, three-way ANOVA only revealed a significant main effect of lesion ($F(1,64)=16.638$, $p<0.01$), and prepulse intensity ($F(2,128)=155.967$, $p<0.01$). For the measure of startle responses to pulse alone as shown in the right panel of Figure 5a, two-way analysis of ANOVA only revealed a significant main effect of treatment ($F(2,64)=12.632$, $p<0.01$) indicating that the treatment of A-437203 significantly reduced startle.

AVE-5997. For the measure of % PPI as shown in the left panel of Figure 5b, three-way ANOVA only revealed a significant main effect of lesion ($F(1,59)=16.732$, $p<0.01$), and prepulse intensity ($F(2,118)=144.165$, $p<0.01$). For the measure of startle responses to pulse alone as shown in the right panel of Figure 5b, two-way analysis of ANOVA only revealed a significant main effect of lesion ($F(1,59)=4.501$, $p<0.05$) indicating that lesion induced a significant enhancement of startle.

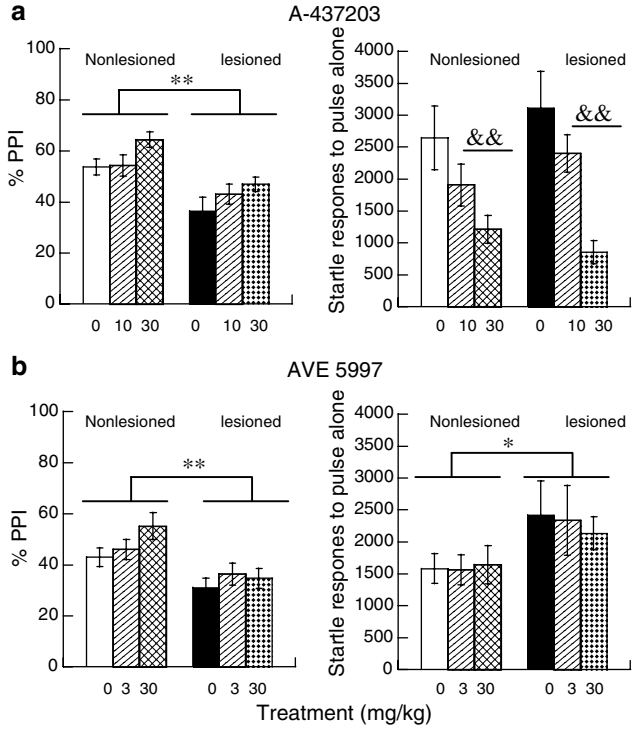

Figure 5 Effect of A-437203 and AVE 5997 on PPI deficits induced by NVH lesions. A-437203 (panel a, $N=11$–12) and AVE 5997 (panel a, $N=9$–12) did not affect PPI in either lesioned or nonlesioned groups. $^{*}p<0.05$ and $^{**}p<0.01$, a significant main effect of lesion on PPI or startle response; $^{\&\&}p<0.01$, a significant main effect of treatment on startle response, indicating that A-437203 significantly affected startle responses.

DISCUSSION

The current study showed that PPI responses in DBA/2J mice exhibiting lower PPI than other mice strains (Paylor and Crawley, 1997) were enhanced by the nonselective D_2/D_3 antagonists, haloperidol, and risperidone, while higher doses were required to make the enhancement by the preferential D_3/D_2 antagonist, BP 897, and the selective D_3 antagonists, SB 277011 and A-437203. No increase of PPI responses in DBA/2J mice was found following the treatment of AVE 5997, another putative selective D_3 antagonist. The PPI deficits induced by NVH lesions were also significantly reversed by haloperidol but not by more selective D_3 antagonists, A-437203 and AVE 5997. Although BP 897 elicited a significant main effect of treatment on PPI, the nonsignificant interaction of treatment and lesion suggested the effect was not selective to the lesioned rats.

Several lines of preclincial evidence have suggested that D_3 antagonists may have antipsychotic potential. For instance, SB 277011 has been demonstrated to reverse PPI deficits induced by social isolation and by the preferential D_3 agonist, PD 128907 (Reavill *et al,* 2000; Zhang *et al,* 2003). Subchronic treatment of SB 277011 preferentially reduced firing rates of DA neurons in ventral tegmental area, a pattern shared by atypical antipsychotics (Ashby *et al,* 2000; Gross *et al,* 1997). Furthermore, a certain degree of interaction of DA and glutamate systems via D_3 receptor has been implicated (Leriche *et al,* 2003). It is also interesting to consider a potential role of D_3 receptor in schizophrenia from the perspective of its involvement in dopamine sensitization, which has been implicated in the disease (Lieberman *et al,* 1997; Richtand *et al,* 2001).

Schizophrenia is a debilitating disease involving dysfunctions in multiple high-order brain areas and numerous neurobiological substrates. Given the complexity of the symptoms in affective, social, motor, and cognitive/perceptive domains, it is impossible to characterize the disease in one animal model, and thus the animal models developed to understand the pathogenesis and identify novel antipsychotics usually only represent specific dysfunctions of schizophrenic patients (Lipska and Weinberger, 2000). Studying the PPI of startle response in rodents has been an approach to investigate neuropathology of impaired sensorimotor gating in schizophrenic patients. Accumulating evidence has strongly implicated an involvement of the dopaminergic system in sensory motor gating. For instance, PPI in rodents can be disrupted by direct dopamine agonists, such as apomorphine and quinpirole, and indirect agonists such as amphetamine and cocaine (Geyer *et al,* 1990; Mansbach *et al,* 1988; Varty and Higgins, 1998). Furthermore, mice lacking the DA transporter also exhibit profound reduction of PPI accompanied by a robust and life-long increase of DA activity in the brain (Giros *et al,* 1996; Ralph *et al,* 2001). Many studies have also been conducted to investigate the relative involvement of subtypes of DA receptors in modulating PPI. There is a general agreement that D_2-like receptors, rather than D_1 receptors, play an important role in modulation of PPI in rats (Geyer *et al,* 2001), although D_1 receptors may indirectly affect PPI by a synergistic interaction with the D_2-like family (Peng *et al,* 1990; Wan *et al,* 1996a). The reversal of PPI-disruptive effects of D_2-like agonists by antipsychotics strongly implicate the D_2-like receptors in PPI modulation. Since the discovery of the D_3 receptor subtype, efforts have been dedicated to dissect the relative involvement of D_2 and D_3 receptor in modulation of PPI and whether D_3 receptors contribute to the clinical effects of antipsychotics. Findings that preferential D_3 receptor agonists, PD 128907 and 7-OH-DPAT, disrupt PPI of the startle response indicate that the D_3 receptor might play a role in sensorimotor gating (Varty and Higgins, 1998; Zhang *et al,* 2003). However, these behavioral effects are confounded by the fact that none of these agonists are highly selective at D_3 receptors in *in vivo* or *in vitro* functional studies, despite their D_3 selectivity reported in radioligand binding studies (Audinot *et al,* 1998; Pugsley *et al,* 1995; Zapata *et al,* 2001). Although PPI deficits in socially isolated rats were attenuated by the selective D_3 receptor antagonist SB 277011, PPI disruption induced by the D_2 receptor preferring agonist apomorphine was not antagonized by SB 277011 (Reavill *et al,* 2000). In contrast to the ambiguous involvement of D_3 receptors in sensorimotor gating, the role of D_2 receptors has been strongly implicated by the finding that amphetamine-induced PPI disruption was diminished in mice lacking the D_2 but not the D_3 or D4 receptor (Ralph *et al,* 1999).

The PPI responding in DBA/2J mice has been found to be enhanced by nonselective D_2/D_3 receptor antagonists both in the current and other published studies from several independent laboratories (McCaughran *et al,* 1997; Olivier *et al,* 2001). However, in the current studies, when the

selectivity of dopamine antagonists shifts towards D_3 receptors, higher doses were required to obtain the PPI enhancing effect following the treatment of two selective D_3 antagonists, A-437203 and SB 277011, and no effect was observed with the treatment of AVE 5997, suggesting a less important role of D_3 receptors in modulation of PPI in DBA/2J mice. However, to our knowledge, nothing is known about D_3 receptors in DBA/2J mice such as receptor distribution, density, and intrinsic activity. Indeed, species differences in D_3 receptor distribution have been reported (Diaz *et al,* 2000; Gurevich and Joyce, 1999). Therefore, it is too early to completely rule out a role of D_3 receptors in sensorimotor gating in general based upon the current study in DBA/2J mice.

As previously reported (Lipska *et al,* 1995), we showed in this study that NVH lesions elicited deficits in sensorimotor gating. An enhancement of postsynaptic DA sensitivity in NVH-lesioned rats has been speculated to underlie the effect given that the lesioned rats show exaggerated responses to DA indirect and direct agonists (Lipska *et al,* 1993; Wan and Corbett, 1997), while no change of basal DA and no further enhancement of DA were observed following amphetamine treatment compared to nonlesioned rats (Wan *et al,* 1996b). The supersensitive postsynaptic DA receptors might contribute to the behavioral alternations observed in NVH-lesioned rats such as impaired sensorimotor gating, enhanced activity in a novel environment, and exaggerated response to stress given the proposed role of DA in gating and motor functions as well as in mediating affective responses to stress. In agreement, we showed in the current study that impaired PPI of startle reflex could be reversed by the D_2/D_3 antagonist, haloperidol. It has been previously reported that NVH lesion-induced PPI deficits were reversible by atypical antipsychotics such as risperidone, clozapine, and olanzapine but not by haloperidol (Le Pen and Moreau, 2002). It is unclear what contributed to this discrepancy between laboratories for the response to haloperidol, although it could be a result of a difference in methodology and dosing. For example, haloperidol was given subcutaneously in the current study, while in Le Pen study, haloperidol was given intraperitoneally. It has been suggested that different administration routes may result in a difference in the pattern of behavioral response (Gentry *et al,* 2004). No significant attenuation of PPI deficits in NVH-lesioned rats was observed following more selective D_3 antagonists, A-437203 and AVE 5997, suggesting that selective blockade of D_3 receptor alone is not sufficient to reverse sensorimotor gating deficits elicited by NVH lesions.

In good agreement with the literature, we also showed that antipsychotics such as risperidone reduced startle responses (Le Pen and Moreau, 2002; Olivier *et al,* 2001) when enhancing PPI. This raises a general concern that the effects on PPI might be secondary to their effects on the startle responses. To address this issue, all of the data of vehicle- and 1 mg/kg risperidone-treated mice from the mouse studies of risperidone, BP 897, SB 277011 and AVE-5997 were pooled and then were sorted by startle responses in ascending values (raw data not shown). In order to obtain equal startle magnitude in the vehicle and risperidone groups, the pairs of individuals with risperidone and vehicle treatment listed next to each other were kept for further analysis. In cases where there were two possible pairs (eg if there was a sequence of vehicle–risperidone–vehicle, risperidone could be paired with the first vehicle and the second vehicle), the pair that had the smaller startle difference between vehicle- and risperidone-treated mice was chosen. This selection resulted in $n = 19$ in water- and risperidone-treated groups, respectively. The remaining mice were excluded. One-way ANOVA revealed (see Figure 6) that there was a significant difference ($p < 0.01$) in % PPI between risperidone- and water-treated mice when their mean values of startle magnitude were equal, suggesting that the effects on PPI responding can be independent of the effects on startle magnitude. A more differentiable effect was observed with haloperidol, which significantly enhanced PPI at 0.3 mg/kg, a dose that only slightly affected basal startle reflex (Figure 2a). Furthermore, haloperidol-induced PPI enhancement did not follow the pattern of its effect on startle reflex. A clearer separation between PPI and startle reflex was demonstrated in rats. For example, A-437203 (Figure 5a) reduced startle responses by more than 50%, while it only slightly increased PPI in both nonlesioned and lesioned rats. There are several lines of evidence supporting the independence of drug effect on PPI and startle reflex. For instance, diazepam significantly reduced startle responses in BALB/cByJ and 129/SvEv without affecting PPI, although it affected PPI and startle response simultaneously in C57 mice (Ouagazzal *et al,* 2001). A study investigating basal acoustic startle response and PPI among several inbred mouse strains showed there was no correlation between the magnitude of basal acoustic startle responses and PPI (Paylor and Crawley, 1997), suggesting differently genetically defined physiological processes are involved in basal acoustic startle and PPI of startle reflex. It is of interest to notice that in DBA/2J mice, D_3 antagonists at a high dose can increase PPI responses without affecting startle reflex as much as their comparator, risperidone. A dissociation expressed in a different way was also observed in NVH studies where some of the selective

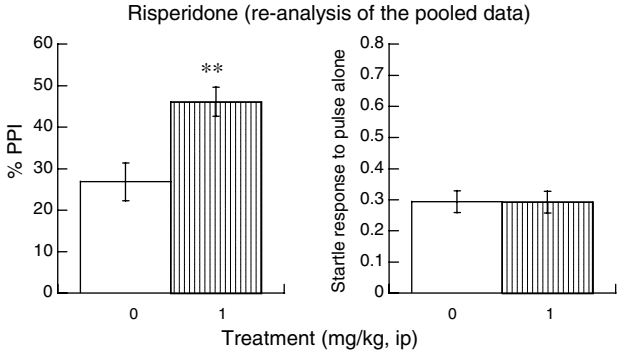

Figure 6 All of the data of vehicle- and 1 mg/kg risperidone-treated mice from the mouse studies of risperidone, BP 897, SB 277011 and AVE-5997 were pooled and then the startle magnitude of vehicle- and risperidone-treated individuals were matched (for details, see paragraph 6 in the Discussion) to compare the effect of vehicle and risperidone on % PPI when the startle magnitude was equal. This selection resulted in $n = 19$ in water- and risperidone-treated groups, respectively. One-way ANOVA revealed a significant difference **($p < 0.01$) between risperidone- and water-treated mice when their mean values of startle magnitude were equal, suggesting that the effects on PPI responding can be independent of the effects on startle magnitude.

D_3 antagonists significantly reduced startle reflex without significantly improving PPI deficit. These observations provide additional evidence supporting the dissociation of the effect on PPI and that on startle reflex, as well as the idea that different neurobiological processes may underlie gating processes and startle reflex.

In summary, the present study indicates that PPI enhancing effects induced by antipsychotics in DBA/2J mice and in NVH-lesioned rats are less likely to be mediated by blockade of D_3 receptors. As mentioned above, there is evidence from clinical and preclinical studies to support a potential use of D_3 antagonists as antipsychotics. It is likely that D_3 antagonists may possess antipsychotic actions via mechanisms that cannot be reflected by the animal models used in the current study, given that any animal model can only represent certain aspects of a very heterogeneous disease. Whether these models *vs* other models/theories are ultimately predictive of efficacy will eventually be obtained from clinical trials.

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