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Antipsychotic property of a muscarinic receptor agonist in animal models for schizophrenia

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ABSTRACT

Pharmacological evidence has implicated cholinergic dysfunction in the manifestation of psychotic symptoms. The purpose of the present study was to clarify the roles of muscarinic and nicotinic receptors in several animal models of schizophrenia. A muscarinic receptor agonist, oxotremorine (0.03–0.3 mg/kg), reversed hyperlocomotion in mice and disruption of prepulse inhibition (PPI) caused by methamphetamine in rats, similar to a typical antipsychotic drug, haloperidol (0.1–0.3 mg/kg). In addition to modulating hyperdopaminergic function, oxotremorine as well as clozapine (3–10 mg/kg) reversed the disruption of PPI caused by ketamine, an *N*-methyl-D-aspartate antagonist in rats, which mimics the clinical symptoms of schizophrenia. One of the spontaneous mouse models, DBA/2J exhibited lower PPI than C57BL/6J. Oxotremorine (0.03–0.06 mg/kg) increased PPI in DBA/2J but not C57BL/6J. On the other hand, a nicotinic receptor agonist, nicotine (0.06–0.6 mg/kg), exhibited no effects on the four animal models of symptoms of schizophrenia we tested. These findings suggest that muscarinic receptors play important roles in animal models to examine sensory gating which is known to be disrupted in schizophrenic patients, and hence activation of muscarinic receptors may provide an alternative approach for the treatment of psychotic symptoms in addition to classical antipsychotics.

1. Introduction

Schizophrenia is a common disorder affecting about 1% of the world population. It has a complex symptomatology, characterized by both positive and negative symptoms as well as cognitive impairment in humans. Although the neurobiological basis of schizophrenia is still unclear, developmental abnormalities of neurotransmission and plasticity are suspected to play roles in it. Dopaminergic activators such as amphetamines and cocaine as well as *N*-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine and ketamine are known to cause psychoses resembling schizophrenia in humans (Luby et al., 1959; Cohen et al., 1962; Snyder, 1973; Brady et al., 1991; Javitt and Zukin, 1991; Krystal et al., 1994; Jentsch and Roth, 1999). Therefore, hyperfunction of the dopaminergic system and hypofunction of the glutamatergic system may be involved in some of the symptoms in schizophrenia (Carlsson, 1988; Jentsch and Roth, 1999; Tsai and Coyle, 2002).

In addition to these neurochemical changes, clinical investigations have suggested that abnormalities of the cholinergic system may be present in schizophrenia (Abood and Biel, 1962; White and Cummings, 1996). It is known that muscarinic receptor antagonists such as scopolamine and atropine elicit psychotic symptoms in normal human subjects and exacerbate the symptoms of schizophrenic patients (Neubauer et al., 1966a,b; Peterson, 1977; Rusted and Warburton, 1988). Furthermore, the muscarinic receptor agonist xanomeline has been reported to be effective in improving some psychotic symptoms in patients with Alzheimer's disease (Bodick et al., 1997). Recent findings suggest that the antipsychotic effects of clozapine might be due to the activation of muscarinic receptors by its major metabolite, desmethylclozapine, in addition to blockade of dopamine and serotonin receptors by clozapine itself (Sur et al., 2003). On the other hand, the activation of nicotinic receptors, another type of cholinergic receptor, by smoking is known to transiently normalize the deficit in auditory sensory gating in schizophrenic patients with overnight deprivation of smoking (Adler et al., 1993). It has been reported that when smoking was curtailed, exacerbation of schizophrenic symptoms occurred in some patients (Greenman and McClellan, 1991). These findings suggest that manipulation of the cholinergic system by activation of both muscarinic and nicotinic receptors is a potential means of treatment of schizophrenia. However, the antipsychotic effects of muscarinic and nicotinic receptor agonists have not been well studied in various pharmacological and non-pharmacological animal models of schizophrenia. Thus, in the present study, we examined the effects of oxotremorine (a muscarinic receptor agonist) and nicotine (a nicotinic receptor agonist) in selected animal models of the symptoms of schizophrenia and compared their effects with those

Abbreviations: ANOVA, analysis of variance; CLZ, clozapine; HPD, haloperidol; KET, ketamine; MAP, methamphetamine; NCT, nicotine; NMDA, *N*-methyl-D-aspartate; OXO, oxotremorine; PPI, prepulse inhibition.

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of haloperidol and clozapine, which are currently available antipsychotic drugs. The animal models used were methamphetamine-induced hyperlocomotion in mice, methamphetamine-induced disruption of prepulse inhibition (PPI) in rats, ketamine-induced disruption of PPI in rats, and PPI in DBA/2J mice, a non-pharmacological model for evaluation of the effects of antipsychotic agents. We found that a muscarinic receptor agonist but not a nicotinic receptor agonist exerted the consistent effects with antipsychotic actions in various animal models of schizophrenia.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (321.1±1.2 g, 7–9 weeks old, Charles River Laboratories Japan, Inc., Kanagawa, Japan), male CD1 (ICR) mice (38.1±0.2 g, 5–7 weeks old, Japan SLC, Inc., Shizuoka, Japan), male DBA/2J mice (20.6±0.2 g, 6–8 weeks old, CLEA Japan, Inc., Shizuoka, Japan), and male C57BL/6J mice (20.9±0.1 g, 8–9 weeks old, CLEA Japan, Inc., Shizuoka, Japan) were used in the present studies. All animals were housed in a climate-controlled animal room (room temperature: 23±2 °C, humidity: 55±15%) with a 12 h light–dark cycle (lights on: 07:00–19:00). DBA/2J and C57BL/6J mice were individually housed after the purchase until the commencement of the study. Sprague–Dawley rats or CD1 (ICR) mice were maintained in groups of 2–3 rats per cage or 5–6 mice per cage, respectively. All animals had access to food (CE-2, CLEA Japan, Inc., Shizuoka, Japan) and water ad libitum. All animal experiments were approved by the Banyu Institutional Animal Care and Use Committee.

2.2. Locomotor activity

Mice were habituated to the experimental room for at least 60 min before testing. Oxotremorine, nicotine, or haloperidol was administered subcutaneously. Methamphetamine (2 mg/kg) was injected subcutaneously 30 min after the administration of test agents in studies of antagonism. Animals were placed in plastic cages (22.5*D*×33.8*W*×14.0*H* cm) immediately after the administration of test agents or of methamphetamine, and locomotor activity was measured during a 60 min observation period using an infrared motion detector system (DAS System, Neuroscience, Inc., Tokyo, Japan).

2.3. Prepulse inhibition (PPI)

An SR-LAB startle chambers (San Diego Instruments, San Diego, CA) was used to perform prepulse inhibition experiments. SR-LAB software controlled and delivered all of the acoustic stimuli to the animals and recorded startle responses. Startle amplitude was measured as the mean value in every 1 ms period of recording during a 100 ms period beginning at stimulus onset in both mouse and rat studies. In each session, animals were randomly assigned to experimental groups, received treatments, and were placed in the testing chambers. Animals were habituated to the experimental room for at least 60 min before commencement of treatment.

2.3.1. Rat study

When oxotremorine, nicotine, haloperidol, and clozapine were tested for effects on PPI, they were administered subcutaneously 30 min before the experiment started. In the combined administration study, methamphetamine (3 mg/kg) or ketamine (5 mg/kg) was injected subcutaneously 30 min after the administration of test agents. The animals were then placed in the chambers 10 min after methamphetamine or immediately after ketamine treatment. Animals had a 5 min acclimation period before starting sessions. A 60 dB background noise was continuously present once animals were placed in the chambers, and was maintained throughout the testing session. The test session consisted of 15 repetitions of a trial, which included 6 different paradigms, which were as follows: 20 ms prepulse at 63, 66, or 72 dB followed by 120 dB, 40 ms startle pulse (3 prepulse pulse conditions), startle pulse alone (pulse alone), a period in which no stimulus was presented (nos), and the 20 ms, 72 dB pulse (i.e., 12 dB above background) (prepulse alone). Our preliminary studies confirmed that these prepulse intensities were themselves insufficient to elicit a significant startle response from rats. With the combination of prepulse and startle pulse, the interval between each onset of two pulses was 100 ms. The stimuli were presented in random order, with inter-stimulus intervals averaging approximately 15–30 s. In order to obtain stable startle responses, data for the first 5 responses to each stimulus were discarded. Ten responses to the same stimulus were averaged, and used for calculation of PPI. PPI levels were determined by the formula ((pulse alone−prepulse pulse)/pulse alone×100) and expressed as the percentage of PPI.

2.3.2. Mouse study

Oxotremorine, nicotine, or haloperidol was administered subcutaneously 30 min before placement in the chamber. Background noise was continuously present at 65 dB. After the acclimation period, animals received a series of three 40 ms, 120 dB bursts of white noise to obtain stable startle responses before subsequent presentation of paradigms. The protocol for the mouse study was identical to that for the rat study, except in the following two respects: 1) the test session consisted of 12 repetitions of a trial which included paradigms: 4 prepulse pulse conditions (70, 75, 80, or 90 dB), a 120 dB pulse alone, nos, and 2 prepulse alone conditions (80 or 90 dB), and 2) inter-stimulus intervals averaged approximately 5–30 s. Twelve responses to the same stimulus were averaged, and used for calculation of the PPI of each animal.

2.4. Drugs

Oxotremorine sesquifumarate salt (Sigma Chemical Co. Ltd., St. Louis, MO, USA), nicotine tartrate dehydrate salt (Nacalai Tesque, Inc., Kyoto, Japan), methamphetamine hydrochloride (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), and ketamine hydrochloride (Sankyo Co., Ltd., Tokyo, Japan) were dissolved in physiological saline. Haloperidol (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) was dissolved in distilled water. Clozapine (Tocris Bioscience, Inc., Ellisville, MO, USA) was dissolved in a minimum amount of 0.1 N HCl and diluted to the required doses with physiological saline. All drugs were freshly prepared before each experiment and subcutaneously injected at a volume of 1 ml/kg for rats or 10 ml/kg for mice.

2.5. Statistical analysis

All values are the mean±S.E.M. Statistical analysis was carried out for comparison between multiple groups with one-way or two-way analysis of variance (ANOVA) followed by post-hoc comparison test (Dunnett's test or *t*-test) if appropriate. A probability level of <0.05 was used to determine statistical significance.

3. Results

3.1. Effects of oxotremorine and nicotine on spontaneous locomotor activity in mice

Effects of oxotremorine (0.03–0.3 mg/kg) and nicotine (0.06–0.6 mg/kg) on spontaneous locomotor activity were examined (Fig. 1A and B, respectively). One-way ANOVA revealed a significant difference among groups [$F(3,20)=52.75$, $P<0.01$ and $F(3,20)=9.77$, $P<0.01$, respectively], and post-hoc analyses showed that oxotremorine at doses of 0.1 and 0.3 mg/kg ($P<0.01$ compared with vehicle-treated group) and nicotine at a dose of

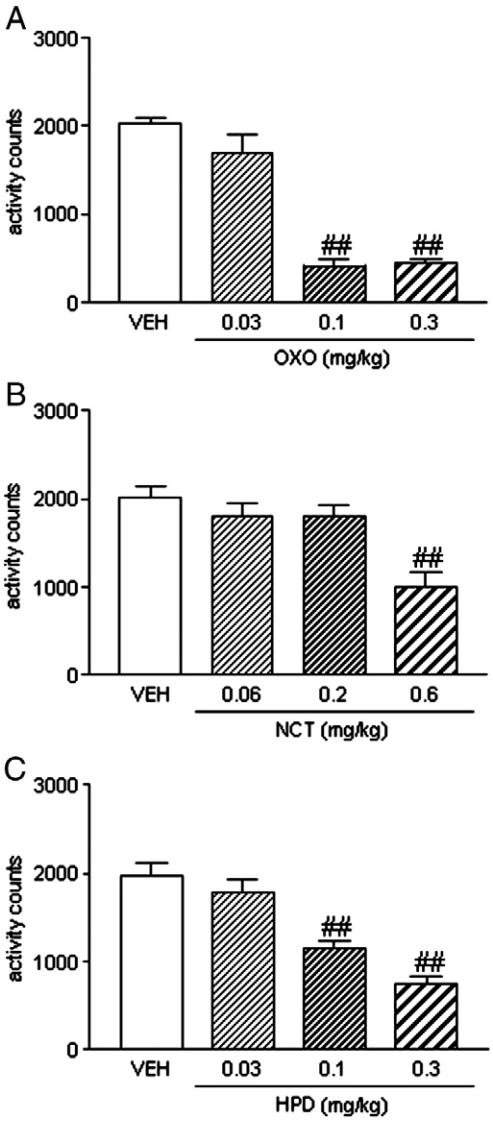

Fig. 1. Effects of oxotremorine (A), nicotine (B), and haloperidol (C) on spontaneous locomotor activity in CD1 (ICR) mice. Oxotremorine (0.03–0.3 mg/kg, s.c.), nicotine (0.06–0.6 mg/kg, s.c.), or haloperidol (0.03–0.3 mg/kg, s.c.) was injected just before initiation of measurement. Locomotor activity was assessed as total activity during a 60 min observation period. VEH = vehicle; OXO = oxotremorine; NCT = nicotine; HPD = haloperidol. Results are the mean ± S.E.M. # represents a significant difference from VEH condition (A, B, and C), ##, $P<0.01$ (Dunnett's test). $n=6$/group in each treatment.

0.6 mg/kg ($P<0.01$ compared with vehicle-treated group) significantly reduced locomotor activity. Likewise, haloperidol at doses of 0.1 and 0.3 mg/kg ($P<0.01$ compared with vehicle-treated group) significantly reduced spontaneous locomotor activity, as shown in Fig. 1C. In addition to suppressing spontaneous locomotor activity, oxotremorine began to elicit several behaviors including salivation, lacrimation, and tremor at a dose of 0.3 mg/kg. On the other hand, nicotine began to elicit crouching with tail rattling at a dose of 0.6 mg/kg. Higher doses were therefore not tested.

3.2. Effects of oxotremorine and nicotine on methamphetamine-induced hyperlocomotion in mice

Subcutaneous administration of oxotremorine (0.03–0.3 mg/kg) dose-dependently antagonized the hyperlocomotion elicited by methamphetamine (2 mg/kg) (Fig. 2A). One-way ANOVA revealed a significant difference among groups [$F(4,25)=64.88$, $P<0.01$], and post-hoc analyses showed that oxotremorine at doses of 0.1 and 0.3 mg/kg ($P<0.01$ compared with methamphetamine-treated group) significantly reversed the effects of methamphetamine. On the other hand, none of the doses of nicotine tested (0.06–0.6 mg/kg) affected the hyperlocomotion elicited by methamphetamine ($P>0.11$ compared with methamphetamine-treated group; Fig. 2B). Haloperidol at doses from 0.1 to 0.3 mg/kg significantly and dose-dependently antagonized methamphetamine-induced hyperlocomotion (Fig. 2C).

3.3. Effects of oxotremorine and nicotine on PPI in rats

We examined whether oxotremorine (0.1–0.3 mg/kg), nicotine (0.06–0.6 mg/kg), haloperidol (0.1–0.3 mg/kg), or clozapine (3–10 mg/kg) affected PPI in rats. None of these agents altered PPI at any doses tested (oxotremorine; [$F(2,81)=2.29$, $P=0.11$], nicotine; [$F(3,132)=1.97$, $P=0.12$],

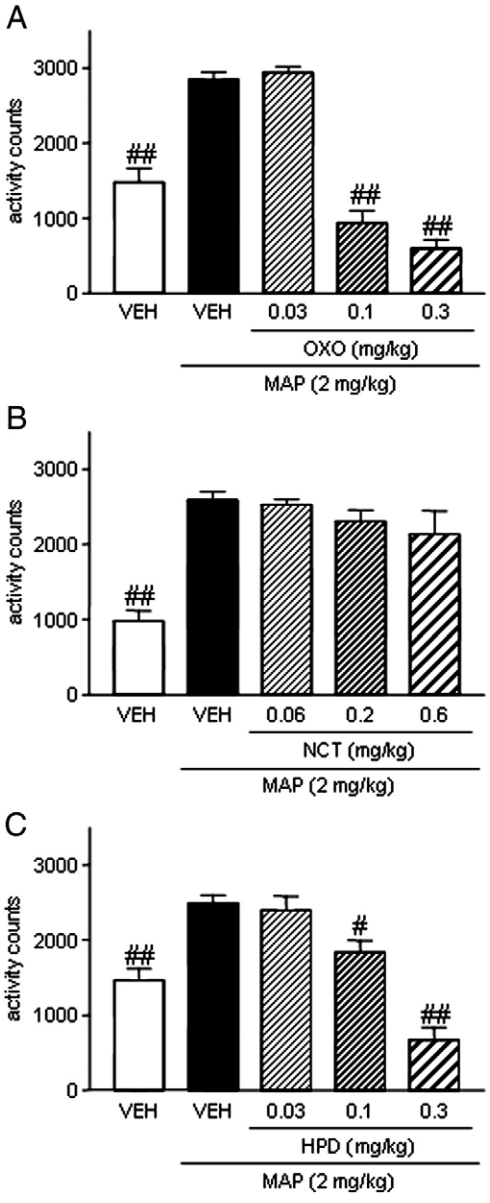

Fig. 2. Effects of oxotremorine (A), nicotine (B), and haloperidol (C) on methamphetamine (2 mg/kg)-induced hyperlocomotion in CD1 (ICR) mice. Oxotremorine (0.03–0.3 mg/kg, s.c.), nicotine (0.06–0.6 mg/kg, s.c.), or haloperidol (0.03–0.3 mg/kg s.c.) was injected 30 min before the administration of methamphetamine (2 mg/kg, s.c.). Locomotor activity was assessed as total activity during a 60 min observation period immediately after the administration of methamphetamine. VEH = vehicle; MAP = methamphetamine; OXO = oxotremorine; NCT = nicotine; HPD = haloperidol. Results are the mean ± S.E.M. # represents a significant difference from VEH + MAP condition (A, B, and C), #, $P<0.05$, ##, $P<0.01$ (Dunnett's test). $n=6$/group in each treatment.

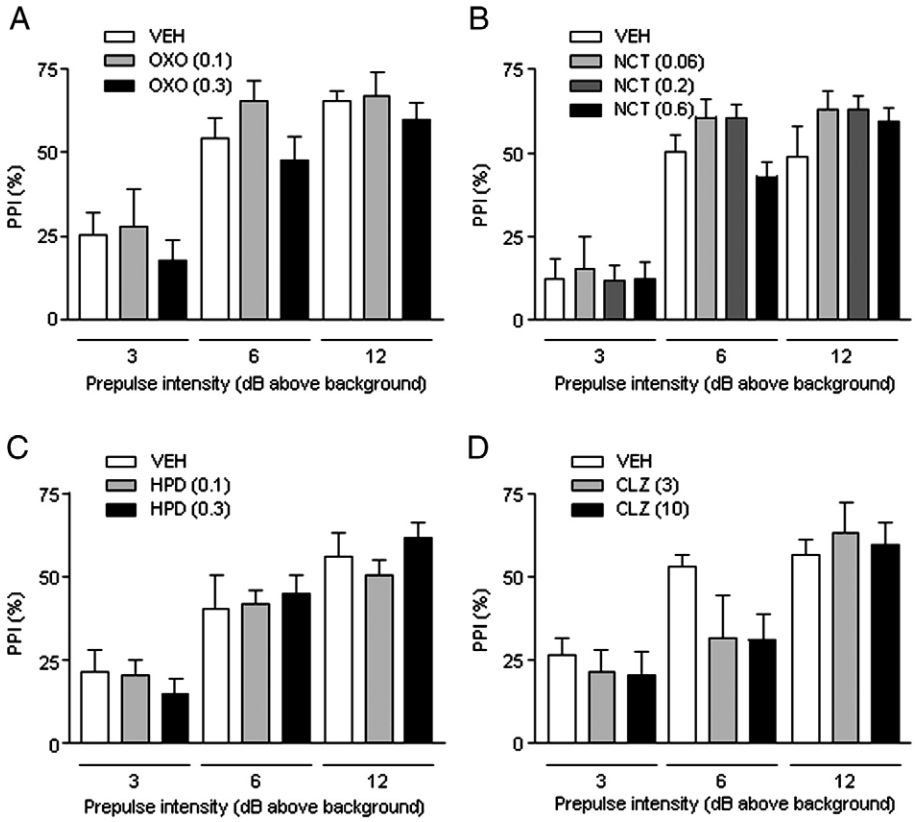

Fig. 3. Effects of oxotremorine (A), nicotine (B), haloperidol (C), and clozapine (D) on PPI in rats at 3 prepulse intensity levels (3, 6, and 12 dB above background). Oxotremorine (0.1–0.3 mg/kg, s.c.), nicotine (0.06–0.6 mg/kg, s.c.), haloperidol (0.1–0.3 mg/kg, s.c.), or clozapine (3–10 mg/kg, s.c.) was administered 30 min before rats were placed in the chambers. VEH = vehicle; OXO = oxotremorine; NCT = nicotine; HPD = haloperidol; CLZ = clozapine. Results are the mean ± S.E.M. (n = 10–12/group).

haloperidol; [$F(2,87)=0.18$, $P=0.83$], clozapine; [$F(2,81)=1.00$, $P=0.37$]) (Fig. 3A, B, C, and D, respectively).

Table 1 shows the effects of test compounds on startle responses with a 120 dB pulse tone in rats. Oxotremorine dose-dependently decreased startle responses in pulse alone condition [$F(2,27)=3.05$, $P=0.06$], and post-hoc analyses showed that it significantly reduced startle responses in this condition at a dose of 0.3 mg/kg ($P<0.05$ compared with vehicle-treated group). Clozapine reduced startle responses in pulse alone condition [$F(2,27)=23.10$, $P<0.01$], and post-hoc analyses showed that at all tested doses ($P<0.01$ compared with vehicle-treated group) it significantly decreased startle responses in this condition. On the other hand, nicotine and haloperidol exhibited no significant effects on startle responses in this condition, although haloperidol tended to reduce startle responses in this condition at a dose of 0.3 mg/kg ($P=0.06$ compared with vehicle-treated group). In these experiments, oxotremorine elicited several behaviors including salivation, lacrimation, and tremor at a dose of 0.3 mg/kg. On the other hand, nicotine began to elicit the reduction of locomotor activity with crouching and tail rattling at a dose of 0.6 mg/kg. Higher doses were therefore not tested.

3.4. Effects of oxotremorine and nicotine on methamphetamine-induced disruption of PPI in rats

Methamphetamine at a dose of 3 mg/kg disrupted PPI with prepulse levels of 63, 66, and 72 dB. Two-way ANOVA for the VEH+VEH and VEH+MAP groups revealed a significant effect of treatment for methamphetamine [$F(1,114)=64.58$, $P<0.01$] but no significant dose × prepulse intensity interaction [$F(2,114)=2.00$, $P>0.13$]. Oxotremorine (0.01–0.3 mg/kg) reversed the methamphetamine-induced disruption of PPI (Fig. 4A). There was a significant main effect of treatment for oxotremorine [$F(4,192)=18.52$, $P<0.01$] and a significant dose × prepulse intensity interaction [$F(8,192)=2.42$, $P<0.05$]. Post-hoc analyses revealed that 0.03, 0.1, and 0.3 mg/kg doses of oxotremorine with prepulses of 66 and 72 dB significantly restored PPI compared with methamphetamine-treated group. On the other hand, nicotine (0.06–0.6 mg/kg) exhibited no effect on the disruption of PPI caused by methamphetamine (Fig. 4B). In haloperidol-treated rats, a significant main effect of treatment was demonstrated for haloperidol [$F(2,96)=10.33$, $P<0.01$], as well as a non-significant dose × prepulse intensity interaction [$F(4,96)=1.42$, $P>0.23$], indicating that haloperidol significantly reversed the impairment of PPI caused by methamphetamine (Fig. 4C). Post-hoc analyses showed that a dose of 0.1 mg/kg with prepulse of 72 dB, and a dose of 0.3 mg/kg with prepulses of 66 and 72 dB significantly reversed PPI compared with methamphetamine-treated group.

Table 1
Effects of tested drugs on the magnitude of startle responses to 120 dB pulse tone in rats

Drug (mg/kg)	Mean ± S.E.M.
VEH	134.8 ± 54.1
OXO (0.1)	61.2 ± 13.9
OXO (0.3)	24.0 ± 2.3*
VEH	101.2 ± 15.3
NCT (0.06)	151.3 ± 23.9
NCT (0.2)	95.4 ± 13.3
NCT (0.6)	163.2 ± 23.4
VEH	147.1 ± 29.1
HPD (0.1)	146.0 ± 20.7
HPD (0.3)	72.7 ± 13.2
VEH	136.0 ± 14.0
CLZ (3)	59.1 ± 11.6**
CLZ (10)	28.3 ± 8.3**

VEH = vehicle; OXO = oxotremorine; NCT = nicotine; HPD = haloperidol; CLZ = clozapine. Results are the mean ± S.E.M. Numbers in parentheses are doses used. Asterisks represent a significant difference from VEH condition, *, $P<0.05$, **, $P<0.01$ (Dunnett's test).

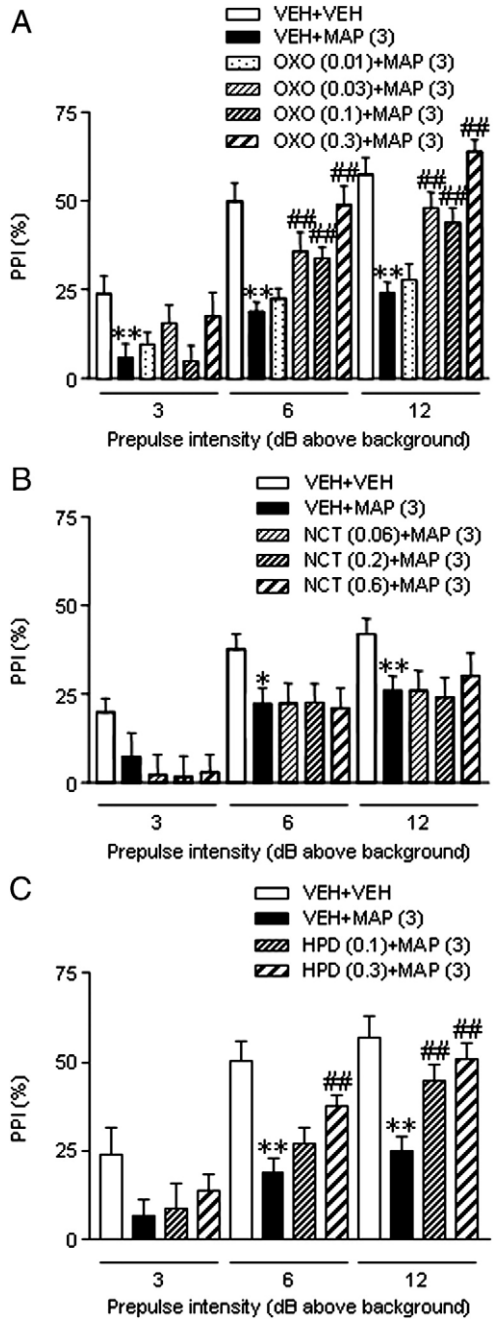

Fig. 4. Effects of oxotremorine (A), nicotine (B), and haloperidol (C) on methamphetamine (3 mg/kg)-induced disruption of PPI in rats at 3 prepulse intensity levels (3, 6, and 12 dB above background). Oxotremorine (0.01–0.3 mg/kg, s.c.), nicotine (0.06–0.6 mg/kg, s.c.), or haloperidol (0.1–0.3 mg/kg, s.c.) was administered 30 min before the administration of methamphetamine. Methamphetamine (3 mg/kg, s.c.) was administered 10 min before rats were placed in the chambers. VEH = vehicle; MAP = methamphetamine; OXO = oxotremorine; NCT = nicotine; HPD = haloperidol. Results are the mean±S.E.M. Asterisks represent a significant difference from VEH+VEH condition (A, B, and C), *, $P<0.05$, **, $P<0.01$ (*t*-test). #, significant difference from VEH+MAP condition (A, B, and C), #, $P<0.05$, ##, $P<0.01$ (Dunnett's test). (n=9–20/group).

Table 2 summarizes the effects of oxotremorine, nicotine, and haloperidol on startle responses with a 120 dB pulse tone in rats. Treatment with methamphetamine increased the startle responses elicited with pulse alone. Significant increases in startle response were observed with methamphetamine ($P<0.01$ compared with VEH+VEH group). Oxotremorine reversed the increase in startle response elicited by methamphetamine in pulse alone condition [$F(4,64)=17.03$, $P<0.01$], and post-hoc analyses showed that oxotremorine at doses of 0.1 and 0.3 mg/kg ($P<0.01$ compared with VEH+MAP group) significantly reduced the exaggeration of startle responses caused by methamphetamine. Likewise, haloperidol significantly attenuated the enhancement of startle responses in pulse alone condition elicited by methamphetamine at all tested doses [$F(2,32)=25.59$, $P<0.01$]. On the other hand, nicotine exhibited no significant effects on startle responses in this condition.

3.5. Effects of oxotremorine and nicotine on ketamine-induced disruption of PPI in rats

Subcutaneous administration of ketamine significantly and dose-dependently impaired PPI across all prepulse levels examined, as indicated by a significant main effect of dose [$F(2,78)=17.92$, $P<0.01$] and a non-significant dose×prepulse intensity interaction [$F(4,78)=0.27$, $P>0.89$], without affecting the magnitude of startle responses (Fig. 5A). Since a dose of 5 mg/kg was found to disrupt PPI at all prepulse levels, it was selected for testing of the effects of oxotremorine, nicotine, haloperidol, and clozapine.

The effects of oxotremorine on ketamine-induced disruption of PPI were examined. Oxotremorine (0.03–0.3 mg/kg) reversed the ketamine-induced disruption of PPI (Fig. 5B). There was a significant main effect of treatment with oxotremorine [$F(3,192)=3.09$, $P<0.05$] but no significant dose×prepulse intensity interaction [$F(6,192)=0.82$, $P>0.55$], indicating that oxotremorine significantly reversed the impairment of PPI caused by ketamine. Post-hoc analyses showed that a dose of 0.3 mg/kg with prepulse of 72 dB significantly reversed PPI compared with ketamine-treated group. Clozapine (3–10 mg/kg), tested as a positive control, reversed the disruption of PPI caused by ketamine (Fig. 5E). There was a significant main effect of treatment with clozapine [$F(2,78)=14.15$, $P<0.01$] but no significant dose×prepulse intensity interaction [$F(4,78)=0.46$, $P>0.76$]. Post-hoc analyses showed that a dose of 10 mg/kg with all prepulse levels significantly reversed PPI compared with ketamine-treated group. On the other hand, neither nicotine (0.06–0.6 mg/kg) nor haloperidol

Table 2
Effects of tested drugs on the magnitude of startle responses by rats to 120 dB pulse tone with methamphetamine or ketamine

Drug (mg/kg)	Mean±S.E.M.	Drug (mg/kg)	Mean±S.E.M.
VEH+VEH	133.0±12.6	VEH	111.0±22.6
VEH+MAP (3)	241.4±13.2**	KET (1.5)	159.0±16.9
OXO (0.01)+MAP (3)	224.9±25.4	KET (5)	112.2±25.1
OXO (0.03)+MAP (3)	194.9±22.2		
OXO (0.1)+MAP (3)	104.2±10.6##	VEH+VEH	141.5±17.6
OXO (0.3)+MAP (3)	128.5±11.6##	VEH+KET (5)	137.5±11.3
		OXO (0.03)+KET (5)	110.2±18.4
VEH+VEH	126.5±23.0	OXO (0.1)+KET (5)	102.2±14.6
VEH+MAP (3)	227.1±41.1*	OXO (0.3)+KET (5)	55.0±8.0##
NCT (0.06)+MAP (3)	211.5±20.4		
NCT (0.2)+MAP (3)	187.8±25.8	VEH+VEH	153.1±17.8
NCT (0.6)+MAP (3)	215.4±31.0	VEH+KET (5)	84.0±11.6**
		NCT (0.06)+KET (5)	156.2±23.4
VEH+VEH	116.9±17.2	NCT (0.2)+KET (5)	196.4±25.9##
VEH+MAP (3)	205.7±12.8**	NCT (0.6)+KET (5)	157.8±21.7
HPD (0.1)+MAP (3)	89.7±15.1##		
HPD (0.3)+MAP (3)	83.5±13.3##	VEH+VEH	110.3±18.7
		VEH+KET (5)	126.4±18.0
		HPD (0.1)+KET (5)	172.1±24.3
		HPD (0.3)+KET (5)	157.6±25.7
		VEH+VEH	164.8±36.1
		VEH+KET (5)	110.5±18.1
		CLZ (3)+KET (5)	52.3±9.8##
		CLZ (10)+KET (5)	61.3±8.2##

VEH = vehicle; MAP = methamphetamine; KET = ketamine; OXO = oxotremorine; NCT = nicotine; HPD = haloperidol; CLZ = clozapine. Results are the mean±S.E.M. Numbers in parentheses are doses used. Asterisks represent a significant difference from VEH or VEH+VEH condition, *, $P<0.05$, **, $P<0.01$ (*t*-test). #, significant difference from VEH+MAP or VEH+KET condition, #, $P<0.05$, ##, $P<0.01$ (Dunnett's test).

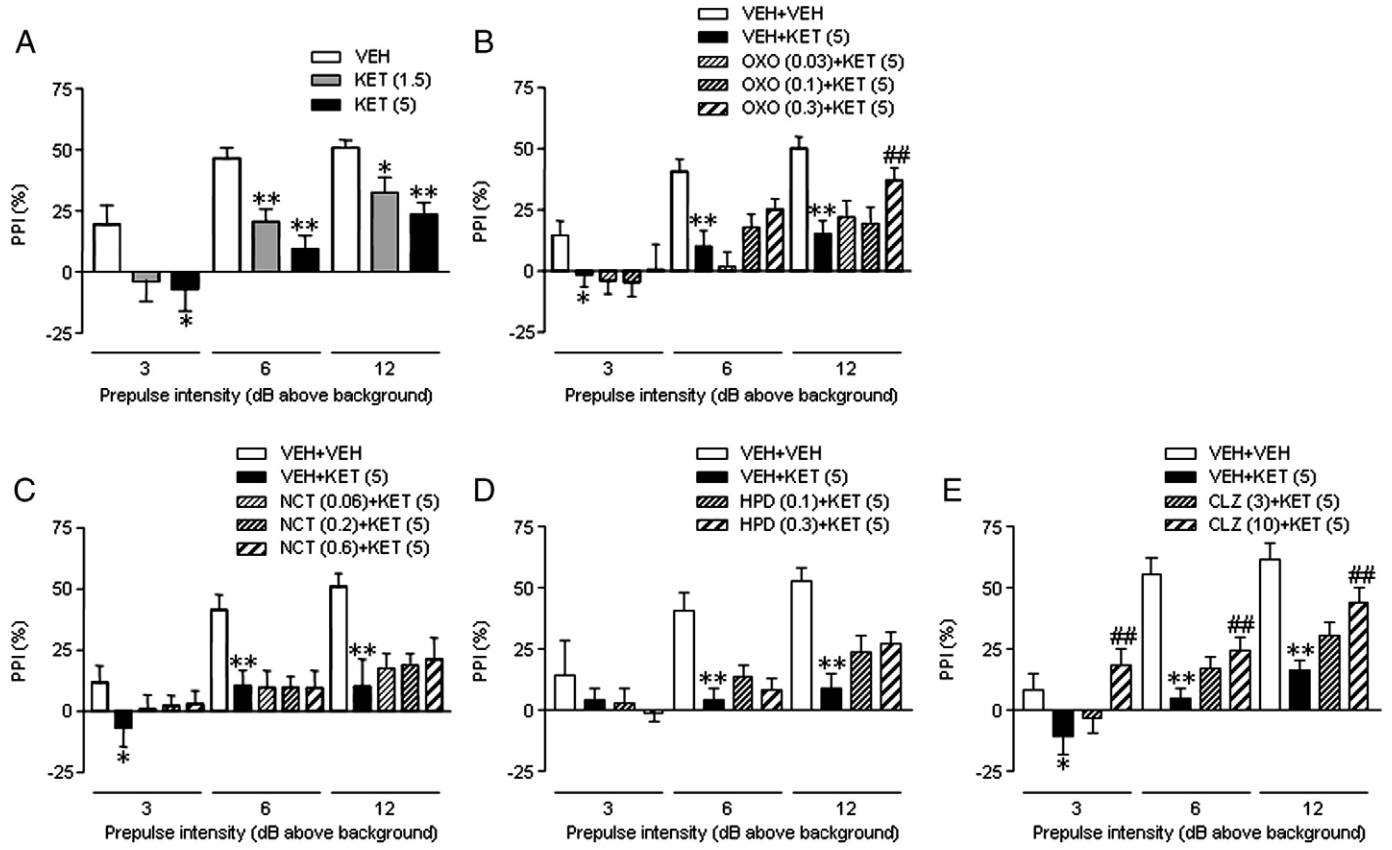

Fig. 5. A, Effects of ketamine (A) on PPI in rats at 3 prepulse intensity levels (3, 6, and 12 dB above background). B, C, and D, Effects of oxotremorine (B), nicotine (C), haloperidol (D), and clozapine (E) on ketamine (5 mg/kg)-induced disruption of PPI in rats at 3 prepulse intensity levels (3, 6, and 12 dB above background). Oxotremorine (0.03–0.3 mg/kg, s.c.), nicotine (0.06–0.6 mg/kg, s.c.), haloperidol (0.1–0.3 mg/kg, s.c.), or clozapine (3–10 mg/kg, s.c.) was injected 30 min before ketamine. Ketamine (5 mg/kg, s.c.) was administered just before rats were placed in the chambers. VEH = vehicle; KET = ketamine; OXO = oxotremorine; NCT = nicotine; HPD = haloperidol; CLZ = clozapine. Results are the mean±S.E.M. Asterisks represent a significant difference from VEH (A) or VEH+VEH condition (B, C, D, and E), *, $P<0.05$, **, $P<0.01$ (*t*-test). #, significant difference from VEH+KET condition (B, C, D, and E), #, $P<0.05$, ##, $P<0.01$ (Dunnett's test). (n=9–20/group).

(0.1–0.3 mg/kg) exhibited to antagonize the disruption of PPI caused by ketamine, as shown in Fig. 5C and D, respectively.

Table 2 shows the effects on startle responses elicited with a 120 dB pulse tone of treatment of rats with ketamine. Ketamine did not affect the magnitude of startle responses at any doses tested. Oxotremorine dose-dependently reduced the startle responses caused by ketamine in pulse alone condition [$F(3,64)=8.29$, $P<0.01$], and post-hoc analyses showed that oxotremorine at a dose of 0.3 mg/kg ($P<0.01$ compared with VEH+KET group) significantly reduced the effects of ketamine. Clozapine decreased the startle responses caused by ketamine in pulse alone condition [$F(2,26)=6.18$, $P<0.01$], and post-hoc analyses showed that clozapine at doses of 3 and 10 mg/kg ($P<0.01$ and $P<0.05$ compared with VEH KET group, respectively) significantly decreased the magnitude of startle responses caused by ketamine. On the other hand, nicotine at a dose of 0.2 mg/kg ($P<0.01$ compared with VEH KET group) increased startle responses in pulse alone condition, while haloperidol exhibited no significant effects on startle responses in this condition.

3.6. Effects of oxotremorine and nicotine on PPI in mice

We examined the effects of oxotremorine, nicotine, and haloperidol on spontaneous PPI in DBA/2J and C57BL/6J mice. When PPI was tested in both DBA/2J and C57BL/6J mice, there was a significant main effect of strain [$F(1,80)=9.06$, $P<0.01$] but no significant strain×prepulse intensity interaction [$F(3,80)=1.28$, $P>0.28$]. Post-hoc analyses showed that DBA/2J mice showed significantly lower PPI than those in C57BL/6J mice with prepulses of 80 and 90 dB (Fig. 6A). In DBA/2J mice, oxotremorine (0.03–0.06 mg/kg) dose-dependently increased PPI (Fig. 6B). There was a significant main effect of treatment with oxotremorine [$F(2,136)=26.01$, $P<0.01$] but no significant dose×prepulse intensity interaction [$F(6,136)=1.00$, $P>0.42$], indicating that oxotremorine significantly enhanced PPI regardless of prepulse level except a dose of 0.03 mg/kg with prepulses of 70 and 75 dB. On the other hand, nicotine (0.06–0.6 mg/kg) had no effect on PPI in DBA/2J mice (Fig. 6C). When the effect of haloperidol (0.03–0.1 mg/kg) on PPI was tested (Fig. 6D), a significant main effect of treatment with haloperidol was found [$F(2,140)=18.97$, $P<0.01$] but no significant dose×prepulse intensity interaction [$F(6,140)=1.08$, $P>0.37$]. Post-hoc analyses showed that 0.1 mg/kg dose of haloperidol with prepulses of 75 and 90 dB significantly increased PPI compared with vehicle-treated group. On the other hand, in C57BL/6J mice, there was a significant main effect of treatment with oxotremorine [$F(2,120)=10.42$, $P<0.01$] but no significant dose×prepulse intensity interaction [$F(6,120)=0.50$, $P>0.81$]. Post-hoc analyses showed that all tested doses of oxotremorine did not altered spontaneous PPI compared with vehicle-treated group (Fig. 6E). Neither nicotine (0.06–0.6 mg/kg) nor haloperidol (0.03–0.1 mg/kg) altered spontaneous PPI at any doses tested in C57BL/6J mice (Fig. 6F and G).

Table 3 shows the effects on startle responses elicited with a 120 dB pulse tone in mice. DBA/2J mice showed a significant lower startle responses in pulse alone condition than C57BL/6J mice. In C57BL/6J but not DBA/2J mice, oxotremorine reduced startle responses in pulse alone condition [$F(2,30)=6.48$, $P<0.01$], and post-hoc analyses showed that it significantly decreased startle responses in this condition at a dose of 0.06 mg/kg ($P<0.01$ compared with vehicle-treated group). On the other hand, neither nicotine nor haloperidol

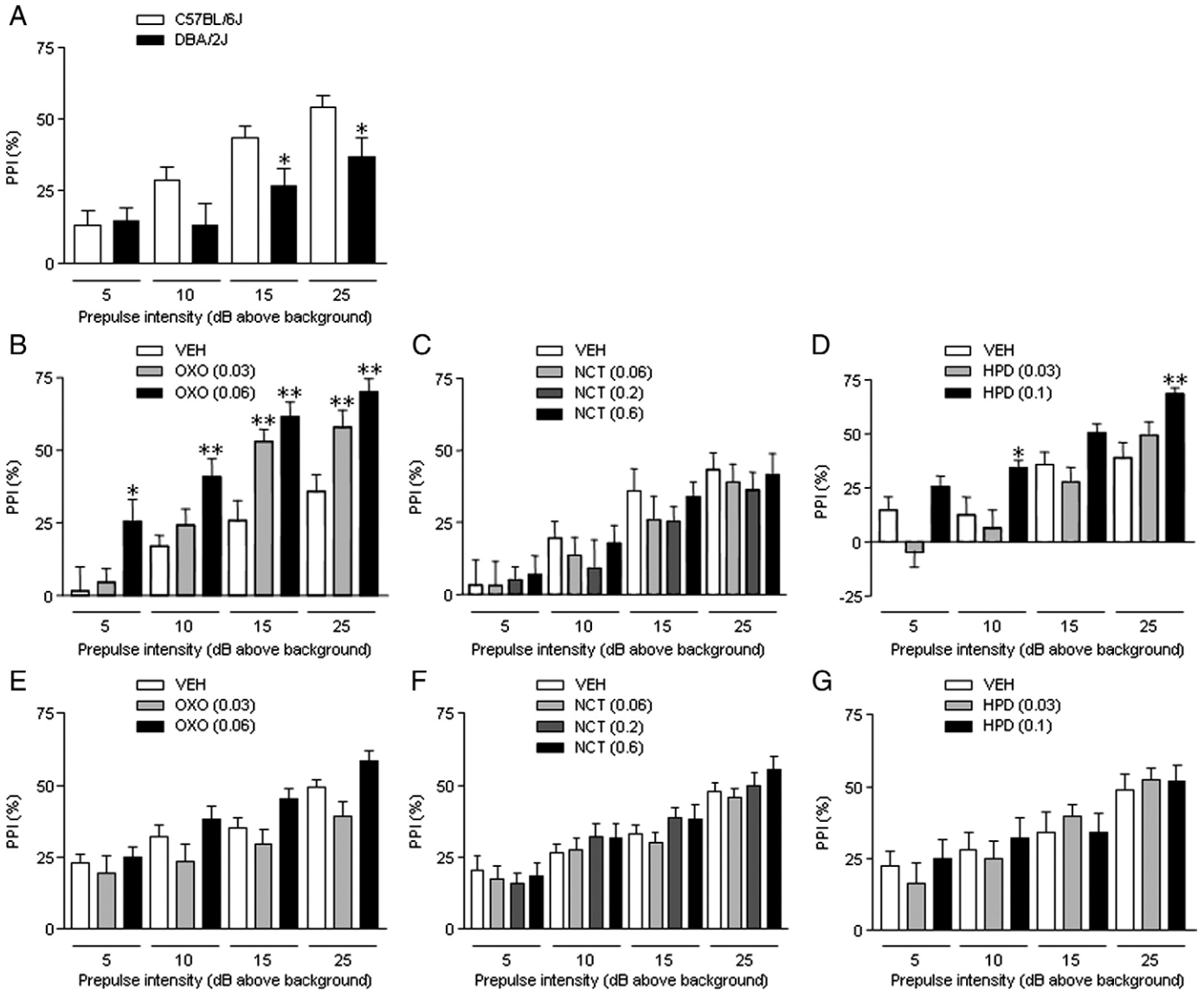

Fig. 6. Spontaneous PPI was tested in both DBA/2J and C57BL/6J mice (A) and effects of oxotremorine (B and E), nicotine (C and F), and haloperidol (D and G) were tested on PPI in both DBA/2J and C57BL/6J mice, respectively. Oxotremorine (0.03–0.06 mg/kg, s.c.), nicotine (0.06–0.6 mg/kg, s.c.), or haloperidol (0.03–0.1 mg/kg, s.c.) was administered 30 min before mice were placed in the chambers. The study was conducted at 4 prepulse intensity levels (5, 10, 15, and 25 dB above background). VEH = vehicle; OXO = oxotremorine; NCT = nicotine; HPD = haloperidol. Results are the mean ± S.E.M. Asterisks represent a significant difference from C57BL/6J mice (A) or VEH condition (B, C, D, E, F, and G), *, $P<0.05$, **, $P<0.01$ (t-test or Dunnett's test). (n = 8–13/group).

affected startle responses in pulse alone condition in both DBA/2J and C57BL/6J mice.

4. Discussion

The present study demonstrated that administration of the potent and selective muscarinic receptor agonist oxotremorine reversed methamphetamine-induced hyperlocomotion as well as disruption of PPI. Oxotremorine also reversed the disruption of PPI caused by ketamine, an NMDA antagonist. In addition, oxotremorine improved naïve PPI in DBA/2J mice, in which PPI is spontaneously less than in C57BL/6J mice. On the other hand, the nicotinic receptor agonist nicotine exhibited no effects on the four animal models of symptoms of schizophrenia examined. These findings indicate that activation of muscarinic receptors but not nicotinic receptors was able to improve behavioral responses in animal models related to schizophrenia.

Previous studies found that the muscarinic receptor antagonist scopolamine caused hyperlocomotion in mice and impaired PPI in rats (van Abeelen and Strijbosch, 1969; Bushnell, 1987; Jones and Shannon, 2000a,b). These effects in both mice and rats were reversed by haloperidol (Fink and Morgenstern 1980; Shannon and Peters, 1990; Jones et al., 2005). It is known that blockade of cholinergic receptors, particularly muscarinic receptors, causes psychosis characterized by

Table 3
Effects of tested drugs on the magnitude of startle responses by DBA/2J and C57BL/6J mice to 120 dB pulse tone

Drug (mg/kg)	Mean ± S.E.M. in DBA/2J	Mean ± S.E.M. in C57BL/6J
Naïve	55.4 ± 6.7**	109.8 ± 12.7
VEH	44.3 ± 6.9	108.6 ± 6.7
OXO (0.03)	51.7 ± 7.8	92.0 ± 8.6
OXO (0.06)	34.0 ± 9.3	72.5 ± 5.7**
VEH	51.2 ± 7.0	116.7 ± 11.0
NCT (0.06)	48.8 ± 8.7	117.5 ± 10.8
NCT (0.2)	60.3 ± 9.2	112.3 ± 8.2
NCT (0.6)	59.5 ± 5.5	115.2 ± 6.8
VEH	42.8 ± 5.6	99.8 ± 9.5
HPD (0.03)	45.6 ± 6.6	95.2 ± 9.6
HPD (0.1)	47.3 ± 5.6	107.3 ± 9.4

VEH = vehicle; OXO = oxotremorine; NCT = nicotine; HPD = haloperidol. Results are the mean ± S.E.M. Numbers in parentheses are doses used. Asterisks represent a significant difference from naïve C57BL/6J mice or VEH condition, **, $P<0.01$ (t-test or Dunnett's test).

hallucinations and cognitive impairment in normal human subjects, and exacerbates symptoms in schizophrenic patients (Neubauer et al., 1966a,b; Peterson, 1977; Rusted and Warburton, 1988), which are similar to those elicited by amphetamines. Recently, the M1/M4 agonist xanomeline was found to reduce hallucinations, agitation, and delusions in patients with Alzheimer's disease (Bodick et al., 1997). This finding indicated that xanomeline behaved like antipsychotic agents. Taken together, these clinical findings suggest that modulation of the muscarinic cholinergic system might be a novel means of treatment of schizophrenia.

In the present study, we found that oxotremorine, a non-selective muscarinic receptor agonist, significantly reversed both the hyperlocomotion and disruption of PPI caused by methamphetamine. Jones et al. (2005) reported that oxotremorine reversed the disruption of PPI by a dopamine receptor agonist, apomorphine. These findings suggested that functional interaction might occur between the cholinergic and dopaminergic systems in the regulation of locomotion and PPI. The cholinergic and dopaminergic neuronal systems exhibit complex relationships in the basal ganglia, and disruption of such relationships could lead to several disorders, such as schizophrenia and parkinsonism (Graybiel, 1990; Di Chiara et al., 1994). Neurons originating from brainstem muscarinic cholinergic nuclei monosynaptically contact mesocortical and mesolimbic dopamine neurons (Bolam et al., 1991) and activate dopamine neurons via cholinergic receptors, probably M1 muscarinic receptors (Lacey et al., 1990), suggesting that the brainstem cholinergic system might play an important role in regulating dopaminergic activity. However, it has remained unclear which subtypes of muscarinic receptors are principally involved in the regulation of dopaminergic transmission.

We also examined the role of activation of muscarinic receptors in dopamine-independent animal models of schizophrenia, including ketamine-induced disruption of PPI in rats. Since the 1990s, Carlsson et al. (1997) and others have proposed that dysfunction of glutamatergic transmission through NMDA receptors may be one of the mechanisms underlying schizophrenia, since deterioration of mental functioning was observed in addicts who used phencyclidine or ketamine (Javitt and Zukin, 1991; Carlsson et al., 1997; Jansen, 2000).

Previous studies found that ketamine-induced disruption of PPI was reversed only by atypical antipsychotics such as clozapine or quetiapine, and not by typical antipsychotics such as haloperidol (Swerdlow et al., 1998), as also found in the present study. It thus appears that the disruption of PPI caused by ketamine may not be the same as that of PPI by dopaminergic activation, which can be reversed by both typical and atypical antipsychotics. This was the first study to demonstrate that oxotremorine can reverse the disruption of PPI elicited by ketamine. These findings suggest that activation of muscarinic receptors can be expected to elicit antipsychotic effects like those of atypical antipsychotics such as clozapine and risperidone.

In addition to pharmacological models, we examined the effects of oxotremorine in a non-pharmacological model of impairment of sensory gating mechanisms. It is known that the DBA/2J strain of mice exhibits lower levels of PPI than other mouse strains (Olivier et al., 2001; Kinney et al., 2003). PPI is significantly enhanced in this mouse strain after treatment with antipsychotics such as clozapine, risperidone, and haloperidol, suggesting that the DBA/2J mouse may be a spontaneous animal model enabling examination of novel antipsychotic activities of drugs (McCaughran et al., 1997; Olivier et al., 2001; Kinney et al., 2003). We confirmed that DBA/2J mice displayed lower basal levels of PPI than C57BL/6J mice but that DBA/2J mice exhibited enhancement of PPI following administration of haloperidol, which were different from the effects of C57BL/6J mice. Oxotremorine significantly enhanced PPI in this strain of mice to the levels observed in C57BL/6J mice. Taken together, these findings in pharmacological and non-pharmacological models suggest that activation of muscarinic receptors could improve symptoms in animal models of schizophrenia, similar to atypical antipsychotic drugs.

In contrast to the effects of oxotremorine, we found that nicotine did not have any antipsychotic effects in various animal models. It was reported that administration of nicotine by cigarette smoking transiently normalized deficient auditory sensory gating in schizophrenic patients (Adler et al., 1993). This clinical finding suggested that nicotinic receptors might be involved in the modulation of the symptoms of schizophrenia. However, only one previous study demonstrated that nicotine, at doses of 0.05 and 0.2 mg/kg, antagonized the disruption of PPI caused by apomorphine, a dopamine receptor agonist, in rats (Suemaru et al., 2004). In the present study, we found no improvement by nicotine of the impairment of PPI caused by methamphetamine in rats in the dose range of 0.06 to 0.6 mg/kg. These discrepancies in findings may be due to differences in prepulse intensity and animal strains between studies. We measured PPI at prepulse levels of 63, 66, and 72 dB with a background noise of 60 dB in Sprague–Dawley rats, while Suemaru et al. (2004) used prepulse levels of 70 and 80 dB with a background noise of 65 dB in Wistar rats. PPI without pharmacological manipulation was increased with doses of 0.001 and 0.01 mg/kg (Acri et al., 1994) and of 0.03 to 0.3 mg/kg (Curzon et al., 1994) of nicotine, while PPI was not affected in the same dose range (0.001–0.3 mg/kg) (Mirza et al., 2000) and at doses of 0.05 to 1 mg/kg of nicotine (Suemaru et al., 2004), and decreased by nicotine at doses of 1 and 3 mg/kg (Schreiber et al., 2002) in rats. In the present study, nicotine did not affect PPI in the dose range of 0.06 to 0.6 mg/kg. These doses were similar dose ranges to those in the previous studies. Thus, it was unlikely to miss the effect of nicotine in the present study. The effects of nicotine on PPI without pharmacological manipulation thus remain unclear, though experimental conditions might alter the effects of nicotine.

We also tested the effects of nicotine on the disruption of PPI caused by ketamine in rats. Our findings were consistent with the previous report that nicotine could not reverse the disruption of PPI caused by another NMDA antagonist, phencyclidine, in Wistar rats (Suemaru et al., 2004).

In addition to the findings obtained in rats, nicotine did not improve PPI in DBA/2J mice in the present study. This finding is consistent with those of Spielewoy and Markou (2004). Interestingly, in the same study (Spielewoy and Markou, 2004), nicotine did reverse the impairment of PPI caused by phencyclidine in DBA/2J mice without affecting spontaneous PPI.

On testing of the effects of subunit-selective nicotinic receptor agonists on PPI, Schreiber et al. (2002) found that the $\alpha4\beta2$ nicotinic receptor agonists epibatidine and A-85380 but not the $\alpha7$ nicotinic receptor agonists GTS-21 and AR-R-17779 impaired spontaneous PPI in Sprague–Dawley rats. In addition, Olivier et al. (2001) reported that GTS-21 had no effects on spontaneous PPI in DBA/2J mice. No study has tested the effects of these subunit-selective nicotinic receptor agonists on the impairment of PPI caused by activation of the dopaminergic system or blockade of NMDA receptors. The effects of nicotine on PPI may result from summation of multiple effects on these subunits.

The non-selective muscarinic receptor agonist oxotremorine exhibited antipsychotic effects on the four animal models of symptoms of schizophrenia we tested. Our findings suggested that activation of muscarinic receptors may be an alternative strategy for the treatment of schizophrenia in addition to classical antipsychotics, although further studies with subunit-selective ligands may be required to clarify the roles of nicotinic receptors in schizophrenia. Molecular cloning studies have shown that genes exist for muscarinic receptors of 5 distinct subtypes, M1 to M5 (Wess, 1996). However, the subtype of muscarinic receptors responsible for mediating the effects of oxotremorine has yet to be determined.

Of the five subtypes, M1 is abundantly expressed in cerebral cortex, hippocampus, and striatum (Weiner et al., 1990; Levey et al., 1991). The density of M1 binding was decreased in cortex, hippocampus, and caudate–putamen in postmortem studies of schizophrenic patients (Dean et al., 1996; Crook et al., 2000,2001; Katerina et al., 2004).

The potential contribution of abnormal M1 muscarinic receptor function to the etiology of schizophrenia is further suggested by the

behavioral abnormalities observed in M1 knockout mice, which have been demonstrated to be hyperactive and to exhibit increased responses to amphetamine challenge (Gerber et al., 2001; Miyakawa et al., 2001). These behavioral abnormalities were also reversed by antipsychotics such as haloperidol and clozapine (Gerber et al., 2001). On the other hand, it was also reported that M4 knockout mice displayed a small but statistically significant increase in basal locomotor activity, and that this spontaneous motor activity was enhanced by a D1 receptor agonist, SKF 38393 (Gomeza et al., 1999). The density and distribution of M4 muscarinic receptors are similar to those of M1 muscarinic receptors, and high levels of M4 muscarinic receptors are found in cerebral cortex, hippocampus and particularly in the striatum on immunocytochemical examination (Levey, 1993). Furthermore, the M1/M4 dual agonist xanomeline exhibited an antipsychotic profile of effects on amphetamine-induced hyperactivity as well as apomorphine-induced disruption of PPI in rats (Stanhope et al., 2001; Jones et al., 2005). These findings suggested that the effects of oxotremorine observed in our studies were probably due to the activation of M1 and/or M4 muscarinic receptors. However, neither selective M1 nor M4 agonists or antagonists are yet available to test this hypothesis. As an alternative approach to test this hypothesis, it would be also worthwhile assessing the effects of oxotremorine in our pharmacological animal models such as methamphetamine and ketamine with M1 and/or M4 knockout mice. Further studies are warranted to clarify the roles of M1 and/or M4 muscarinic receptors in schizophrenia once such selective M1 and/or M4 muscarinic receptor ligands become available.

In summary, the present study demonstrated that the muscarinic receptor agonist oxotremorine but not the nicotinic receptor agonist nicotine ameliorated abnormal behavioral changes caused by psychostimulants such as methamphetamine and ketamine and improved spontaneous PPI in DBA/2J mice. These findings suggest that modulation of muscarinic receptors could be an alternative approach for the treatment of schizophrenia, psychosis, and related disorders in addition to classical antipsychotics.

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