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ORIGINAL INVESTIGATION

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Drug-induced potentiation of prepulse inhibition of acoustic startle reflex in mice: a model for detecting antipsychotic activity?

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Abstract *Rationale:* Schizophrenic patients typically have impaired startle habituation (SH) and prepulse inhibition of the startle reflex (PPI). PPI can be disrupted in rats by psychomimetics, and drug-induced reversal of this deficit is considered to predict potential antipsychotic properties. Certain strains of mice, such as C57BL/6J, naturally display poor PPI. *Objective:* To test whether mice spontaneously showing low levels of PPI might prove a useful tool for detecting novel antipsychotics. *Methods:* PPI and SH were evaluated in four strains of mice: BALB/cByJ, MORO, 129/SvEv and C57BL/6J. The effects of antipsychotic [haloperidol (1, 3 and 6 mg/kg), clozapine (0.3, 1, 3 and 30 mg/kg) and risperidone (0.1, 0.3 and 1 mg/kg,)] and non-antipsychotic [diazepam (3, 10 and 30 mg/kg), buspirone (1, 3 and 10 mg/kg), desipramine (3, 10 and 30 mg/kg), morphine (3, 10 and 30 mg/kg) and scopolamine (0.3, 1 and 3 mg/kg)] drug treatments were studied on PPI. *Results:* Haloperidol (6 mg/kg), clozapine (3 and 30 mg/kg), and risperidone (1 mg/kg) all significantly enhanced PPI in C57BL/6J. All non-antipsychotics failed to improve PPI in this strain, except diazepam. Facilitation of PPI was also obtained in the other strains; however, clear inter-strain differences were observed depending on the class of antipsychotic used and on the level of prepulse intensity. *Conclusion:* Antipsychotic-induced facilitation of PPI is clearly detected in mice naturally exhibiting poor levels of sensorimotor gating (e.g., C57BL/6J), but is also observed in other strains of mice. The use of this procedure as a potential screening test for detecting novel antipsychotic medications is discussed.

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Introduction

Schizophrenia is a chronic and debilitating syndrome, which is generally associated with a wide range of cognitive and emotional alterations. Pathological processes that underlie the profound neuropsychiatric disturbances in schizophrenia are poorly understood. However, the symptom heterogeneity observed in schizophrenic patients suggests that different etiologic and pathogenic causes (e.g., genetic, developmental, environmental and social) may contribute to schizophrenia disorders (Freedman et al. 1999; Harrison 1999). Although there has been major advances in the pharmacological management of schizophrenia with atypical antipsychotics, there is still substantial need for novel antipsychotic agents with improved efficacy and side-effect profiles. Towards this goal, significant efforts have been devoted to the development of preclinical models that can have face, construct and predictive validity to schizophrenia. One such model, which has received growing interest in the recent years, is the prepulse inhibition paradigm. Prepulse inhibition (PPI) refers to the inhibition of a startle reflex that occurs when an intense startling stimulus (acoustic or tactile) is preceded by a barely detectable prepulse. PPI provides an operational measure of sensorimotor gating and may reflect the ability to screen exteroceptive stimuli for their physiological or cognitive relevance (for review, see Swerdlow and Geyer 1998). Several clinical studies have shown that schizophrenic patients have deficient PPI and startle habituation (SH) (Geyer and Braff 1987; Braff et al. 1992). Habituation is viewed as the simplest form of non-associative learning and reflects decreased responding to repeated presentation of an initially novel exteroceptive stimulus. Common neuropathological mechanisms were proposed to underlie clinical signs and reduced PPI and habituation in the schizophrenic patients (Geyer and Braff 1987; Braff et al. 1992).

Multiple approaches have been developed in rats to mimic the sensorimotor gating deficits exhibited by schizophrenic patients. PPI can be disrupted pharmacologically with administration of psychomimetics, such as dopaminergic agonists (amphetamine and apomorphine), glutamatergic antagonists (PCP and MK-801) and serotoninergic agonists (DOI). The restoration of psychomimetic-induced disruption of PPI is widely used to screen for new antipsychotic medications (Varty and Higgins 1995; Swerdlow and Geyer 1998). However, this approach focuses on particular neurotransmitter systems and therefore may be primarily sensitive to agents operating through the same systems. PPI can be also disrupted non-pharmacologically by early developmental insults such as isolation rearing (Geyer et al. 1993) or neonatal brain lesions (Lipska et al. 1995). In keeping with the neurodevelopmental hypothesis of schizophrenia, these perturbations were shown to lead to postpubertal emergence of sensorimotor gating impairment (Geyer et al. 1993; Lipska et al. 1995). Interestingly, the deficit of PPI induced by isolation rearing could be reversed with a wide range of antipsychotics, irrespective of their pharmacological properties, indicating that non-pharmacological approaches to disrupting PPI may be the most promising way to identify antipsychotics with new mechanisms of action (Varty and Higgins 1995; Bakshi et al. 1998).

The aim of the present study was to evaluate a new procedure for screening antipsychotics, which would bypass the need for pharmacological disruption of PPI. In recent years, the development of new molecular techniques to manipulate the mouse genome, and the close correspondence between the mouse and human genomes have strongly encouraged researchers to extend the acoustic PPI paradigm to mice. As shown by a number of studies, reliable startle reflex and PPI can be obtained in mice using stimulus parameters almost identical to those used in rats (Dulawa and Geyer 1996; Logue et al. 1997; Paylor and Crawley 1997; Geyer 1999). More importantly, marked genetic differences in PPI were also reported across strains of mice, with the C57BL/6J strain showing a poor PPI (Paylor and Crawley 1997). Thus, in the present study we tested the hypothesis that various doses of antipsychotics could improve PPI in mice showing poor sensorimotor gating. We first evaluated PPI and startle habituation in our laboratory with four strains of mice, C57BL/6J, BALB/cByJ, MORO and 129/SvEv. Given that C57BL/6J mice showed the lowest degree of PPI and rate of habituation compared to all other strains, we studied the effects of antipsychotics (haloperidol, risperidone and clozapine) and non-antipsychotics (diazepam, buspirone, desipramine, morphine and scopolamine) on PPI in this particular strain. The effects of the active doses of antipsychotics on PPI in the other strains were also studied for comparison.

Materials and methods

Animals

Adult male mice of the following strains, C57BL/6J, Swiss (MORO), 129/SvEv and BALB/cByJ were used. Animals weighing between 20 and 24 g were housed four per cage with water and food ad lib. They were allowed 1 week of acclimation prior to testing. The experiment took place between 0800 hours and 1800 hours.

Apparatus

Testing was conducted in eight startle devices (SRLAB, San Diego Instruments, San Diego, Calif., USA) each consisting of a 5.1 cm (outside diameter) Plexiglas cylinder mounted on a Plexiglas platform in a ventilated, sound-attenuated cubicle with a high frequency loudspeaker (28 cm above the cylinder) producing all acoustic stimuli. The background noise of each chamber was 70 dB. Movements within the cylinder were detected and transduced by a piezoelectric accelerometer attached to the Plexiglas base, digitized and stored by a computer. Beginning at the stimulus onset, 65 readings of 1 ms duration were recorded to obtain the animal's startle amplitude.

Drugs

Haloperidol, clozapine, risperidone, diazepam, buspirone, desipramine, scopolamine and morphine (synthesized at Roche) were dissolved in NaCl 0.9% containing 0.3% Tween and administered intraperitoneally (IP) in an injection volume of 10 ml/kg, with 30-min pretreatment time.

General procedure

The experimental procedures used in this study have received approval from a local committee based on adherence to Swiss federal regulations and guidelines on animal experimentation.

Experiment I

Prepulse inhibition. Twelve naive mice of each strain were tested. Each session was initiated with a 5-min acclimation period followed by five successive 110 dB trials. These trials were not included in the analysis. Six different trial types were then presented: startle pulse (ST110, 110 dB/40 ms), low prepulse stimulus given alone (P74, 74 dB/20 ms), high prepulse stimulus given alone (P90, 90 dB/20 ms), P74 or P90 given 100 ms before the onset of the startle pulse (PP74 and PP90, respectively), and finally a trial where only the background noise was presented (NST) in order to measure the baseline movement in the cylinders. All trials were applied 10 times and presented in random order (P74 and P90 were only given 5 times) and the average inter-trial interval (ITI) was 15 s (10–20 s).

Startle habituation. Twelve naive mice of each strain were used in this experiment. Following a 5-min acclimation period, 111 trials of 110 dB were presented over a 45-min test session. The intertrial interval varied randomly from 10 to 20 s, with an average of 15 s. The data from the first trial were analyzed separately, because the startle responses to the first stimulus presentation were considered to reflect initial reactivity to a unique event (Adams and Geyer 1981; Markou et al. 1994). The remaining 110 trials were grouped in blocks of ten trials each (11 blocks). The amount of habituation (percent habituation) was calculated by the following equation: 100×[(mean amplitude startle for block 1–mean amplitude startle for block11)]/mean amplitude startle for block 1. A high percentage value reflects a high degree of habituation.

Experiment II

Effects of antipsychotics on PPI in C57BL/6J mice. Separate group of animals received an injection of haloperidol (1, 3 and 6 mg/kg), clozapine (0.3, 1, 3 and 30 mg/kg) or risperidone (0,1, 0.3 and 1 mg/kg,) and were tested 30 min later. The procedure for testing was the same as in experiment I.

Effects of non-antipsychotics on PPI in C57BL/6J mice. Five groups of naive animals received an injection of diazepam (3, 10 and 30 mg/kg,), buspirone (1, 3 and 10 mg/kg), desipramine (3, 10 and 30 mg/kg), morphine (3, 10 and 30 mg/kg) and scopolamine (0.3, 1 and 3 mg/kg). The procedure for testing was the same as in experiment I.

Experiment III

Effects of antipsychotics on MORO, BALB/cByJ and 129/SvEv. Naive animals of each strain were treated with haloperidol (6 mg/kg), risperidone (1 mg/kg), clozapine (30 mg/kg) or diazepam (10 mg/kg), and tested 30 min later. The procedure for testing was the same as in experiment I.

Statistical analysis

Analysis of data was carried out with one-way or two-way ANOVA followed by Duncan test for post-hoc comparisons whenever the ANOVAs indicated statistically significant main or interaction effects. The startle and %PPI were analyzed with a two-way ANOVA with strain (or drug dose) as the between-subject factor and the stimuli as the repeated measure. The analysis of the startle habituation over the session was carried out using two-way ANOVA with strain as the between-subject factor and block as the repeated measure (11 levels). The percent startle habituation was analyzed with one-way ANOVA with the strain as between-subject factor.

Results

Acoustic startle response and prepulse inhibition

The acoustic startle responses of the four mouse strains are illustrated in Fig. 1A. There was no significant difference in the non-stimulus trials, thus indicating that the four strains have comparable basal activity in the cylinder. In addition, no startle response was displayed by any of the strains to 74 or 90 dB. In contrast, a significant difference in the startle response to a 110 dB pulse was noted between the strains. The overall ANOVA revealed a significant main effect of strain [$F(3,43)=3.28$, $P<0.05$], and the post hoc comparisons indicated that BALB/cByJ mice had a significantly higher startle response than MORO and C57BL/6J mice ($P<0.05$, Duncan's test). Even though 129/SvEv mice showed a lower startle response than BALB/cByJ mice, no significant difference was noted between the two strains. Similarly, no significant difference was observed between MORO, 129/SvEv and C57BL/6 strains.

Figure 1B illustrates the magnitude of PPI in BALB/cByJ, C57BL/6J, MORO and 129/SvEv mice. The two-way ANOVA revealed a significant main effect of prepulse intensity [$F(1,43)=142.2$, $P<0.01$], thus indicating that there was a significant increase in the level of PPI with increasing prepulse intensity. More importantly,

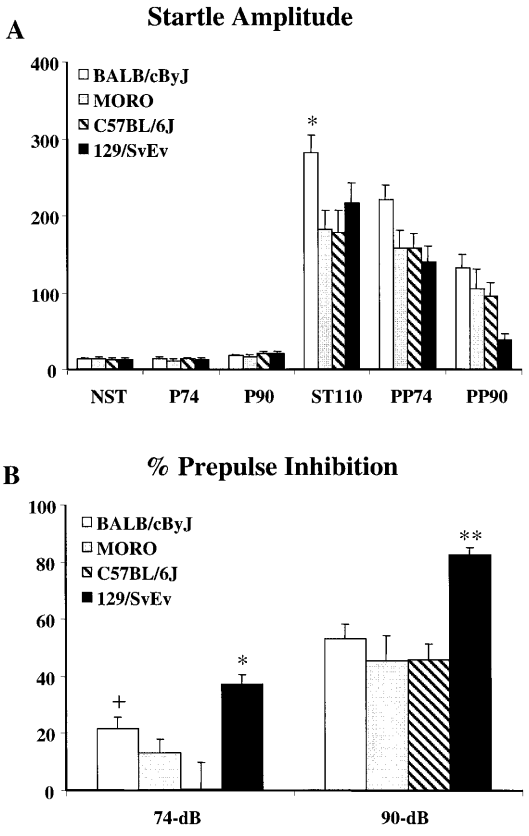

Fig. 1A, B Startle responses and prepulse inhibition in BALB/cByJ, MORO, C57BL/6J and 129/SvEv mice. **A** Mean startle response±SEM (n=12 per strain) for no-stimulus trial (*NST*), low and high prepulse stimulus given alone (P74 and P90, respectively), startle pulse (*ST110*) and P74 or P90 given 100 ms before the onset of the startle (PP74 and PP90, respectively). *$P<0.05$, significantly different from MORO and C57BL/6J mice (Duncan's test after significant ANOVA). **B** Percent prepulse inhibition at 74 and 90 dB prepulse intensities (n=12 per strain). **$P<0.05$, significantly different from BALB/cByJ, MORO and C57BL/6J mice; *$P<0.05$, significantly different from MORO and C57BL/6J mice and $^{+}P<0.05$, significantly different from C57BL/6J (Duncan's test after significant ANOVA)

a significant main effect of strain was noted [$F(3,43)=10.6$, $P<0.01$], reflecting that the four mouse strains displayed a different level of PPI. At 74 dB prepulse intensity, C57BL/6J and 129/SvEv mice showed the smallest and the greatest degree of PPI, respectively. Posthoc comparisons indicated that C57BL/6J mice had a significantly lower level of PPI than 129/SvEv and BALB/cByJ mice ($P<0.05$, Duncan's test). The MORO mice also showed a significantly lower level of PPI as compared to 129/SvEv mice ($P<0.05$, Duncan's test). At the 90 dB prepulse intensity, no difference in the magnitude of PPI was noted between BALB/cByJ, MORO and C57BL/6J mice. However, 129/SvEv mice displayed a significantly higher level of PPI compared to all other strains ($P<0.05$, Duncan's test).

Acoustic startle habituation

The mean startle responses of the different strains to repeated presentation of 110 dB stimulus is illustrated in Fig. 2A. The ANOVA revealed a statistically significant difference among the four strains on initial startle reactivity [$F(3,43)=3.99$, $P<0.05$). Post hoc tests indicated that BALB/cByJ displayed the greatest startle reactivity compared to all other strains ($P<0.05$, Duncan's test). No significant differences on initial startle reactivity were noted between C57BL/6J, MORO and 129/SvEv mice.

All mouse strains showed a gradual decrease of the startle response to the acoustic stimuli over the course of the session (Fig. 2A). The overall ANOVA revealed a significant main effect of strains [$F(3,430)=4.03$, $P<0.05$], block [$F(10,430)=13.37$, $P<0.01$] and near effect of strain×block interaction [$F(30,430)=1.39$, $P=0.08$]. Because BALB/cByJ mice displayed a higher startle response over the test session compared to all other strains, the comparisons of inter-strain differences at each block were not carried out. We have used the percentage of habituation to analyze the variation of degree of habituation between strains (Fig. 2B). The one-way ANOVA revealed a significant strain effect [$F(3,42)=4.02$, $P<0.01$], and post hoc tests indicated that the MORO mice have significantly higher degree of habituation than C57BL/6J and 129/SvEv mice ($P<0.05$, Duncan's test). Although BALB/cByJ mice tended to show a higher rate of habituation than C57BL/6J and MORO mice, no significant difference was noted between the three strains.

Effects of haloperidol, risperidone and clozapine on prepulse inhibition and startle response in C57BL/6J mice

As C57BL/6J mice showed the poorest degree of both PPI and startle habituation compared to all other strains, we selected this strain to establish the minimum doses of

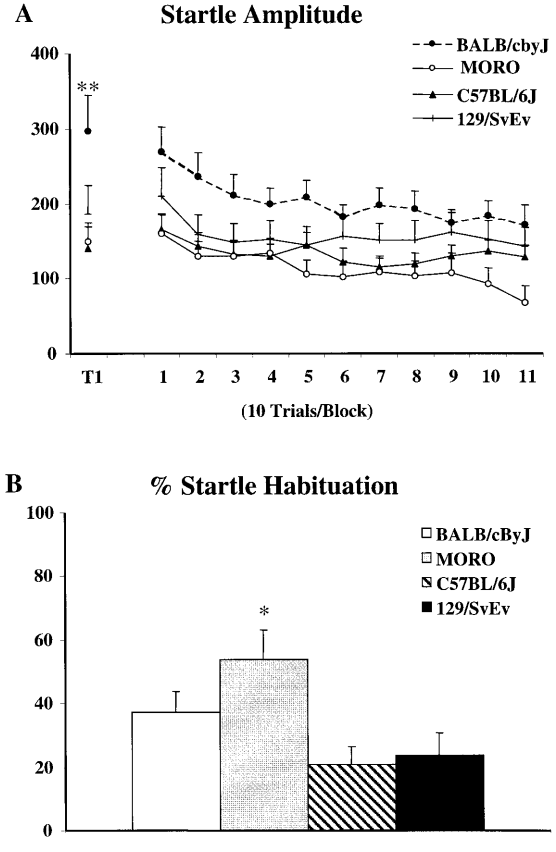

Fig. 2A, B Startle habituation of BALB/cByJ, MORO, C57BL/6J and 129/SvEv mice. **A** Mean startle response±SEM to 110 dB acoustic stimuli over the course of the session (n=12 per strain). Data from trial 1 (*T1*) are analysed separately. Each block is the mean of ten consecutive trials. $^{**}P<0.05$, significantly different from MORO, C57BL/6J and 129/SvEv mice (Duncan's test after significant ANOVA). **B** Percent startle habituation of BAB/cByJ, MORO, C57BL/6J and 129/SvEv mice (n=12 per strain). $^{*}P<0.05$, significantly different from C57BL/6J and 129/SvEv mice (Duncan's test after significant ANOVA)

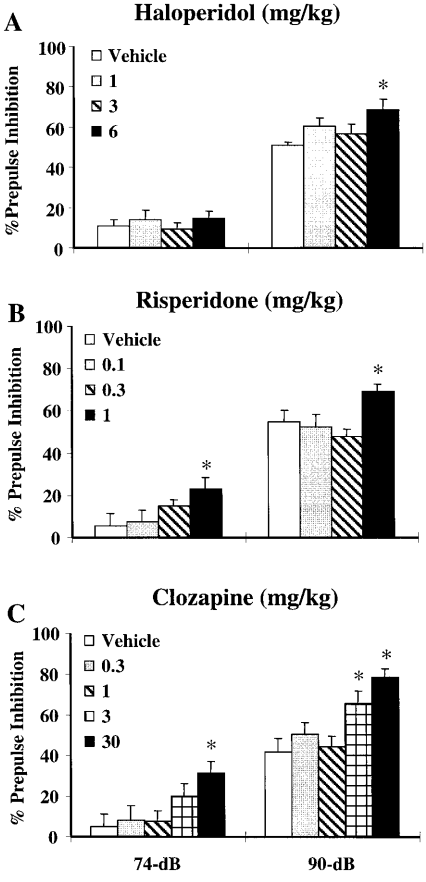

Fig. 3 Effects of haloperidol (n=12 per dose, **A**), risperidone (n=7–8 per dose, **B**) and clozapine (n=8–7 per dose, **C**) on prepulse inhibition in C57BL/6J mice at 74 and 90 dB prepulse intensities. $^{*}P<0.05$, significantly different from vehicle (Duncan's test after significant ANOVA)

Table 1 Effects of haloperidol, risperidone and clozapine on the mean baseline startle amplitude in C57BL/6J mice

	Startle response
Haloperidol (mg/kg)	
0	185.5±15.6
1	186.5±16.1
3	138.8±17.2*
6	78.5±15.5*
Risperidone (mg/kg)	
0	204.7±21.7
0.1	174.5±17.3
0.3	130.7±23.4*
1	110.9±17.7*
Clozapine (mg/kg)	
0	232.9±31.2
0.3	203.5±29.2
1	185.3±22.1
3	90.2±25.9*
30	42.7±9.3*

*P<0.05, significantly different from vehicle-treated animals (Duncan's test after significant ANOVA)

antipsychotics needed to potentiate the PPI. Figure 3A illustrates the effects of various doses of haloperidol on PPI. The two-way ANOVA revealed a near main effect of treatment [F(3,44)=2.38, P=0.08)]. Because haloperidol tended to increase PPI at the loudest prepulse intensity (90 dB) separate analysis was then carried out. The one-way ANOVA revealed a significant main effect of treatment [F(3,44)=3.46, P<0.05] at the 90 dB prepulse. The post hoc comparisons indicated a significant effect on PPI at the highest dose of haloperidol (6 mg/kg) (P<0.05, Duncan's test). As shown in Fig. 3B and C, both risperidone and clozapine induced a dose-dependent potentiation of PPI, and this was evident at both 74 and 90 dB prepulse intensities. In line with this trend, the overall ANOVA revealed a significant main effect of treatment for risperidone [F(3,27)=3.63, P<0.05] and clozapine [F(4,32)=7.52, P<0.01]. Post hoc comparisons indicated that the highest dose of risperidone (1 mg/kg) significantly potentiated PPI at both prepulse intensities (P<0.05, Duncan's test). In the clozapine experiment, both 3 and 30 mg/kg clozapine significantly potentiated the PPI at 90 dB prepulse intensity, but only the highest dose had a significant effect on PPI at the 74 dB prepulse intensity (P<0.05, Duncan's test).

Table 1 illustrates the effects of antipsychotics on the startle response alone. A significant decrease of the startle response was noted with haloperidol (3 and 6 mg/kg), risperidone (0.3 and 1 mg/kg,) and clozapine (3 and 30 mg/kg) (P<0.05, Duncan's test following significant ANOVA).

Effects of non-antipsychotic drugs on prepulse inhibition in C57BL/6J mice

Diazepam produced a dose-dependent potentiation of PPI (Fig. 4A). The overall ANOVA revealed a significant main effect of treatment [F(3,28)=8.46, P<0.01]. The post hoc comparisons indicated that all doses of diazepam (3, 10 and 30 mg/kg) significantly potentiated PPI at both 74 and 90 dB prepulse intensities (P<0.05, Duncan's test). In contrast, buspirone (1, 3 and 10 mg/kg), desipramine (3, 10 and 30 mg/kg), morphine (1, 3, and 10 mg/kg) and scopolamine (3, 10 and 30 mg/kg) failed to affect PPI at any of the prepulse intensities (Fig. 4 and Table 2). Overall ANOVA failed to reveal significant

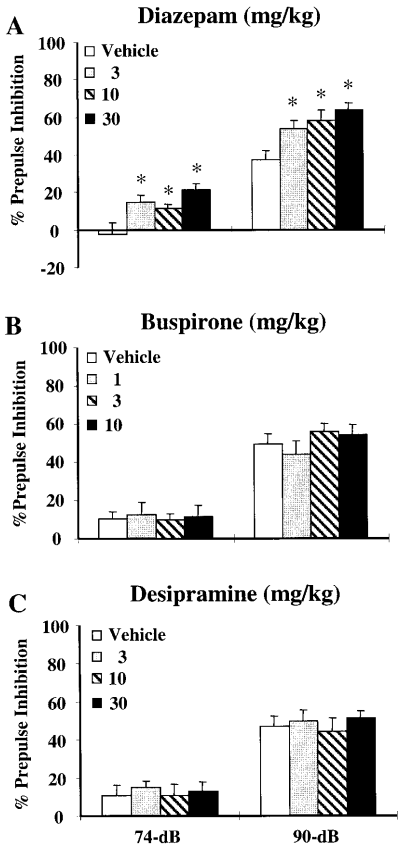

Fig. 4 Effects of diazepam (n=8 per dose, **A**), buspirone (n=12 per dose, **B**) and desipramine (n=6 per dose, **C**) on prepulse inhibition in C57BL/6J mice at 74 and 90 dB prepulse intensities. *P<0.05, significantly different from vehicle (Duncan's test after significant ANOVA)

Table 2 Effects of morphine and scopolamine on the mean baseline startle amplitude and PPI in C57BL/6J mice

	Startle response	PPI 74 dB	PPI 90 dB
Morphine (mg/kg)			
0 (n=12)	159.9±22.0	9.4±7.3	43.6±7.4
1 (n=12)	182.9±25.6	16.9±4.5	53.3±6.1
3 (n=12)	249.3±23.2	9.7±6.1	39.2±5.2
10 (n=12)	161.4±16.4	5.6±4.5	50.6±4.5
Scopolamine (mg/kg)			
0 (n=8)	190.0±30.7	14.9±4.57	55.5±5.6
3 (n=8)	206.5±21.5	14.7±6.43	62.2±4.0
10 (n=7)	262.5±45.2	4.9±5.65	52.8±5.6
30 (n=7)	272.6±50.7	5.4±4.9	55.6±4.9

Table 3 Effects of diazepam, buspirone and desipramine on the mean baseline startle amplitude in C57BL/6J mice

	Startle response
Diazepam (mg/kg)	
0	172.6±17.5
3	206.2±21.7
10	194.5±25.3
30	71.2±13.0*
Buspirone (mg/kg)	
0	227.8±12.7
1	177.8±24.4
3	194.4±7.2
10	159.7±19.3*
Desipramine (mg/kg)	
0	186.2±32.9
3	220.0±14.3
10	186.9±25.1
30	192.8±17.8

*P<0.05, significantly different from vehicle-treated animals (Duncan's test after significant ANOVA)

main effect of treatment (buspirone: [F(3,44)=0.88, P>0.05]; desipramine: [F(3,40)=0.43, P>0.05]; morphine: [F(3,56)=1.26, P>0.05] and scopolamine: [F(3,54)= 1.36, P>0.05]).

Table 3 illustrates the effects of diazepam, buspirone and desipramine on the mean basal startle response in C57BL/6J. Both diazepam and buspirone significantly reduced the startle at the highest doses tested (30 and 10 mg/kg, respectively) (P<0.05, Duncan's test). Desipramine, morphine and scopolamine had no effects on the startle response at any of the doses tested (Table 2 and Table 3).

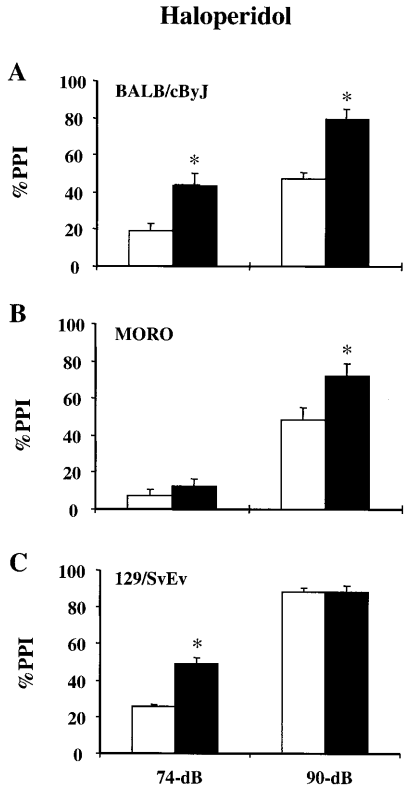

Fig. 5 Effects of haloperidol (6 mg/kg) on PPI in BALB/cByJ (n=8 per dose, **A**), MORO (n=12 per dose, **B**) and 129/SvEv (n=6 per dose, **C**) mice at 74 and 90 dB prepulse intensities. *P<0.05, significantly different from vehicle (Duncan's test after significant ANOVA)

Effects of haloperidol, risperidone, clozapine and diazepam on PPI in BALB/cByJ, MORO and 129/SvEv mice

Following the determination of the effective dose of haloperidol, risperidone, clozapine and diazepam on PPI in C57BL/6J strain, we examined their effects in all other strains in order to determine whether the ability of the different drugs to improve PPI is restricted to the C57BL/6J mice or could be generalized to other strains.

Haloperidol

As can be seen from Fig. 5, haloperidol at the dose of 6 mg/kg differentially potentiated PPI depending of the strain and prepulse intensity. The two-way ANOVA revealed a significant main effect of treatment for all three strains [BALB/cByJ: F(1,28)=30.27, P<0.01; MORO: F(1,44)=7.04, P<0.05; 129/SvEv: F(1,10)=14.15, P<0.01]. Analysis of simple main effects at each prepulse intensity followed by Duncan's test indicates that haloperidol significantly potentiated PPI in BALB/cByJ mice at both prepulse intensities. In MORO and 129/SvEv strains, haloperidol significantly potentiated PPI at 90 and 74 dB, respectively. Haloperidol (6 mg/kg) significantly reduced the startle response in all mouse strains (P<0.05, Duncan's test, Table 4).

Risperidone

Figure 6 illustrates the effects of risperidone (1 mg/kg) on PPI in the various mouse strains. The two-way ANOVA revealed a significant main effect of treatment for all three strains [BALB/cByJ: F(1,28)=19.65, P<0.01; MORO: F(1,44)=4.47, P<0.05; 129/SvEv: F(1,10)=18.20, P<0.01]. Analysis of simple main effects at each prepulse intensity followed by Duncan's test indicated that risperidone significantly potentiated PPI in BALB/cByJ and MORO mice at the highest prepulse intensities. In contrast, in 129/SvEv mice significant potentiation of PPI was noted at the 74 dB prepulse intensity. Risperidone 1 mg/kg significantly reduced the startle response in all three mouse strains (P<0.05, Duncan's test, Table 4).

Clozapine

Figure 7 illustrates the effects of clozapine at the dose of 30 mg/kg on PPI in the three mouse strains. The two-way ANOVA revealed a significant main effect in

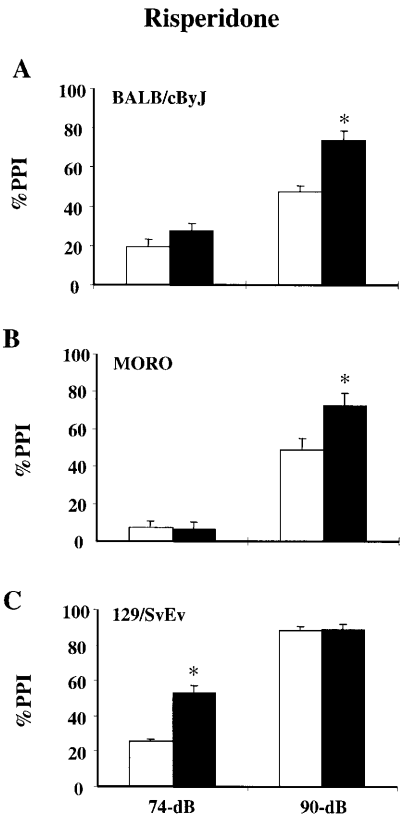

Fig. 6 Effects of risperidone (1 mg/kg) on PPI in BALB/cByJ (*n*=8 per dose, **A**), MORO (*n*=12 per dose, **B**) and 129/SvEv (*n*=6 per dose, **C**) mice at 74 and 90 dB prepulse intensities. **P*<0.05, significantly different from vehicle (Duncan's test after significant ANOVA)

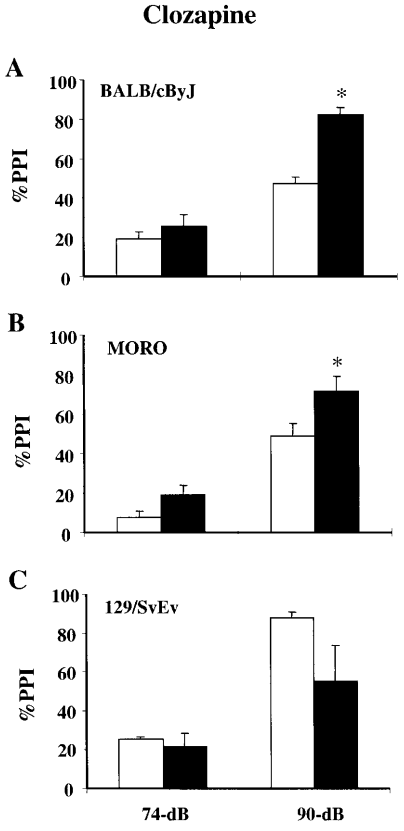

Fig. 7 Effects of clozapine (30 mg/kg) on PPI in BALB/cByJ (*n*=8 per dose, **A**), MORO (*n*=12 per dose, **B**) and 129/SvEv (*n*=6 per dose, **C**) mice at 74 and 90 dB prepulse intensities. **P*<0.05, significantly different from vehicle (Duncan's test after significant ANOVA)

Table 4 Effects of haloperidol, risperidone, clozapine and diazepam on the mean baseline startle amplitude in BALB/cJ, MORO and 129/SVEV mice

	BALB/cJ	MORO	129/SVEV
Vehicle	328.6±15.3	275.8±33.7	248.6±34.5
Haloperidol (6 mg)	113.6±24.7*	85.4±15.7*	150.3±15.8*
Risperidone (1 mg)	166.4±15.3*	157.7±26.7*	125.0±21.4*
Clozapine (30 mg)	68.2±0.9*	94.9±16.5*	60.2±7.2*
Diazepam (10 mg)	262.4±14.7*	192.2±47.3	74.1±27.9*

**P*<0.05, significantly different from vehicle-treated animals (Duncan's test after significant ANOVA)

BALB/cByJ and MORO mice but not in the 129/SvEv strain [BALB/cByJ: $F(1,28)=20.7$, $P<0.01$; MORO: $F(1,44)=9.64$, $P<0.01$; 129/SvEv: $F(1,10)=2.39$, NS]. Analysis of simple main effects at each prepulse intensity followed by Duncan's test indicates that clozapine significantly potentiated PPI in BALB/cByJ and MORO mice at the highest prepulse intensities. In contrast, in 129/EVEV mice, clozapine failed to increase PPI at any of the prepulse intensities. Clozapine significantly reduced the startle response in all three strains ($P<0.05$, Duncan's test, Table 4).

Diazepam

Figure 8 illustrates the effects of diazepam (10 mg/kg) on PPI in the various mouse strains. In MORO and 129/SvEv mice, no significant effect was noted at any of the prepulse intensities [$F(1,34)=2.42$, NS and $F(1,10)=3.31$, NS, respectively]. In contrast, diazepam tended to decrease PPI in BALB/cByJ mice. The two-way ANOVA failed to reveal a significant main effect of treatment [$F(1,28)=0.01$, NS], but a significant effect of treatment×prepulse intensity interaction was noted [$F(1,28)=6.35$, $P<0.05$]. Post hoc test indicates that this effect is related to a significant decrease of PPI at 74 dB prepulse intensity. As can be seen in Table 4, diazepam was without any effect on the startle response in the MORO mice. In contrast, significant reduction of the startle response was noted in both BALB/cByJ and 129/SvEv mice ($P<0.05$, Duncan's test).

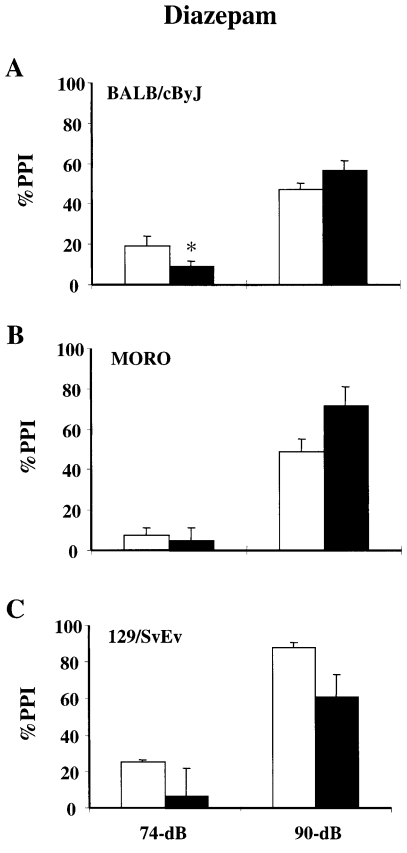

Fig. 8 Effect of diazepam (10 mg/kg) on PPI in BALB/cByJ (n=8 per dose, **A**), MORO (n=7–12 per dose, **B**) and 129/SvEv (n=6 per dose, **C**) mice at 74 and 90 dB prepulse intensities. *P<0.05, significantly different from vehicle (Duncan's test after significant ANOVA)

Discussion

Acoustic startle response, PPI and startle habituation in C57BL/6J, BALB/cByJ, MORO and 129/SvEv mice

The present study confirms clear strain differences in both the startle response and PPI. Among the four strains tested, BALB/cByJ mice displayed the greatest initial startle reactivity and the greatest startle response amplitude over the test session. The level of PPI also varied between the strains, with the widest range of responses seen with the lowest prepulse intensity (74 dB). Overall, C57BL/6J and MORO strains showed the lowest levels of PPI while 129/SvEv strain displayed the highest level. These findings are consistent with those previously reported by Paylor and Crawley (1997), who compared the startle response and PPI of 13 different mouse strains including C57BL/6J, BALB/cByJ and 129/SvEv mice. We have also found clear strain differences in startle habituation: C57BL/6J and 129/SvEv mice had the lowest rate of habituation, whilst MORO mice displayed the greatest rate of habituation.

Interestingly, the degrees of PPI and startle habituation seem independent of the magnitude of the startle response. For instance, 129/SvEv, C57BL/6J and MORO strains all had comparable startle responses. However, the former strain displayed the greatest level of PPI, and this was consistent across the two prepulse intensities (74 and 90 dB). These variations in startle response and PPI observed between the strains confirm previous findings suggesting that the genetic substrates of these two behavioral responses may be different (Logue et al. 1997; Paylor and Crawley 1997). Similarly, there was no correlation between variations in the startle response and startle habituation, indicating that the inter-strain variation in the rate of habituation more likely reflects differences in the processing of exteroceptive stimuli rather than sensorimotor responsiveness. More importantly, we failed to find an association between the degree of PPI and the rate of startle habituation. Indeed, the 129/SvEv strain, which displayed the greatest degree of PPI, had a poor rate of habituation. In contrast, MORO mice that had poor sensorimotor gating displayed the greatest rate of habituation. Overall, these data suggest that the acoustic startle response, startle habituation and PPI involve different genetically defined physiological processes.

Drugs effects on PPI in C57BL/6J strain

Our major objective in this study was to test the hypothesis that antipsychotics may be effective in potentiating PPI in mice naturally displaying poor sensorimotor gating such as C57BL/6J strain (Paylor and Crawley 1997; present study). We thus evaluated the effects of various doses of antipsychotics on PPI in this strain. The atypical antipsychotics, risperidone and clozapine improved PPI at both prepulse intensities 74 and 90 dB. In contrast, the typical antipsychotic haloperidol was only active at the highest prepulse intensity. These data are in line with those reported by McCaughran et al. (1997), who have shown that antipsychotics could facilitate PPI in C57BL/6J strain. However, in contrast to our finding they found that low doses of haloperidol (0.4 mg/kg) and risperidone (0.4 mg/kg) could improve PPI across three prepulse intensities, 56, 68 and 80 dB. In the present study, lower doses of haloperidol (1 and 3 mg/kg) and risperidone (0.1 and 0.3 mg/kg) were also tested; however, no facilitation of PPI was detected. It is possible that differences in the parameters used to establish PPI and the experimental design between our study and that of McCaughran and colleagues may contribute to these discrepancies. It is also important to mention that in their experiment, haloperidol (0.2–4.8 mg/kg) was found to increase the startle response, while in our conditions haloperidol reduced the startle response at similar doses (3–6 mg/kg). It is thus possible that the discrepancies between the two studies may be due to substrain differences in drug responses. Nevertheless, the fact that in both studies haloperidol had opposite effects on basal startle but similar effects on PPI (facilitation) suggests that the effects of neuroleptics on startle response and PPI may be independent.

A range of non-antipsychotic molecules was also studied to examine whether the facilitation of PPI is specific to compounds possessing antipsychotic properties. As predicted, morphine, scopolamine, desipramine and buspirone failed to improve PPI, even though the latter drug significantly reduced startle at the highest dose tested. Surprisingly, we found a clear PPI facilitation with diazepam, at doses (3 and 10 mg/kg) that had no effects on the basal startle response. This strongly suggests that the effects of diazepam do not reflect non-specific effects of sedation on PPI performance. In addition, at 10 mg/kg, diazepam markedly reduced startle amplitude in BALB/cByJ and 129/SvEv strains without improving PPI. Altogether, these data argue for specific effects of diazepam on PPI. It is unlikely that the effects of diazepam on PPI are due to its anxiolytic properties, since the doses of buspirone that failed to affect PPI were reported to produce anxiolytic effects in mice across several anxiety tests (Costall 1989; Lecci et al. 1990; Grewal et al. 1997). These data provide further evidence for the role of benzodiazepine-$GABA_A$ receptors in the modulation of sensorimotor gating. In line with our findings, blockade of $GABA_A$ receptors within the prefrontal cortex, ventral hippocampus or ventral pallidum was reported to disrupt PPI in rat (Swerdlow et al. 1990; Kodsi and Swerdlow 1995; Japha and Koch 1999). Thus, it is most likely that these brain areas may mediate the PPI facilitation observed with diazepam.

Drugs effects on PPI: strain comparisons

The effects of the active doses of antipsychotics (haloperidol, risperidone and clozapine) as well as the anxiolytic (diazepam) on PPI in C57BL/6J mice were also studied in the other mouse strains for comparison. Only single dose of each antipsychotic was used and thus, comparisons of drug effects across strains should be made cautiously as different strains may have different dose-response curves to individual drugs. At the low prepulse (74 dB), C57BL/6J and 129/SvEv mice were the most sensitive, since two of the three antipsychotics used tested positive. Indeed, both risperidone and clozapine were able to improve PPI in C57BL/6J strain while in 129/SvEv mice both haloperidol and risperidone were effective. This finding indicates that the potentiation of PPI by antipsychotics is not limited to strains with poor sensorimotor gating. We also found that the efficacy of antipsychotics is independent of the baseline level of PPI displayed by each strain. In the risperidone study, the values of PPI displayed by C57BL/6J and 129/SvEv mice (5.57±5.76 and 25.33±1.15) were respectively comparable to the values of PPI displayed by MORO and BALB/cByJ mice (7.4±3.54 and 19.17±3.88); however, the antipsychotic-induced potentiation of PPI was only observed in the two former strains. On the other hand, among the four strains of mice tested, only C57BL/6J strain was sensitive to the action of clozapine at the low prepulse intensity.

Interestingly, we also noted a possible relationship between the rate of startle habituation and the strain sensitivity to antipsychotics at the low prepulse intensity. Indeed, the most sensitive strains were the ones that displayed the lowest rate of startle habituation (C57BL/6J and 129/SvEv) while the less sensitive strain was the one that displayed the highest rate of habituation (MORO mice). Thus, sensitivity to antipsychotics may depend in part on the inability to habituate to repeated exteroceptive stimuli. However, this finding needs to be confirmed in other mouse strains. Several other factors seem also to influence the sensitivity to antipsychotics. For instance, at the low prepulse intensity, C57BL/6J mice were most sensitive to atypical antipsychotics (risperidone and clozapine), while BALB/cByJ mice were most sensitive to haloperidol. These subtle inter-strain differences in the sensitivity to antipsychotics could be related to congenital differences in the function of certain neurotransmitters (e.g., dopamine and serotonine) as previously revealed in C57BL/6J and BALB/cByJ strains (Helmeste and Seeman 1982; Daszuta and Portalier 1985; Kanes et al. 1993). Another factor that clearly determines the sensitivity to antipsychotics is the intensity level of the prepulse. Indeed, at the high prepulse intensity (90 dB) all three antipsychotics tested were able to facilitate PPI in all strains except in 129/SvEv mice. The lack of effects in the latter strain is likely due to a ceiling effect since nearly a maximal level of PPI (82%) was reached at the 90 dB prepulse. This finding suggest that the salience of the prepulse is also a critical factor to be taken into consideration when studying antipsychotic-induced improvement of PPI in naive mice.

It should be noted that the PPI facilitation was only obtained with doses of antipsychotics that markedly reduced the basal startle response. Thus, the possibility arises that the improvement of PPI is simply an artifact of drug-induced reductions of the basal startle. The independence of drug effects on PPI and basal startle has been demonstrated by several studies conducted in both rats and mice (Varty and Higgins 1995; Depoortere et al. 1997; Swerdlow et al. 1998; Furuya et al. 1999). Our data obtained with the low prepulse intensity also argue in this direction. Indeed, we found that a decrease in startle is not systematically associated with an increase in PPI. For instance, risperidone reduced startle to a similar extent in all strains (C57BL76J: 54.97±10.19; BALB/cByJ: 49.37±4.65; 129/SvEv: 49.71±8.63; MORO: 42.82± 9.70; percent of startle reduction relative to the vehicle-treated animals), but at the 74 dB prepulse, an increase in PPI was only observed in two strains, C57BL/6J and 129/SvEv. Similarly, the PPI facilitation induced by clozapine was restricted to C57BL/6J, whereas significant reduction of the startle response was observed in all strains. At the high prepulse intensity, the inter-strain differences in the sensitivity to antipsychotics were, however, no longer observed, and therefore it cannot be excluded that in this case drug effects on baseline startle and PPI may be somehow linked.

Finally, we found that diazepam-induced PPI facilitation was restricted to C57BL/6J strain. Indeed, no improvement of PPI was detected in any of the other mouse strains even though diazepam significantly reduced the startle in BALB/cByJ and 129/SvEv mice. Once again, this finding provides further evidence for the independence of drug effects on baseline startle and PPI. Consistent with our data, previous studies have shown that C57BL/6J strain is more sensitive to the anxiolytic effects of benzodiazepines (e.g., diazepam and alprazolam) as compared to other strains (e.g. BALB/cByJ, A/J and DBA2 strains) (Mathis et al. 1994; Garrett et al. 1998; Weizman et al. 1999). The marked differences between the mouse strains in the effects of diazepam on PPI could be related to structural diversity and/or function of benzodiazepine/$GABA_A$ receptors in the brain as previously suggested (Garrett et al. 1998; Weizman et al. 1999). In the past few years the involvement of central GABAergic systems in the pathophysiology of schizophrenia has attracted a great deal of attention. Numerous clinical studies have suggested that a deficiency of central GABAergic transmission may contribute to the neuropathology of schizophrenia (Mohler 1997; Keverne 1999; Lewis et al. 1999; Ohnuma et al. 1999). Furthermore, benzodiazepines were reported to be effective in many cases as sole agents in the treatment of schizophrenic patients, thus suggesting that they may have by themselves clinically relevant antipsychotic effects (Wolkowitz and Pickar 1991; Delini-Stula et al. 1992; Kimhi et al. 1995; Delini-Stula and Berdah-Tordjman 1996; Wassef et al. 1999). In this respect, understanding the mechanisms underlying the PPI facilitation observed with diazepam in C57BL/6J mice, which naturally display a low level of PPI, may perhaps aid in the elucidation of the potential involvement of benzodiazepine/$GABA_A$ receptor dysfunction in the development of sensorimotor gating deficits revealed in schizophrenic patients.

In conclusion, the present study shows that typical and atypical antipsychotics can improve PPI in naive mice. The use of this PPI procedure may therefore represent a useful screening test for detecting antipsychotic activity. Among the four strains studied, C57BL/6J mice appear to be the most interesting, as they are the only one (1) concomitantly exhibiting low PPI and startle habituation, (2) sensitive to atypical antipsychotics at both 74 and 90 dB PPI, and (3) able to reveal antipsychotic-like activity of diazepam confirming previous clinical studies (Wolkowitz and Pickar 1991; Delini-Stula et al. 1992; Kimhi et al. 1995; Delini-Stula and Berdah-Tordjman 1996; Wassef et al. 1999). At 90 dB prepulse intensity, BALB/cByJ and MORO mice were also sensitive to all antipsychotics, which suggests that other mouse strains could be also used if appropriate PPI parameters are selected. However, given the possibility that the facilitation of PPI at 90 dB prepulse intensity and reduction of the baseline startle may be linked, it would be better to show that PPI could be improved at more than one prepulse intensity. Finally, as compared to other screening procedures used in mice (e.g., psychomimetic-induced hyperactivity, sterotypies or disruption of PPI), the improvement of PPI is only obtained with higher doses of antipsychotics that affected baseline startle (but see McCaughran et al. 1997). Most of the traditional screening tests are, however, based on pharmacological manipulation of single neurotransmitter system (e.g., dopamine) and therefore it is not surprising that they show better sensitivity to antipsychotics, which act at the same systems. This is clearly illustrated by the differential effects of typical antipsychotics on the PPI disruption induced by dopamine agonists and glutamate antagonists (for review see Swerdlow and Geyer 1998). Basal PPI is most likely dependent on tonic activity of a number of neurotransmitters (e.g., dopamine, serotonine, glutamate, GABA), and therefore it is conceivable that high doses of antipsychotics are necessary to change the balance between the activity of these various neurotransmitters and improve basal PPI. The sensitivity of the procedure for detecting antipsychotics may require further improvement, possibly by selecting more appropriate PPI parameters and/or strains of mice.

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