Typical and atypical antipsychotic drug effects on locomotor hyperactivity and deficits in sensorimotor gating in a genetic model of NMDA receptor hypofunction

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Abstract

Psychotomimetic effects of NMDA antagonists in humans suggest that NMDA receptor hypofunction could contribute to the pathophysiology of schizophrenia. A mouse line that expresses low levels of the NMDA R1 subunit (NR1) of the NMDA receptor was generated to model endogenous NMDA hypofunction. These mutant mice show increased locomotor activity, increased acoustic startle reactivity and deficits in prepulse inhibition (PPI) of acoustic startle. The present study examined effects of a typical antipsychotic drug, haloperidol, and two atypical antipsychotic drugs (olanzapine and risperidone) on behavioral alterations in the NR1 hypomorphic (NR1−/−) mice. Haloperidol significantly reduced activity in the wild type controls at each dose tested (0.05, 0.1, and 0.2 mg/kg). The NR1−/− mice were less sensitive to the haloperidol-induced locomotor inhibition in comparison to the NR1+/+ mice. In contrast to haloperidol, olanzapine reduced the hyperactivity in the NR1−/− mice at a dose that produced minimal effects on locomotor activity in the wild type mice. These data suggest that non-dopaminergic blocking properties of olanzapine contribute to the drug's ability to reduce hyperactivity in the NR1 deficient mice. In the PPI paradigm, haloperidol (0.5 mg/kg) did not affect the increased startle reactivity in the NR1−/− mice, but did reduce startle amplitude in the NR1+/+ mice. Haloperidol increased PPI in both the mutant and wild type strains. Unlike haloperidol, risperidone (0.3 mg/kg) and olanzapine (3 mg/kg) reduced startle magnitude in both NR1+/+ and NR1−/− mice. Like haloperidol, risperidone and olanzapine increased PPI in both NR1+/+ and NR1−/− mice. The similar effects of these atypical antipsychotic drugs in wild type mice and mice with markedly reduced NR1 expression suggest that the drugs were not working by a NMDA receptor-dependent mechanism to increase PPI. Since both haloperidol and the atypical drugs increased PPI, it is likely that D_2 dopamine receptor blockade is responsible for the drug effects on sensorimotor gating.
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1. Introduction

NMDA receptor antagonists such as ketamine and phencyclidine (PCP) induce behaviors in healthy humans that mimic positive, negative and cognitive symptoms of schizophrenia (Cohen et al., 1962; Krystal et al., 1994; Lahti et al., 2001; Luby et al., 1959; Malhotra et al., 1996). In addition, schizophrenia patients challenged with ketamine have symptom exacerbation similar to that experienced during active phases of their illness (Lahti et al., 1995a,b; Malhotra et al., 1997). These data provide support for the hypothesis that reduced NMDA receptor function could contribute to the pathophysiology of schizophrenia.

If reduced function of NMDA receptors is involved in schizophrenia, investigating neurobiological and behavioral consequences of experimentally-induced NMDA receptor hypofunction could contribute to understanding the disease. To study the effects of endogenous NMDA receptor hypofunction, a mouse genetic model of reduced NMDA receptor expression was generated in which the NMDA R1 (NR1) subunit of the NMDA receptor is reduced markedly (Mohn

et al., 1999). These mice are characterized as NR1 hypomorphic, since the genetic alteration (insertion of a neomycin resistance gene into intron 20 of the *NR1* locus) results in dramatic under-expression, but not elimination, of the NR1 gene. Western blot analysis of cerebral cortical homogenates indicated almost 90% reduction in the expression of the NR1 protein in the mutant mice (Mohn et al., 1999). Autoradiographic analysis of ^{3}H-MK-801 binding demonstrated global reduction in ligand binding with decreases of 60–85% binding found among different brain regions. The hippocampal formation exhibited the greatest reduction in ^{3}H-MK-801 binding in the mutant mice.

The NR1 hypomorphic mice (NR1−/−) exhibit altered phenotypes that include increased locomotor activity (Mohn et al., 1999), reduced metabolic activity in the medial prefrontal cortex, anterior cingulate cortex, and the hippocampus (Duncan et al., 2002), deficits in social interactions (Duncan et al., 2004; Mohn et al., 1999), and deficits in prepulse inhibition of acoustic startle (PPI) (Duncan et al., 2006, 2004; Fradley et al., 2005). In addition, the NR1−/− mice show enhanced sensitivity to amphetamine-induced PPI disruption (Moy et al., 2006), but not to the locomotor stimulatory or Fos-inducing effects of amphetamine (Miyamoto et al., 2004).

Schizophrenia patients show deficits in sensorimotor gating in acoustic startle and tactile startle PPI paradigms (Braff et al., 2001). NMDA antagonists disrupt PPI in rodents, and the prototypical "atypical" antipsychotic drug clozapine is more effective than the typical antipsychotic drug haloperidol in blocking the consequence of this pharmacologically-induced NMDA receptor hypofunction (Bakshi et al., 1994; Keith et al., 1991). Some of the newer "atypical" or second generation antipsychotic drugs (e.g. olanzapine, quetiapine) also block NMDA antagonist-induced deficits in PPI (Bakshi and Geyer, 1995; Swerdlow et al., 1996). In a previous study, we found that clozapine and quetiapine, but not haloperidol, reduced the acoustic startle responses in the NR1 hypomorphic mice (Duncan et al., 2006). However, all three of the antipsychotics tested reduced PPI in the mutant mice. The present study compared effects of haloperidol with two other atypical antipsychotic drugs, olanzapine and risperidone, on deficits in PPI in the NR1 hypomorphic mice, to determine if these atypical antipsychotics exhibit profiles similar to clozapine and quetiapine in the model. In addition, effects of haloperidol and olanzapine were compared on locomotor activity in the NR1+/+ and NR1−/− mice.

2. Methods

2.1. Generation of F1 hybrid NR1 hypomorphic mice

The NR1-deficient mice were created initially on a mixed genetic background consisting of alleles derived from 129/SvEv, C57BL/6, and DBA/2 (Mohn et al., 1999). It is clear that modifier alleles present in various inbred mouse lines can dramatically alter the impact of primary genetic lesions. Therefore, it was important to obtain populations of mice that differ genetically only at the NR1 locus. A strategy was devised

to produce NR1 hypomorphs and genetically identical wild type populations by generating F1 hybrid mice from C57BL/6 and 129/ SvEv inbred strains. C57BL/6 heterozygous (NR1+/−) animals were intercrossed with 129/SvEv heterozygous animals. All of the F1 offspring of these litters are genetically identical at all loci except at the *NR1* gene. This approach was taken because the NR1-/- homozygotes do not breed and the NR1-/- mice must be bred from heterozygote NR1+/- mice to obtain NR1-/- progeny. When attempts were made to breed NR1+/- mice from parents of the same inbred strain, the mutation produced stunted growth and lower than expected yield of the NR1-/- genotype. These problems were not found for the F1 hybrid NR1−/− mice. The NR1 mutation was maintained on the C57BL/6 and 129/ SvEv genetic backgrounds by breeding heterozygous animals to commercially available stock (Jackson Laboratories). Resulting heterozygous offspring from these crosses were used to maintain the lines and to provide heterozygous breeders for the generation of the F1 hybrid homozygous mice (NR1−/−) and their control populations (NR1+/+).

All procedures in the present study were conducted in strict compliance with the policies on animal welfare of the National Institutes of Health and the University of North Carolina (stated in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, 1996 edition), and approved by the University of North Carolina Institutional Animal Care and Use Committee.

2.2. Drugs

Drugs were administered IP either immediately before testing, for the activity measure, or 30 min before testing, for the PPI measure. Doses of haloperidol (Pharmaceutical Associates, Inc.) were derived by diluting an oral solution of haloperidol lactate with 0.9% saline. Risperidone (Sigma) was suspended in a 23% (w/v) solution of cyclodextrin (Sigma). Olanzapine (Eli Lilly) was dissolved in vehicle containing 5 μl of 20% acetic acid/ml 0.9% saline.

2.3. Assessment of locomotor activity and drug administration

Locomotor activity was assessed in photocell-based activity chambers under standardized environmental conditions, using a TruScan activity monitor (Coulbourn Instruments, Allentown, PA) with a 25.8×25.8 cm Plexiglas chamber and a beam spacing of 1.52 cm. Mice were acclimated to the room in which testing was carried out for at least at least 7 days before testing. Mice were injected with vehicle, haloperidol (0.05–0.2 mg/kg), or olanzapine (0.625 or 1.25 mg/kg) immediately prior to placement in the activity chambers. Separate cohorts of mice were used for each dose of each drug examined. Activity data were collected for each mouse over a 180 min time course, beginning when the mouse was first placed in the testing chamber. Data were collected in five-minute intervals. The distance traveled in each five-minute interval was measured as the total of all vectored $X–Y$ coordinate changes. For each group of mice, the mean±SEM was calculated for each five-minute time interval.

2.4. Acoustic startle and prepulse inhibition

The acoustic startle measure was based on the reflexive whole-body flinch, or startle response, following exposure to a sudden noise. Animals were tested with a San Diego Instruments SR-Lab system using the procedure described by Paylor and Crawley (1997). Briefly, mice (4–7 months of age) were placed in a small Plexiglas cylinder within a larger, sound-attenuating chamber (San Diego Instruments). The cylinder was seated upon a piezoelectric transducer, which allowed vibrations to be quantified and displayed on a computer. The chamber included a house light, fan, and a loudspeaker for the

acoustic stimuli (bursts of white noise). Background sound levels (70 dB) and calibration of the acoustic stimuli were confirmed with a digital sound level meter (San Diego Instruments).

Drugs were administered 30 min before the start of testing in the apparatus. Each mouse was tested in 2 sessions using a cross-over design, where for the 1st testing, one-half of the mice of each genotype received vehicle and the other half received drug treatment (0.5 mg/kg haloperidol, 0.3 mg/kg risperidone, or 1 or 3 mg/kg olanzapine). The 2nd testing was conducted 7 days after the 1st testing. Mice that received drug during the 1st testing session received vehicle in the second session. The doses of haloperidol, risperidone, and olanzapine were chosen based on efficacy in animal models of antipsychotic drug action (Ouagazzal et al., 2001; Simon et al., 2000; Tada et al., 2004; Zhang and Bymaster, 1999). An additional consideration was the effects of the drugs on startle reactivity. Accordingly, the maximal dose chosen for risperidone and olanzapine was constrained by dose-related effects on startle responsivity.

Each test session consisted of 42 trials, presented following a five-minute habituation period. Seven different types of trials were presented: no-stimulus trials, trials with the acoustic startle stimulus (40 ms; 120 dB) alone, and trials in which a prepulse stimulus (20 ms; either 74, 78, 82, 86, or 90 dB) had onset 100 ms before the onset of the startle stimulus. The different trial types were presented in blocks of 7, in randomized order within each block, with an average intertrial interval of 15 s (range: 10 to 20 s). Measures were taken of the startle amplitude for each trial, defined as the peak response during a 65-ms sampling window that began with the onset of the startle stimulus. An overall analysis was performed for each subject's data for levels of prepulse inhibition at each prepulse sound level (calculated as $100 - [$(response amplitude for prepulse stimulus and startle stimulus together/response amplitude for startle stimulus alone) $\times 100]$).

A separate experiment was conducted to determine the startle amplitudes to the prepulse stimuli alone. The same procedure was used as described above, but with the following seven different types of trials: no-stimulus trials, and trials with acoustic startle stimuli at sound levels of 74, 78, 82, 86, 90, and 120 dB. Again, mice received 42 trials in all, in the design described above.

2.5. Statistical analyses

For locomotor activity experiments, data from each drug dose were first tested by repeated measures Analysis of Variance (ANOVA), with the factors genotype (wild type or NR1$-/-$), drug treatment, and time (in five-minute intervals). Since the main effect of genotype was significant for every drug dose, separate repeated measures ANOVAs were then conducted within each genotype, with the factors drug treatment and time. A separate analysis of the locomotor data was performed in order to more clearly present overall drug effects. In this case, data from each drug dose were summed for each hour of the three-hour session, and were tested by repeated measures ANOVAs, with the factors genotype and time (h).
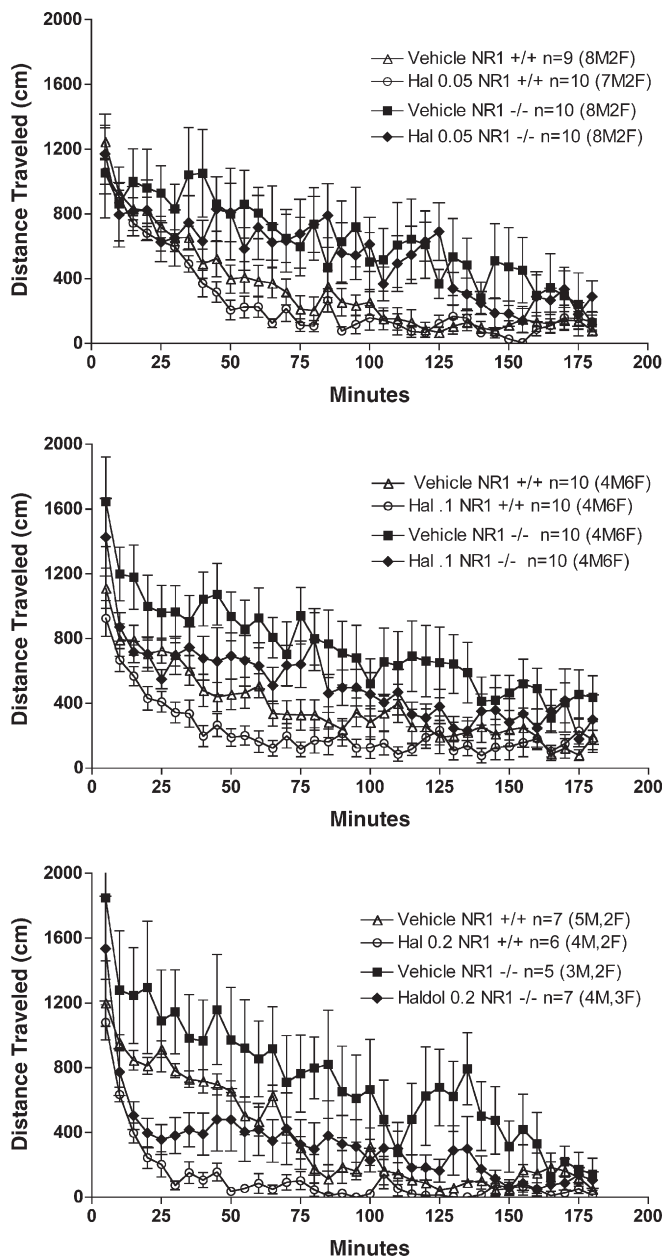

Fig. 1. Locomotor activity after injection of haloperidol in NR1+/+ and NR1−/− mice. Top panel, 0.05 mg/kg; middle panel, 0.1 mg/kg; bottom panel, 0.2 mg/kg. Mice were injected with the drug immediately before placement in the activity chambers. Data are means±SEM.
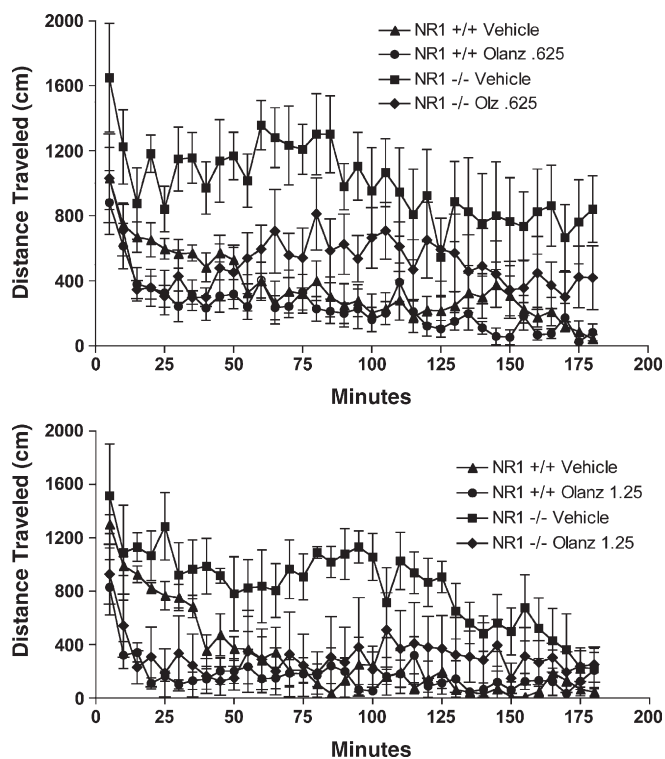
Fig. 2. Effects of olanzapine on locomotor activity in NR1+/+ and NR1−/− mice. Mice were injected with olanzapine immediately before placement in the activity chambers. Top panel, 1.25 mg/kg; bottom panel, 0.625 mg/kg. Data are means±SEM.

respect to the magnitude of hyperactivity of the mutant mice relative to controls. However, the basic behavioral phenotype of increased activity and reduced habituation during time course of testing was apparent in all cohorts (Figs. 1 and 2), with significant main effects of genotype observed for all doses of haloperidol [0.05 mg/kg, $F(1,35)=7.23$, $p=0.0109$; 0.1 mg/kg,
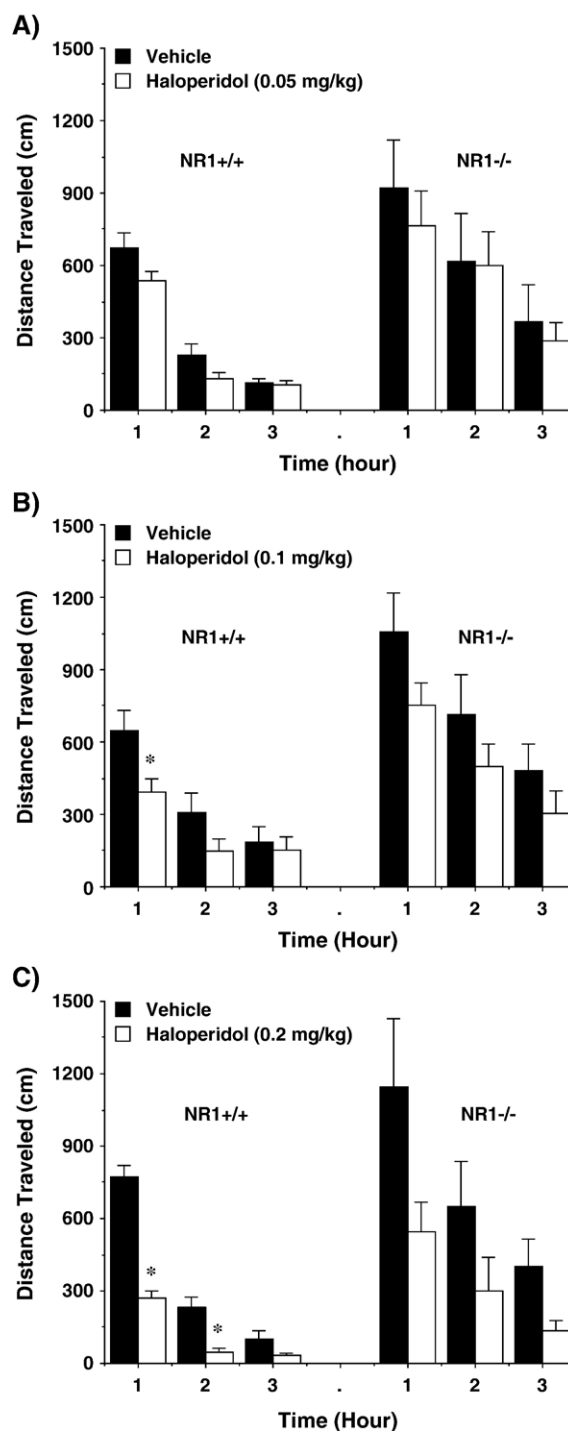

Fig. 3. Effects of haloperidol on locomotor activity in wild type (NR1+/+) and NMDA receptor-deficient (NR1−/−) mice during one-hour periods. Data from Fig. 1 were summarized for each hour of a three-hour test session. Subjects were injected immediately before hour 1. *$p<0.05$.

Post-hoc comparisons were performed using Bonferroni/Dunn tests.

For acoustic startle PPI studies, overall repeated measures ANOVAs were used for each drug dose, with the factors genotype, drug treatment, and intensity level of the acoustic stimulus (decibel or dB level; the repeated measure). Next, separate repeated measures ANOVAs were conducted within each genotype, using the factors drug treatment and stimulus dB level. Post-hoc repeated measures ANOVAs were used to determine drug effects at each dB level only when the within-group ANOVA indicated a significant effect of drug treatment. For all comparisons, significance was set at $p<0.05$.

3. Results

3.1. Effects of haloperidol and olanzapine on locomotor activity

3.1.1. Overall differences between NR1−/− mice and wild type controls

In each of the overall repeated measures ANOVAs, the effect of time was highly significant [$p<0.0001$ for every drug dose], reflecting changes in locomotion across the three-hour period for every test. In general, the NR1−/− mice exhibited increased activity and showed less habituation over the course of the three-hour test session, in comparison to the wild type mice. There was some variability across the different cohorts of mice tested with
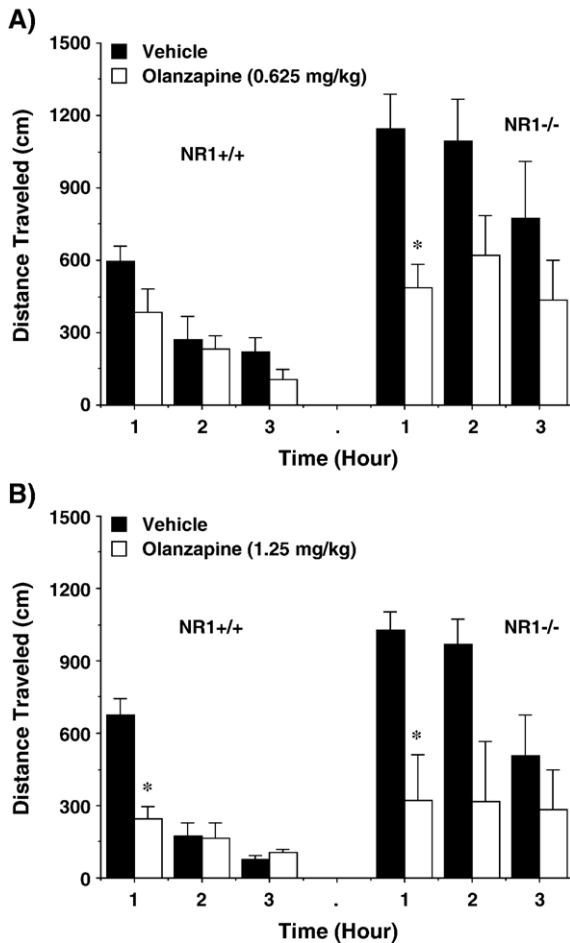
Fig. 4. Effects of olanzapine on locomotor activity in wild type (NR1+/+) and NMDA receptor-deficient (NR1−/−) mice during one-hour periods. Data from Fig. 2 were summarized for each hour of a three-hour test session. Subjects were injected immediately before hour 1. *$p<0.05$.

$F(1,36)=14.13$, $p=0.0006$, and 0.2 mg/kg, $F(1,21)=10.419$, $p=0.004$] and olanzapine [0.625 mg/kg, $F(1,22)=16.21$, $p=0.0006$, and 1.25 mg/kg, $F(1,12)=8.24$, $p=0.0141$]. Genotype differences in habituation across the test sessions were demonstrated by significant genotype×time interactions for every drug dose except for the higher dose of olanzapine [haloperidol; 0.05 mg/kg, $F(1,35)=3.6$, $p<0.0001$; 0.1 mg/kg, $F(1,35)=1.7$, $p=0.0073$, and 0.2 mg/kg, $F(1,35)=1.56$, $p=0.0212$; and olanzapine, 0.625 mg/kg, $F(1,35)=1.89$, $p=0.0016$]. Because of these significant differences between the two genotypes, drug treatment effects were investigated separately for the NR1+/+ and NR1−/− groups.

3.1.2. Haloperidol

In the wild type mice (Fig. 1), haloperidol led to significant overall reductions in locomotor activity for each dose [0.05 mg/kg, main effect of treatment, $F(1,17)=4.56$, $p=0.0476$; 0.1 mg/kg, treatment×time interaction, $F(35,630)=2.31$, $p<0.0001$; 0.2 mg/kg, main effect of treatment, $F(1,11)=60.27$, $p<0.0001$, treatment×time interaction, $F(35,385)=6.53$, $p<0.0001$]. In the NR1−/− mice, haloperidol at doses of 0.05

and 0.1 mg/kg had no apparent effects in the NR1 hypomorphic mice [$p>0.05$ for treatment main effects and interactions, overall repeated measures ANOVAs for each dose]. For the 0.2 mg/kg dose of haloperidol, the main effect for drug treatment closely approached significance [$F(1,10)=4.88$, $p=0.0517$] (Fig. 1). For all comparisons, the effect of time was highly significant [$p<0.0001$].

3.1.3. Olanzapine

Unlike the findings with haloperidol, the NR1−/− mice did not exhibit subsensitivity to the effects of olanzapine (Fig. 2). At the 0.625 mg/kg dose of olanzapine, there was a significant main effect of treatment for the NR1−/− mice [$F(1,11)=5.42$, $p=0.0401$], but not for the NR1+/+ mice. At the higher dose
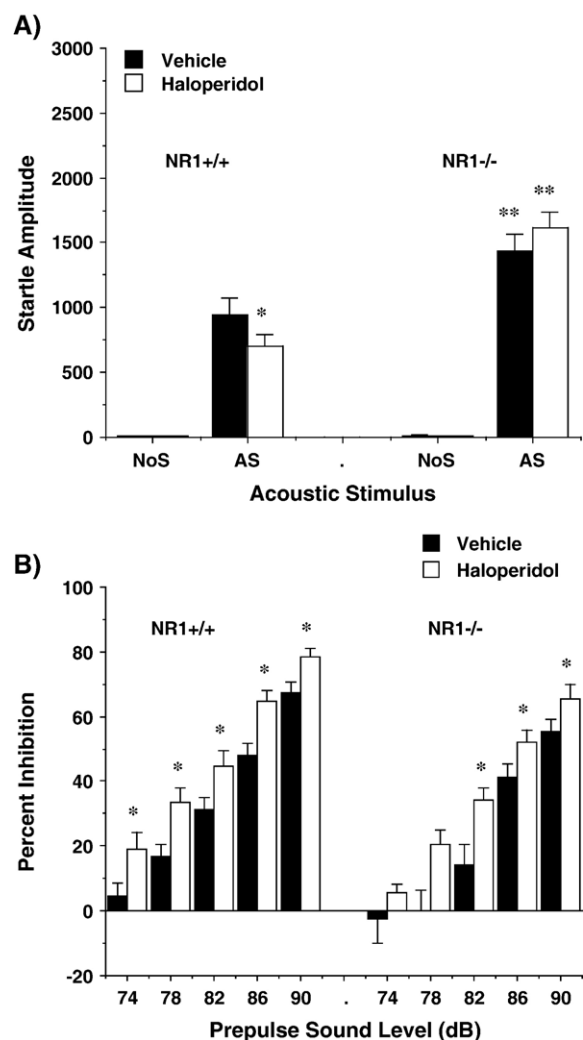

Fig. 5. Amplitude of the startle response (A) and percent prepulse inhibition (B) following treatment with haloperidol (0.5 mg/kg). Data shown are means (+SEM) for each group. Subject numbers were 4 male NR1+/+, 6 female NR1+/+, 4 male NR1−/−, and 5 female NR1−/−. Trials included no stimulus (NoS) and acoustic stimulus (AS, 120 dB) trials. Significant main effects of genotype were found for amplitude [$F(1,17)=20.81$ $p=0.0003$] and percent inhibition [$F(1,17)=12.38$, $p=0.0026$]. *$p<0.05$; within-group comparison between means for vehicle and drug. **$p<0.05$, comparison to corresponding NR1+/+ mean (for A only).

haloperidol [0.05 mg/kg, main effect of treatment, $F(1,17) = 4.56$, $p = 0.0476$; 0.1 mg/kg, treatment × time interaction, $F(2,36) = 5.19$, $p = 0.0105$; 0.2 mg/kg, main effect of treatment, $F(1,11) = 60.27$, $p < 0.0001$, treatment × time interaction, $F(2,22) = 26.34$, $p < 0.0001$]. For all comparisons, the effect of time (h) was highly significant [$p < 0.0001$].

As depicted in Fig. 4, olanzapine lowered rates of locomotor activity in the mutant group at both doses [0.625 mg/kg, main effect of treatment, $F(1,11) = 5.42$, $p = 0.0401$; 1.25 mg/kg, treatment × time interaction, $F(2,12) = 6.36$, $p = 0.0131$], but only had significant effects in the wild type mice at the highest dose [main effect of treatment, $F(1,6) = 7.52$, $p = 0.0337$, and treatment × time interaction, $F(2,12) = 18.78$, $p = 0.0002$].
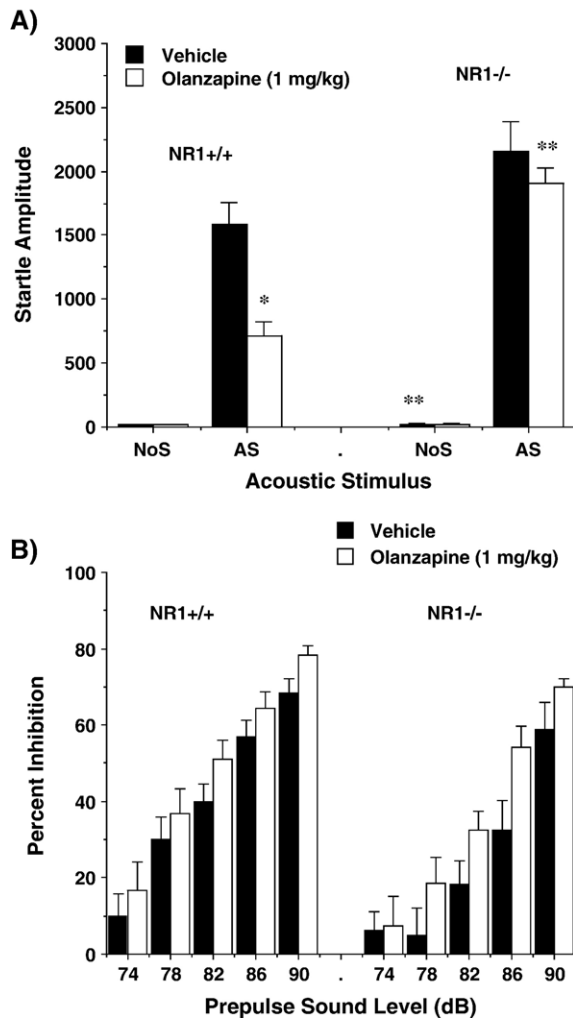

Fig. 6. Amplitude of the startle response (A) and percent prepulse inhibition (B) following treatment with olanzapine (1.0 mg/kg). Data shown are means (+SEM) for each group. Subject numbers were 15 NR1+/+ (5 males and 10 females), and 9 NR1−/− (3 males and 6 females). Trials included no stimulus (NoS) and acoustic stimulus (AS, 120 dB) trials. Significant main effects of genotype were found for amplitude [$F(1,22) = 19.93$, $p = 0.0002$] and percent inhibition [$F(1,22) = 8.18$, $p = 0.0091$]. *$p < 0.05$; within-group comparison between means for vehicle and drug. **$p < 0.05$, comparison to corresponding NR1+/+ mean (for A only).

of olanzapine (1.25 mg/kg), significant effects of treatment were observed in both genotypes [NR1+/+, main effect of treatment, $F(1,6) = 7.52$, $p = 0.0337$, and treatment × time interaction, $F(35,210) = 3.85$, $p < 0.0001$; and NR1−/−, treatment × time interaction, $F(35,210) = 1.7$, $p = 0.0124$]. For all comparisons, the effect of time was highly significant [$p < 0.0001$].

3.1.4. Summarized results for locomotor activity

Figs. 3 and 4 present the same data for locomotor activity, collapsed across each hour of the three-hour session. As noted above, haloperidol did not induce significant decreases in activity in the NR1−/− mice, although the main effect of treatment approached significance at the highest dose [$F(1,10) = 4.88$, $p = 0.0517$] (Fig. 3). In contrast, the wild type mice showed significant locomotor decreases with every dose of
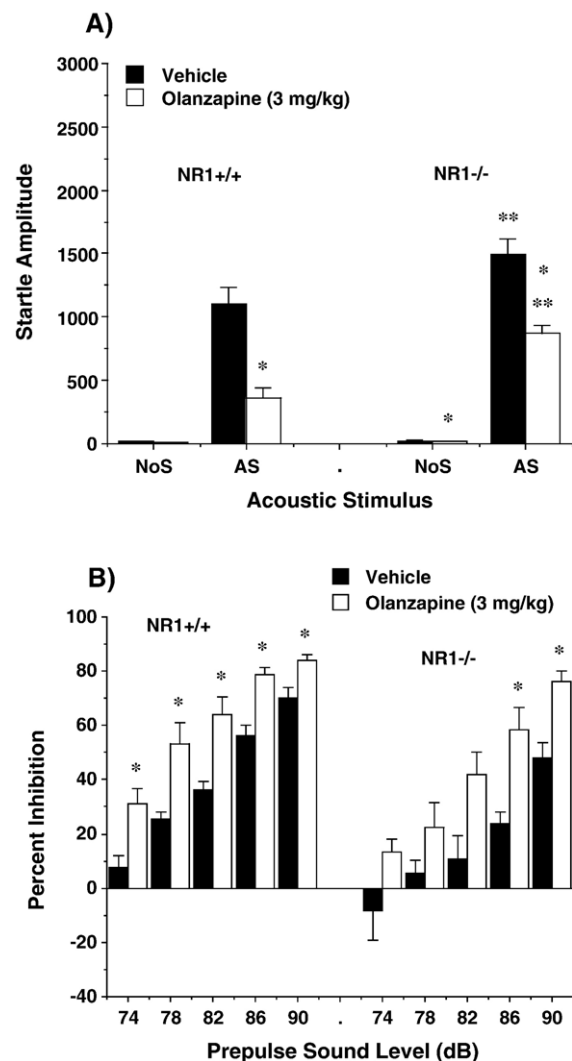

Fig. 7. Amplitude (A) and percent prepulse inhibition (B) of the startle response following treatment with olanzapine (3 mg/kg). Data shown are means (+SEM) for each group. Subject numbers were 10 NR1+/+ (4 male and 6 female), and 8 NR1−/− (4 male and 4 female). Trials included no stimulus (NoS) and acoustic startle stimulus (AS, 120 dB) trials. Significant main effects of genotype were found for amplitude [$F(1,16) = 18.47$, $p = 0.0006$] and percent inhibition [$F(1,16) = 18.15$, $p = 0.0006$]. *$p < .05$; within-group comparison between means for vehicle and drug. **$p < 0.05$, comparison to corresponding NR1+/+ mean (for A only).
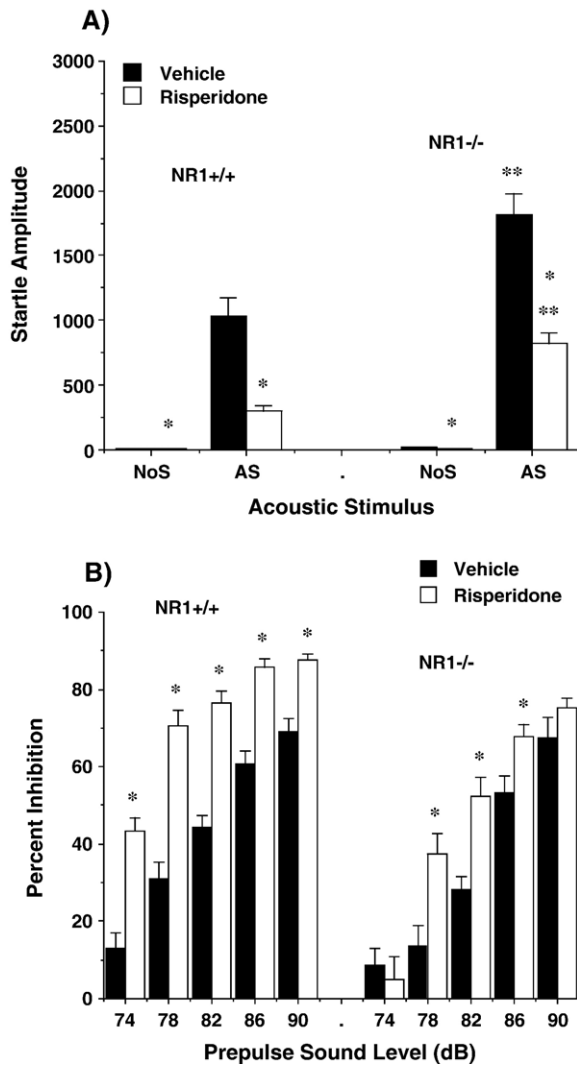
Fig. 8. Amplitude of the startle response (A) and percent prepulse inhibition (B) following treatment with risperidone (0.3 mg/kg). Data shown are means (+SEM) for each group. Subject numbers were 12 NR1+/+ (7 males and 5 females), and 11 NR1−/− (7 males and 4 females). Trials included no stimulus (NoS) and acoustic stimulus (AS, 120 dB) trials. Significant main effects of genotype were found for amplitude [$F(1,21)=29.61$, $p<0.0001$] and percent inhibition [$F(1,21)=54.71$, $p<0.0001$]. *$p<0.05$; within-group comparison between means for vehicle and drug. **$p<0.05$, comparison to corresponding NR1+/+ mean (for A only).

For all comparisons, the effect of time (h) was significant [$p<0.02$].

3.2. Effects of haloperidol, olanzapine, and risperidone on startle and PPI

3.2.1. Overall effects of genotype and drug treatment

As observed with the activity measure, significant main effects of genotype on amplitude and PPI were found for each drug dose (respective F and p values given in captions for Figs. 5–8). Overall, the NR1−/− mice had consistently higher startle amplitudes and lower levels of prepulse inhibition, in comparison to the NR1+/+ mice. The effect of drug treatment

varied with compound and dose, but the only significant genotype×treatment interactions were found for the effects of haloperidol on startle amplitude [$F(1,17)=9.74$, $p=0.0062$]. These results indicate that, in general, the different drugs tested had similar effects in the wild type and mutant mice. For all overall comparisons, the effect of acoustic decibel level (the repeated measure) was highly significant [$p<0.0001$]. Detailed descriptions of the individual drug effects for each genotype are given below.

3.2.2. Effects of haloperidol on startle amplitude and PPI

Treatment with haloperidol (0.05 mg/kg) significantly reduced startle amplitude in the NR1+/+ mice [main effect of treatment, $F(1,9)=18.33$, $p=0.002$], but had no significant effects in the NR1−/− mice (Fig. 5). However, haloperidol enhanced PPI in both the NR1+/+ and NR1−/− groups [main effect of treatment, NR1+/+, $F(1,9)=24.42$, $p=0.0008$; and NR1−/−, $F(1,8)=7.15$, $p=0.0282$]. For all comparisons, the effect of acoustic decibel level (the repeated measure) was highly significant [$p<0.0001$].

3.2.3. Effects of olanzapine on startle amplitude and PPI

The results of the startle testing indicated that, at a dose of 1 mg/kg, olanzapine decreased response to the startle stimulus in the NR1+/+ mice [main effect of treatment, $F(1,14)=56.13$, $p<0.0001$; and treatment×decibel level interaction, $F(1,14)=57.07$, $p<0.0001$], but did not have significant effects in the NR1−/− group (Fig. 6A). The 3 mg/kg dose of olanzapine significantly reduced the amplitude of the startle response in both genotypes (Fig. 7A) [main effect of treatment, NR1+/+, $F(1,9)=31.28$, $p=0.0003$; and NR1−/−, $F(1,7)=19.77$, $p=0.003$]. Wild type mice given 3 mg/kg olanzapine showed decreased startle amplitude after the acoustic stimulus, but not under baseline (no stimulus) conditions [post-hoc tests, and treatment×dB interaction, $F(1,9)=31.38$, $p=0.0003$]. In the mutant group, olanzapine (3 mg/kg) significantly decreased both baseline amplitude and startle amplitude [post-hoc tests, and treatment×dB interaction, $F(1,7)=18.79$, $p=0.0034$]. For all comparisons, the effect of acoustic decibel level (the repeated measure) was highly significant [$p<0.0001$].

Olanzapine, at a dose of 1 mg/kg, had no significant effect on PPI in the wild type or mutant mice (Fig. 6B). As shown in Fig. 7B, the higher olanzapine dose (3 mg/kg) enhanced prepulse inhibition in the NR1+/+ group at every sound level of the prepulse stimulus [post-hoc tests following significant main effect of treatment, $F(1,9)=27.9$, $p=0.0005$]. In the NR1−/− group, olanzapine tended to increase PPI at all prepulse levels, but significant increases were apparent only at the two highest prepulse sound levels [post-hoc tests following significant main effect of treatment, $F(1,7)=9.66$, $p=0.0171$]. For all comparisons, the effect of acoustic decibel level (the repeated measure) was highly significant [$p<0.0001$].

3.2.4. Effects of risperidone on startle amplitude and PPI

Risperidone (0.3 mg/kg) significantly reduced the amplitude of the startle response in both experimental groups (Fig. 8A) [main effect of treatment, NR1+/+, $F(1,11)=20.97$, $p=0.0008$;

and NR1−/−, $F(1,10)=52.92$, $p<0.0001$]. Following drug treatment, the wild type and mutant mice demonstrated decreased amplitudes under baseline conditions (no stimulus), and after the acoustic stimulus [post-hoc tests, and treatment×dB level interaction, NR1+/+, $F(1,11)=20.21$, $p=0.0009$; and NR1−/−, $F(1,10)=53.51$, $p<0.0001$].

Risperidone also increased prepulse inhibition in both experimental groups [main effect of treatment, NR1+/+, $F(1,11)=52.65$, $p<0.00001$; and NR1−/−, $F(1,10)=7.64$, $p=0.02$]. As shown in Fig. 8B, enhanced prepulse inhibition was observed in the NR1+/+ group at every sound level of the prepulse stimulus, while in the NR1−/− group, risperidone increased percent inhibition at three prepulse sound levels [post-hoc tests, and treatment×dB level interaction, NR1+/+, $F(4,44)=4.39$, $p=0.0045$; and NR1−/−, $F(4,40)=5.99$, $p=0.0007$]. For all comparisons, the effect of acoustic decibel level (the repeated measure) was highly significant [$p<0.0001$].

3.3. Startle responses following presentation of the prepulse acoustic stimuli alone

As shown in Fig. 9, all of the experimental groups demonstrated slight, but significant, startle responses to acoustic stimuli at the decibel levels of the prepulses used in the present study [post-hoc comparisons following significant effects of decibel level for NR1+/+ males, $F(6,60)=324.38$, $p<0.0001$; NR1−/− males, $F(6,30)=22.88$, $p<0.0001$; NR1+/+ females, $F(6,54)=124.39$, $p<0.0001$; and NR1−/− females, $F(6,54)=70.07$, $p<0.0001$]. However, it is notable that the amplitude levels of the startle responses at the prepulse-decibel levels (74 to 90
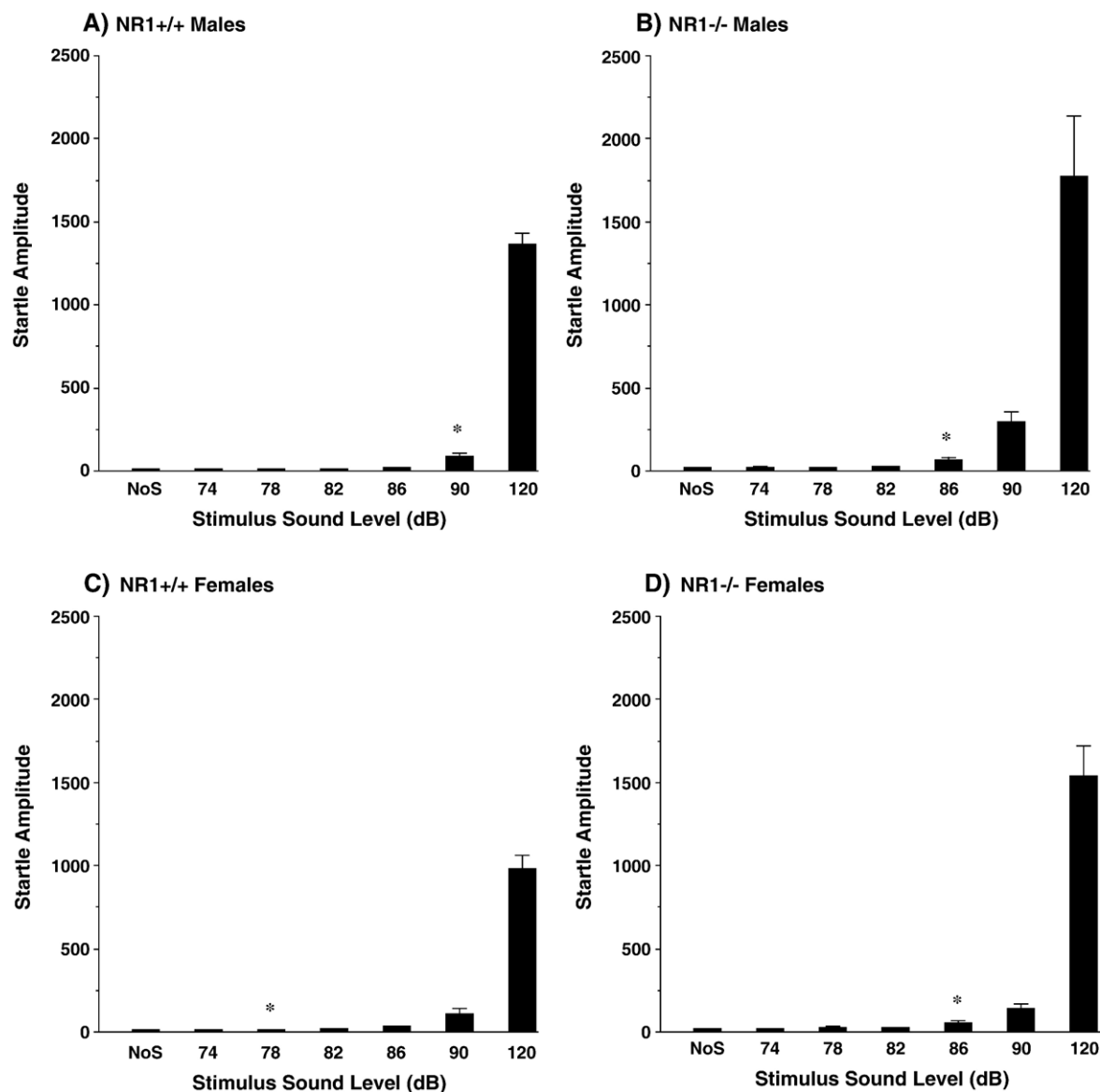

Fig. 9. Lowest stimulus sound level producing a significant startle response. Data shown are means (+SEM) for each group. Subject numbers were 21 NR1+/+ (11 males and 10 females), and 16 NR1−/− (6 males and 10 females). Trials included no stimulus (NoS) trials. *$p<0.05$, comparison with amplitude during the NoS trials.

decibels) were very small in comparison to the magnitude of startle observed following the 120 decibel stimulus.

4. Discussion

As previously reported, reductions in PPI and increased locomotor activity were observed in NR1−/− mice (Duncan et al., 2004; Fradley et al., 2005; Mohn et al., 1999). The present study was designed to determine the effects of antipsychotic drugs with differing pharmacological properties on these behavioral alterations. The larger goal of the work was to assess the potential utility of the NR1−/− mice as screens for new antipsychotic drugs with "atypical" properties. All of the antipsychotics tested (haloperidol, olanzapine, risperidone) increased PPI in both wild type and NR1−/− mice. These data, together with the previously published findings with clozapine and quetiapine (Duncan et al., 2006) indicate that the NR1−/− mice do not provide a model to distinguish effects of typical and atypical drugs on sensorimotor gating. Olanzapine and risperidone, but not haloperidol, reduced the increased startle reactivity in the mutant mice, similar to effects observed previously for clozapine and quetiapine (Duncan et al., 2006).

In the locomotor activity test, olanzapine, but not haloperidol, reduced activity in the NR1−/− mice at a dose that produced minimal effects on activity in the wild type mice. In this regard, the effects of olanzapine are similar to those of clozapine reported by Mohn et al. (1999). These findings suggest differential effects of typical and atypical antipsychotic drugs on the enhanced locomotor activity in NR1−/− mice. Comparison of additional drugs will be necessary before definitive conclusions can be made about the utility of the NR1−/− mice to distinguish typical and atypical antipsychotic in the locomotor activity test.

Differential effects of olanzapine and risperidone, in comparison to haloperidol, were found on startle reactivity in NR1+/+ and NR1−/− mice. These data suggest that properties of olanzapine and risperidone in addition to D_2 dopamine receptor blockade likely contribute to the actions of these drugs on startle response in the model. Both olanzapine and risperidone are potent D_2 dopamine and serotonin 2A antagonists and also affect noradrenergic and histamine receptor systems (for review, see Duncan et al., 1999). Further work will be required to determine the specific pharmacological properties of olanzapine and risperidone responsible for the reduction of startle amplitude in the mice.

The fact that all of the antipsychotics tested produced similar effects on PPI in the NR1+/+ and in the NR1−/− mice suggests that NMDA receptor mechanisms were not critically involved in the effects of the drugs. The increased PPI in the wild type mice after administration of antipsychotics in the present study is in accord with other studies demonstrating effects of antipsychotics on PPI in different mouse strains (Kinkead et al., 2005; Lipina et al., 2005; Olivier et al., 2001; Ouagazzal et al., 2001).

It is unlikely that reduced PPI in the NR −/− mice is simply related to the increased startle reactivity. Relationships between startle reactivity and PPI under different experimental condi-

tions are complex, and increased startle reactivity is not necessarily associated with decreased PPI. For example, in an assessment of 13 strains of mice with widely varying startle response magnitudes and amount of PPI, there was no clear relationship between magnitude of the startle response and percent PPI (Paylor and Crawley, 1997). Also, doses of nicotine that increase startle reactivity actually increase PPI (Acri et al., 1994; Schreiber et al., 2002). Furthermore, in previous work (Duncan et al., 2006) and in the present study, haloperidol increased PPI in the NR1−/− mice, but did not alter the exaggerated startle reactivity in the mutants when no prepulse was presented. Also, in the present study, olanzapine at a dose of 1 mg/kg reduced startle responses in the wild type mice but did not affect PPI at that dose. Therefore, there does not appear to be a predictable relationship between reduced startle amplitude and increased PPI. The very slight startle response induced by the prepulse stimuli in both wild type and mutant mice suggest that mutant mice do not exhibit a degree of exaggerated startle response to the prepulses that would confound results.

The reduced PPI in the NR1 hypomorphic mice is consistent with studies in rats and mice (Brody et al., 2003b; Lipina et al., 2005) showing that NMDA antagonists reduce PPI (Mansbach, 1991; Mansbach and Geyer, 1989). In rats, most studies find that atypical, but not typical, antipsychotic drugs reverse or attenuate NMDA antagonist-induced disruption of PPI (Bakshi and Geyer, 1995; Bakshi et al., 1994; Brody et al., 2004; Mansbach et al., 2001; Swerdlow et al., 1998, 1996). By contrast, in the NR1−/− mice, haloperidol increased PPI in a similar fashion as olanzapine and risperidone in the present work, and clozapine and quetiapine in our previous studies (Duncan et al., 2006).

For mice, there is only one published study comparing effects of haloperidol with an atypical antipsychotic drug (clozapine) in regard to reversal of NMDA antagonist-induced PPI disruption. That study found that neither haloperidol nor clozapine reversed the effects of MK-801 on PPI. However clozapine has been reported by others to attenuate the effects of MK-801 on PPI in mice (Lipina et al., 2005; Long et al., 2006) but those studies did not test effects of a typical antipsychotic.

There are marked differences between brain metabolic effects produced by acute administration of NMDA antagonist and those that result from the chronic reduction of NMDA receptor function in the NR1 hypomorphic mice. NMDA antagonists produce a marked activation of certain brain regions, whereas neuroanatomically selective reduction in brain metabolic activity was found in the NR1 hypomorphic mice (Duncan et al., 2002). Thus the physiological state induced by the chronic deficit in NMDA receptor function is distinct from that induced by acute administration of NMDA antagonists. The NR1−/− mouse provides a model for effects of chronic developmental NMDA hypofunction whereas models involving acute administration of NMDA antagonists may model acute psychotic exacerbation.

In addition to the NR1 hypomorphic model examined in the present work, a variety of other approaches have also been used to create genetic mouse models of reduced NMDA and

metabotropic glutamate receptor hypofunction. Mice with dysfunction of the NMDA receptor have been created by deletion of the NR2A (ε1) subunit (Miyamoto et al., 2001). The NR2A knockout mice survive and exhibit increased locomotor activity, impairment of latent learning in a water-finding task, and increased turnover of dopamine and serotonin in frontal cortex and striatum. Another genetic approach to disrupt NMDA receptor function was to generate mice with targeted point mutations of the glycine regulatory site of the receptor. Such mice exhibited sustained non-habituating hyperactivity and increased startle response, but normal PPI (Ballard et al., 2002). Deficits in PPI have been observed after deletion of mGluR1 and mGluR5 metabotropic glutamate receptors (Brody et al., 2003a, 2004). In the mGluR1 knockout mice, a D_2 dopamine antagonist (raclopride) did not reverse the PPI deficits but lamotrigine, an antiepileptic drug that is effective as a mood stabilizer, did reduce the PPI deficits (Brody et al., 2003a). For the mGluR5 knockout mice, neither raclopride, clozapine, nor lamotrigine altered the PPI deficits (Brody et al., 2004).

In summary, the NR1 hypomorphic mouse provides a genetic model of endogenously reduced NMDA receptor function associated with increased locomotor activity and PPI deficits that can be attenuated by clinically effective antipsychotic drugs. Olanzapine (but not haloperidol) reduced the locomotor hyperactivity in the NR1−/− mice at a dose that did not affect activity in the wild type mice. Olanzapine and risperidone reduced the exaggerated startle response and PPI deficits in the NR1−/− mice. In contrast, haloperidol reduced the PPI deficits but not the increased startle response in the mutant mice.

Activity of known antipsychotic drugs in the NR1 hypomorphic mouse model provides validating evidence for the utility of the model to "detect" agents with antipsychotic effects. All of the antipsychotics tested in NR1 hypomorphic mice to date have effects on the dopaminergic system and it is likely that dopamine receptor antagonism contributes to their actions in this model of endogenous NMDA hypofunction. It is possible that the NR1 hypomorphic mice may provide a model to test potential therapeutic agents for schizophrenia that work by novel mechanisms that do not involve dopamine receptor antagonism. It will be especially interesting to explore behavioral effects of drugs that modulate glutamate-mediated neurotransmission in the NR1 hypomorphic mice.

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