

Gary E. Duncan · Sheryl S. Moy ·  
Jeffery A. Lieberman · Beverly H. Koller

## Effects of haloperidol, clozapine, and quetiapine on sensorimotor gating in a genetic model of reduced NMDA receptor function

Received: 25 May 2005 / Accepted: 20 September 2005 / Published online: 16 December 2005  
© Springer-Verlag 2005

**Abstract** *Rationale:* Reduced N-methyl D-aspartate (NMDA) receptor function is hypothesized to contribute to the pathophysiology of schizophrenia. In order to model chronic and developmental NMDA receptor hypofunction, a mouse line was developed that expresses low levels of the NMDA R1 (NR1) subunit of the NMDA receptor. These mice show increased acoustic startle reactivity and deficits in prepulse inhibition (PPI) of acoustic startle. *Objectives:* The present study tested the hypothesis that these altered acoustic startle responses in the NR1 hypomorphic (NR1<sup>-/-</sup>) mice would be affected by antipsychotic drug treatment. *Methods:* Mice were injected with drugs 30 min before assessment of acoustic startle responses with and without prepulse stimuli. *Results:* Haloperidol (0.5 or 1.0 mg/kg) did not reduce the increased startle reactivity in the NR1<sup>-/-</sup> mice, but did increase PPI in both the mutant and wild type mice. Clozapine (3 mg/kg) and quetiapine (20 mg/kg) reduced startle magnitude and increased PPI in both the wild type and mutant mice. The antidepressant drug imipramine (10 and 20 mg/kg) had minimal effects on startle amplitude in NR1<sup>-/-</sup> or wild type mice. However, for the 20-mg/kg dose of imipramine, a significant increase in PPI was observed in the wild type animals, but not in the mutant mice. *Conclusions:* The results demonstrate that PPI can be increased in a mouse model of chronic NMDA receptor hypofunction by typical and atypical antipsychotic drugs. The similar effects of

typical and atypical antipsychotic drugs to increase PPI in the wild type and mutant mice indicates that the assessment of behavior of the NR1 hypomorphic mice in the PPI paradigm offers no advantage over the wild type controls for identifying new clozapine-like drugs.

**Keywords** Acoustic startle · Antipsychotic · NMDA receptor · Mouse model · Prepulse inhibition · Schizophrenia

### Introduction

Neuropsychological effects induced by subanesthetic doses of N-methyl D-aspartate (NMDA) receptor antagonists in humans suggest that reduced NMDA receptor function could contribute to the pathophysiology of schizophrenia. NMDA receptor antagonists such as ketamine and phencyclidine induce a constellation of effects 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). Furthermore, stabilized schizophrenia patients challenged with ketamine experience symptoms that are similar to those occurring in active phases of their illness (Lahti et al. 1995a; Lahti et al. 1995b; Malhotra et al. 1997). Behavioral effects induced by challenge with ketamine mimic a broader and more complete range of schizophrenic-like symptoms, compared to treatment with dopaminergic psychostimulants. For example, drugs such as amphetamine and methylphenidate induce positive symptoms, but do not produce the cognitive deficits and affective withdrawal characteristic of schizophrenia (Angrist et al. 1980; Lieberman et al. 1987).

The hypothesis that NMDA hypofunction contributes to the symptoms of schizophrenia is supported by studies demonstrating beneficial effects of compounds that potentiate NMDA receptor function. Glycine is a positive allosteric modulator and coagonist of the NMDA receptor (Leeson and Iversen 1994). Administration of high doses of glycine (Heresco-Levy et al. 1996; Heresco-Levy et al. 1999; Javitt et al. 1994), and other agonists of the glycine

G. E. Duncan (✉) · S. S. Moy · J. A. Lieberman  
Department of Psychiatry, CB# 7090,  
University of North Carolina School of Medicine,  
Chapel Hill, NC 27599-7090, USA  
e-mail: gduncan@med.unc.edu  
Tel.: +1-919-9668237  
Fax: +1-919-9661856

B. H. Koller  
Department of Genetics,  
University of North Carolina School of Medicine,  
Chapel Hill, NC 27599-7090, USA

regulatory site such as D-cycloserine (Goff et al. 1999; Goff et al. 1995; Heresco-Levy et al. 2002) and D-serine (Tsai et al. 1998), can reduce symptoms in schizophrenia. Although the reported effects of glycine site agonists have been modest, these pioneering studies provide a proof of principle that augmenting NMDA receptor function has therapeutic potential.

Given the findings in humans described above, it is possible that defining the neurobiological consequences of experimentally induced NMDA receptor hypofunction could contribute to the understanding of the neurobiology of schizophrenia. Toward this aim, a mouse genetic model of chronic and developmentally reduced NMDA receptor dysfunction was generated in which levels of the NMDA R1 (NR1) subunit of the NMDA receptor were markedly reduced (Mohn et al. 1999). These mice are best characterized as NR1 hypomorphic since the genetic alteration (insertion of a neomycin resistance gene into intron 20 of the NR1 locus) results in dramatic underexpression (80–90% reduction), but not elimination, of the *NR1* gene. Autoradiographic analysis of <sup>3</sup>H-MK-801 binding demonstrated that the NR1 hypomorphic mice had an 80–85% reduction in hippocampal binding and 60–70% reduction in the binding of the ligand in other brain regions. However, there was no reduction of <sup>3</sup>H-kainic acid or <sup>3</sup>H-AMPA binding in any brain region of the mutant mice, in comparison to the wild type mice. The NR1 hypomorphic mice exhibited altered behavioral phenotypes that include reduced locomotor habituation in an activity chamber, deficits in social interactions (Duncan et al. 2004; Mohn et al. 1999), and deficits in prepulse inhibition (PPI) of acoustic startle (Duncan et al. 2004). The mutant mice also exhibited altered patterns of regional brain metabolism, including reduced activity in the medial prefrontal and anterior cingulate cortices, and in the hippocampus (Duncan et al. 2002). The NR1 hypomorphic mouse thus serves an animal model with intrinsic deficits, potentially relevant to symptoms of schizophrenia, that are produced by a targeted neurochemical alteration based on a specific pathophysiological hypothesis of the human disease.

Deficits in sensorimotor gating are observed in schizophrenia patients in acoustic and tactile startle PPI paradigms (Braff et al. 2001). The cross-species potential of such PPI paradigms make them attractive models to explore actions of drugs in animal models that have direct relevance to clinical situations. 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 experimentally 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) are also effective in the preclinical models involving acute challenge with NMDA antagonists (Bakshi and Geyer 1995; Swerdlow et al. 1996). In the present study, the effects of haloperidol, clo-

zapine, and quetiapine were examined in the wild type control and NR1 hypomorphic mice in the acoustic startle PPI paradigm. In addition, the tricyclic antidepressant drug, imipramine, that exhibits a similar side-effect profile as clozapine with regard to anticholinergic and antihistaminic actions, was tested to assess specificity of antipsychotic action in the model.

---

## Methods

### 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 is important to obtain populations of mice that differ genetically only at the NR1 locus. A strategy was devised to generate NR1 hypomorphs and genetically identical wild type populations. C57BL/6 heterozygous 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. The 129-SvEv mice that were coisogenic were used as moms. C57BL/6 mice were backcrossed >14 generations from C57BL/6 mice purchased from Jackson Laboratories. Analysis of the F1 hybrid litters showed that mice homozygous for the mutation were present at the expected frequency and that these NR1 hypomorphic mice were very close in size to their wild type littermates. The NR1 mutation was maintained on the C57BL/6 and 129/SvEv genetic backgrounds by breeding heterozygous animals. 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<sup>-/-</sup>) and their control populations (NR1<sup>+/+</sup>). At total of 63 separate litters of the F1 hybrid mice were used in the present study.

### Acoustic startle and PPI

The acoustic startle measure was based on the reflexive whole-body flinch, or startle response, after exposure to a sudden noise. The 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). The background sound levels (70 dB) and the calibration of

the acoustic stimuli were confirmed with a digital sound level meter (San Diego Instruments).

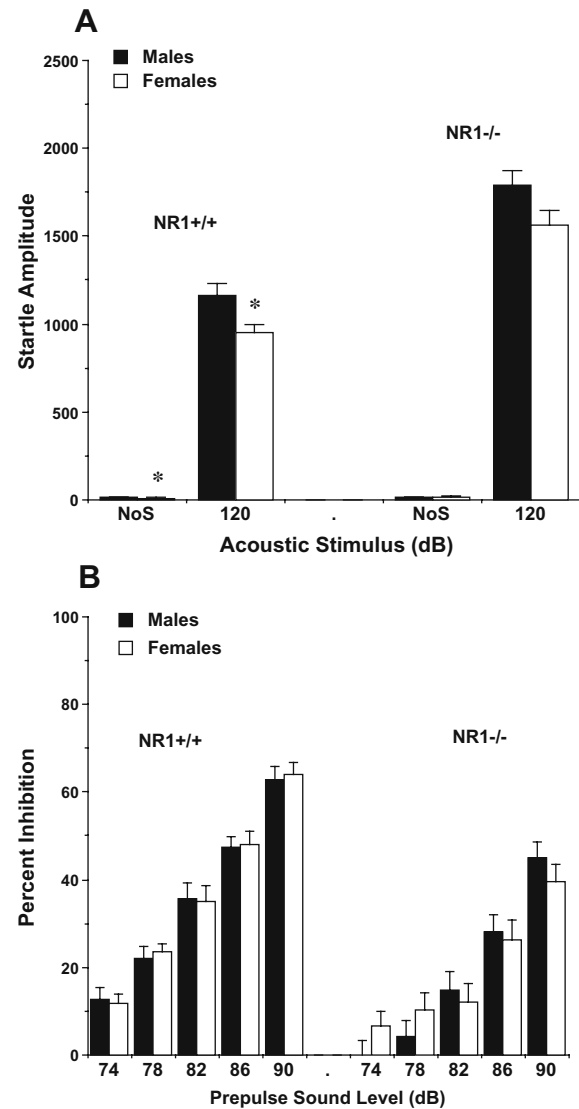
Separate groups of mice were used to test each drug dose. Drugs were administered 30 min before the start of testing in the apparatus. Each mouse was tested in two sessions using a cross-over design where, for the first testing, one-half of the mice of each genotype received vehicle and the other half received drug treatment (haloperidol 0.5 or 1.0 mg/kg, diluted with 0.9% saline from an oral solution of haloperidol lactate; clozapine 1, 2, or 3 mg/kg, dissolved with 5  $\mu$ l of 20% acetic acid/ml of 0.9% saline; quetiapine 10 or 20 mg/kg, same vehicle as clozapine; or imipramine 10 or 20 mg/kg, dissolved in saline). The second test session was conducted 7 days after the first test session. The mice that received a drug during the first test session received a vehicle in the second session. The doses of haloperidol, clozapine, and quetiapine were chosen based on efficacy in animal models of anti-psychotic drug action (Ouagazzal et al. 2001; Simon et al. 2000; Tada et al. 2004; Zhang and Bymaster 1999). Doses of imipramine were chosen based on efficacy in animal models of depression (Bai et al. 2001; David et al. 2003). An additional consideration was the effects of the drugs on startle reactivity. Accordingly, the maximal dose chosen for clozapine and quetiapine were constrained by dose-related effects on startle responsiveness.

Each test session consisted of 42 trials, presented after a 5-min habituation period. Seven different types of trials were presented: no-stimulus (NoS) 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 started 100 ms before the onset of the startle stimulus. The different trial types were presented in blocks of seven, in randomized order within each block, with an average intertrial interval of 15 s (range: 10 to 20 s). 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, was measured. An overall analysis was performed for each subject's data for levels of PPI at each prepulse sound level (calculated as  $100 - [(response\ amplitude\ for\ prepulse\ stimulus\ and\ startle\ stimulus\ together / response\ amplitude\ for\ startle\ stimulus\ alone) \times 100]$ ).

## Statistical Analyses

Data were compared using three separate analyses, in order to determine: (1) overall effects of genotype (wild type and NR1<sup>-/-</sup>) and sex across the entire study; (2) genotype and drug effects at each separate drug dose and (3) drug effects within each genotype at each separate drug dose. First, an overall repeated measures analysis of variance (ANOVAs) was used to determine the effects of genotype and sex on responses observed after vehicle treatment. Then further repeated measures ANOVAs were used to determine the main effects of genotype and drug treatment, and the genotype  $\times$  treatment interaction, for each drug dose.

After these initial analyses, separate repeated measures ANOVAs were conducted to compare the data within each of the genotype groups, using the factors of drug treatment and acoustic stimulus decibel (dB) level (the repeated measure). These within-group ANOVAs were used to determine drug treatment effects in either the wild type or NR1<sup>-/-</sup> mice at each separate drug dose. Post hoc repeated measures ANOVAs were used to determine drug effects at each sound level only when the within-group ANOVA indicated a significant effect of drug treatment. For all comparisons, significance was set at  $p < 0.05$ .



**Fig. 1** Amplitude of the startle response (a) and percent prepulse inhibition (b) after the presentation of acoustic stimuli in male and female NR1<sup>+/+</sup> and NR1<sup>-/-</sup> mice. Data shown are means (+SEM) for each group. Trials included no stimulus (NoS) trials and acoustic startle stimulus (120) alone trials. Data were combined from vehicle groups of seven different cohorts. Subject numbers were 37 male and 23 female NR1<sup>+/+</sup> mice, and 31 male and 19 female NR1<sup>-/-</sup> mice. \* $p < 0.05$ , post hoc comparison at one stimulus intensity level following significant  $F$  value, repeated measures ANOVA

**Results**

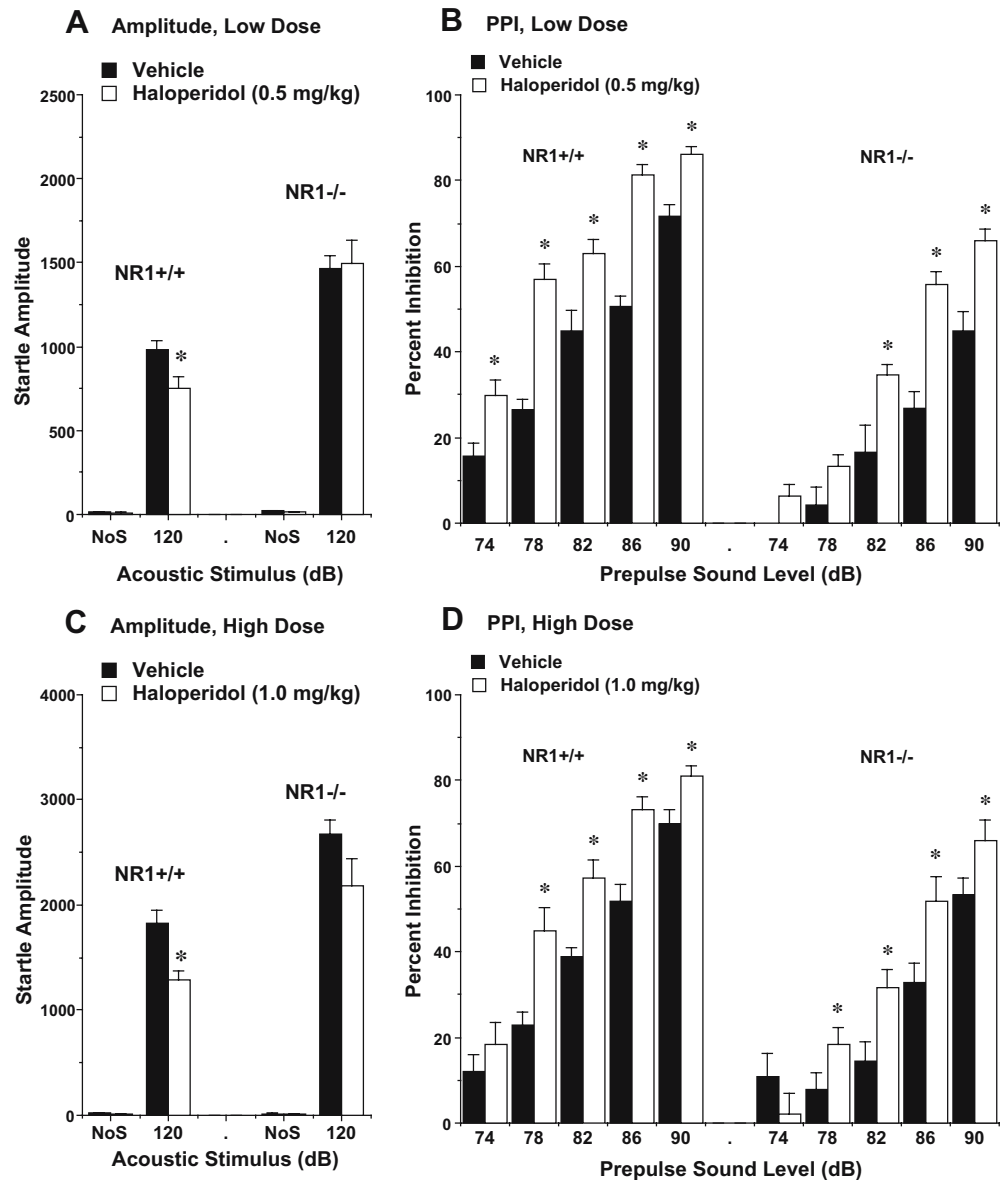
Assessment of gender effects on startle magnitude and PPI

Because of the labor- and resource-intensive breeding required to generate the NR1<sup>-/-</sup> mice and their wild type NR1<sup>+/+</sup> controls, it is desirable to use both males and females in the experiments. To determine if gender effects exist for the alterations in startle magnitude and/or PPI in the NR1<sup>-/-</sup> mice, the data for vehicle-treated wild type and mutant mice from seven experiments were combined. The results from this analysis on data from the entire study are described below.

*Amplitude* Overall, as shown in Fig. 1a, the NR1<sup>-/-</sup> mice had higher startle amplitudes in comparison to the wild

type controls [main effect of genotype,  $F(1,106)=63.6$ ,  $p<0.0001$ ]. There were also significant differences between male and female startle responses [main effect of sex,  $F(1,106)=7.8$ ,  $p=0.006$ ]. Separate within-group analyses revealed that, in the NR1<sup>+/+</sup> group, females had significantly lower amplitudes than the males for both baseline (NoS) trials and for the 120-dB startle stimulus [main effect of sex,  $F(1,58)=4.4$ ,  $p=0.0397$ ]. The lower baseline amplitude might have reflected the lower weights of the female mice, rather than the less general movement within the testing chamber. However, in the NR1<sup>-/-</sup> animals, differences between the sexes only approached significance [main effect of sex,  $F(1,48)=3.4$ ,  $p=0.0688$ ]. Since there were modest but significant sex differences in startle magnitude, the number of male and female mice in the different genotype groups was balanced as closely as possible for all experiments.

**Fig. 2** Amplitude of the startle response (a, c) and percent prepulse inhibition (b, d) after a low dose (0.5 mg/kg) or high dose (1.0 mg/kg) of haloperidol. Data shown are means (+SEM) for each group. Drug or vehicle was administered 30 min before testing. Trials included no stimulus (NoS) trials and acoustic startle stimulus (120 dB) trials. Subject numbers for the low-dose group were 21 NR1<sup>+/+</sup> (12 males and nine females) and 16 NR1<sup>-/-</sup> (eight males and eight females). Subject numbers for the high-dose group were ten NR1<sup>+/+</sup> and ten NR1<sup>-/-</sup> (seven males and three females in each group). \* $p<0.05$ , post hoc comparison at one stimulus intensity level following significant  $F$  value, repeated measures ANOVA



**Prepulse inhibition** There was a clear reduction in percent inhibition in the NR1<sup>-/-</sup> group [Fig. 1b, main effect of genotype,  $F(1,106)=38.3$ ,  $p<0.0001$ ]. However, no significant effects of sex on PPI were observed in the overall repeated measures analysis. Further analyses confirmed that sex did not have a significant effect on PPI in the wild type or mutant mice.

### Overall effects of genotype and drug treatment

Replicating previous work, each set of NR1<sup>-/-</sup> mice in the present study demonstrated exaggerated startle responses and deficits in PPI, in comparison to the wild type controls (i.e., significant main effects of genotype on amplitude and PPI were found for each experiment). While the overall effect of drug treatment varied with compound and dose, the only significant genotype x treatment interactions were found for the effect of haloperidol (0.5 mg/kg) on startle amplitude with no prepulse stimulus [ $F(1,35)=5.4$ ,  $p=0.0257$ ], and clozapine (2 mg/kg) on PPI [ $F(1,22)=6.4$ ,  $p=0.0185$ ]. These results indicate that, overall, the different drugs tested had similar effects in the wild type and mutant mice, with the exception of the lower dose of haloperidol (0.5 mg/kg) and the moderate (2 mg/kg) dose of clozapine.

Detailed descriptions of the individual drug effects within each group, wild type and mutant, are given below. The  $F$  values for the main effects of drug, and drug x dB level interactions, were obtained from within-group repeated measures ANOVAs. Significant effects at each dB level of the acoustic stimuli were determined by post hoc comparisons.

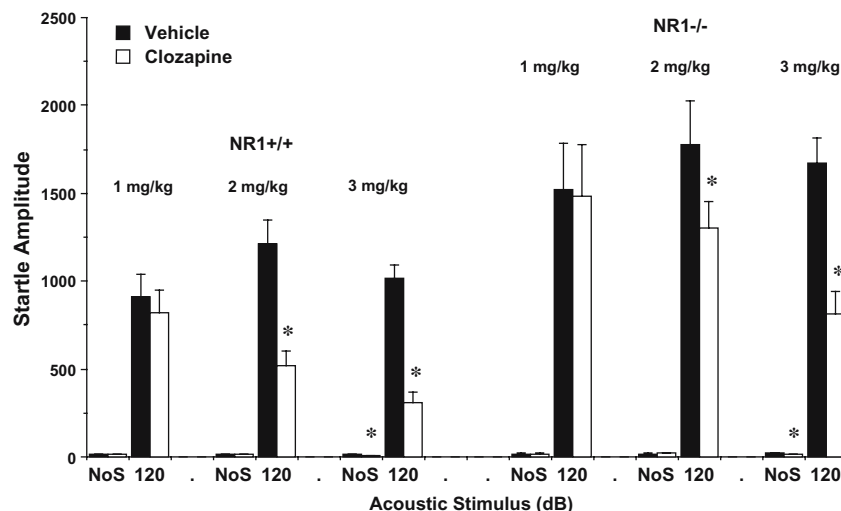
### Effects of haloperidol

**Amplitude** As shown in Fig. 2a and c, treatment with haloperidol at both 0.5 and 1.0 mg/kg significantly reduced the startle amplitude in the NR1<sup>+/+</sup> mice [lower dose, main effect of drug,  $F(1,20)=18.6$ ,  $p=0.0003$ , and drug x dB interaction,  $F(1,20)=18.2$ ,  $p=0.0004$ ; higher dose,  $F(1,9)=17.7$ ,  $p=0.0023$  and  $F(1,9)=17.6$ ,  $p=0.0023$ ]. Haloperidol did not change startle amplitudes in the NR1<sup>-/-</sup> mice at either dose tested.

**Prepulse inhibition** As presented in Fig. 2b and d, haloperidol (0.5 and 1.0 mg/kg) induced significant increases in PPI in both the NR1<sup>+/+</sup> and NR1<sup>-/-</sup> groups. The levels of PPI were enhanced by drug treatment in the wild type animals at almost every prepulse sound level [post hoc tests after repeated measures ANOVA, lower dose, main effect of drug,  $F(1,20)=35.7$ ,  $p<0.0001$ , and drug x dB interaction,  $F(4,80)=9.6$ ,  $p<0.0001$ ; and higher dose,  $F(1,9)=15.2$ ,  $p=0.0037$  and  $F(4,36)=3.7$ ,  $p=0.0126$ ]. In the mutant mice, PPI was increased only at the higher prepulse decibel levels (82, 86, and 90 dB) with the 0.5 mg/kg dose of haloperidol [main effect of drug,  $F(1,15)=12.8$ ,  $p=0.0028$ ]. The 1.0 mg/kg dose of haloperidol led to increases at all but the lowest prepulse sound level in the NR1<sup>-/-</sup> group [main effect of drug,  $F(1,9)=8.1$ ,  $p=0.0192$ , and drug x dB interaction,  $F(4,36)=4.0$ ,  $p=0.0088$ ].

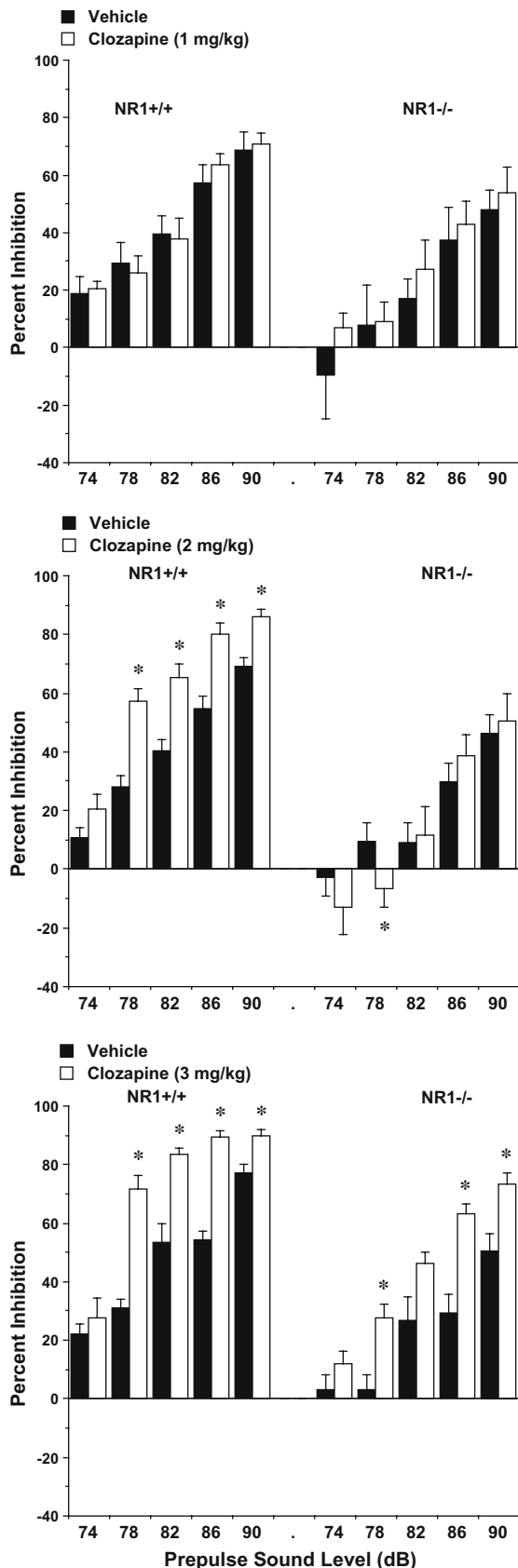
### Effects of clozapine

**Amplitude** As shown in Fig. 3, treatment with clozapine produced dose-dependent effects on startle amplitude in



**Fig. 3** Amplitude of the startle response after treatment with clozapine (1, 2 or 3 mg/kg). Data shown are means (+SEM) for each group. Drug or vehicle was administered 30 min before testing. Trials included no stimulus (NoS) trials and acoustic startle stimulus (120 dB) trials. Separate cohorts were tested for each dose. Subject numbers for each dose were: 1 mg/kg, eight NR1<sup>+/+</sup> (six males and

two females) and six NR1<sup>-/-</sup> (four males and two females); 2 mg/kg, 14 NR1<sup>+/+</sup> (seven males and seven females) and ten NR1<sup>-/-</sup> (five males and five females); 3 mg/kg, 13 NR1<sup>+/+</sup> (eight males and five females) and ten NR1<sup>-/-</sup> (six males and four females). \* $p<0.05$ , post hoc comparison at one stimulus intensity level following significant  $F$  value, repeated measures ANOVA



◀ **Fig. 4** Percent prepulse inhibition after treatment with clozapine (1, 2 or 3 mg/kg). Data shown are means (+SEM) for each group. Drug or vehicle was administered 30 min before testing. For subject numbers, see caption for Fig. 3. \* $p < 0.05$ , post hoc comparison at one prepulse intensity level following significant  $F$  value, repeated measures ANOVA

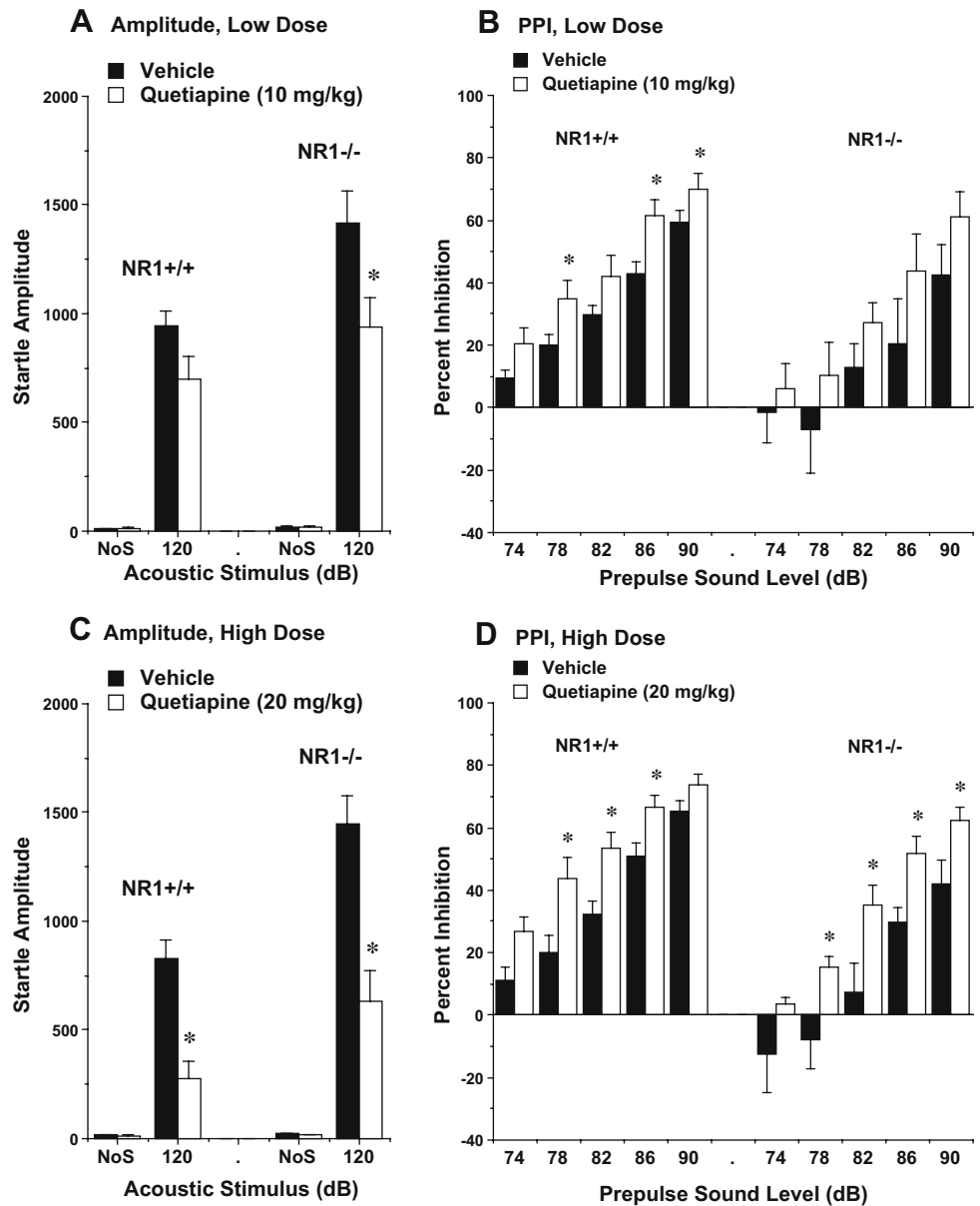
both NR1+/+ and NR1-/- mice. The lowest dose (1 mg/kg) did not have significant effects on amplitude in either the NR1+/+ or NR1-/- mice. At a dose of 2 mg/kg, amplitude was reduced in both the wild type group [main effect of drug,  $F(1,13)=21.9$ ,  $p=0.0004$ ; and drug $\times$ dB interaction,  $F(1,13)=22.0$ ;  $p=0.0004$ ] and the mutant group [main effect of drug,  $F(1,9)=9.9$ ,  $p=0.0118$ ; and drug $\times$ dB interaction,  $F(1,9)=10.3$ ,  $p=0.0106$ ]. Post hoc tests indicated that significant drug effects were evident only for the acoustic startle stimulus, and not for baseline amplitude.

The highest dose of clozapine (3 mg/kg) significantly reduced the response amplitudes for the baseline activity (with no presentation of the acoustic stimulus) and for startle after the acoustic stimulus in both the NR1+/+ animals [main effect of drug,  $F(1,12)=120.4$ ,  $p < 0.0001$  and drug $\times$ dB interaction,  $F(1,12)=117.8$ ,  $p < 0.0001$ ] and the NR1-/- mice [main effect of drug,  $F(1,9)=25.4$ ,  $p=0.0007$ , and drug $\times$ dB interaction,  $F(1,9)=25.6$ ,  $p=0.0007$ ].

**Prepulse inhibition** The results for the three doses of clozapine tested are presented in Fig. 4. At the lowest dose (1 mg/kg), clozapine did not induce significant changes in either experimental group. At 2 mg/kg, clozapine enhanced the percent inhibition at every prepulse decibel except at the lowest (74 dB) level in the NR1+/+ group [main effect of drug,  $F(1,13)=21.9$ ,  $p=0.0004$ ; and drug $\times$ dB interaction,  $F(4,52)=6.8$ ,  $p=0.0002$ ]. In contrast to this effect in the wild type mice, in the mutant mice the 2 mg/kg dose of clozapine did not alter PPI in four of the five prepulse levels tested and led to a significant decrease in PPI at one dB level [drug $\times$ dB level interaction,  $F(4,36)=4.6$ ,  $p=0.0044$ ].

Both NR1+/+ and NR1-/- mice showed enhanced levels of PPI at the highest clozapine dose (3 mg/kg) with more change detected in the wild type mice. As observed with the 2 mg/kg dose, clozapine enhanced the percent inhibition at every prepulse decibel level except at the lowest (74 dB) in the NR1+/+ group [main effect of drug,  $F(1,12)=27.5$ ,  $p=0.0002$ , and drug $\times$ dB interaction,  $F(4,48)=13.3$ ,  $p < 0.0001$ ]. It is notable that the high degree of percent inhibition seen with the two highest dB levels may reflect a ceiling on further increases in this group. The mutant animals also showed significant enhancements in PPI at the 3 mg/kg dose of clozapine [main effect of drug,  $F(1,9)=9.3$ ,  $p=0.0136$ , and drug $\times$ dB interaction,  $F(4,36)=3.2$ ,  $p=0.0225$ ], and post hoc comparisons indicated significant effects at three of the five prepulse sound levels.

**Fig. 5** Amplitude of the startle response (a, c) and percent prepulse inhibition (b, d) after treatment with quetiapine (10 and 20 mg/kg). Data shown are means (+ SEM) for each group. Drug or vehicle was administered 30 min before testing. Trials included no stimulus (NoS) trials and acoustic startle stimulus (120 dB) trials. Subject numbers for the low dose were 13 NR1<sup>+/+</sup> and eight NR1<sup>-/-</sup> (all female) mice. Subject numbers for the high dose were ten NR1<sup>+/+</sup> (four males and six females) and nine NR1<sup>-/-</sup> (four males and five females). \* $p < 0.05$ , post hoc comparison at one stimulus intensity level following significant  $F$  value, repeated measures ANOVA



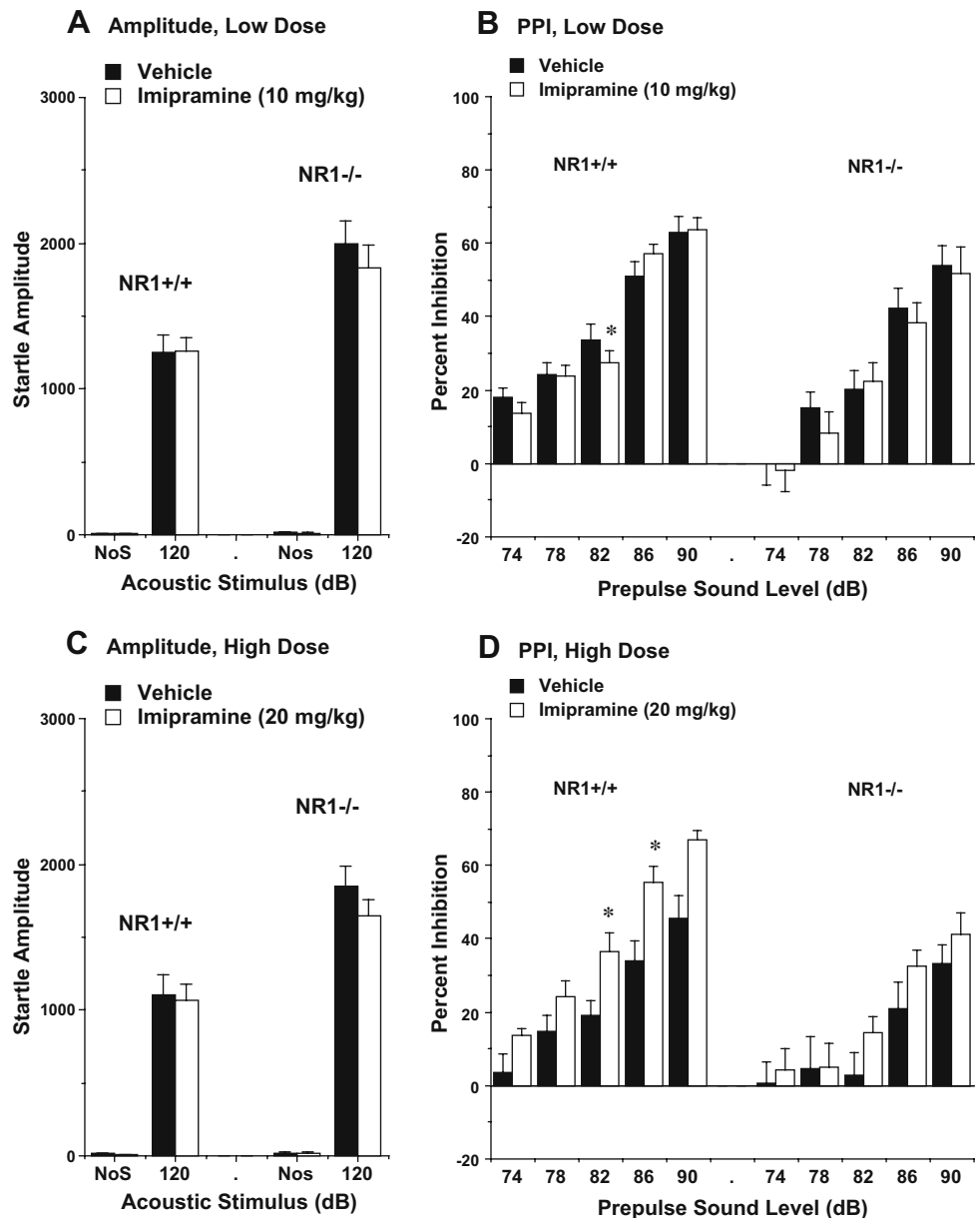
## Effects of quetiapine

**Amplitude** Quetiapine significantly reduced the startle response amplitudes to the acoustic stimulus in both experimental groups (Fig. 5a and c). This effect was dose-dependent in the wild type animals. At the lower dose tested (10 mg/kg), the effect of quetiapine closely approached significance in the NR1<sup>+/+</sup> group [main effect of drug,  $F(1,12)=4.5$ ,  $p=0.0557$ ]. At the higher dose (20 mg/kg), quetiapine reduced the startle response to the acoustic stimulus in the wild type mice [main effect of drug,  $F(1,9)=14.3$ ,  $p=0.0043$ , and drug $\times$ dB interaction,  $F(1,9)=14.5$ ,  $p=0.0042$ ]. Significant decreases in amplitude were observed in the NR1<sup>-/-</sup> mice with both doses

of quetiapine: 10 mg/kg [main effect of drug,  $F(1,7)=10.9$ ,  $p=0.0131$ , and drug $\times$ dB interaction,  $F(1,7)=10.7$ ,  $p=0.0138$ ] and 20 mg/kg: [ $F(1,8)=24.3$ ,  $p=0.0011$  and  $F(1,8)=23.6$ ,  $p=0.0013$ ].

**Prepulse inhibition** Quetiapine significantly enhanced the PPI of the startle response to an acoustic stimulus at both 10 and 20 mg/kg doses in the wild type mice [low dose, main effect of drug,  $F(1,12)=6.1$ ,  $p=0.0297$ ; high dose,  $F(1,9)=6.8$ ,  $p=0.028$ ], but only at the higher dose in the mutant animals (Fig. 5b and d). At the 20 mg/kg dose of quetiapine, the mutant animals had enhanced PPI at all but the lowest prepulse sound level [main effect of drug,  $F(1,8)=8.6$ ,  $p=0.019$ ].

**Fig. 6** Amplitude of the startle response (**a, c**) and prepulse inhibition (**b, d**) after treatment with imipramine (10 and 20 mg/kg). Data shown are means (+SEM) for each group. Drug or vehicle was administered 30 min before testing. Trials included no stimulus (NoS) and acoustic startle stimulus (120 dB) trials. Subject numbers for the low dose were ten NR1<sup>+/+</sup> and ten NR1<sup>-/-</sup> (all male) mice. Subject numbers for the high dose were nine NR1<sup>+/+</sup> and nine NR1<sup>-/-</sup> (five males and four females in each group). \* $p < 0.05$ , post hoc comparison at one stimulus intensity level following significant  $F$  value, repeated measures ANOVA



## Effects of imipramine

**Amplitude** Imipramine had no significant effects on startle amplitude in either the wild type or mutant mice at the two doses tested (Fig. 6a and c).

**Prepulse inhibition** The NR1<sup>+/+</sup> group showed a small, but significant, decrease in percent inhibition at one prepulse sound level (82 dB) at the lower dose (10 mg/kg) of imipramine [drug×dB level interaction,  $F(4,36)=3.0$ ,  $p=0.0301$ ]. In contrast, imipramine increased PPI at the higher dose in the wild type animals [main effect of drug,  $F(1,8)=10.6$ ,  $p=0.0116$ ], but had no significant effects in the mutant animals at either dose (Fig. 6b and d).

## Discussion

The PPI paradigm in mice provides a model system that may be directly relevant to the deficits in PPI observed in schizophrenia patients. As previously reported, robust reductions in PPI were observed in NR1<sup>-/-</sup> mice (Duncan et al. 2004). The present study was designed to assess the effects of antipsychotic drugs with differing pharmacology on alterations in acoustic startle and PPI in the mutant mice. All of the antipsychotics tested increased PPI in both wild type and NR1<sup>-/-</sup> mice at the highest doses examined. For clozapine and quetiapine, the doses that effectively increased PPI in the wild type did not significantly increase PPI in the mutant mice. These data suggest reduced sensitivity of the NR1<sup>-/-</sup> mice to the PPI-enhancing actions of



these drugs. The relationship of these findings to the under expression of NR1 subunits in the mutants will require further study.

The antidepressant drug imipramine, at doses of 10–20 mg/kg, did not alter PPI in the NR1<sup>-/-</sup> mice, while the higher dose did increase PPI in the wild type mice. Imipramine was selected for study as a comparator nonantipsychotic drug that has sedative and other side-effect properties related to anticholinergic and antihistaminic actions similar to clozapine. The dose range of imipramine tested was chosen to represent moderate and high doses based on efficacy in mouse models of antidepressant activity. The inability of imipramine to reverse the PPI deficits in the NR1<sup>-/-</sup> mice suggests some specificity for antipsychotic drugs in the model. However, before firm conclusions can be drawn regarding antipsychotic specificity for the model, it will be important to examine a range of drugs from other therapeutic classes. In addition, it will be important to determine if imipramine is effective in tests of antidepressant activity in the mutant mice.

It was surprising that haloperidol increased PPI in the NR1<sup>-/-</sup> mice since the drug is inactive or less active than clozapine and second-generation antipsychotic drugs in numerous NMDA antagonist challenge models (Arvanov et al. 1997; Arvanov and Wang 1999; Bakshi et al. 1994; Corbett et al. 1995; Duncan et al. 1998; Duncan et al. 2000). Although haloperidol increased PPI to a similar extent as clozapine and quetiapine, the latter drugs attenuated the exaggerated startle response in the NR1<sup>-/-</sup> mice, whereas haloperidol did not. For clozapine and quetiapine, the effects of the drugs on startle response magnitude and PPI were to essentially change the measures to levels seen in the wild type mice, in a dose-dependent way.

For haloperidol, D2 dopamine receptor blockade is most likely responsible for the observed effects on PPI in the NR1<sup>-/-</sup> and NR1<sup>+/+</sup> mice. For clozapine and quetiapine, it is likely that pharmacological properties other than D2 blockade contribute to the effects of the drugs, since reduction in startle reactivity and increased PPI were found in both strains. As noted previously, the effects of clozapine and quetiapine were apparent in wild type mice at lower doses than in the mutant mice. The relationship of these later findings to the under expression of NR1 subunits in the mutants will require further study.

The increase in startle magnitude observed in the NR1<sup>-/-</sup> mice does not reflect the startle responsiveness of schizophrenia patients who do not generally exhibit increased acoustic startle reactivity under standardized testing conditions (Geyer et al. 2001). In the NR1<sup>-/-</sup> mice, it is unlikely that reduced PPI is simply related to the increased startle reactivity. The relationship between startle reactivity and PPI under different experimental conditions is 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 the present study, haloperidol increased PPI in the NR1<sup>-/-</sup> mice, but did not alter the exaggerated startle reactivity in the mutants when no prepulse was presented.

The effects of antipsychotic drugs on PPI in the NR1<sup>-/-</sup> mice are consistent with studies on rodents showing that atypical antipsychotic drugs can attenuate the disruptive effects of NMDA antagonists on PPI (Bakshi and Geyer 1995; Bakshi et al. 1994; Brody et al. 2004; Mansbach et al. 2001; Swerdlow et al. 1998; Swerdlow et al. 1996). The increased PPI in the wild type mice after the administration of antipsychotics in the present study is in accord with other studies demonstrating the effects of antipsychotics on baseline PPI in different strains of mice (Lipina et al. 2005; McCaughran et al. 1997; Olivier et al. 2001; Ouagazzal et al. 2001).

In addition to the NR1 hypomorphic mice, there have been several other genetic mouse models created to produce NMDA and metabotropic glutamate receptor hypofunction. Mice with dysfunction of the NMDA receptor have been created by the deletion of the NR2A ( $\epsilon 1$ ) 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 used 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 nonhabituating 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. 2003, 2004). In the mGluR1 knockout mice, a D2 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. 2003). For the mGluR5 knockout mice, raclopride, clozapine, or lamotrigine did not alter the PPI deficits (Brody et al. 2004).

In conclusion, the NR1 hypomorphic mice provide a genetic model of reduced NMDA receptor function demonstrating intrinsic deficits in PPI that can be attenuated by a range of clinically effective antipsychotic drugs. Unlike models involving challenge with NMDA antagonists, the genetic model of reduced NMDA receptor function did not evidence differential sensitivity for the effects of typical vs atypical antipsychotic agents. However, it is possible that the NR1 hypomorphic mouse model may provide a way to test potential therapeutic agents for schizophrenia that work by novel (non-dopaminergic) mechanisms. All of the currently available antipsychotic drugs have actions at dopamine receptors, but all of such drugs were developed and identified in part from their actions in screens for antidopaminergic activity. As speculated, the identification of drugs that work by non-dopaminergic mechanisms to correct the PPI deficits in the NR1 subunit-deficient mice could promote the discovery of agents that have clinical properties different from and better than currently used antipsychotic drugs.

**Acknowledgements** This research was supported by MH063398, the UNC Neurodevelopmental Disorders Research Center (HD03110), the UNC Silvio O. Conte Center for the Neuroscience of Mental Disorders (MH064065), and was given a grant from the Investigator Sponsored Studies Program of AstraZeneca.

## References

- Acri JB, Morse DE, Popke EJ, Grunberg NE (1994) Nicotine increases sensory gating measured as inhibition of the acoustic startle reflex in rats. *Psychopharmacology* 114:369–374
- Angrist B, Rotrosen J, Gershon S (1980) Responses to apomorphine, amphetamine, and neuroleptics in schizophrenic subjects. *Psychopharmacology* 67:31–38
- Arvanov VL, Liang X, Schwartz J, Grossman S, Wang RY (1997) Clozapine and haloperidol modulate N-methyl-D-aspartate- and non-N-methyl-D-aspartate receptor-mediated neurotransmission in rat prefrontal cortical neurons in vitro. *J Pharmacol Exp Ther* 283:226–234
- Arvanov VL, Wang RY (1999) Clozapine, but not haloperidol, prevents the functional hyperactivity of N-methyl-D-aspartate receptors in rat cortical neurons induced by subchronic administration of phencyclidine. *J Pharmacol Exp Ther* 289:1000–1006
- Bai F, Li X, Clay M, Lindstrom T, Skolnick P (2001) Intra- and interstrain differences in models of “behavioral despair”. *Pharmacol Biochem Behav* 70:187–192
- Bakshi VP, Geyer MA (1995) Antagonism of phencyclidine-induced deficits in prepulse inhibition by the putative atypical antipsychotic olanzapine. *Psychopharmacology* 122:198–201
- Bakshi VP, Swerdlow NR, Geyer MA (1994) Clozapine antagonizes phencyclidine-induced deficits in sensorimotor gating of the startle response. *J Pharmacol Exp Ther* 271:787–794
- Ballard TM, Pauly-Evers M, Higgins GA, Ouagazzal AM, Mutel V, Borroni E, Kemp JA, Bluethmann H, Kew JNC (2002) Severe impairment of NMDA receptor function in mice carrying targeted point mutations in the glycine binding site results in drug-resistant nonhabituating hyperactivity. *J Neurosci* 22:6713–6723
- Braff DL, Geyer MA, Swerdlow NR (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology* 156:234–258
- Brody SA, Conquet F, Geyer MA (2003) Disruption of prepulse inhibition in mice lacking mGluR1. *Eur J Neurosci* 18:3361–3366
- Brody SA, Conquet F, Geyer MA (2004) Effect of antipsychotic treatment on the prepulse inhibition deficit of mGluR5 knockout mice. *Psychopharmacology* 172:187–195
- Cohen BD, Rosenbaum G, Luby ED, Gottlieb JS (1962) Comparison of phencyclidine hydrochloride (sernyl) with other drugs: simulation of schizophrenic performance with phencyclidine hydrochloride (sernyl) lysergic acid diethylamide (LSD-25), and amobarbital (Amytal) sodium, II — symbolic and sequential thinking. *Arch Gen Psychiatry* 6:79–85
- Corbett R, Camacho F, Woods AT, Kerman LL, Fishkin RJ, Brooks K, Dunn RW (1995) Antipsychotic agents antagonize non-competitive N-methyl-D-aspartate antagonist-induced behaviors. *Psychopharmacology* 120:67–74
- David DJP, Renard CE, Jolliet P, Hascoet M, Bourin M (2003) Antidepressant-like effects in various mice strains in the forced swimming test. *Psychopharmacology* 166:373–382
- Duncan GE, Leipzig JN, Mailman RB, Lieberman JA (1998) Differential effects of clozapine and haloperidol on ketamine-induced brain metabolic activation. *Brain Res* 812:65–75
- Duncan GE, Miyamoto S, Gu HB, Lieberman JA, Koller BH, Snouwaert JN (2002) Alterations in regional brain metabolism in genetic and pharmacological models of reduced NMDA receptor function. *Brain Res* 951:166–176
- Duncan GE, Miyamoto S, Leipzig JN, Lieberman JA (2000) Comparison of the effects of clozapine, risperidone, and olanzapine on ketamine-induced alterations in regional brain metabolism. *J Pharmacol Exp Ther* 293:8–14
- Duncan GE, Moy SS, Perez A, Eddy DM, Zinzow WM, Lieberman JA, Snouwaert JN, Koller BH (2004) Deficits in sensorimotor gating and tests of social behavior in a genetic model of reduced NMDA receptor function. *Behav Brain Res* 153:507–519
- Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology* 156:117–154
- Goff DC, Tsai G, Levitt J, Amico E, Manoach D, Schoenfeld DA, Hayden DL, McCarley R, Coyle JT (1999) A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. *Arch Gen Psychiatry* 56:21–27
- Goff DC, Tsai G, Manoach DS, Coyle JT (1995) Dose-finding trial of D-cycloserine added to neuroleptics for negative symptoms in schizophrenia. *Am J Psychiatry* 152:1213–1215
- Heresco-Levy U, Ermilov M, Shimoni J, Shapira B, Silipo G, Javitt DC (2002) Placebo-controlled trial of D-cycloserine added to conventional neuroleptics, olanzapine, or risperidone in schizophrenia. *Am J Psychiatry* 159:480–482
- Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Horowitz A, Kelly D (1996) Double-blind, placebo-controlled, crossover trial of glycine adjuvant therapy for treatment-resistant schizophrenia. *Br J Psychiatry* 169:610–617
- Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Silipo G, Lichtenstein M (1999) Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. *Arch Gen Psychiatry* 56:29–36
- Javitt DC, Zylberman I, Zukin SR, Heresco-Levy U, Lindenmayer JP (1994) Amelioration of negative symptoms in schizophrenia by glycine. *Am J Psychiatry* 151:1234–1236
- Keith VA, Mansbach RS, Geyer MA (1991) Failure of haloperidol to block the effects of phencyclidine and dizocilpine on prepulse inhibition of startle. *Biol Psychiatry* 30:557–566
- Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB Jr, Charney DS (1994) Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry* 51:199–214
- Lahti AC, Holcomb HH, Medoff DR, Tamminga CA (1995a) Ketamine activates psychosis and alters limbic blood flow in schizophrenia. *Neuroreport* 6:869–872
- Lahti AC, Koffel B, LaPorte D, Tamminga CA (1995b) Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology* 13:9–19
- Lahti AC, Weiler MA, Tamara MB, Parwani A, Tamminga CA (2001) Effects of ketamine in normal and schizophrenic volunteers. *Neuropsychopharmacology* 25:455–467
- Leeson PD, Iversen LL (1994) The glycine site on the NMDA receptor: structure-activity relationships and therapeutic potential. *J Med Chem* 37:4053–4067
- Lieberman JA, Kane JM, Alvir JAJ (1987) Provocative tests with psychostimulant drugs in schizophrenia. *Psychopharmacology* 91:415–433
- Lipina T, Labrie V, Weiner I, Roder J (2005) Modulators of the glycine site on NMDA receptors, D-serine and ALX 5407, display similar beneficial effects to clozapine in mouse models of schizophrenia. *Psychopharmacology* 179:54–67
- Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, Kelley R (1959) Study of a new schizophrenomimetic drug-sernyl. *Arch Neurol Psych* 81:363–369
- Malhotra AK, Pinals DA, Adler CM, Elman I, Clifton A, Pickar D, Breier A (1997) Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. *Neuropsychopharmacology* 17:141–150

- Malhotra AK, Pinals DA, Weingartner H, Sirocco K, Missar CD, Pickar D, Breier A (1996) NMDA receptor function and human cognition — the effects of ketamine in healthy volunteers. *Neuropsychopharmacology* 14:301–307
- Mansbach RS, Carver J, Zorn SH (2001) Blockade of drug-induced deficits in prepulse inhibition of acoustic startle by ziprasidone. *Pharmacol Biochem Behav* 69:535–542
- McCaughran J, Mahjubi E, Decena E, Hitzemann R (1997) Genetics, haloperidol induced catalepsy and haloperidol-induced changes in acoustic startle and prepulse inhibition. *Psychopharmacology* 134:131–139
- Miyamoto Y, Yamada K, Noda Y, Mori H, Mishina M, Nabeshima T (2001) Hyperfunction of dopaminergic and serotonergic neuronal systems in mice lacking the NMDA receptor epsilon 1 subunit. *J Neurosci* 21:750–757
- Mohn AR, Gainetdinov RR, Caron MG, Koller BH (1999) Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. *Cell* 98:427–436
- Olivier B, Leahy C, Mullen T, Paylor R, Groppi VE, Sarnyai Z, Brunner D (2001) The DBA/2J strain and prepulse inhibition of startle: a model system to test antipsychotics? *Psychopharmacology* 156:284–290
- Ouagazzal AM, Jenck F, Moreau JL (2001) Drug-induced potentiation of prepulse inhibition of acoustic startle reflex in mice: a model for detecting antipsychotic activity? *Psychopharmacology* 156:273–283
- Paylor R, Crawley JN (1997) Inbred strain differences in prepulse inhibition of the mouse startle response. *Psychopharmacology* 132:169–180
- Schreiber R, Dalmus M, De Vry J (2002) Effects of alpha(4)/beta(2)- and alpha(7)-nicotine acetylcholine receptor agonists on prepulse inhibition of the acoustic startle response in rats and mice. *Psychopharmacology* 159:248–257
- Simon VM, Parra A, Minarro J, Arenas MC, Vinader-Caerols C, Aguilar MA (2000) Predicting how equipotent doses of chlorpromazine, haloperidol, sulpiride, raclopride and clozapine reduce locomotor activity in mice. *Eur Neuropsychopharmacol* 10:159–164
- Swerdlow NR, Bakshi V, Waikar M, Taaid N, Geyer MA (1998) Seroquel, clozapine and chlorpromazine restore sensorimotor gating in ketamine-treated rats. *Psychopharmacologia* 140:75–80
- Swerdlow NR, Bakshi VP, Geyer MA (1996) Seroquel restores sensorimotor gating in phencyclidine-treated rats. *J Pharmacol Exp Ther* 279:1290–1299
- Tada M, Shirakawa K, Matsuoka N, Mutoh S (2004) Combined treatment of quetiapine with haloperidol in animal models of antipsychotic effect and extrapyramidal side effects: comparison with risperidone and chlorpromazine. *Psychopharmacology* 176:94–100
- Tsai G, Pinchen Y, Chung L-C, Lange N, Coyle JT (1998) D-serine added to antipsychotics for the treatment of schizophrenia. *Biol Psychiatry* 44:1081–1089
- Zhang W, Bymaster FP (1999) The in vivo effects of olanzapine and other antipsychotic agents on receptor occupancy and antagonism of dopamine D-1, D-2, D-3, 5HT(2A) and muscarinic receptors. *Psychopharmacology* 141:267–278