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Preweaning Sensorimotor Deficits and Adolescent Hypersociability in *Grin1* Knockdown Mice

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Abstract

Mice with knockdown of the *N*-methyl-D-aspartate (NMDA) receptor NR1 subunit, encoded by the gene *Grin1*, have been investigated as a model for the intrinsic NMDA hypofunction hypothesized for schizophrenia. Previous work has shown that adult *Grin1* mutant mice have overt deficits in habituation and sensorimotor gating, exaggerated reactivity to environmental stimuli, reduced social approach, and other alterations that reflect behavioral manifestations of schizophrenia. In humans, the emergence of overt symptoms of the disorder typically occurs in adolescence or early adulthood, suggesting a role for aberrant maturation of NMDA receptor signaling in symptom onset. The following study evaluated *Grin1* mutant mice for abnormal behavioral phenotypes during the preweaning, adolescent, and adult periods. Measures included open field activity, prepulse inhibition of acoustic startle responses, and social preference in a three-chamber choice task. Mice from the C57BL/6J inbred strain, one of the parental strains for the *Grin1* line, were also tested. The results showed that developmental reduction of NMDA receptor function led to significant alterations in behavior during the second and third weeks of life, including exaggerated startle responses and sensorimotor gating deficits on postnatal day 13, and pronounced hypersociability in adolescence. Male *Grin1* mutant mice were more susceptible than female mice to the detrimental effects of decreased NMDA signaling. Overall, these findings provide evidence that reduced *Grin1* function leads to abnormal phenotypes in the preweaning period, and that deficient NMDA signaling can lead to both overt hypersociability or marked asociality, dependent upon sex and age.

Keywords

hyperactivity; impulsive behavior; NMDA receptor; postnatal; prepulse inhibition; schizophrenia; social approach; startle

Introduction

Antagonists of the *N*-methyl-D-aspartate (NMDA) receptor, such as ketamine and phencyclidine, can induce a schizophrenia-like syndrome in adults, suggesting that disruption of NMDA receptor signaling could play a role in symptoms of schizophrenia [1–5]. The psychotomimetic effects of ketamine, a dissociative anesthetic, appear to be age-

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dependent. A substantial percentage of adults over the age of 30 show adverse emergence reactions following ketamine anesthesia, while children and adolescents have a much lower incidence of detrimental psychological effects [6]. Interestingly, symptoms of schizophrenia typically emerge in late adolescence and early adulthood [7], the time points of increasing susceptibility to ketamine effects, implicating aberrant maturation of neurocircuitry involving NMDA receptor signaling in onset of the disorder [5].

Mice with reduced levels of the obligatory NR1 subunit (*Grin1*) of the NMDA receptor have been proposed as an animal model for intrinsic NMDA hypofunction [8–11]. These mice show profound changes in behavior that parallel symptoms in schizophrenia, including deficits in prepulse inhibition of acoustic startle responses and reduced social approach [8–13]. *Grin1* knockdown mice also have altered exploration in novel environments, exaggerated startle responses, and perseverative over-grooming [8–10,12,14]. Further, in a resident-intruder assay for social interaction and aggression, the *Grin1* mutant mice show significantly blunted neuronal activation across several brain regions, including cingulate cortex, amygdala, and the dentate gyrus of the hippocampal formation [15].

Although these studies have clearly established aberrant behavioral profiles in adult mice with reduced *Grin1* expression, a remaining question is whether the onset of phenotypic changes has a developmental time course that reflects the typical emergence of symptoms in schizophrenia. A recent intriguing report by Ramsey and colleagues [16] showed that *Grin1* knockdown mice have significant decreases in striatal spine density, a marker for synapse number, during the adolescent period (postnatal day (PD) 40), but not on PD14, providing evidence for detrimental effects of abnormal maturation of NMDA receptor signaling. There is additional evidence from other mouse models that the postweaning stage in mice (PD24 to PD28) could be a sensitive period for disruption of sensorimotor gating. Nakamura et al. [17], using a panel of inbred mouse strains, investigated the role of tryptophan hydroxylase in the maturation of prepulse inhibition. The researchers found that intact serotonergic function was critical for the development of prepulse inhibition during the postweaning period, but not the preweaning period. Similarly, the emergence of abnormal startle reactivity in *Fmr1*-null mice, a model of fragile X syndrome, does not occur until the third or fourth postnatal week [18].

The focus of the present studies was to determine whether aberrant behavioral phenotypes were present in preweaning or adolescent *Grin1* mice, which could be informative for maturational events in NMDA receptor signaling relevant to schizophrenia onset, or for the more subtle prodromal changes evident before first episodes of the disorder. We conducted the startle test at PD13, when startle responses to acoustic stimuli are often first evident in mouse pups, and again at PD17, when significant maturation of the auditory system has occurred [17–21]. Open field tests were conducted at PD12 and PD18-20, to encompass a period when mouse pups show rapid development of locomotor abilities (i.e. [22]). Social approach was examined on PD34-38, in periadolescence, before the post-puberty period of late adolescence and young adulthood that, in humans, is associated with increased vulnerability to schizophrenia [7]. Finally, the open field and social approach tests were conducted in a separate set of adult animals, to confirm previous findings of abnormal exploration and deficient sociability in *Grin1* mutant mice [8–10,28].

Methods

Subjects

NR1-NMDA receptor subunit (*Grin1*) knockdown mice and littermate controls served as subjects. The mutant mice were engineered by incorporating a neomysin resistance gene (neo) into intron 20 of the *Grin1* locus, leading to an underexpression, but not elimination,

of the *Grin1* gene [10]. The *Grin1*$^{+/+}$, *Grin1*$^{+/neo}$, and *Grin1*$^{neo/neo}$ subjects for the present studies were generated from heterozygous breeder pairs. Briefly, offspring were F1 hybrid mice, obtained by the intercross of 129S6 (Taconic) -*Grin1*$^{+/neo}$ female mice with C57BL/6-*Grin1*$^{+/neo}$ males. The 129-*Grin1*$^{+/neo}$ mice were coisogenic for the *Grin1*^{neo} mutation. The C57BL/6-*Grin1*$^{+/neo}$ mice were derived from twelve generations of backcrossing to C57BL/6J (Jackson Laboratories, Bar Harbor, Maine). Subjects tested during the preweaning and adolescent periods were taken from 10 litters, while subjects tested in adulthood were taken from 20 litters. In addition, subjects included offspring of 4 litters from C57BL/6J (B6) breeding stock (Jackson Laboratory). The B6 group was evaluated to provide a profile of behavior in one of the parental strains of the *Grin1* line (and not as a direct comparison group for statistical analyses). For all litters, day of birth was considered postnatal day (PD) 0.

Animals were maintained on a 12L:12D circadian schedule with lights on at 7 AM, in a 20–24°C housing room, and provided water and mouse chow *ad libitum*. A small section of PVC pipe was present in each cage for enrichment, and dams were provided cotton nestlets for nesting material. All breeding and testing procedures were conducted in strict compliance with the “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, National Research Council, 1996) and approved by the Institutional Animal Care and Use Committee of the University of North Carolina.

Study Design

One set of mice was tested for exploration in a novel environment and sensorimotor gating during the preweaning period, and social approach in adolescence. All pups were given two tests in the open field, one on PD12 and one on PD18-20, and two tests for acoustic startle responses, one on PD13 and one on PD17. The three-chamber social choice test was conducted on PD34-38. Numbers of mice were 15 *Grin1*$^{+/+}$, 25 *Grin1*$^{+/neo}$, 15 *Grin1*$^{neo/neo}$, and 23 B6 offspring. A separate set of adult mice (21 *Grin1*$^{+/+}$ and 21 *Grin1*$^{neo/neo}$) were tested in the open field and social approach assays at the age of 3–6 months, with 18–24 days between the two procedures. For all groups, both male and female mice were evaluated, with a range of 6–16 of each sex per group.

Open Field Test in Preweaning Mice

On PD12 and again on PD18-20, the litter was removed from the home cage and placed into a beaker. One at a time, each pup was placed into the center of a Phenotyper box (30.5 cm×30.5 cm×43.5 cm; Noldus Information Technology, Wageningen, The Netherlands). The mouse was allowed to acclimate to the apparatus for 30 sec and then freely explore for 10 min., before being returned to a separate beaker. When all pups had been tested, the litter was returned to the home cage. The integrated camera system in the Phenotyper was connected to a computer and the video was scored for distance traveled (cm) in real time using Ethovision tracking software (Noldus Information Technology, Wageningen, The Netherlands). In addition, the experimenter viewed the trial in real time and recorded the number of rearing movements. The Phenotyper box was wiped down with water and dried with a paper towel after every trial, and cleaned with 70% ethanol at the end of each testing day.

Open Field Test in Adult Mice

Exploratory activity in a novel environment was assessed for one hour in a photocell-equipped automated open field (41 cm×41 cm×30 cm height; Versamax system, Accuscan Instruments). Measures were taken of total distance traveled and time spent in the center during the test. Activity chambers were contained inside sound-attenuating boxes, equipped with houselights and fans. Removable floors were washed with hot soapy water between

each trial. Chamber walls were wiped down with water and dried with a paper towel after every trial, and cleaned with 70% ethanol at the end of each testing day.

Acoustic Startle Procedure

The acoustic startle assay 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 published procedures [23,24]. Briefly, mice 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. Sensitivity calibration of the transducer was constant between PD13 and PD17 (i.e. sensitivity was not increased to accommodate smaller pup sizes). The chamber included a houselight, 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).

Each test session consisted of 42 trials, presented following a 5-min habituation period. There were 7 different types of trials: the 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 sec (range: 10 to 20 sec). Measures were taken of the startle amplitude for each trial, defined as the peak response during a 65-msec sampling window that began with the onset of the startle stimulus. Levels of PPI at each prepulse sound level were calculated as 100 - [(response amplitude for prepulse stimulus and startle stimulus together / response amplitude for startle stimulus alone)×100].

Three-chamber social choice test

Mice were tested in an automated three-chamber box, using methods developed by our laboratory [25–27]. Dividing walls had retractable doorways allowing access into each chamber. The automated box had photocells embedded in each doorway to allow quantification of entries and duration in each chamber of the social test box. The chambers of the apparatus were cleaned with water and dried with paper towels between each trial. At the end of each test day, the apparatus was sprayed with 70% ethanol and wiped clean with paper towels.

The choice test had three 10-min phases:

1. *Habituation.* The test mouse was first placed in the middle chamber and allowed to explore, with the doorways into the two side chambers open.
2. *Sociability.* After the habituation period, the test mouse was enclosed in the center compartment of the social test box, and an unfamiliar mouse (stranger 1; a sex-matched C57BL/6J adult) was enclosed in a wire cage (11 cm H, 10.5 bottom diameter, bars spaced 1 cm apart; Galaxy Cup, Spectrum Diversified Designs, Inc., Streetsboro, Ohio) and placed in a side chamber. All stranger mice had been previously habituated to the wire cage across at least five days. The location for stranger 1 alternated between the left and right sides of the social test box across subjects. An empty wire cage was placed in the opposite side, to serve as a novel object control. Following placement of stranger 1, the doors were re-opened, and the subject was allowed to explore the entire social test box. Measures were taken of the amount of time spent in each chamber and the number of entries into each

chamber by the automated testing system. In addition, the amount of time spent sniffing each of the wire cages was recorded by a human observer.

3. *Preference for social novelty.* At the end of the sociability test, each mouse was further tested for preference to spend time with a more-novel stranger. A new unfamiliar mouse (stranger 2; a sex-matched C57BL/6J adult from a home cage different from that of stranger 1) was placed in the wire cage that had been empty during the previous session. The test mouse then had a choice between the first, already-investigated mouse (stranger 1) and the novel unfamiliar mouse (stranger 2). The same measures were taken as with the sociability test.

Statistics

Statview (SAS Inc., Cary, NC) was used for data analyses. Two-way or repeated measures Analysis of Variance (ANOVA) were used to determine effects of genotype (wild type, heterozygous, or *Grin1* knockdown) and sex. Separate analyses for males and females were then conducted, in order to determine the effects of genotype within each sex. Post-hoc comparisons were conducted with Fisher's Protected Least Significant Difference (PLSD) tests following significant effects in the overall ANOVAs. Within-group repeated measures ANOVAs were used to determine side preferences in the social approach assay. Data from the separate set of C57BL/6J mice were not included in the analyses with the *Grin1* mice. For all comparisons, significance was set at $p < 0.05$.

Results

Body Weight (PDs 12, 17, 34–38)

Differences in body weight were observed in the male groups, starting at PD17 (Fig. 1A). The male *Grin1*$^{neo/neo}$ mice were smaller than both the wild type and heterozygous males at both PD17 and PD34-38 [post-hoc tests following main effect of genotype, $F(2,29)=8.03$, $p=0.0017$; genotype×time interaction, $F(4,58)=3.77$, $p=0.0086$]. These significant differences were not found in the female *Grin1* groups (Fig. 1B). In the C57BL/6J mice (Fig. 1C), the male mice weighed significantly more than the female mice by the adolescent period [post-hoc tests following main effect of sex, $F(1,21)=9.37$, $p=0.0059$; sex×time interaction, $F(2,42)=57.8$, $p<0.0001$].

Open Field Test (PDs 12 and 18–20)

Distance traveled—As previously observed in adult *Grin1*$^{neo/neo}$ mice [8,10,28], the loss of NMDA function led to overt increases in locomotor activity (Fig. 2). 2-way ANOVAs confirmed significant main effects of genotype on PD12 [$F(2,49)=14.31$, $p<0.0001$] and PD18-20 [$F(2,49)=66.16$, $p<0.0001$]. Separate analyses for the male and female mice revealed that significant differences were only observed in the male *Grin1* mice on PD12 [$F(2,30)=16.77$, $p<0.0001$], but were seen in both male and female mice on PD18-20 [males, $F(2,30)=98.22$, $p<0.0001$; females, $F(2,19)=13.48$, $p=0.0002$]. The male and female C57BL/6J mouse pups tested at the same ages traveled similar distance in the open field. Overall, there were no significant main effects or interactions for sex in the measures from the open field test.

Time in center regions—In addition to increased ambulation, the *Grin1*$^{neo/neo}$ mice also spent more time in the center of the open field, an indication of decreased anxiety-like behavior (Fig. 3). Significant main effects of genotype were evident on PD12 [$F(2,49)=10.17$, $p=0.0002$] and PD18-20 [$F(2,49)=7.54$, $p=0.0014$]. Although the amount of time spent in the center by the *Grin1*$^{neo/neo}$ mice was higher, dependent on sex and age, than

time spent by the wild type and heterozygous groups, it is notable that the levels were comparable to the highly-exploratory C57BL/6J mice.

Open Field Test in Adult Mice

A separate set of mice (age 3–6 months) were tested in a larger open field than used with the preweaning groups. During the first intervals of the one-hour test, the wild type and mutant mice had similar levels of locomotion (Fig. 4). However, the *Grin1*$^{neo/neo}$ group failed to demonstrate a typical pattern of habituation [main effect of genotype, $F(1,37)=8.71$, $p=0.0055$; genotype×time interaction, $F(11,407)=4.54$, $p<0.0001$]. The *Grin1* knockdown mice also had small, albeit significant, increases in time spent in the center region [main effect of genotype, $F(1,37)=8.21$, $p=0.0068$; data not shown]. The repeated measures ANOVAs did not indicate any significant main effects or interactions for sex in the measures from the open field test.

Acoustic Startle Test (PDs 13 and 17)

Startle response amplitude—Previous studies have found markedly exaggerated startle responses in adult *Grin1*$^{neo/neo}$ mice [8,12,13]. This same enhancement of startle was evident in both male and female mutant groups during the preweaning period (Fig. 5). Repeated measures ANOVAs for startle amplitudes indicated highly significant main effects of genotype [PD13; $F(2,49)=21.28$, $p<0.0001$; PD17; $F(2,49)=17.78$, $p<0.0001$] and genotype×decibel level interactions [PD13; $F(12,294)=7.39$, $p<0.0001$; PD17; $F(12,294)=8.4$, $p<0.0001$]. Overall, there were no significant main effects or interactions for sex on startle amplitudes. In the C57BL/6J group, male and female mice showed similar levels of startle amplitude, with marked increases from PD13 to PD17 (data not shown).

Prepulse inhibition of startle responses—During adulthood, both male and female *Grin1*$^{neo/neo}$ mice have impaired prepulse inhibition [29]. However, in the present study, only the male mutant mice demonstrated the characteristic deficits, and the reductions appeared more severe on PD17, in contrast to the earlier age (Fig. 6). The milder phenotype on PD13 was reflected in the lack of a genotype main effect in the overall repeated measures ANOVA [main genotype effect, $F(2,49)=1.54$, $p=0.2248$; genotype×decibel level interaction, $F(8,196)=2.95$, $p=0.0039$]. However, a strong main effect of genotype was evident four days later, on PD17 [$F(2,49)=7.38$, $p=0.0016$], as well as a genotype×decibel level interaction [$F(8,196)=2.26$, $p=0.0246$]. There was also a significant main effect of sex on PD17 [$F(1,49)=4.06$, $p=0.0495$], in line with the lack of prepulse inhibition deficits in the female *Grin1*$^{neo/neo}$ mice.

As found with the measure for startle amplitude, there were no significant main effects or interactions for sex on percent inhibition in the C57BL/6J groups (data not shown).

Social Approach (PDs 34–38)

Time spent in each side of the three-chamber test box—Overt deficits in social approach and interaction have been reported for adult *Grin1*$^{neo/neo}$ mice [8–10]. However, in the present study, the male mutant mice demonstrated a striking and unexpected enhancement of sociability (Fig. 7). An overall repeated measures confirmed a significant interaction between genotype and side of the test box [$F(2,49)=5.39$, $p=0.0077$]. Within-group comparisons indicated that only the *Grin1*$^{neo/neo}$ males had a significant preference for spending time in the proximity of a stranger mouse, versus an empty cage.

In the subsequent test for social novelty, each mouse was given a choice between spending time in the side with the first stranger mouse, versus a newly-introduced second stranger. Although the repeated measures analyses did not reveal any genotype effects during this

phase of the social approach test, the *Grin1*$^{neo/neo}$ males were the only group to demonstrate a significant preference for the more novel conspecific. Thus, the mutant males not only showed a general increase in social interest, but also recognized and preferred a less-familiar stranger.

Time spent sniffing each cage—The measure for sniffing provides a more specific index of attention directed toward the social targets in the approach test [26,27,30]. In the present study, the results supported an aberrant hypersociability in the adolescent *Grin1*$^{neo/neo}$ males (Fig. 8). Again, the *Grin1*$^{neo/neo}$ males had significantly enhanced approach to the stranger mouse [main effect of genotype, $F(2,49)=3.64$, $p=0.0335$; genotype×side interaction, $F(2,49)=9.89$, $p=0.0002$]. The main effect of sex approached significance [$F(1,49)=3.92$, $p=0.0533$], reflecting the lower levels of sniffing observed in the female mice.

In the test for social novelty preference, significant effects of genotype were evident in both male and female mice, confirming the results from the overall repeated measures ANOVA [main effect of genotype, $F(2,48)=23.4$, $p<0.0001$]. However, the two sexes showed different patterns: the male *Grin1*$^{neo/neo}$ mice again exhibited enhanced preference for the more novel stranger, while the female *Grin1*$^{neo/neo}$ mice had general increases in sniffing directed towards both cages [main effect of sex, $F(1,48)=16.43$, $p=0.0002$; sex×side interaction, $F(1,48)=5.45$, $p=0.0237$].

Entries into each side of the three-chamber test box—Both the male and female NMDA-receptor mutant mice had similar significant increases in number of entries into each side of the test box, indicating that the differential profiles for social preference were not dependent upon differential levels of exploration or activity during the test (Fig. 9). The overall repeated measures ANOVAs revealed significant main effects of genotype for both the sociability test [$F(2,49)=24.47$, $p<0.0001$] and the preference for social novelty test (data not shown) [$F(2,49)=10.02$, $p=0.0002$]. There were no main effects or interactions for the variables sex or side of chamber for the entries measures.

Social approach in adolescent C57BL/6J mice—As shown in Fig. 10, the C57BL/6J mice showed mild sociability with the measures of time in each side [within-group comparisons following significant effect of side, $F(1,21)=7.85$, $p=0.0107$] and time spent sniffing each cage [within-group comparisons following significant effect of side, $F(1,21)=36.77$, $p<0.0001$]. When the second stranger was introduced to the social test box, the adolescent mice showed a shift toward the more-novel conspecific [time in each side, effect of side, $F(1,21)=9.3$, $p=0.0061$; time spent sniffing each cage, $F(1,21)=21.85$, $p=0.0001$]. Although the within-group comparisons were not consistently significant in both male and female mice, the overall ANOVAs did not reveal any effects of sex on social approach in the C57BL/6J mice. As observed with the *Grin1* groups, no side preferences were evident with the measure for entries.

Social Approach in Adult Mice

In confirmation of previous findings, adult *Grin1*$^{neo/neo}$ mice (3–6 months in age) had significant decreases in social preference (Fig. 11). During the test for sociability, significant main effects of genotype were found for the measure of time spent in each side [$F(1,38)=14.41$, $p=0.0005$] and time spent sniffing each cage [$F(1,38)=15.99$, $p=0.0003$; genotype×side interaction, $F(1,38)=4.42$, $p=0.0422$]. Post-hoc analyses indicated that both male and female *Grin1* knockdown mice spent less time in the proximity of the stranger mouse, in comparison to the wild type mice. In contrast to findings from the open field, the *Grin1*$^{neo/neo}$ mice had reduced exploration in the social approach task, measured by entries

into each side [main genotype effect, F(1,38)=11.22, p=0.0018; genotype×side interaction, F(1,38)=5.98, p=0.0095]. Thus, the adult *Grin1*$^{neo/neo}$ mice did not demonstrate the hypersociability or increased exploration observed in the adolescent mutant mice.

In the test for social novelty preference, neither the male or female groups showed significant preference for the more novel stranger 2, versus the previously-investigated stranger 1 (data not shown). Although the test did not reveal any deficiencies in discrimination, the *Grin1* knockdown mice had general decreases in time spent in the side chambers [main genotype effect, F(1,38)=7.16, p=0.0109], time spent sniffing the two stranger cages [main genotype effect, F(1,38)=5.35, p=0.0263], and entries during the test [main genotype effect, F(1,38)=5.77, p=0.0213].

Discussion

The first episode of schizophrenia typically occurs during late adolescence or early adulthood [7]. Olney and Farber [5] proposed the time of onset for the disorder is linked to maturation of neural circuitry that is dependent upon intact NMDA receptor function. However, the present study found that abnormal behavioral phenotypes were clearly evident on postnatal days (PDs) 12 and 13 in mice with reduced expression of *Grin1*. Preweaning male and female *Grin1* mutant pups exhibited hyperactivity in an open field and marked exaggeration of acoustic startle responses, similar to *Grin1*$^{neo/neo}$ adults. The male *Grin1*$^{neo/neo}$ mice had mild impairment of prepulse inhibition at PD13, and more overt impairment by PD17. Further, the *Grin1*$^{neo/neo}$ males also had overt hypersociability in adolescence, in contrast to the deficits in social approach observed in adult mutant mice in the present study, and in previous reports [8–10].

In humans, the startle reflex can be elicited in newborn infants [31]. However, prepulse inhibition may be reduced or absent in neonates, indicating that further maturation of brain structures is necessary to obtain levels of sensorimotor gating found in school-age children and adults [32–34]. Similarly, startle responses to an acoustic stimulus can be found in mice at the time of ear opening (PDs 12–13) [20], while the time course for maturation of prepulse inhibition can extend beyond the postweaning period [17]. The present study found a marked increase in the magnitude of startle responses across days PD13 to PD17 in C57BL/6J mice, without overt changes in prepulse inhibition, except at the lowest prepulse sound level. Aberrant acoustic startle responses and prepulse inhibition could be observed by PD13 in the male *Grin1*$^{neo/neo}$ mice. These alterations were exacerbated by PD17, suggesting that neural development was further disrupted by the NMDA receptor hypofunction across the four-day span. Only the male *Grin1*$^{neo/neo}$ mice demonstrated significant deficits in prepulse inhibition, although a previous study found similar decreases in prepulse inhibition in adult male and female *Grin1*$^{neo/neo}$ mice [29]. Overall, male mutant mice had a more severe phenotype than the females for most measures. These findings are in line with results from epidemiological studies on schizophrenia prevalence rates, which suggest that males have an earlier onset of psychosis and a more severe symptomatology [35–37].

Previous studies have reported marked social approach deficits in adult *Grin1*$^{neo/neo}$ mice [8–10], as well as mice with decreased NMDA receptor glycine affinity (*Grin1*^{D481N} mice) [38]. Therefore, we were surprised to find *enhanced* sociability in the mutant mice during the adolescent period. It is possible that an aberrant increase in social approach indicates a lack of normal inhibitory processes governing interaction with unfamiliar and potentially dangerous adult mice. Similar to the findings from the open field test, in which the *Grin1*$^{neo/neo}$ preweaning mice spent more time in the center region, the hypersociability could be an index for deficient environmental constraints on responses, reminiscent of

inappropriate and impulsive behavior. Halene and colleagues [9] also found reduced behavioral inhibition in adult *Grin1*$^{neo/neo}$ mice tested in a novel open field and zero maze, but in their study, the mutant mice had significantly decreased sociability in a three-chamber choice task.

Altered signaling by the schizophrenia candidate gene *Neuregulin 1* (*Nrg1*) has been implicated in the reduced NMDA-receptor function proposed for schizophrenia [39]. We have found that adult male mice with reduced expression of *Nrg1*, or the *Nrg1* receptors, *Erbb3* and *Erbb4*, have significant hypersociability in the three-chamber task [40]. The increased social approach was not accompanied by other behavioral changes observed in the adolescent *Grin1*$^{neo/neo}$ mice, including enhanced preference for social novelty or increased entries during the task. Abnormal increases in sociability have also been reported for mice with deletion of *Dlg4* (PSD-95), a model for dysfunction of excitatory synapses relevant to autism and other neurodevelopmental disorders [41]. Mice with reduced *MeCP2*, a model for Rett syndrome, can show hypersociability in both adolescence (PD 32–34) [42] and adulthood (12–13 weeks in age) [43]. A recent study reported that age-dependent alterations in cortical NMDA receptor density could be found in both post-mortem samples from Rett syndrome subjects, and in the *MeCP2*-null mouse model [44]. Thus, alterations in several genes linked to NMDA receptor function result in a hypersociability phenotype, similar to the increased social approach we observed in the male *Grin1*$^{neo/neo}$ mice.

One goal for the present study was to determine age-dependent vulnerability to disruption of *Grin1*. Other researchers have investigated critical periods in susceptibility to NMDA receptor hypofunction. Belforte et al. [45] used elegant engineering techniques to selectively disrupt *Grin1* in GABAergic neurons during either the postnatal period or postadolescence. The postnatal ablation led to an abnormal behavioral profile starting at 8 weeks of age, including transient hyperlocomotion, decreased center time in an open field, and reduced prepulse inhibition, without concomitant changes in startle amplitudes. These aberrant phenotypes were not observed with the postadolescent ablation, providing evidence that the behavioral effects were dependent upon a developmental dysregulation of NMDA function. As previously noted, Ramsey et al. [16] reported that *Grin1*$^{neo/neo}$ mice have significant loss of synaptic spine density in striatum at 6 weeks of age, but not at 2 weeks. The loss of striatal synapses was associated with a 50% to 80% decrease in synaptic Disc1 (Disrupted-in-Schizophrenia-1) protein in the 6-week-old mice. Notably, the Disc1 depletion was also detected in the neonatal *Grin1*$^{neo/neo}$ mice, although to a lesser extent (25%) than the adolescent mice. Other research groups have found that mutations in *Disc1* can lead to hyperactivity and impaired prepulse inhibition in adult mice [46–48], suggesting that reduced *Disc1* expression in *Grin1*$^{neo/neo}$ mice could play a role in abnormal phenotypes.

The maturational events in the preweaning and adolescent periods include high levels of synaptogenesis in multiple brain regions, followed by refinement and pruning of synaptic connections [49]. Disruption of the processes regulating synapse density has been implicated in the developmental pathology of schizophrenia [50–53]. Given that NMDA receptors have a critical role in dendritic spine motility and density [16,54], NMDA hypofunction could have a profound impact on the maintenance of appropriate synaptic connections. In particular, Hayashi-Takagi et al. [55] have shown that NMDA receptors regulate the ability of Disc1 to stabilize synaptic structural plasticity by binding to Kalirin-7 (Kal-7), which controls a rho-family small GTP-binding protein, Rac1, important for dendritic spine morphology and function. Thus, abnormalities in either NMDA receptor function or Disc1 could be a mechanistic basis for aberrant synaptic refinement and pruning, relevant to the emergence and progression of symptoms in schizophrenia [50].

An important caveat for this study is that repeated testing in behavioral assays could be especially stressful for preweaning mice. In particular, repeated maternal separation has been associated with both short-term and long-term negative effects on mouse offspring, including increased anxiety-like and depression-like responses, alterations in social behavior, and learning deficits [56–58]. Although some researchers have found that negative outcomes from maternal separation can be non-significant or strain-dependent [59–62], it is possible that *Grin1*$^{neo/neo}$ mice could have a differential susceptibility to the effects of preweaning stress, in comparison to wild type mice. Therefore, the hypersociability phenotype in the adolescent mutant mice might be dependent on both genetic and environmental factors, in line with the detrimental interactions between genetic vulnerability and stress proposed for schizophrenia [63–65].

The identification of prodromal indicators for the emergence of schizophrenia could be especially important if early intervention can attenuate progression of the disorder [66]. For example, treatment with an antipsychotic compound, beginning at the first psychotic episode, has been shown to prevent the reductions in gray matter volume associated with schizophrenia [67,68]. In this regard, mouse models of developmental onset are valuable screens to determine preclinical efficacy of early treatment [11,69]. The present results suggest that aberrant phenotypes in preweaning and adolescent *Grin1*$^{neo/neo}$ mice could be used to model pre-morbid abnormalities that signal increased susceptibility for the onset of schizophrenia [70–72].

Acknowledgments

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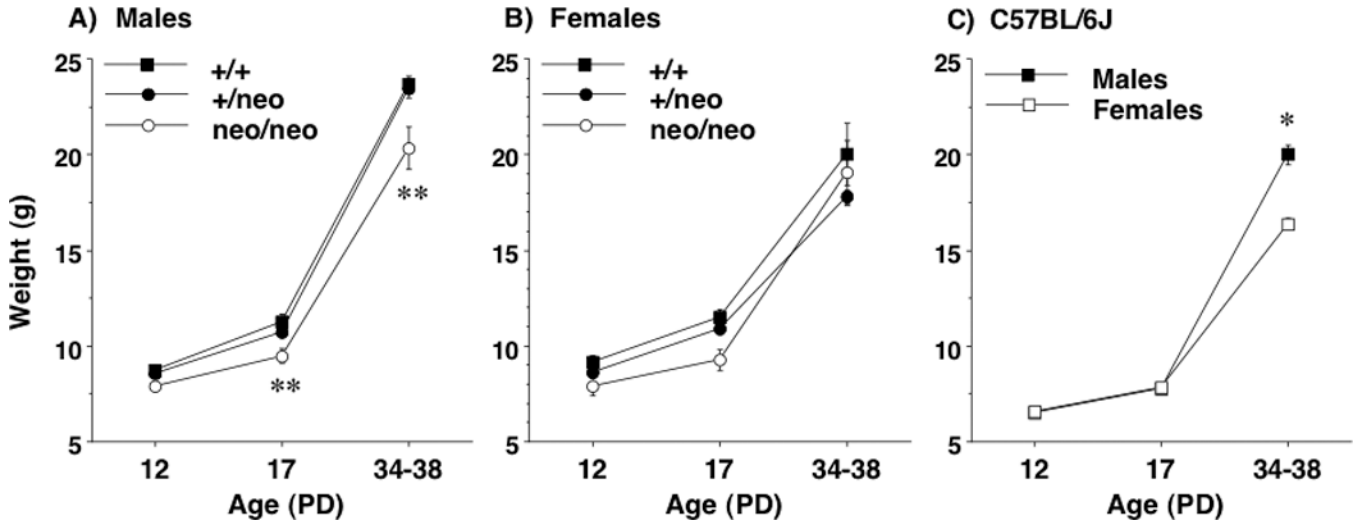

Fig. 1.

Body weight and growth. Data shown are means ± SEM. Numbers of mice tested were 15 *Grin1*$^{+/+}$ (9 males and 6 females), 25 *Grin1*$^{+/neo}$ (16 males and 9 females), 15 *Grin1*$^{neo/neo}$ (8 males and 7 females), and 23 B6 offspring (13 males and 10 females). **p<0.05, comparison to both wild type and heterozygous mice. *p<0.05, comparison to female mice.

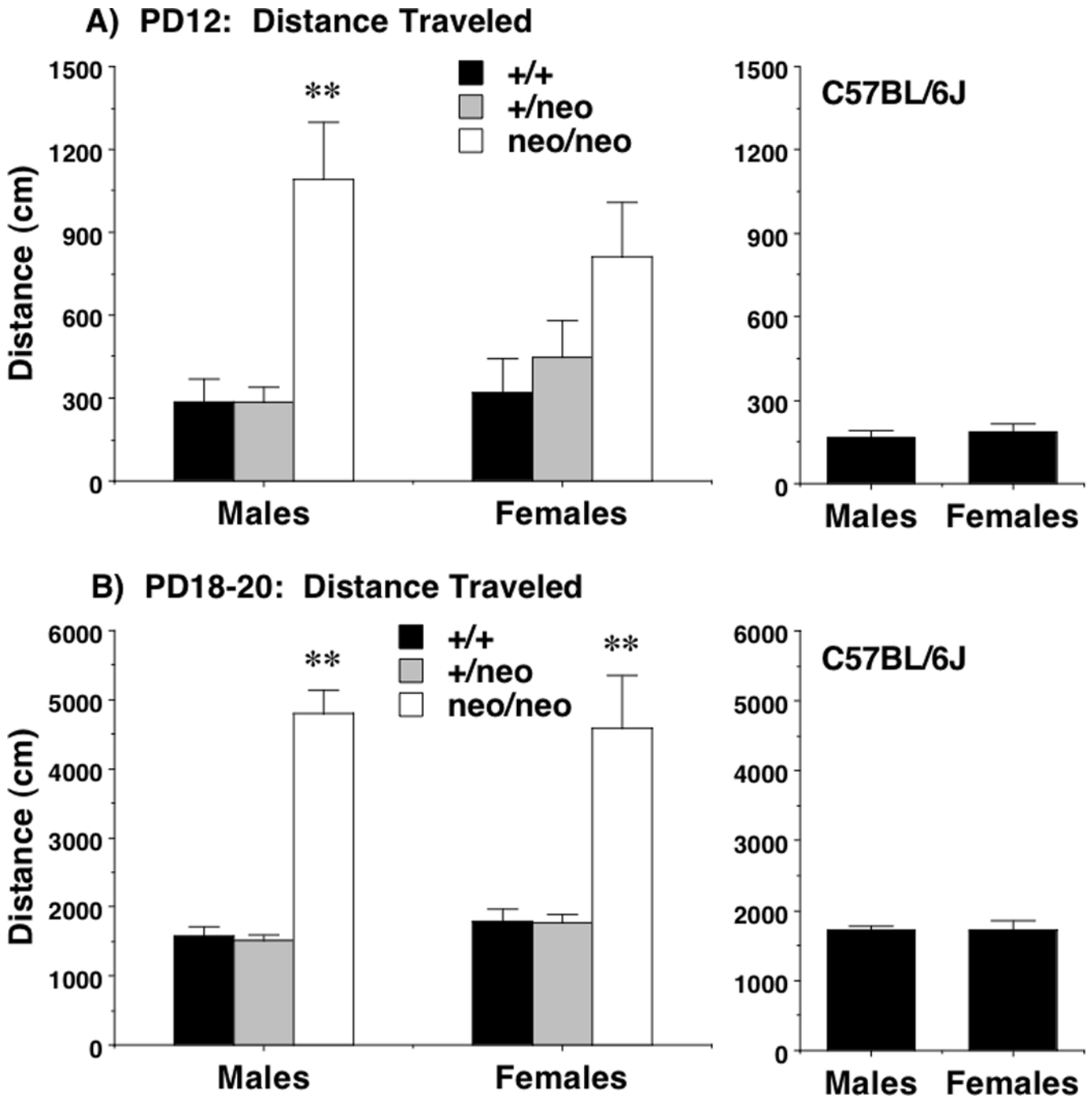

Fig. 2.

Increased distance traveled by preweaning *Grin1*$^{neo/neo}$ mice in an open field. Data shown are means + SEM for a 10-min test. Scales for Panels A and B are different, to accommodate changes across age. **p<0.05, comparison to both wild type and heterozygous mice.

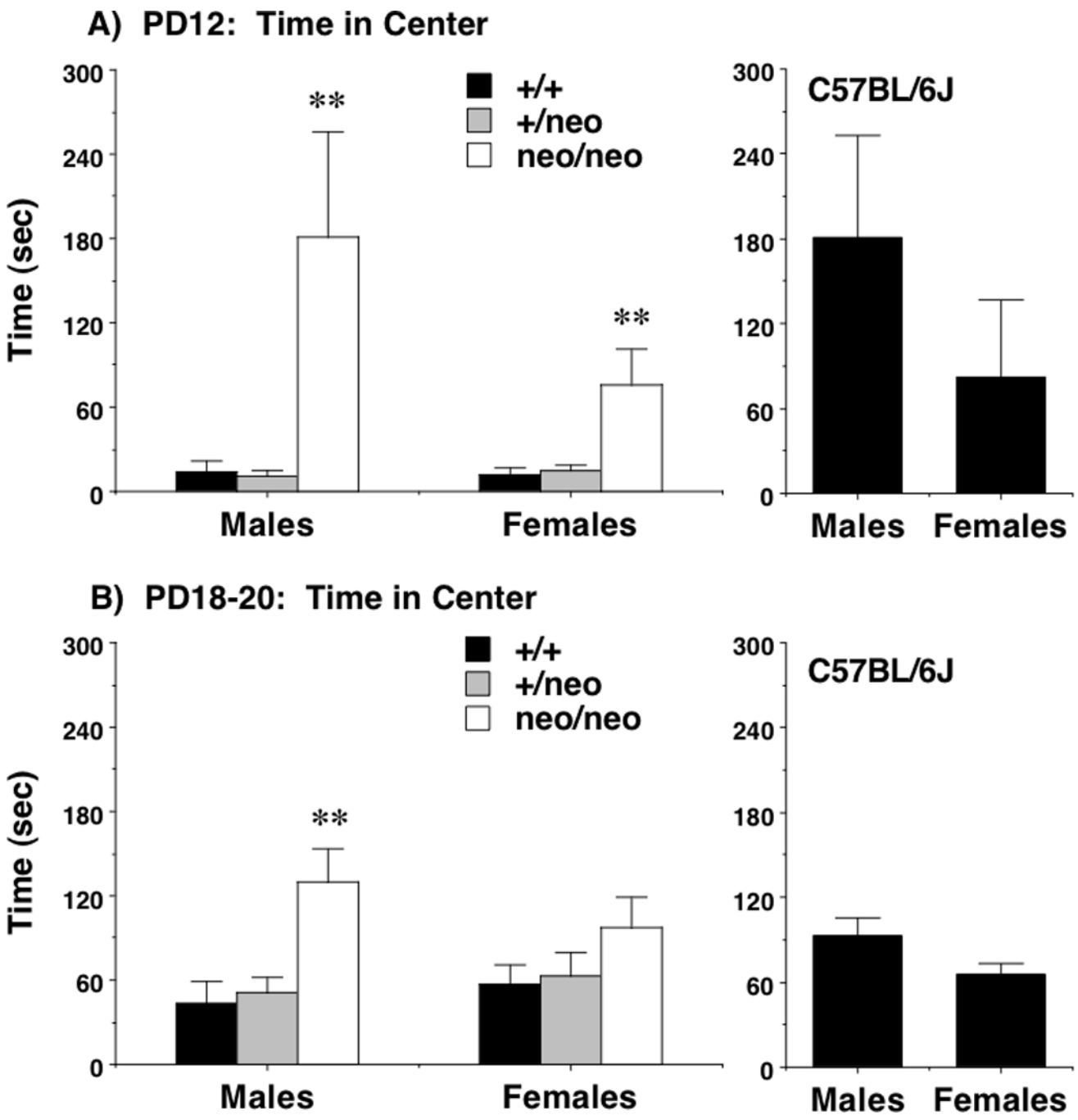

Fig. 3.

Increased time in the center regions of an open field in preweaning *Grin1*$^{neo/neo}$ mice. Data shown are means + SEM for a 10-min test. **p<0.05, comparison to both wild type and heterozygous mice.

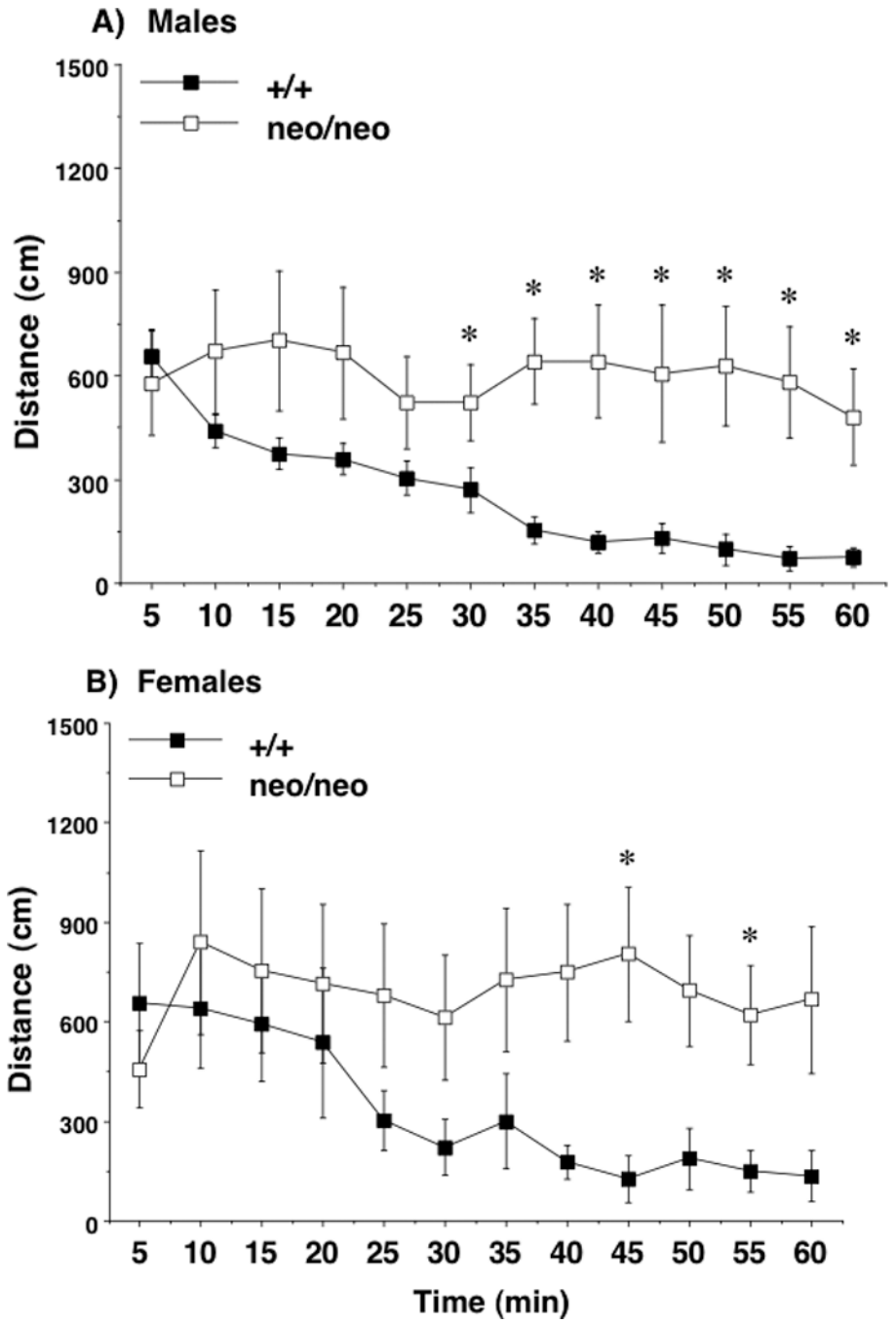

Fig. 4.

Habituation deficits in adult *Grin1*$^{neo/neo}$ mice in an open field. Data shown are means + SEM for a 1-hr test. Numbers of mice were 21 *Grin1*$^{+/+}$ (15 males and 6 females) and 21 *Grin1*$^{neo/neo}$ (10 males and 11 females). Data from one *Grin1*$^{neo/neo}$ male were lost when the mouse escaped from the open field. *p<0.05.

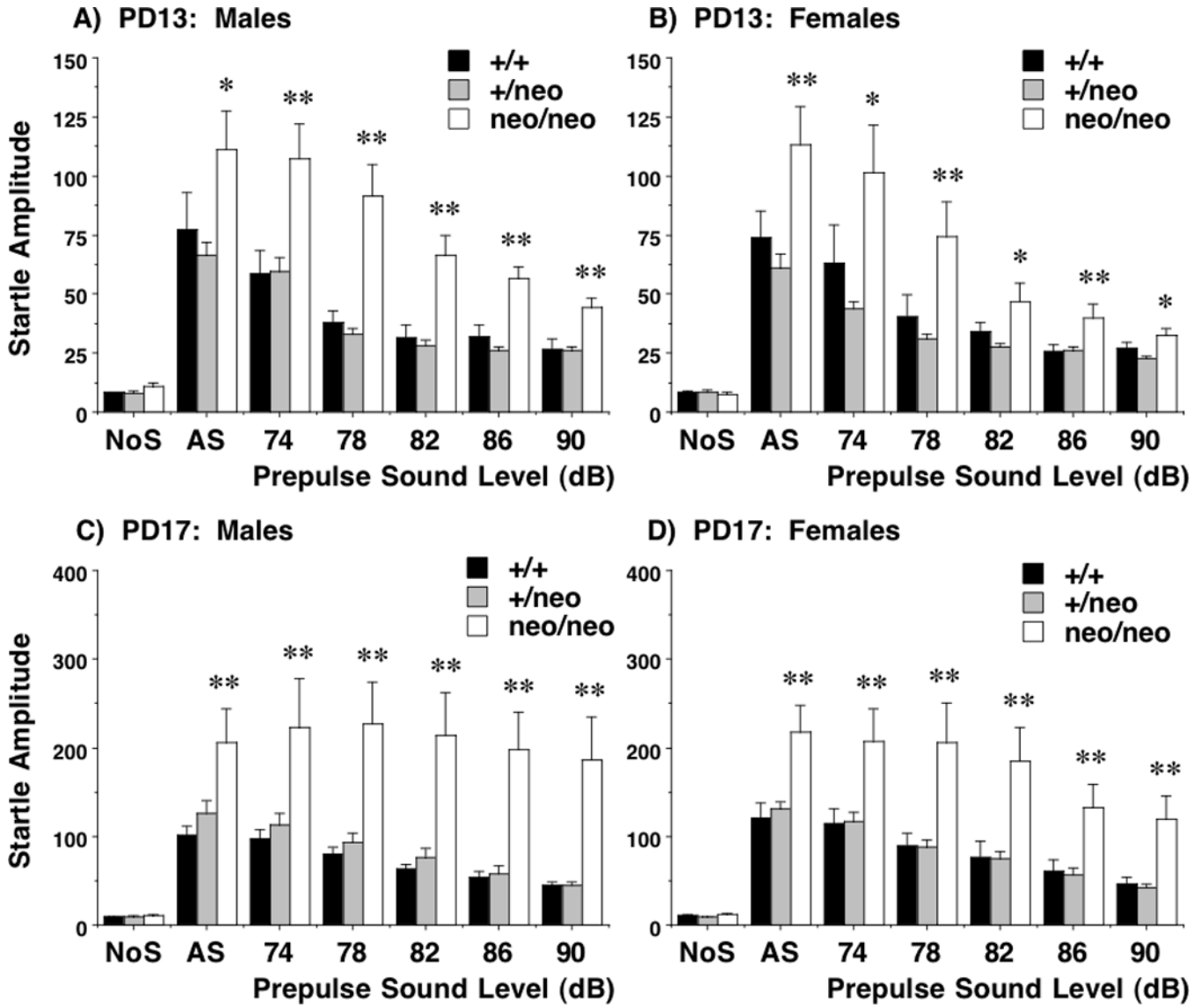

Fig. 5.

Exaggerated startle responses following presentation of acoustic stimuli in *Grin1*$^{neo/neo}$ mice. Data shown are means + SEM for each group. Scales for Panels A and B are different from Panels C and D, to accommodate increased startle amplitude across age. Trials included no stimulus (No S) trials and acoustic startle stimulus (AS) alone trials. *p<0.05, comparison to heterozygous mice. **p<0.05, comparison to both wild type and heterozygous mice.

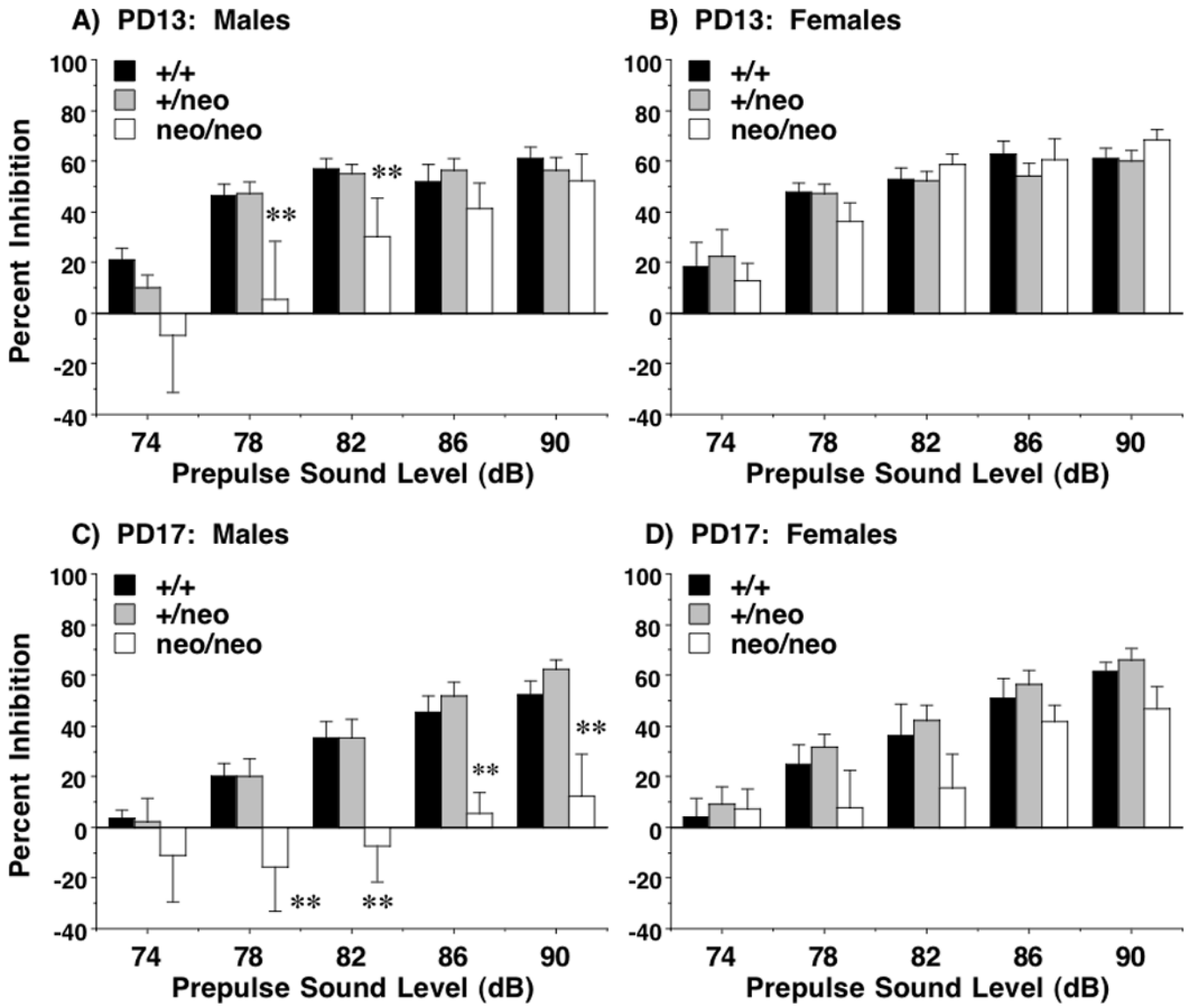

Fig. 6.

Reduced prepulse inhibition in male, but not female, *Grin1*$^{neo/neo}$ mice. Data shown are means + SEM for each group. **p<0.05, comparison to both wild type and heterozygous mice.

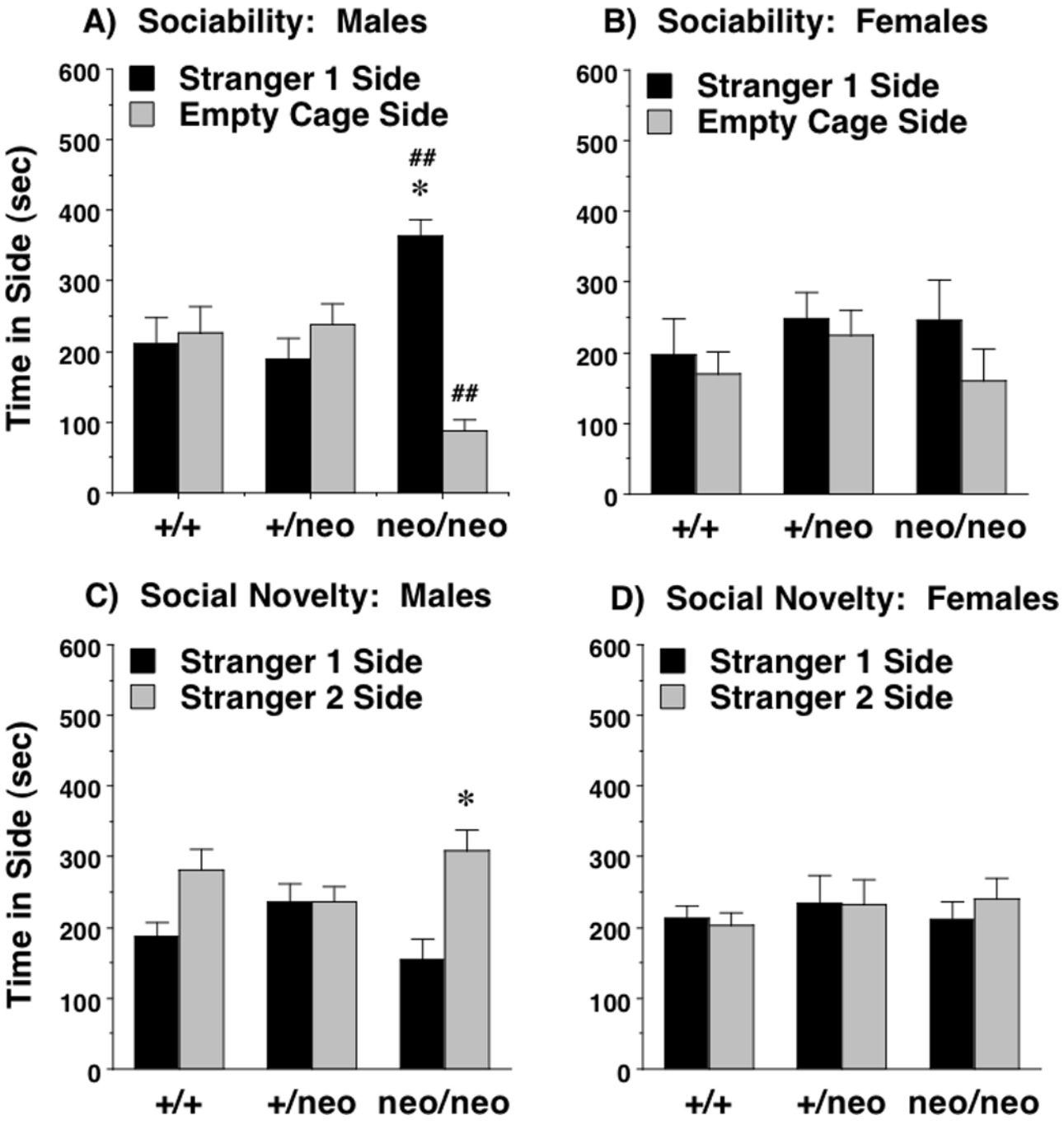

Fig. 7.

Time spent in each of the side chambers during the test for sociability (A, B) and social novelty (C, D). Data shown are mean + SEM for each group for a 10-min test. * $p < 0.05$, within-group comparison between stranger 1 side and opposite side. ## $p < 0.05$, comparison to both wild type and heterozygous mice.

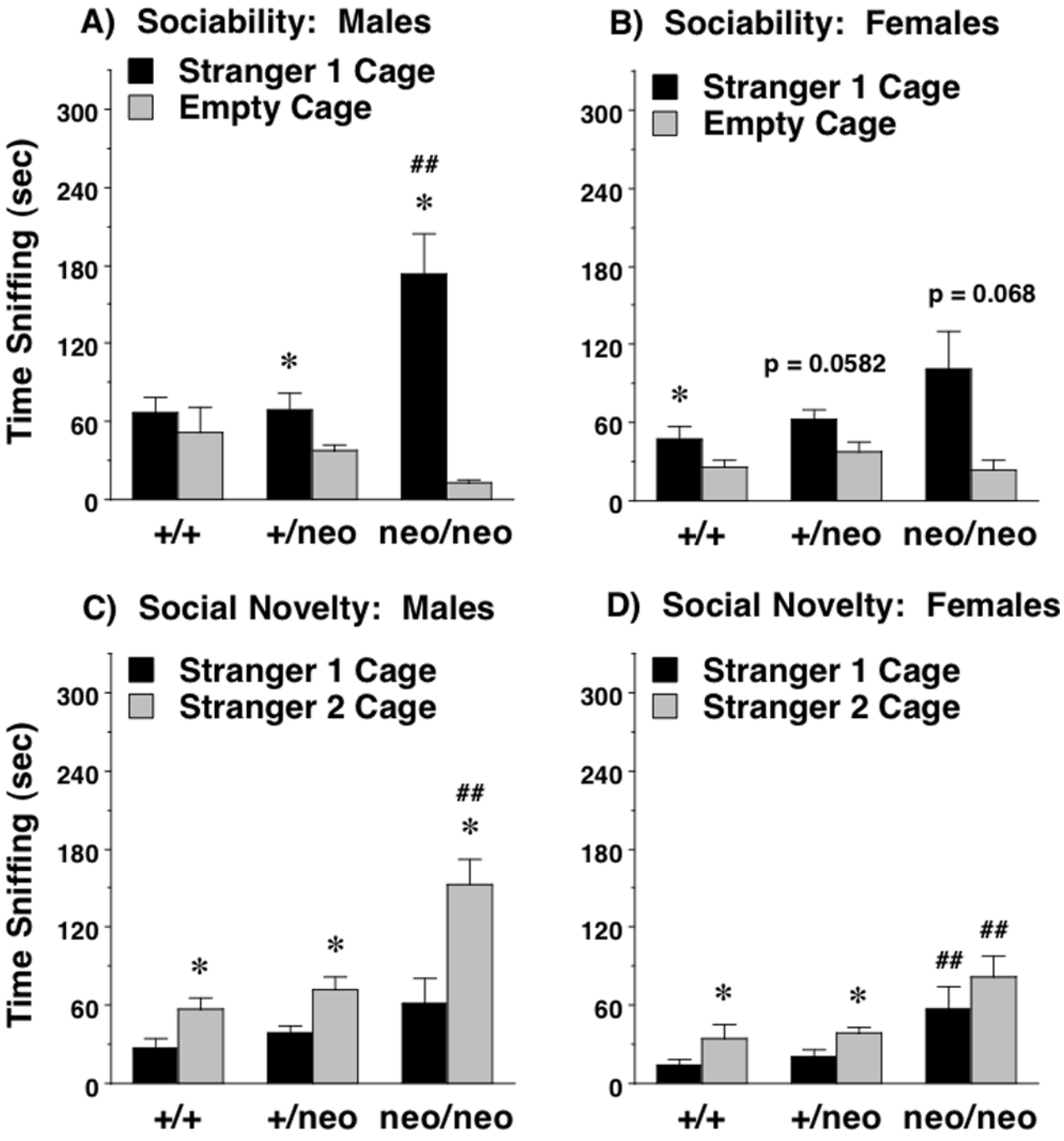

Fig. 8.

Time spent sniffing each of the side chambers during the test for sociability (A, B) and social novelty (C, D). Data shown are mean + SEM for each group for a 10-min test. * $p < 0.05$, within-group comparison between stranger 1 side and opposite side. ## $p < 0.05$, comparison to both wild type and heterozygous mice. P values above bars indicate non-significant trends for within-group sociability.

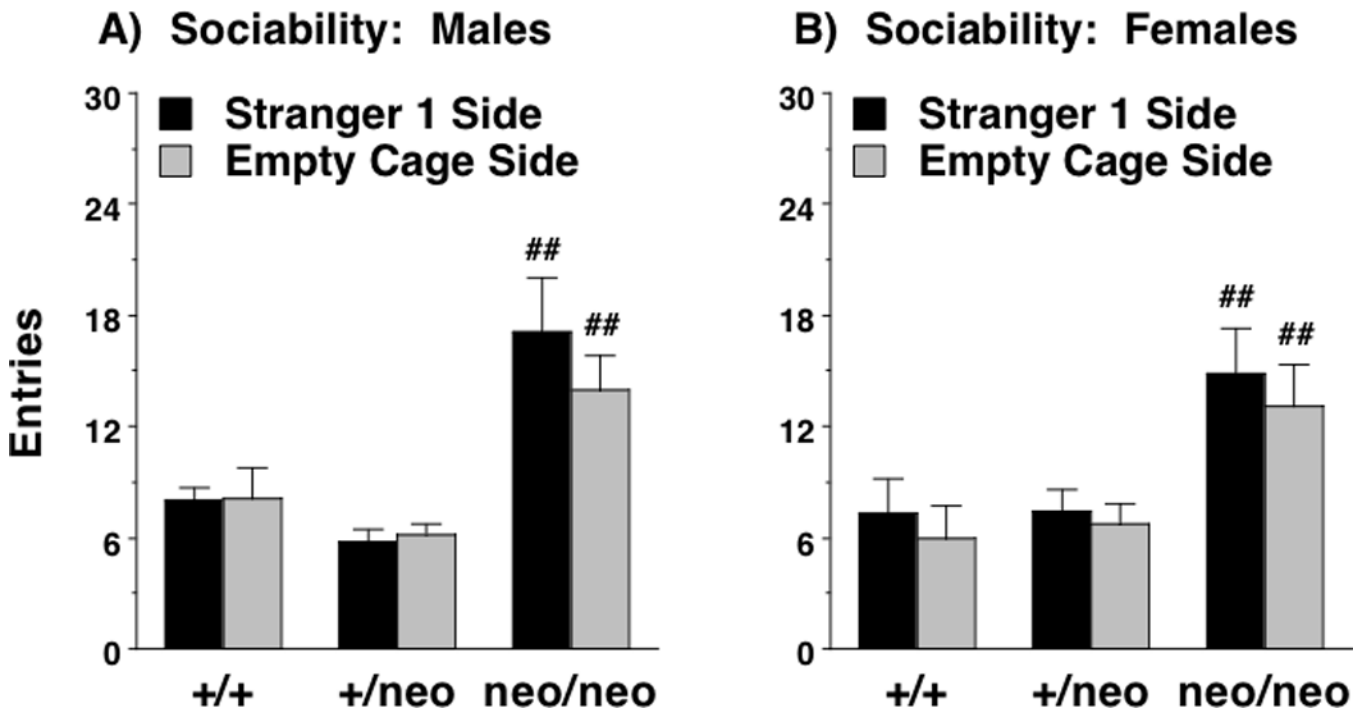

Fig. 9.

Entries into each of the side chambers during the test for sociability). Data shown are mean + SEM for each group for a 10-min test. ## $p < 0.05$, comparison to both wild type and heterozygous mice.

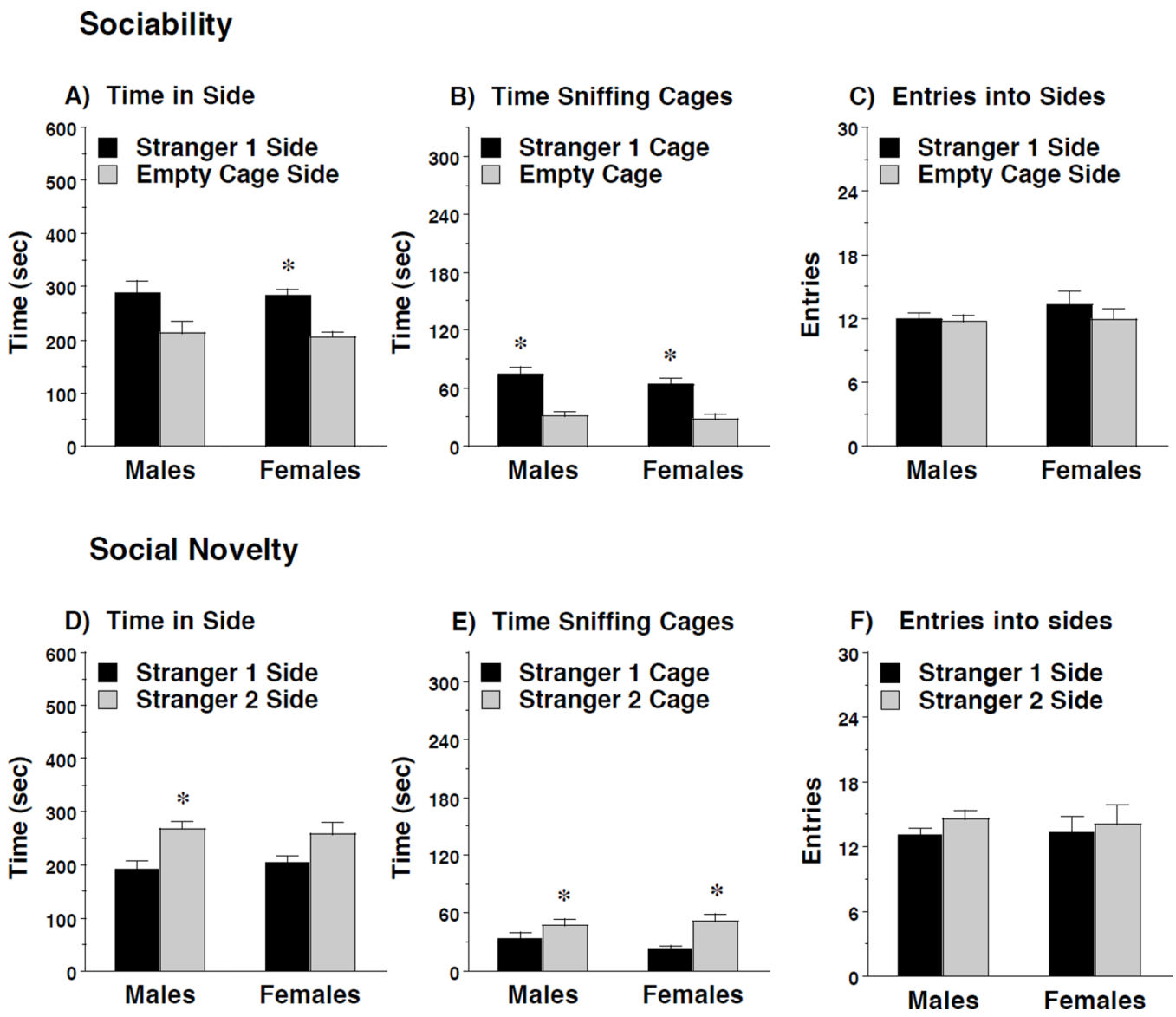

Fig. 10.

Social approach in C57BL/6J mice on postnatal days 34–38. Data shown are mean + SEM for each group for a 10-min test. * $p < 0.05$, within-group comparison between stranger 1 side and opposite side.

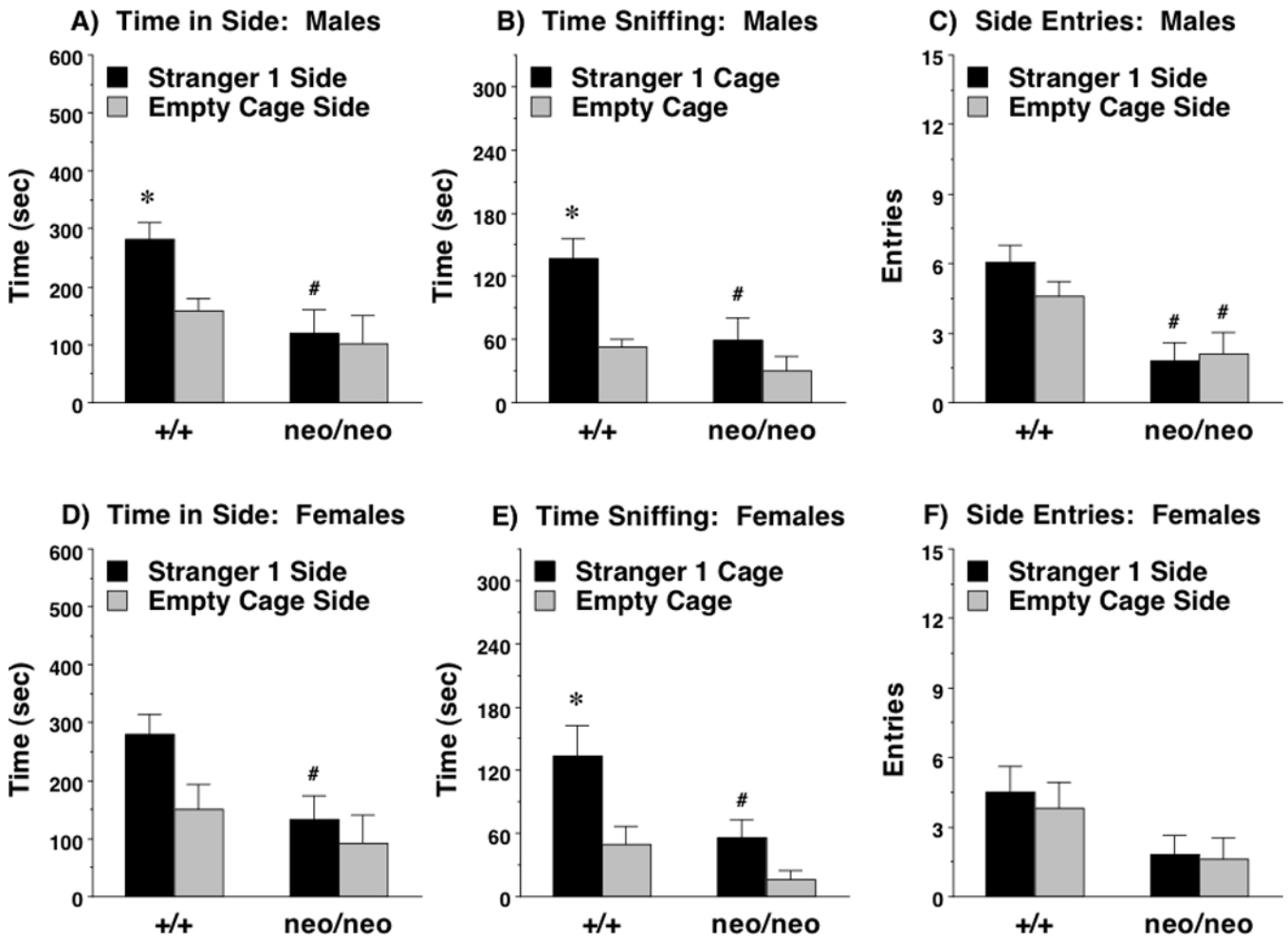

Fig. 11.

Social approach deficits in adult *Grin1*$^{neo/neo}$ mice. Data shown are mean + SEM. * $p < 0.05$, within-group comparison between stranger 1 side and empty cage side. # $p < 0.05$, comparison to *Grin1*$^{+/+}$ mice.