Neuropharmacology 105 (2016) 61–71

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Reversal of social deficits by subchronic oxytocin in two autism mouse models

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ARTICLE INFO

Article history:
Received 23 August 2015
Received in revised form
28 November 2015
Accepted 29 December 2015
Available online 31 December 2015

Keywords:
Autism spectrum disorders
Clozapine
Oxytocin
Risperidone
Schizophrenia
Sociability

ABSTRACT

Social deficits are a hallmark feature of autism spectrum disorder (ASD) and related developmental syndromes. Although there is no standard treatment for social dysfunction, clinical studies have identified oxytocin as a potential therapeutic with prosocial efficacy. We have previously reported that peripheral oxytocin treatment can increase sociability and ameliorate repetitive stereotypy in adolescent mice from the C58/J model of ASD-like behavior. In the present study, we determined that prosocial oxytocin effects were not limited to the adolescent period, since C58/J mice, tested in adulthood, demonstrated significant social preference up to 2 weeks following subchronic oxytocin treatment. Oxytocin was also evaluated in adult mice with underexpression of the *N*-methyl-D-aspartate receptor NR1 subunit (encoded by *Grin1*), a genetic model of autism- and schizophrenia-like behavior. Subchronic oxytocin had striking prosocial efficacy in male *Grin1* knockdown mice; in contrast, chronic regimens with clozapine (66 mg/kg/day) or risperidone (2 mg/kg/day) failed to reverse deficits in sociability. Neither the subchronic oxytocin regimen, nor chronic treatment with clozapine or risperidone, reversed impaired prepulse inhibition in the *Grin1* knockdown mice. Overall, these studies demonstrate oxytocin can enhance sociability in mouse models with divergent genotypes and behavioral profiles, adding to the evidence that this neurohormone could have therapeutic prosocial efficacy across a spectrum of developmental disorders.

1. Introduction

Oxytocin is a neuropeptide hormone with a long-recognized role in maternal responses and mother-infant bonding. Clinical studies in subjects with autism spectrum disorder (ASD) have found that acute oxytocin can improve social function and decrease motor stereotypy and other forms of repetitive behavior (Andari et al., 2010; Guastella et al., 2010; Hollander et al., 2003, 2007). Further, Hall et al. (2012) observed that acute oxytocin could ameliorate indicators of social anxiety in male adolescents and adults with fragile X syndrome. One recent study using a 5-week regimen with intranasal oxytocin in young children (3–8 years in age) with ASD found improved social responsivity, although no concomitant reduction in abnormal repetitive behavior (Yatawara et al., 2015). These initial reports also suggest that oxytocin might not have the same potential for adverse events as found with more powerful psychoactive agents, such as risperidone or fluoxetine, used to treat co-morbid symptoms in ASD (Mahajan et al., 2012; West et al., 2009; Yatawara et al., 2015). However, not all clinical trials using intranasal application of oxytocin to ameliorate social deficits or other symptoms have proven successful (Anagnostou et al., 2012; Cacciotti-Saija et al., 2015; Dadds et al., 2014),

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http://dx.doi.org/10.1016/j.neuropharm.2015.12.025

indicating the need for further investigation of oxytocin as a therapeutic agent.

Our research group has reported that peripheral administration of oxytocin can alleviate sociability deficits in two mouse models of autism-like behavior, the BALB/cByJ and C58/J inbred strains (Teng et al., 2013). Previous work has shown that BALB/cByJ and the related substrain, BALB/cJ, are characterized by a lack of social preference in a three-chambered choice task and by anxiety-like behavior in an elevated plus maze (Brodkin et al., 2004; Moy et al., 2007; Sankoorikal et al., 2006). Our previous study showed that, while acute oxytocin treatment did not reverse social deficits, a subchronic regimen of four injections, given across 8–9 days, led to significant sociability in adolescent BALB/cByJ mice, tested 24 h following the final dose (Teng et al., 2013).

Our group has also investigated oxytocin effects in the C58/J inbred strain, which has low sociability in a three-chambered task, deficits in social transmission of food preference, and overt repetitive behavior (Moy et al., 2008b, 2014; Muehlmann et al., 2012; Ryan et al., 2010; Silverman et al., 2012). We found that a subchronic oxytocin regimen had prosocial effects in adolescent male and female C58/J mice, with increases in social preference emerging one or two weeks following treatment (Teng et al., 2013). Acute, but not subchronic, administration of oxytocin led to significant decreases in abnormal repetitive behavior.

In the present studies, we investigated whether oxytocin would exert prosocial effects in adult C58/J mice, similar to our findings in adolescents. Motivation for social affiliation and regulation of social interactions can differ between adolescents and adults (Spear, 2011; see also Ernst et al., 2006). For example, Morales and Spear (2014) reported that, in a two-chamber test box, adolescent rats had higher levels of social interaction and a higher frequency of crossovers toward an unfamiliar social partner than adult rats. These data support a greater sensitivity to the rewarding aspects of novel social stimuli during adolescence, and raise the possibility that oxytocin might be most effective at this stage of development, while long-term social deficits in adults could be more recalcitrant to reversal.

The present studies also extended the evaluation of oxytocin to a third model of ASD-like behavior and synaptopathology, the *Grin1* knockdown mouse. Although the mechanistic basis for ASD is not known, genetic analyses in human populations have implicated several genes important for synaptic function, including *GRIN1*, which encodes the obligatory NMDAR1 subunit of the *N*-methyl-D-aspartate (NMDA) receptor (Abrahams and Geschwind, 2008; Voineagu et al., 2011; Zeidán-Chuliá et al., 2014); however, not all studies have found an positive association between ASD and *GRIN1* (e.g. Sanders et al., 2015; Tarabeux et al., 2011). There is growing evidence that alterations in NMDA receptor signaling play a role in ASD and other neurodevelopmental disorders (for recent reviews, see Burnashev and Szepetowski, 2015; Lee et al., 2015), including reports that autism candidate genes, such as *NEUROLIGIN-1* and *SHANK3*, serve as regulators of NMDA receptor function (Budreck et al., 2013; Duffney et al., 2013). Mice with reduced *Grin1* expression recapitulate many ASD features, including overt social deficits, inappropriate social interaction, abnormal repetitive behavior, self-injurious responses, and impaired sensorimotor gating (Billingslea et al., 2014; Duncan et al., 2004; Finlay et al., 2015; Gandal et al., 2012; Milenkovic et al., 2014; Mohn et al., 1999; Moy et al., 2008a, 2012, 2014; Saunders et al., 2013). We determined the effects of oxytocin on social deficits, reduced prepulse inhibition, and hyperactivity in *Grin1* knockdown mice. We also examined whether chronic regimens with atypical antipsychotics, initiated in early adolescence or young adulthood, have prosocial efficacy in the *Grin1* knockdown model.

2. Methods and materials

2.1. Animals

C58/J mice were offspring of breeding pairs obtained from Jackson Laboratories (Bar Harbor, ME). *Grin1*$^{neo/neo}$ mice engineered with a neomycin resistance gene (neo) in intron 20 of the *Grin1* locus and *Grin1*$^{+/+}$ littermate controls were generated from heterozygous breeder pairs, as previously described (Mohn et al., 1999; Moy et al., 2012). Experimenters conducting the behavioral tests were blind to genotype.

Mice were maintained in groups of 2–4 animals per polycarbonate mouse cage, in a room under a 12-h light/dark cycle (lights off at 7pm). ProLab RMH 3000 chow and water were provided ad libitum. All animal procedures were conducted in strict compliance with the animal welfare policies set by the National Institutes of Health and the University of North Carolina (UNC), and were approved by the UNC Institutional Animal Care and Use Committee.

2.2. Drug treatment regimens

2.2.1. Oxytocin

Oxytocin (Bachem, Torrance, CA) was dissolved in saline containing 0.002% glacial acetic acid. All injections were administered IP (intraperitoneal) in a volume of 10.0 ml/kg. For the subchronic regimen, mice were given four injections of vehicle or oxytocin (1.0 or 2.0 mg/kg) across 8–9 days, with at least 48 h between each injection (i.e. mice were injected on sequential weekdays WFMW or WFTTh). Experimenters conducting the behavioral tests were blind to drug treatments.

2.2.2. Chronic clozapine and risperidone regimens in Grin1 mice

Chronic regimens with clozapine (30 days; 66 mg/kg/day) or risperidone (21 days; 2.0 mg/kg/day) were initiated with a preliminary ramping up of drug dose to minimize sedative or other side effects at the beginning of treatment. Doses were selected to reflect therapeutic dosage in humans, determined by clinical levels of dopamine D_2 receptor occupancy (Kapur et al., 2003; Wadenberg et al., 2001).

Slow-release pellets were utilized for chronic clozapine administration because of difficulties in higher-dose drug solubility for osmotic minipumps (Kapur et al., 2003) and issues with variable plasma levels during administration in drinking water (Perez-Costas et al., 2008). At 11–14 weeks of age, mice were briefly anesthetized by isoflurane and implanted, using a trocar injector, with subcutaneous 30-day slow-release clozapine or sham tablets (Innovative Research of America, Sarasota, FL). The target dosage of 66 mg/kg/day was reached by incremental stages across 8 days, with 2–3 total pellet implants per subject. Following each implant, the trocar injection site was sealed using Tissuemend (Jeffers Inc., Dothan, AL).

For the initial acclimation to risperidone (Sigma–Aldrich, St. Louis, MO), adolescent mice (starting at age 33–38 days) received 3 IP injections of either saline vehicle containing 1% glacial acetic acid (adjusted to pH 5.5) or risperidone (0.3 mg/kg), with 2–3 days between each injection. One day following the third injection, mice were briefly anesthetized by isoflurane and implanted with a subcutaneous osmotic minipump (Model 1002; Alzet; Braintree Sci. Inc., Braintree, MA) containing either risperidone (2.0 mg/kg/day) or vehicle, for a 14-day delivery. At the end of the 14-day period, mice were again anesthetized, and the depleted 14-day pump was replaced by a new 7-day pump (Model 1007D) for the final phase of the 21-day regimen. This pump replacement allowed dosage to be adjusted for increased body weight during the chronic risperidone

treatment.

2.3. C58/J inbred strain model

Oxytocin has persistent effects on social behavior in adolescent C58/J mice (Teng et al., 2013). In this study, we investigated whether social deficits in adult C58/J mice could also be reversed by oxytocin treatment. Subjects were male and female mice (7–8 of each sex per treatment group; 5–6 months of age at time of testing), treated using a subchronic regimen of vehicle or 1.0 mg/kg oxytocin. Mice were tested in the 3-chamber choice task at two time points, 24 h and 2 wk post-treatment.

2.4. Grin1 knockdown model

2.4.1. Acute oxytocin effects on open field activity

Subjects were 7–9 male mice and 6–9 female mice of each genotype (*Grin1*$^{+/+}$ and *Grin1*$^{neo/neo}$) per treatment group, 8–11 months in age, taken from 12 litters. Each mouse was given 3 1-hr tests, one with vehicle pretreatment and one with each dose of oxytocin (0.5 and 1.0 mg/kg), with 1 week between each test. A balanced treatment design was used, so that order of treatments was balanced for genotype and sex across the 3 tests. Mice were placed into the activity chambers immediately after each treatment.

2.4.2. Acute oxytocin effects on sensorimotor gating

Subjects were 12–13 male mice and 10–13 female mice of each genotype (*Grin1*$^{+/+}$ and *Grin1*$^{neo/neo}$), 5–7 months of age, taken from 23 litters. The acoustic startle test was conducted 50 min following treatment with vehicle or oxytocin (1.0 mg/kg). Each mouse was given 2 sessions, one with vehicle pretreatment and one with oxytocin pretreatment, with 1 week between each session. A balanced treatment design was used, so that order of treatment was balanced by genotype and sex across the 2 tests.

2.4.3. Subchronic oxytocin regimen

Subjects were 8–9 male mice and 8–10 female mice of each genotype (*Grin1*$^{+/+}$ and *Grin1*$^{neo/neo}$) per treatment group (vehicle or 1.0 mg/kg oxytocin), tested at 3–5 months of age, taken from 29 litters. Each subject was tested in the 3-chamber choice task approximately 24 h following the final treatment. Mice were further tested in an acoustic startle assay 48 h post-treatment, and the marble-burying assay 4–5 days post-treatment. Because no genotype or treatment effects were observed in the female groups, an additional set of female *Grin1* mice (6–8 of each genotype, 7 months in age, taken from 5 litters) were given subchronic treatment with a higher dose of oxytocin (2.0 mg/kg) and tested in the 3-chamber choice task.

2.4.4. Chronic clozapine

Subjects were 4–8 male mice and 4–6 female mice of each genotype per treatment group, 3–4 months of age at time of behavioral testing, taken from 23 litters. Mice were tested in the 3-chamber choice task 32–35 days following the final subcutaneous implant of a 30-day slow-release pellet. Mice were also tested in an acoustic startle assay at two time points, 15–16 days and 35–38 days following the final pellet implantation.

2.4.5. Chronic risperidone

Subjects were 6–9 male mice and 5–8 female mice of each genotype per treatment group, 60–70 days of age at time of behavioral testing, taken from 23 litters. Mice were tested in the 3-chamber choice task 23–26 days following the start of a 21-day chronic regimen (administered by osmotic minipump). Mice were also tested in an acoustic startle assay 24–27 days and a marble-burying task 28–29 days after initiation of the chronic regimen.

2.5. Behavioral testing procedures

2.5.1. Three-chamber social choice test

Social approach was assessed in a 3-chamber Plexiglas box (procedure modified from Moy et al., 2007). The test started with a 10-min habituation phase, with free exploration of the empty test box, followed by a 10-min test for sociability. During the sociability phase, the test mouse was given a choice between an unfamiliar stranger mouse (a sex-matched C57BL/6J adult), contained in a Plexiglas cage placed in one side chamber, or an empty Plexiglas cage in the opposite side chamber. Cages were drilled with holes to allow investigation of the stranger. Measures were taken of the time spent in each chamber, time spent in 5 cm proximity to each cage, and number of entries into each chamber, by an automated image tracking system (Ethovision, Noldus Information Technology, Wageningen, the Netherlands).

2.5.2. Open field test

Activity was assessed in a photocell-equipped automated open field (41 cm × 41 cm × 30 cm; Versamax System, AccuScan Instruments, Columbus, OH). Measures were taken of total distance traveled, rearing movements, and time spent in the center region of the chamber, for each 1-hr test.

2.5.3. Acoustic startle test

Mice were evaluated for acoustic startle responses with an SR-Lab system (San Diego Instruments). Each test session consisted of a 5-min habituation period, followed by 42 trials: 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. Measures were taken of startle amplitude, defined as the peak response during a 65-msec sampling window following onset of the startle stimulus. PPI was calculated as 100 - [(response amplitude for prepulse stimulus and startle stimulus together/response amplitude for startle stimulus alone) x 100].

2.5.4. Marble-burying assay

Each subject was tested in a polycarbonate mouse cage located in a sound-attenuating chamber with ceiling light and fan. The cage contained 5 cm deep clean corncob bedding, with 20 black glass marbles (14 mm diameter) arranged in an equidistant 5 × 4 array on top of the bedding. Measures were taken of the number of marbles covered 2/3 or more by the bedding after a 30 min test.

2.6. Statistical analysis

Data were analyzed with one-way, two-way, or repeated measures analysis of variance (ANOVA), with factors treatment, sex, and genotype (dependent on experiment), using Statview software (SAS, Cary, NC). Repeated measures included side of social test box, test session, or prepulse sound level. Separate repeated measures ANOVAs were conducted for each sex to determine oxytocin effects on sociability in male and female mice. Within-treatment repeated measures ANOVAs were used to determine side preference in the social choice test. Fisher's protected least-significant difference (PLSD) tests were used for comparing group means only when a significant F value was determined by ANOVA. Significance was set at $p < 0.05$.

3. Results

3.1. Prosocial oxytocin effects in adult C58/J mice

3.1.1. Sociability in male and female C58/J mice

Previous work in our laboratory has shown that subchronic oxytocin has persistent prosocial effects in adolescent C58/J mice (Teng et al., 2013). In the present study, both male and female adult C58/J mice (ages 5–6 months) treated with oxytocin, but not vehicle, had significant preference for spending time in the stranger side of a 3-chamber box (Fig. 1). In the male mice, the prosocial oxytocin effects were evident at 24 h and 2 wk post-treatment [within-treatment group post-hoc analyses following significant effect of side, $F(1,14) = 9.38$, $p = 0.0084$; determined by a 3-way repeated measures ANOVA, with factors treatment, side, and time point for testing] (Fig. 1A, B). In the male groups, there was a non-significant trend for a treatment × side interaction [$F(1,14) = 3.8$, $p = 0.0717$]. Female mice treated with oxytocin demonstrated significant preference for the stranger side only at the 24-h time point [within-treatment group post-hoc analyses following significant effect of side, $F(1,13) = 11.40$, $p = 0.005$] (Fig. 1C).

3.1.2. Preference for social proximity in male and female C58/J mice

We have previously reported that adolescent C58/J male mice have positive sociability with the measure of sniffing directed towards a stranger mouse (Moy et al., 2008b). The present study used a similar measure, proximity to each cage. In the first test, given 24 h following the subchronic regimen, the C58/J male mice treated with vehicle, but not oxytocin, spent significantly more time in proximity to the stranger mouse than the empty cage (Fig. 2A). However, during second test, only the oxytocin-treated group demonstrated significant social preference [within-treatment group post-hoc analyses following significant effect of side, $F(1,14) = 6.66$, $p = 0.0218$] (Fig. 2B). In the female groups, mice treated with oxytocin demonstrated significant preference for the stranger side at both the 24-hr and 2-wk time points [within-treatment group post-hoc analyses following significant effect of side, $F(1,13) = 34.29$, $p < 0.0001$] (Fig. 2C, D). In contrast, vehicle-treated female mice failed to demonstrate positive sociability with the proximity measure in either test.

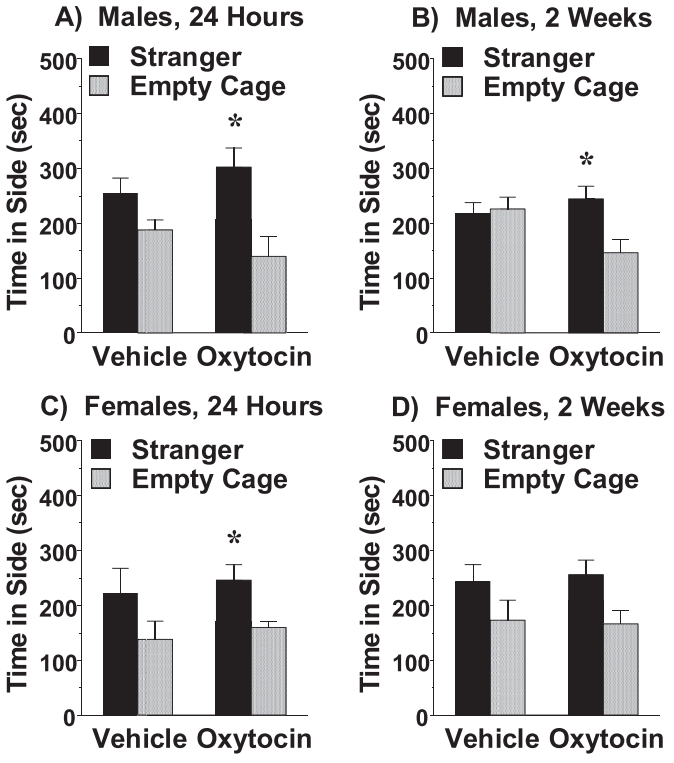

Fig. 1. Persistent prosocial effects of subchronic oxytocin in adult C58/J mice. Subjects were tested for sociability in a 3-chamber choice task. The subchronic regimen consisted of 4 treatments with either vehicle or oxytocin (1.0 mg/kg, IP) across an 8–9 day period. Mice were tested at 24 h, and again at 2 wk, following the final treatment. *$p < 0.05$, within-group comparison to empty cage side.

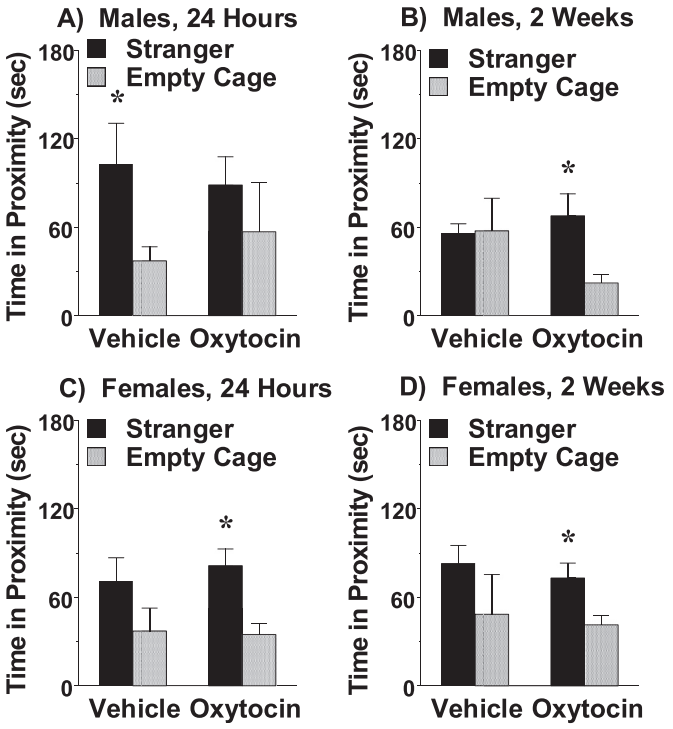

Fig. 2. Effects of subchronic oxytocin on time spent in proximity to a stranger mouse. Testing occurred at 24 h, and again at 2 wk, following the final treatment. During the second test, only the oxytocin treatment groups had significant social preference. *$p < 0.05$, within-group comparison to empty cage side.

3.1.3. Oxytocin effects on entries in the 3-chamber test

Subchronic oxytocin did not alter number of entries during the test in the C58/J groups, indicating prosocial effects were not due to general changes in activity or exploration (Fig. 3). Number of entries is not typically used as an index for social approach; however, it is notable that the male C58/J mice treated with oxytocin, but not vehicle, showed significantly more entries into the side containing the stranger mouse during the first social test [effect of side, $F(1,14) = 8.89$, $p = 0.0099$] (Fig. 3A).

3.2. Acute oxytocin effects in *Grin1*$^{+/+}$ and *Grin1*$^{neo/neo}$ mice

3.2.1. Acute oxytocin effects on hyperactivity in an open field

This study determined whether acute oxytocin could reduce the overt hyperactivity previously reported in *Grin1* knockdown mice (Duncan et al., 2004; Mohn et al., 1999; Moy et al., 2014). Three-way repeated measures ANOVAs did not reveal significant effects of sex on performance in the open field; therefore, data for males and females were combined for analysis. In line with previous findings, the *Grin1*$^{neo/neo}$ mice had higher levels of activity than wild-type mice (Fig. 4A, B). Highly significant main effects of genotype were found for each measure [distance traveled, $F(1,29) = 14.04$, $p = 0.0008$; rearing movements, $F(1,29) = 21.07$, $p < 0.0001$; and

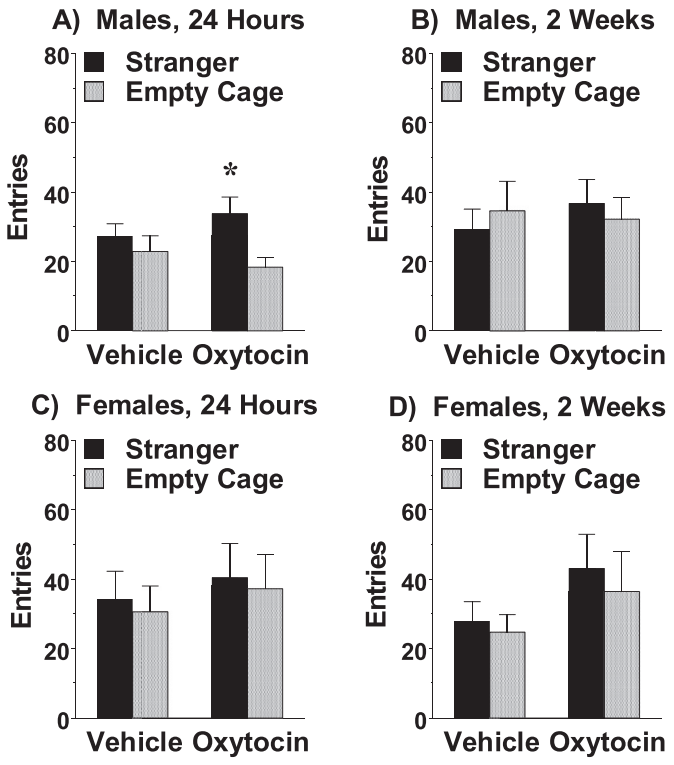

Fig. 3. Effects of subchronic oxytocin on side-chamber entries by C58/J mice. Overall number of entries was not changed by oxytocin in either male or female mice. Testing occurred at 24 h, and again at 2 wk, following the final treatment. *p < 0.05, within-group comparison to empty cage side.

center time, F(1,29) = 13.16, p = 0.0011 (Fig. 4C)]. In addition, a significant effect of treatment was revealed for distance traveled [F(2,58) = 3.26, p = 0.0456]. Post-hoc comparisons confirmed that the higher dose of oxytocin (1.0 mg/kg) led to a significant decrease in locomotor activity in the *Grin1*$^{neo/neo}$ mice. No effects of oxytocin on activity were observed in the wild-type group.

3.2.2. Acute oxytocin effects on sensorimotor gating

Previous studies have shown that oxytocin and oxytocin receptor agonists can rescue sensorimotor gating deficits in rodents (Feifel et al., 2012; Ring et al., 2010). In this study, we determined if acute administration of oxytocin could reverse impaired prepulse inhibition in the *Grin1*$^{neo/neo}$ mice. A 3-way repeated measures ANOVA did not indicate any significant effects of sex; therefore, data from males and females were combined. As shown in Fig. 4D and E, the *Grin1*$^{neo/neo}$ mice demonstrated deficits in prepulse inhibition at almost every decibel level, which were not reversed by acute treatment with oxytocin [main effect of genotype, F(1,46) = 18.48, p < 0.0001; genotype × decibel interaction, F(4,184) = 7.59, p < 0.0001; no significant effects of treatment].

3.3. Prosocial effects of subchronic oxytocin in *Grin1*$^{neo/neo}$ mice

3.3.1. Oxytocin effects on sociability in male Grin1 mice

Subchronic oxytocin (1.0 mg/kg) led to a striking increase in sociability in male *Grin1*$^{neo/neo}$ mice, but not wild-type controls (Fig. 5). As shown in Fig. 5A, only the *Grin1* knockdown mice treated with oxytocin had significant social preference 24 h following the final injection. In contrast, male *Grin1*$^{+/+}$ mice had similar positive sociability after either the vehicle or oxytocin regimen (Fig. 5B). A repeated measures ANOVA on time spent in each chamber indicated significant 2-way interactions between genotype and side [F(1,29) = 5.92, p = 0.0214], treatment and side [F(1,29) = 10.04, p = 0.0036], and a 3-way interaction between genotype, treatment,

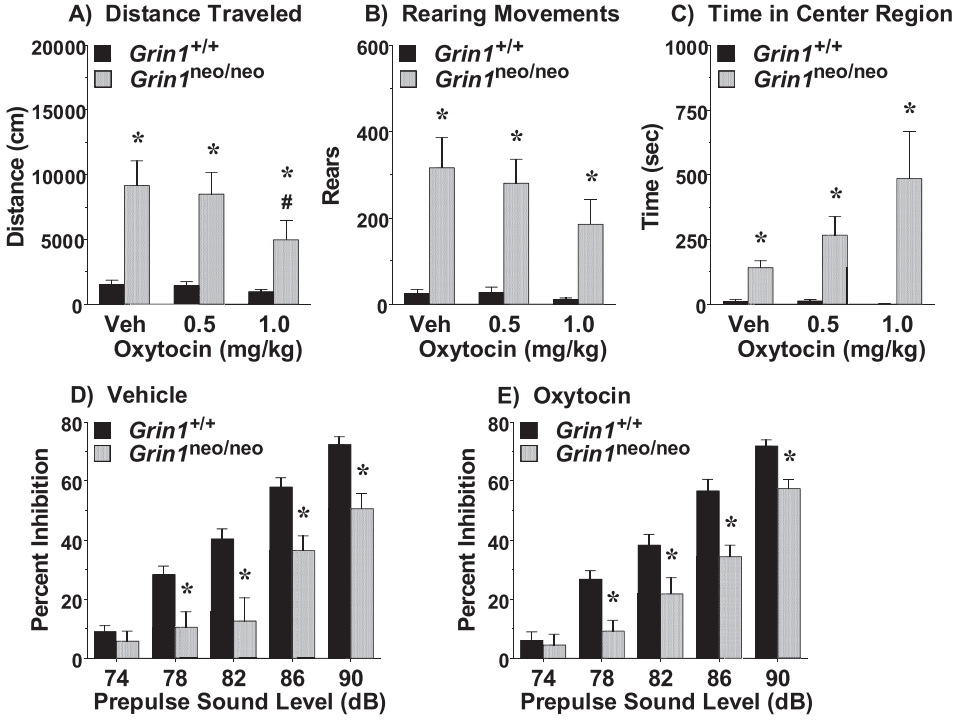

Fig. 4. Effects of acute oxytocin on hyperactivity and sensorimotor gating deficits in *Grin1*$^{neo/neo}$ mice. (A-C) Vehicle (Veh) or oxytocin (0.5 or 1.0 mg/kg, IP) was administered immediately before a 1-hr open field test. D, E) Vehicle or oxytocin (1.0 mg/kg, IP) was administered 50 min before an acoustic startle test. *p < 0.05, comparison to *Grin1*$^{+/+}$ mice. #p < 0.05, within-genotype comparison to vehicle (Panel A).

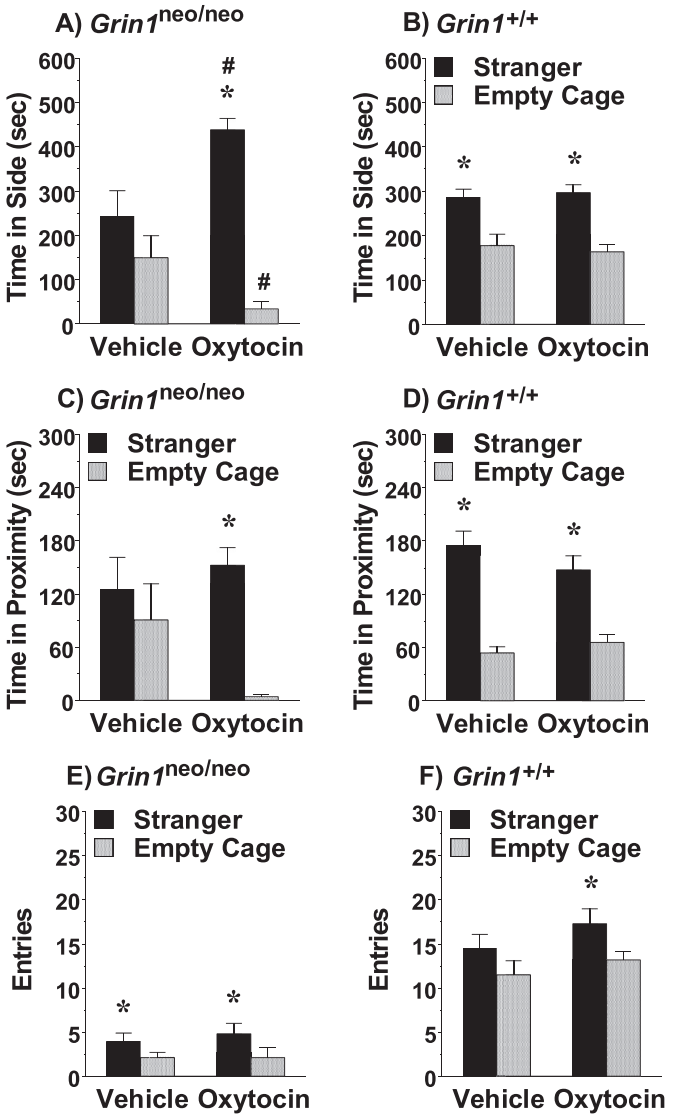

Fig. 5. Prosocial effects of subchronic oxytocin in male *Grin1*$^{neo/neo}$ mice. Subjects were tested for sociability in a 3-chamber choice task. The subchronic regimen consisted of 4 treatments with either vehicle or oxytocin (1.0 mg/kg, IP) across an 8–9 day period. *Grin1* mice were tested 24 h following the final treatment. *p < 0.05, within-group comparison to empty cage side. #p < 0.05, comparison to vehicle-treated group (Panel A).

and side [F(1,29) = 7.46, p = 0.0106]. A similar 3-way interaction for genotype, treatment, and side emerged for the measure of proximity to each cage (Fig. 5C and D) [F(1,24) = 4.99, p = 0.0351; data missing for 5 mice tested before cage-zone tracking available].

3.3.2. Lack of oxytocin effects on entries in male Grin1 mice

Subchronic oxytocin did not alter number of entries during the test in the *Grin1* groups, indicating prosocial effects were not due to general changes in activity or exploration (Fig. 5). In the *Grin1*$^{neo/neo}$ mice, oxytocin did not rescue deficits in entry numbers (Fig. 5E) [main effect of genotype, F(1,29) = 97.02, p < 0.0001; and side, F(1,29) = 21.46, p < 0.0001]. In the *Grin1*$^{+/+}$ mice, only the oxytocin-treated group showed more entries into the stranger side, versus the empty cage side (Fig. 5F) [within-treatment group post-hoc analyses following significant effect of side, F(1,14) = 9.91, p = 0.0071].

3.3.3. Oxytocin (1.0 and 2.0 mg/kg) effects on sociability in female Grin1 mice

In contrast to the results from the male *Grin1* mice, there were no significant effects of genotype or treatment (1.0 mg/kg oxytocin) for measures of sociability in the female groups. Therefore, an additional set of female mice was tested with a higher dose of oxytocin, 2.0 mg/kg, using a subchronic regimen. Measures of proximity are presented in Fig. 6A, since wild-type female mice (from either treatment group) did not have significant sociability by the measure of time spent in each side of the test box. As shown in Fig. 6A, the *Grin1*$^{+/+}$ and *Grin1*$^{neo/neo}$ mice treated with vehicle or with 1.0 mg/kg oxytocin spent similar amounts of time in proximity to the stranger mouse, although preference was only significant in the wild-type females [within-treatment group post-hoc analyses following significant effect of side, F(1,27) = 23.11, p < 0.0001; no effect of genotype or treatment; data missing for 4 mice tested before cage-zone tracking available]. In contrast, following treatment with the higher dose, the female *Grin1*$^{neo/neo}$ mice demonstrated overt social preference and spent significantly more time than the wild-type mice in proximity to the stranger mouse [main effect of genotype, F(1,12) = 9.51, p = 0.0095; effect of side, F(1,12) = 17.49, p = 0.0013, and genotype × side interaction, F(1,12) = 6.42, p = 0.0262].

3.3.4. Lack of oxytocin effects on entries in female Grin1 mice

As observed in the male *Grin1* groups, subchronic oxytocin did not alter number of entries during the test in the female mice, or reverse marked deficits in the *Grin1*$^{neo/neo}$ groups (Fig. 6B). Highly significant main effects of genotype were observed in the mice

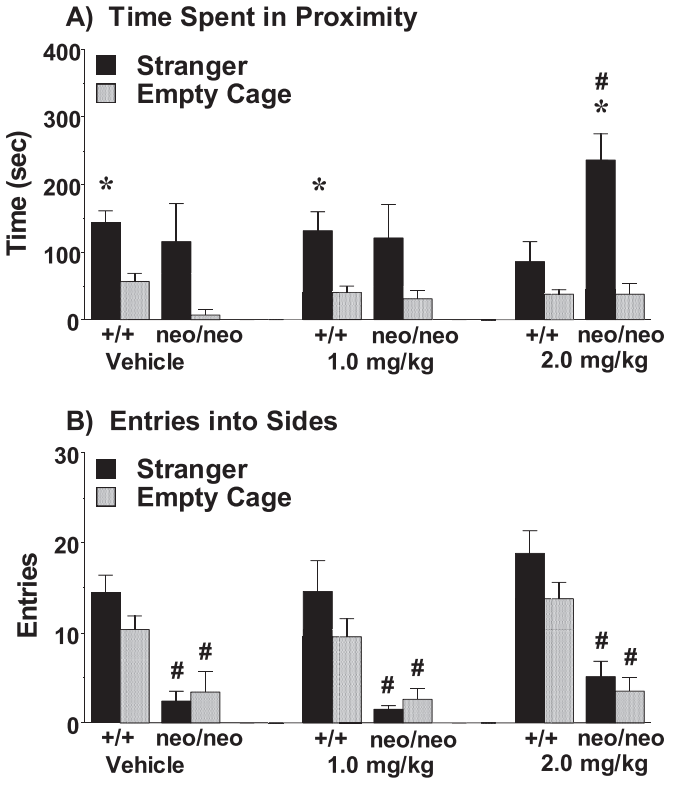

Fig. 6. Increased sociability in female *Grin1*$^{neo/neo}$ mice following subchronic treatment with 2.0 mg/kg oxytocin. Subjects were tested for social preference in a 3-chamber choice task. The subchronic regimen consisted of 4 treatments with either vehicle, 1.0 mg/kg, or 2.0 mg/kg oxytocin (IP) across an 8–9 day period, with testing 24 h following the final treatment. *p < 0.05, within-group comparison to empty cage side. #p < 0.05, comparison to *Grin1* wild-type (+/+).

treated with 1.0 mg/kg oxytocin [$F(1,31) = 34.16$, $p < 0.0001$] and 2.0 mg/kg oxytocin [$F(1,12) = 25.7$, $p = 0.0003$].

3.3.5. Lack of prosocial effects of chronic clozapine or risperidone in *Grin1*$^{neo/neo}$ mice

In a search for therapeutic agents with prosocial efficacy, our research group evaluated chronic regimens with two atypical antipsychotics, clozapine and risperidone. Overall, neither chronic regimen reversed social deficits in the *Grin1*$^{neo/neo}$ mice (Fig. 7). Separate repeated measures ANOVAs for clozapine and risperidone did not indicate any significant effects of treatment or sex; therefore, data were combined for vehicle groups, and for males and females. An overall repeated measures ANOVA for time spent in each side revealed highly significant effects of genotype [$F(1,93) = 32.88$, $p < 0.0001$] and side [$F(1,93) = 13.20$, $p = 0.0005$], but not treatment. Interestingly, neither the wild-type nor *Grin1*$^{neo/neo}$ groups demonstrated significant social preference following the chronic antipsychotic regimens (Fig. 7B and C). Further, the chronic clozapine and risperidone treatments failed to alleviate the low numbers of entries observed in the *Grin1*$^{neo/neo}$ groups [main effect of genotype, $F(1,93) = 6.55$, $p = 0.0121$; and side, $F(1,93) = 6.42$, $p = 0.0129$; but not treatment] (Fig. 7E and F).

3.4. Failure to reverse sensorimotor gating deficits in *Grin1*$^{neo/neo}$ mice

On the day following the social approach test (48 h following the final injection with oxytocin or vehicle), the *Grin1* mice were further evaluated in an acoustic startle test. An overall repeated measures ANOVA for prepulse inhibition did not indicate any significant effects for sex; therefore, data for male and female mice were combined. As shown in Fig. 8A and B, the *Grin1*$^{neo/neo}$ mice had overt deficits in prepulse inhibition, which were not rescued by the subchronic oxytocin regimen [main effect of genotype, $F(1,64) = 31.64$, $p < 0.0001$; genotype x sound level interaction, $F(4,246) = 5.87$, $p = 0.0002$; no effects of treatment].

Similarly, chronic treatment with the antipsychotic drugs did not reverse sensorimotor gating deficits in the *Grin1*$^{neo/neo}$ mice. Repeated measures ANOVAs did not indicate any significant effects for sex; therefore, data for male and female mice were combined. As presented in Fig. 8C and D, the *Grin1*$^{neo/neo}$ mice in both the vehicle and clozapine groups had reduced prepulse inhibition, in comparison to wild-type [main effect of genotype, $F(1,39) = 14.56$, $p = 0.0005$; genotype x sound level interaction, $F(4,156) = 3.06$, $p = 0.0184$; no effects of treatment]. In the clozapine study, mice were given a re-test on days 36–38 of the acclimated chronic regimen; no significant effects of clozapine were observed at this additional time point (data not shown). A similar pattern was observed following chronic risperidone, i.e. *Grin1*$^{neo/neo}$ mice in both treatment groups had comparable deficits in prepulse inhibition (Fig. 8E, F) [main effect of genotype, $F(1,52) = 52.18$, $p < 0.0001$; genotype x sound level interaction, $F(4,208) = 5.63$, $p = 0.0003$; no effects of treatment].

3.5. Failure to reverse deficits in marble-burying by *Grin1*$^{neo/neo}$ mice

We have previously reported that *Grin1* knockdown mice have overt deficits in a marble-burying task (Moy et al., 2014). In the present study, *Grin1* mice were tested for marble-burying 4–5 days after the final treatment in a subchronic oxytocin regimen. As shown in Fig. 9, oxytocin did not rescue deficits in the *Grin1*$^{neo/neo}$ mice. A 3-way ANOVA indicated a highly significant effect of genotype [$F(1,50) = 1755.56$, $p < 0.0001$], with no effects of treatment or sex. Similarly, chronic risperidone did not have significant effects in a marble-burying test, conducted 28–29 days after the first pump implant. Both the vehicle- and risperidone-treated *Grin1*$^{neo/neo}$ mice had profound deficits in marble-burying, in comparison to the wild-type mice [main effect of genotype, $F(1,52) = 1371.76$, $p < 0.0001$].

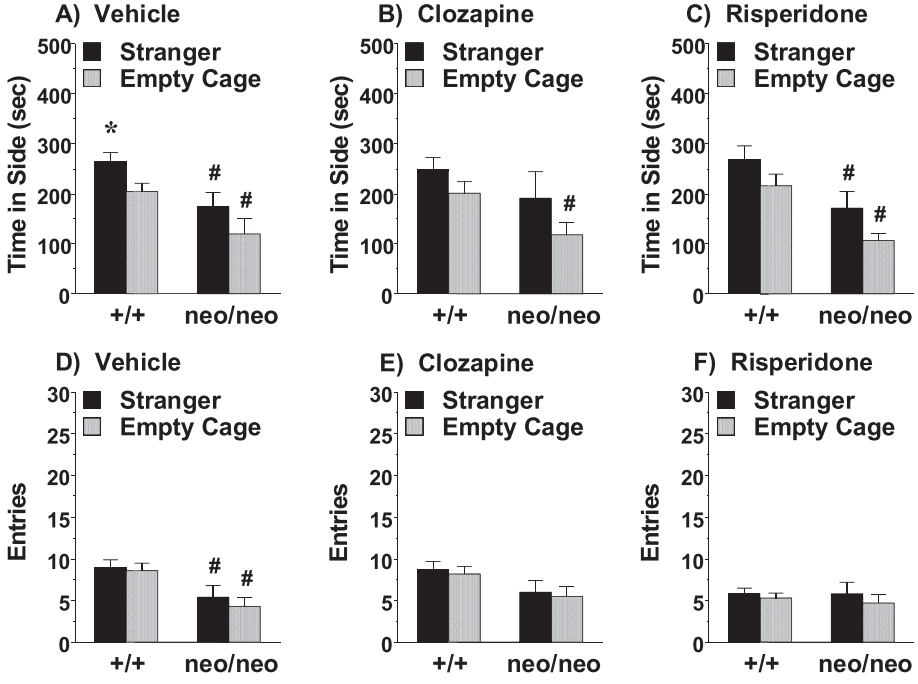

Fig. 7. Lack of significant prosocial effects of chronic clozapine or chronic risperidone in *Grin1*$^{neo/neo}$ mice. Testing occurred following chronic treatment with either clozapine (acclimated 30-day regimen; 66 mg/kg/day) or risperidone (acclimated 21-day regimen; 2.0 mg/kg/day). *$p < 0.05$, within-genotype comparison to empty cage side (Panel A). #$p < 0.05$, comparison to wild-type (+/+) group.

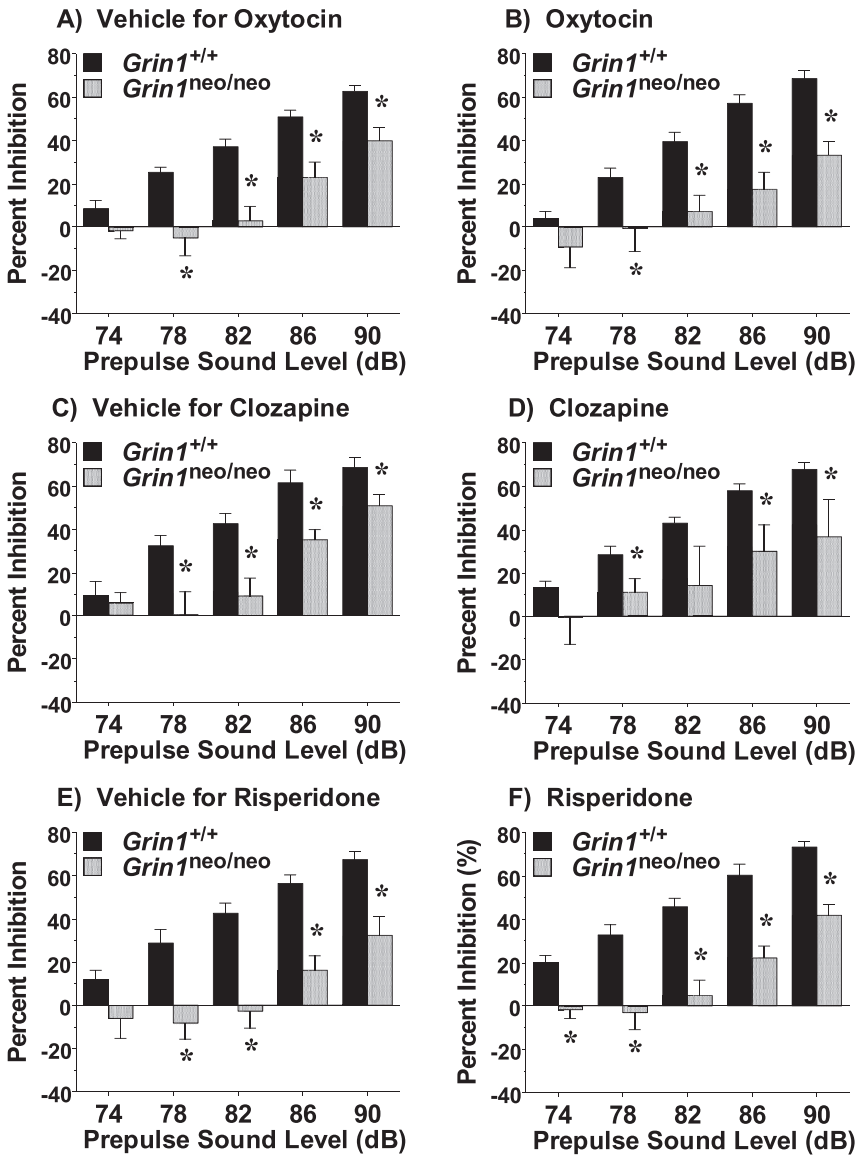

Fig. 8. No effects of subchronic oxytocin, or chronic clozapine or risperidone, on impaired sensorimotor gating in *Grin1*$^{neo/neo}$ mice. (A and B) *Grin1* wild-type (+/+) and knockdown (neo/neo) mice were tested for prepulse inhibition of acoustic startle responses 48 h after the final treatment in the subchronic regimen. C and D) *Grin1* mice were tested on day 15–16 of the chronic clozapine regimen (66 mg/kg/day). Data were omitted from one male *Grin1*$^{neo/neo}$ mouse in the clozapine-treated group with extremely low startle amplitudes. E and F) *Grin1* mice were tested 3–6 days following the chronic risperidone (21 day; 2.0 mg/kg/day) regimen. *$p < 0.05$.

4. Discussion

The present studies demonstrated that oxytocin has prosocial effects in C58/J and *Grin1* knockdown mice, two genetically-divergent mouse models of neurodevelopmental disorders. In C58/J, significant sociability was found up to 2 weeks following a subchronic oxytocin regimen, supporting the premise that repeated treatment with oxytocin can induce persistent alterations in neural circuitry underlying aspects of social perception, motivation, or reward. In the *Grin1* knockdown model, subchronic oxytocin led to a striking increase in sociability in the *Grin1*$^{neo/neo}$ mice, without altering social approach in the wild-type group. In both models, the enhanced social approach was observed in adult mice, indicating that chronic social deficits maintained beyond adolescence are not recalcitrant to reversal. Together with our previous findings in BALB/cByJ (Teng et al., 2013), these results provide evidence that prosocial effects of oxytocin can be observed in genetically- and phenotypically-diverse mouse models of autism-relevant behaviors, suggesting oxytocin could have generalized efficacy across subtypes of the autism spectrum disorders.

In mice, the IP route of injection has been shown to induce a rapid increase in oxytocin levels of amygdala and hippocampus, measured by sequential microdialysates across a 2 h period (Neumann et al., 2013). Our studies used a subchronic regimen with four IP injections of oxytocin. Sobota et al. (2015) found that a similar subchronic regimen with oxytocin increases social approach, reduces anxiety-like behavior, and decreases amygdalar activation in adolescent C57BL/6J mice. Other researchers have reported that, in adolescent rats, more extended regimens (10 IP injections of oxytocin) can lead to persistent increases in social preference and decreases in anxiety-like behavior (Bowen et al., 2011; Suraev et al., 2014). More recently, a study using mice with targeted disruption of *Cntnap2*, an ASD candidate gene, found increased sociability following a daily oxytocin regimen (from

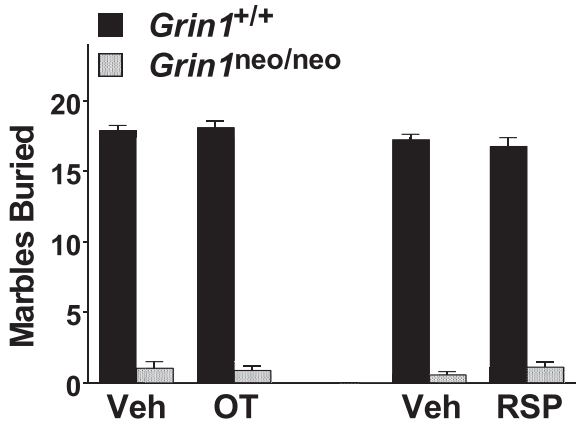

Fig. 9. No effects of subchronic oxytocin or chronic risperidone treatment on marble-burying deficits in *Grin1*$^{neo/neo}$ mice. The marble-burying assay was conducted 4–5 days following the end of the subchronic regimen of oxytocin (OT) or vehicle (Veh), or 7–8 days following a chronic risperidone (RSP, 21 day; 2.0 mg/kg/day) regimen. Post-hoc tests were not conducted, due to number of zero scores in the *Grin1*$^{neo/neo}$ groups.

postnatal day 7–21) in adolescent knockout mice (Peñagarikano et al., 2015). The investigators also showed that acute treatment with oxytocin had significant effects on social approach, but not on hyperactivity or perseverative responses, in the *Cntnap2* model. These results are in contrast to the present findings, in which acute oxytocin significantly attenuated overt hyperactivity in *Grin1* knockdown mice. It is notable that, in the C58/J model, we have previously reported that acute oxytocin significantly decreases abnormal repetitive behavior at a dose that does not reduce general locomotion (Teng et al., 2013).

Not all studies have reported positive effects from chronic oxytocin regimens in rodents. Bales et al. (2014) did not observe enhanced sociability in BTBR *T*$^{+}$*Itpr3*^{tf}/J mice following daily treatment with oxytocin across 30 days. In monogamous prairie voles, a 21-day regimen, from weaning age to puberty, led to *decreased* time spent by male voles in side-to-side contact with a familiar female partner (Bales et al., 2013). Huang et al. (2014) found that 7-to-21 day regimens of intranasal oxytocin in C57BL/6J led to decreased affiliative behavior, as well as decreased oxytocin receptor binding in brain regions implicated in social behavior and reward, including amygdala and nucleus accumbens. A similar reduction in oxytocin receptor binding has been reported following 15-day central infusion of oxytocin in C57BL/6 (Peters et al., 2014). These latter studies suggest that hyperstimulation of normal social circuitry with oxytocin might have detrimental consequences in wild-type or control mice. Although Sobota et al. (2015) were able to demonstrate prosocial effects of subchronic oxytocin in adolescent C57BL/6J mice, the oxytocin treatment did not reverse social deficits induced by the NMDA antagonist ketamine, in contrast to our present findings with the NMDA receptor knockdown mice.

The *Grin1*$^{neo/neo}$ mouse was originally proposed as a model of schizophrenia (Mohn et al., 1999). Recent clinical studies in subjects with schizophrenia have suggested that oxytocin could have therapeutic efficacy for deficits in emotion recognition, attribution bias, and other aspects of social cognition, following acute (Davis et al., 2013; Fischer-Shofty et al., 2013; Woolley et al., 2014) or chronic (Pedersen et al., 2011) treatment. In addition, 2-to-8 week regimens of intranasal oxytocin have been reported to alleviate more general positive and negative symptoms in schizophrenia (Feifel et al., 2010; see Pedersen, 2014 for review), suggesting that oxytocin might have broader antipsychotic-like activity. Work in rodent models has demonstrated antipsychotic-like effects of acute oxytocin or oxytocin agonists on sensorimotor gating deficits (Feifel et al., 2012; Ring et al., 2010), although Huang et al. (2014) did not observe changes in prepulse inhibition following chronic oxytocin in C57BL/6J. In the present studies, neither acute nor subchronic oxytocin reversed impaired prepulse inhibition in *Grin1*$^{neo/neo}$ mice, providing evidence that antipsychotic-like oxytocin action is dependent upon the particular animal model.

We also evaluated the effects of chronic antipsychotic treatment on social deficits in the *Grin1* knockdown model. Currently, only two drugs have FDA approval for treatment of ASD, risperidone and aripiprazole. While these drugs have been found to have some benefits against irritability in ASD, there is still no standard treatment for impaired social behavior (Chadman, 2014). Similarly, atypical antipsychotics generally have modest-to-poor efficacy against social deficits in schizophrenia (Penn et al., 2009; Roberts et al., 2010). Studies in the BTBR *T*$^{+}$*Itpr3*^{tf}/J and *Cntnap2*$^{-/-}$ mouse models of ASD-like behavior have shown that acute risperidone does not reverse sociability deficits (Chadman, 2011; Gould et al., 2011; Peñagarikano et al., 2011; Silverman et al., 2010). Further, Mielnik et al. (2014) did not observe increased sociability following acute clozapine in mice with deficient NMDA receptor function. However, the question remained whether chronic antipsychotic intervention, initiated in early adolescence or young adulthood, could alleviate the severity of social impairment. In the present study, we found that chronic clozapine or risperidone failed to rescue social deficits in *Grin1*$^{neo/neo}$ mice. Further, although acute treatment with these agents can ameliorate impaired sensorimotor gating in the *Grin1* model (Duncan et al., 2006a,b), chronic exposure did not alter performance in the acoustic startle task in either wild-type or knockdown mice.

Overall, these results point to a unique and selective prosocial efficacy for oxytocin. Together with our previous findings in BALB/cByJ, we have demonstrated that a subchronic oxytocin regimen can lead to persistent enhancement of sociability across three models with divergent genotypes and behavioral profiles, suggesting the possibility of generalized therapeutic benefits across the autism spectrum disorders, as well as other neurodevelopmental disorders characterized by social impairment. Further studies with this panel of models could identify common abnormalities in signaling pathways underlying deficits in social approach, as well as the mechanism of action for adaptive changes in brain with subchronic oxytocin intervention.

Acknowledgments

We sincerely thank Rebecca Dye and Tamara T. Davis for their valuable contributions to this research. Support for this project was provided by the Department of Defense (AR1002312P1, AR1002312P2, and AR100231P3), the National Institute for Mental Health (RO1 MH080069 and K01 MH094406), and the National Institute of Child Health & Human Development (P30 HD03110; U54 HD079124). Dr. Teng was supported by an Autism Speaks Translational Postdoctoral Fellowship (#7952). These funding sources were not involved in the study design, the collection, analysis or interpretation of data, writing of the report, or in the decision to submit the article for publication.

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