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Behavioral abnormalities exhibited by reelin deficient mice may model behavioral deficit characteristics that are associated with schizophrenia, bipolar disorder, autism, and/or attention deficit hyperactivity disorder (ADHD). To expand upon the behavioral phenotype of reelin deficient mice, the effects of methylphenidate (MPH) and nicotine (NIC) were evaluated on specific behaviors in homozygous (*rl/rl*, 100% deficient), heterozygous (*+/rl*, 50% deficient), and wild-type (+/+, 0% deficient) mice to determine if drugs would differentially influence behavior depending on level of reelin deficiency, and to determine if responses to drug treatments modeled effects that would be predicted, assuming mice modeled impairments associated with schizophrenia, bipolar disorder, autism, or ADHD. In addition, NIC and saccharin vehicle (S-VEH) intake levels were measured to determine if mice self-administer NIC differently depending on genotype. A non-linear gene dosage effect of NIC, but not MPH, was observed in prepulse inhibition (PPI), such that NIC improved PPI in +/+ and *rl/rl* mice, while NIC tended to reduce abnormally elevated startle response and PPI in *+/rl* mice. During NIC self-administration trials, *+/rl* mice decreased NIC intake when concentration of NIC increased to 60 mg/kg/BW, while *rl/rl* mice tended to increase NIC intake with increased NIC concentration. Moreover, *+/rl* and *rl/rl* mice exhibited elevated levels of S-VEH intake, and *rl/rl* mice exhibited an intensified fluid dispenser side bias compared to +/+ mice. In tests of social interaction, *rl/rl* mice displayed impaired social recognition. In

addition, NIC *+/rl* mice exhibited increased social behavior, while NIC *rl/rl* mice displayed decreased social behavior. In the passive avoidance (PA) test, all genotypes demonstrated the ability to withhold a response—which is a measure executive functioning—and *rl/rl* mice demonstrated enhanced performance. In addition, MPH produced enhanced performance in *+/rl* mice and *rl/rl* compared to VEH condition. Thus, behavioral outcomes and pharmacological responses in the present study expand upon profiles of *+/rl* and *rl/rl* mice, offer guidance about which behaviors across and/or within diagnostic categories may be most profitably modeled by behavioral features of these mice, and may provide insight into underlying neurobiological pathology associated with altered cholinergic and dopaminergic pathways as a consequence of disrupted reelin production.

THE EFFECTS OF NICOTINE AND METHYLPHENIDATE ON ABNORMAL BEHAVIORS IN REELIN DEFICIENT MICE: POTENTIAL ANIMAL MODELS FOR NEURODEVELOPMENTAL DISORDERS

By

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APPROVAL PAGE

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CHAPTER I

INTRODUCTION

Animal models of neurodevelopmental disorders provide opportunities to investigate biological mechanisms of abnormal behaviors. However, although neurodevelopmental disorders are classified by distinct diagnostic criteria, specific behavioral impairments may transcend diagnostic categories. In addition, the degree of impairment may vary across subtypes within diagnostic categories. Thus, recent directions in research involve the identification of animal models of shared behavioral impairments across and within subtypes of diagnostic categories (Geyer, 2006; Tordjman et al., 2007) to unravel structure–function relationships of behavioral impairments in neuropsychiatric disorders. In this approach, using animal models to gain knowledge of both shared and non-shared structure–function relationships may advance efforts to determine neurobiological targets for preventive strategies and interventions via pharmacological treatments in specific behavioral domains. Thus, in the present study, the pharmacological effects of methylphenidate (MPH) and nicotine (NIC) on the behavioral phenotype of reelin deficient mice were investigated in the context of behavioral domains that are relevant to schizophrenia, bipolar disorder, autism, and ADHD.

Reelin Deficits and Abnormal Neurobiology in Mice and Patient Populations

Reelin, an extracellular matrix protein (RELN), plays a key role in neuronal migration (D'Arcangelo et al., 1995) and a putative role in neural plasticity in adulthood (see review, Levenson, Qiu, & Weeber, 2008). Disruption of reelin pathways and abnormal neurobiology of reelin deficient mice bear similarities to abnormalities reported in neuropsychiatric populations. For example, the absence of reelin in homozygous reelin mutant mice (*rl/rl*) (D'Arcangelo et al., 1995) and reduced (~50%) levels of reelin in heterozygous reelin deficient mice *(+/rl*) (Costa et al., 2001) mirror deficiencies of reelin that have been reported in individuals with autism (up to ~80%), schizophrenia (~50%), and bipolar disorder (~50%) (Fatemi, Earle, & McMenomy, 2000). Reelin deficient mice also exhibit neuroanatomical anomalies, such as reduced dendritic branching (Pappas, Kriho, & Pesold, 2001), which bear similarities to neurodevelopmental abnormalities found in brains of individuals with schizophrenia, bipolar disorder (Costa et al., 2001), and autism (Pardo & Eberhart, 2007). Central to the pharmacological focus of the present study are reports of altered pathways involving the neurotransmitters acetylcholine (ACh) and dopoamine (DA) in reelin deficient mice (Tueting et al., 1999) and individuals with schizophrenia (Freedman, Hall, Adler, & Leonard, 1995; Stone, Morrison, & Pilowsky, 2007), bipolar disorder (Torrey et al., 2005), autism (Pardo & Eberhart, 2007), and ADHD (Kuntsi, McLoughlin, & Asherson, 2006).

Cholingergic Abnormalities, Reelin Deficient Mice, and Neuropsychiatric Disorders

Acetylcholine is a key excitatory neurotransmitter, and as such, activation of ACh

receptors (AChRs) results in increased neural signaling (McKay, Placzek, & Dani, 2007). Importantly, abnormalities in ACh pathways have been reported in reelin deficient mice. For example, *+/rl* mice exhibit reduced cholingergic cell numbers and decreased cholinergic innervation in selective areas of the brain (Sigala et al., 2007). In *rl/rl* mice, evidence of elevated expression of acetylcholinesterase (AChE), an enzyme involved in the hydrolysis of acetylcholine, suggests reduced availability of acetylcholine in the cerebellum and hippocampus (Garcia-Ayllon et al., 2003). Conversely, the absence of AChE-positive fibers elsewhere suggests potentially high levels of acetylcholine in other areas of the brain in *rl/rl* mice (Martin, 1981). In addition, prenatal administration of AChE blocker in *rl/rl* mice reversed certain behavioral effects (i.e., ultrasound vocalization, amphetamine-induced locomotion, and stereotypy) associated with the absence of reelin (Laviola, Adriani, Gaudino, Marino, & Keller, 2006). Thus, reelin deficiency appears to be associated with altered AChE and ACh activity, which in turn results in behavioral abnormalities.

The aforementioned reports of cholinergic abnormalities in reelin deficient mice are of particular interest in light of reports of altered ACh pathways in neuropsychiatric disorders. For example, altered expression of nicotinic ACh receptors (nAChr) in the hippocampus, frontal cortex, and other areas have been associated with schizophrenia (see Reviews, Gotti & Clementi, 2004; Olincy & Stevens, 2007), while under- and over-expression of nAChr subtypes have been associated with autism (Martin-Ruiz et al., 2004; Perry et al., 2001). While ADHD has not been associated with altered reelin pathways, it has been associated with altered nAChr expression as well (Potter,

Newhouse, & Bucci, 2006). Importantly, altered ACh pathways in reelin deficient mice and in neuropsychiatric patients may be relevant to compromised neural plasticity, including disruption of pathways involving other key neurotransmitters.

Dopaminergic Abnormalities, Reelin Deficient Mice, and Neuropsychiatric Disorders

Activation of ACh receptors results in indirect excitatory and/or inhibitory influences on pathways involving other neurotransmitters, including (but not limited to) dopamine (McKay, Placzek, & Dani, 2007). Thus, reports of abnormal dopamine functioning in reelin deficient mice are also central to this study. For example, young adult *+/rl* mice exhibit alterations in the mesolimbic dopamine pathway, including reduced DA2 receptor mRNA, increased DA3 receptor mRNA levels, and increased D3 binding sites (Ballmaier, Zoli, Leo, Agnati, & Spano, 2002). Furthermore, since ACh receptors are typically present on dopamingergic neurons in the mesolimbic pathway (Wooltorton, Pidoplichko, Broide, & Dani, 2003), these findings also suggest possible disturbances in ACh functioning. In *rl/rl* mice, DA transporters are significantly reduced (Giompres & Delis, 2005), and the absence of reelin signaling has been associated with a reduction of DA receptors and receptor-mediated dopaminergic functions, and with reduced methamphetamine-induced hyperlocomotion (Matsuzaki et al., 2007). In addition, disruption of reelin in +/+ mice results in reduced methamphetamine-induced hyperlocomotion, which suggests that reelin signaling plays a key role in the dopaminergic related behavior in adult mice (Matsuzaki et al., 2007).

Altered dopamine functioning has also been reported in individuals with schizophrenia, autism, and ADHD. For example, decreased DA activity in the prefrontal cortex and increased DA activity in the striatum have been reported in individuals with schizophrenia (Stone, Morrison, & Pilowsky, 2007). In autism, recent reports of impaired neural synchronization, which relies on signaling mediated by D4 receptor activation, is impaired (Deth, Muratore, Benzecry, Power-Charnitzky, & Waly, 2008), and in ADHD, altered DA activation and DA transporters have been reported (see review Swanson et al, 2007). Taken together, evidence of altered neurobiology, including pathways involving ACh and DA, in reelin deficient mice and in patient populations, suggests that investigation of behavioral impairments in reelin deficient mice may be relevant to the development of models for behavioral impairments associated with human disorders such as schizophrenia, autism, and ADHD.

Behavioral Abnormalities in Reelin Deficient Mice: Potential Behavioral Models for Human Disorders

Behavioral abnormalities exhibited by reelin deficient mice may model core diagnostic criteria and related characteristics for human psychopathology. For example, impaired sensorimotor gating, as measured by prepulse inhibition (PPI) of the startle response in *+/rl* mice (Tueting et al., 1999), and deficits in PPI in *rl/rl* mice (Salinger, Ladrow, & Wheeler, 2003) may model impaired PPI associated with schizophrenia, bipolar disorder (Perry, Minassian, Feifel, & Braff, 2001), and autism (Perry, Minassian, Lopez, Maron, & Lincoln, 2007). Similarly, reports of abnormal social interactions in

reelin deficient mice, such as severe impairments in maternal behavior (Salinger, not published) and abnormally dominant behavior for *rl/rl* mice (Salinger et al., 2003) suggest that social behavior in reelin deficient mice may model behavioral impairments associated with schizophrenia, bipolar disorder, autism, and ADHD. In addition, reports of abnormal response inhibition in reelin deficient mice (Ognibene, Adriani, Granstrem, Pieretti, & Laviola, 2007) suggest that these mice may be useful to model impairments of executive functioning reported in individuals with schizophrenia, bipolar, ADHD, and autism (Russo et al., 2007).

Thus, behavioral abnormalities exhibited by reelin deficient mice may model behavioral impairments that are observed within and/or across diagnostic categories of schizophrenia, bipolar disorder, autism, and ADHD. If so, reelin deficient mice may provide opportunities to delineate abnormal neurobiological mechanisms associated with behavioral impairments associated with neuropsychiatric disorders. Therefore, this study expanded upon earlier research to further characterize the behavioral phenotypes of reelin deficient mice as potential models for behavioral characteristics associated with schizophrenia, bipolar disorder, autism, and possibly ADHD. To do so, the effects of nicotine (NIC), a direct acetylcholine agonist, and methylphenidate (MPH), a direct dopamine agonist, were evaluated on measures of sensorimotor gating and learning in *rl/rl, +/rl* and +/+ mice. NIC self-administration and effects of NIC in tests of sociability and social recognition (memory) were also evaluated.

Pharmacological Manipulations and Behavioral Assays

PPI. The first goal of this study was to evaluate the effects of pharmacological manipulations of prepulse inhibition (PPI) of the startle response. PPI is frequently used to operationally measure sensorimotor gating, or the ability to filter sensory stimuli, across different species (Braff, Geyer, & Swerdlow, 2001). In PPI, when a prepulse is presented, the size of the startle response that is produced by a loud, startling stimulus (e.g.120 dB) is reduced compared to when the startle stimulus occurs without a prepulse. This phenomenon is modulated, in part, by dopaminergic (Ballmaier et al., 2001) and cholinergic pathways (Swerdlow et al., 2001). Thus, modulation of dopaminergic and cholinergic pathways is of particular importance to the pharmacological manipulations in the present study.

Methylphenidate (MPH) directly targets DA transporters to increase the availability of DA, and as previously discussed, *rl/rl* and *+rl* mice exhibit abnormalities in pathways involving DA (Giompres & Delis, 2005; Yamashita et al., 2006). In human populations, while MPH attenuates some behavioral deficits associated with ADHD, MPH exacerbates behaviors that are characteristic of other disorders such as schizophrenia (Koreen, Lieberman, Alvir, & Chakos, 1997). Thus, if MPH alters PPI, the direction of change in PPI could be informative in terms of reelin deficiency and behavioral impairments associated with neuropsychiatric disorders.

Nicotine (NIC), an agonist of acetylcholine, binds to and activates nicotinic acetylcholine receptors (nAChRs). Importantly, *rl/rl* and *+rl* mice exhibit abnormalities in pathways involving acetylcholine, and *rl/rl* mice reliably exhibit impaired PPI

(Salinger, Ladrow & Wheeler, 2003; Goode, 2006). In addition, NIC has been shown to improve PPI in mice (Ingram et al., 2005). In schizophrenic populations, administration of NIC via smoking improves PPI (Postma et al., 2006) and normalizes abstinence-induced PPI deficits (George et al., 2006). Although effects of NIC on impaired PPI in individuals with bipolar disorder and autistic populations have not been reported, it has recently been suggested that NIC may exert negative effects on individuals with autism (Lippiello, 2006). Thus, NIC could normalize PPI abnormalities in *rl/rl* mice, as it does in individuals with schizophrenia. On the other hand, NIC could exacerbate PPI in *rl/rl* mice, which would be consistent with ACh-antagonist theories about autism (Lippiello, 2006).

Nicotine self-administration. The second objective of this study was to determine if *rl/rl*, *+/rl*, and +/+ mice differ in patterns of self-administration of NIC, based in part on reports that individuals in the aforementioned diagnostic categories exhibit different prevalence rates of smoking. For example, compared to the general population (~25-30%), people with schizophrenia exhibit higher prevalence rates (67% - 87%) of smoking (Poirier et al., 2002; Vanable, Carey, Carey, & Maisto, 2003). Furthermore, severity of symptoms and intensity of smoking are positively correlated in schizophrenia (Patkar et al., 2002). Prevalence rates for bipolar disorder (70%) (Hughes, Hatsukami, Mitchell, & Dahlgren, 1986) and ADHD (40%) (Pomerleau, Downey, Stelson, & Pomerleau, 1995) are also higher compared to the general population. Conversely, the prevalence rate of smoking in autism is low (12.5%) (Bejerot & Nylander, 2003) compared to the general population. Although the latter observation could be reflective of limited access to

cigarettes, Lippiello (2006) theorizes that, based on evidence of neuroanatomical abnormalities with potential hypercholinergic consequences, individuals with autism may actively avoid NIC. Thus, in the present study, if *rl/rl, +/rl,* and +/+ mice displayed differences in NIC self-administration, outcomes may be informative in terms of potential relationships between reelin deficiency NIC, the degree of nicotine abuse, and reelin-related pathologies (i.e., schizophrenia, bipolar disorder, autism) as well as ADHD.

Social interactions. The third objective of this study was to determine if *rl/rl* and *+/rl* mutant mice exhibit abnormalities in social activity. The rationale for this assay was based, in part, on similarities in reelin disruption between reelin deficient mice and individuals with disorders that are characterized by reelin disruption and abnormal social behavior. In addition, the relationship, if any, between degree of reelin deficiency and the severity and direction of social impairment was also of interest based on the heterogeneity in social impairment observed in schizophrenia, bipolar disorder, and autism. For example, although schizophrenia is characterized by poor social skills and/or social withdrawal, schizoaffective subtypes of schizophrenia may display more outgoing behavior during manic-like episodes (American Psychiatric Association, 2000). Similarly, individuals with bipolar disorder may exhibit social withdrawal during depressive episodes; however, during manic episodes, the opposite may be observed (American Psychiatric Association, 2000). Conversely, autism is characterized by a profound lack of sociability (American Psychiatric Association, 2000). Thus, models to elucidate neurobiological and behavioral components that underlie the heterogeneity of social impairment within and across diagnostic categories may be useful.

Animal models involving attention and responsiveness to social cues may be useful to model social impairments, based on reports that individuals with autism and schizophrenia differ in orienting responses to social stimuli (Sasson et al., 2007). In addition, nicotine has been reported to enhance social memory in mice (Manrique, Miquel, & Aragon, 2005). Thus, the effect of NIC on mice in tasks dependent on socially relevant cues was evaluated in a modified version of a behavioral assay designed to evaluate social activity (Crawley, 2004). In the modified paradigm, if mice spend more time with a novel non-social stimulus (inanimate object) compared to a novel social stimulus (stranger mouse), they model diminished social behavior. In the subsequent trial, if mice spend more time with a mouse to which they have had recent exposure (familiar mouse) compared to a novel social stimulus (new stranger mouse), or if they exhibit no preference between social stimuli, the lack of normal preference for novel social stimuli reflects impaired social recognition (memory).

Passive avoidance (PA). The fourth objective of this study was to assess executive functioning of reelin deficient mice in an assay that requires animals to learn an association between a response (moving from a light chamber into a dark chamber) and an aversive stimulus (mild foot shock delivered if animal enters dark chamber). This test measures emotional learning and memory, and the ability to withhold the response when exposed to the chamber during the second trial. In previous studies conducted in this lab (Goode, 2006), reelin deficient mice tended to exhibit decreased latency (compared to +/+ mice) to enter the dark chamber (where previously shocked). Furthermore, Ognibene et al. (2007) recently reported that *+/rl* mice exhibited an inability to inhibit motor

responses in operant conditioning. Taken together, these findings suggest that reelin deficiency in mice may be associated with deficits of executive functioning.

Importantly, PA is, in part, modulated by DA (Ichihara, Nabeshima, & Kameyama, 1988), and MPH, a direct agonist of DA, has been reported to improve performance on tasks that require response inhibition in humans (Aron, Dowson, Sahakian, & Robbins, 2003). However, nicotinic acetylcholine receptors (nAChRs)—which are important for processes involved in learning and memory—appear to play a role in normal performance in the passive avoidance as well (King et al., 2003; Picciotto et al., 1995). Furthermore, this assay has been shown to be sensitive to the effects of NIC such that NIC can enhance passive avoidance learning in adult wild-type mice (King et al., 2003). Thus, both MPH and NIC effects were assessed on performance in the passive avoidance test.

Summary of Predictions

Pharmacological manipulations of prepulse inhibition (PPI). MPH was not predicted to improve PPI. In addition, if MPH exacerbated performance, as other direct dopamine agonists do, then PPI deficits of reelin deficient mice could potentially model schizophrenia (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001; Koreen et al., 1997).

NIC was predicted to enhance normal PPI in +/+ and *+/rl* mice, and normalize impaired PPI in *rl/rl* mice. If so, PPI response to NIC in *rl/rl* mice may model NIC attenuation of PPI deficits in individuals with schizophrenia (Postma et al., 2006). If

however, NIC exacerbated PPI deficits, mice may more appropriately model autism (Lippiello, 2006).

Self-administration of nicotine. If NIC intake levels were elevated and/or if levels increased over time compared to +/+ mice, then NIC self administration in reelin deficient mice may model abnormally high nicotine consumption exhibited by individuals with schizophrenia (Poirier et al., 2002; Vanable et al., 2003), bipolar disorder (Hughes et al., 1986) and/or ADHD (Pomerleau et al., 1995). If mice exhibited lower NIC intake levels and/or decreased NIC intake over time compared to +/+ mice, then they would be viewed more appropriately as avoiding NIC as do individuals with autism (Bejerot & Nylander, 2003).

Social interaction paradigm. If mice displayed a preference for an inanimate stimulus versus a social stimulus, they would exhibit decreased sociability (Crawley, 2004), as do individuals with schizophrenia and autism (American Psychiatric Association, 2000). If mice displayed a preference for a recently-familiar social stimulus versus a novel social stimulus, they would model a lack of normal preference for novel social stimuli or be displaying social recognition/memory deficits associated with schizophrenia or autism (Crawley, 2004). In addition, if NIC enhanced social memory (Manrique et al., 2005), then improved performance may mirror NIC-enhanced cognitive functioning associated with schizophrenia. However, if NIC diminished performance, the findings may more appropriately support theories associated with autism (Lippiello, 2006).

Pharmacological manipulations of passive avoidance (PA). MPH improves performance on tasks that require response inhibition in individuals with ADHD (Aron et al., 2003). Therefore, if reelin deficient mice exhibited inferior learning in PA, and if MPH improved passive avoidance performance, then mice would model impaired inhibition associated with ADHD. If, on the other hand, MPH exacerbated PA performance deficits, the effects of MPH on PA performance of reelin deficient mice would model MPH effects on cognitive performance in individuals with schizophrenia (Koreen et al., 1997).

NIC was predicted to enhance cognitive performance in the passive avoidance test (King et al., 2003). If mice exhibited impaired PA learning, and if NIC enhanced performance of *+/rl* mice, then NIC effects on PA performance of *+/rl* mice would model NIC effects on executive functioning deficits associated with schizophrenia and/or ADHD. If NIC exacerbated PA performance, NIC effects on PA performance of reelin deficient mice would support theories related to autism (Lippiello, 2006).

CHAPTER II

METHOD

Subjects

Experimentally naïve mice (B6C3FE strain, Jackson Laboratories, Bar Harbor, Maine, U.S.A.), bred in our animal colony, were utilized for the behavioral assessments. Mice were weaned at 21 days or at body weight of 10–12 grams, whichever came later and were housed socially, in same-sex groups of 2-4 littermates. A 10/14 hour lights-on/ lights-off schedule at a constant room temperature of ~23°C was maintained. Rat chow and water were available ad libitum before and after all of the experiments. The mice were between 3-6 months of age at the beginning of assessment procedures. Two cohorts of mice were tested (see Table 2). In the first cohort of mice (n = 60), approximately half of the mice of each genotype and gender were assigned to either MPH or VEH conditions. Testing of the second cohort (n = 95) followed upon completion of testing for MPH mice, and approximately half of the mice of each genotype and gender were assigned to either NIC or VEH. All experiments were conducted between 1100h and 1700h in a testing laboratory with lighting and room temperature conditions similar to those of the vivarium. The procedures for all paradigms were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of North Carolina at Greensboro.

Drugs and Methods of Administration

MPH preparation for gavage administration (PPI and Passive avoidance trials). MPH (30 mg) was dissolved in 2 ml in tap water (VEH) and administered 30 minutes prior to trials by gavage at a dose of 30 mg/kg/BW. The time of administration of 30 minutes prior to trials was based on pilot trials. Two methods of administering drug treatments to mice for PPI are prevalent in the literature: Intraperitoneal (IP) and subcutaneous (SC). Although there is greater bioavailability of a drug in a shorter period of time when administered IP or SC, MPH was administered by gavage to minimize stress related to repeated injections. Doses used in behavioral studies with mice have varied, depending on the experimental design and method of administration, with intraperitoneal (IP) doses up to 30 mg/kg/BW (Gainetdinov et al., 1999; Rhodes & Garland, 2003; Trinh, Nehrenberg, Jacobsen, Caron, & Wetsel, 2003). Pilot trials conducted in this lab (MPH 3, 10, 20, 30 mg/kg/BW) determined 30 mg/kg/BW to be an effective dose.

NIC preparation for gavage administration for PPI, social activity and PA trials. Nicotine (NIC) -(-) NIC Hydrogen tartrate (Sigma), 3 mg and 6 mg, was dissolved in 1 ml tap water (VEH) and administered 20 minutes prior to trials by gavage at doses of 3 mg/kg/BW or 6 mg/kg/BW. In previous studies, time of administration prior to testing varied from no time delay to 15 minutes prior to testing. Whereas IP and SC injections result in shorter latency of bioavailability, oral and gastric administration reported in other types of drug studies indicate that longer time periods between dosing and behavioral trials may be necessary to maximize bioavailability (Petersen, Norris, &

Thompson, 1984; Svensson, 1987). All mice in NIC condition received both 3 mg and 6 mg NIC and order of administration was counterbalanced; however, no order effects of treatment were detected, and 3 mg/kg/BW trials yielded non-significant results (data not shown) and thus, are not included in results or discussion.

NIC preparation for self-administration trials. Nicotine (NIC) -(-) NIC Hydrogen tartrate (Sigma) was dissolved saccharin (0.4%) vehicle (S-VEH), and mice were provided with NIC and/or S-VEH solutions for 10 consecutive days (days 1-5 at NIC 30 mg/L and days 6-10 at NIC 60 mg/L). The concentration of 30 mgl/L was based on methods reported by Adriani, Macri, Pacifici, and Laviola (2002), and the concentration of 60 mg/L was based on reports of plasma levels of NIC in mice following chronic, oral, ad libitum, self-administration of NIC (60 mg/L) of 34.4 ng/ml (Rowell, Hurst, Marlowe, & Bennett, 1983), which is comparable to steady-state levels of about 40 ng/ml reported in chronic smokers (McNabb, 1984; Russell, Jarvis, Iyer, & Feyerabend, 1980).

Behavioral Assays

Sequence of behavioral assays. The sequence of trials for all mice was acoustic startle response (ASR) and prepulse inhibition (PPI), social activity, passive avoidance (PA), and NIC self-administration trials (10 days).

Acoustic startle response (ASR) and prepulse inhibition (PPI). Prepulse inhibition (*PPI*) of the acoustic startle response (ASR) measures an unlearned, involuntary reflex modulation that is thought to underlie the ability to engage in selective attention (Braff et

al., 2001). More specifically, when an acoustic prepulse is presented, the size of the startle response that is produced by a loud, startling stimulus is reduced compared to when the startle stimulus occurs without a prepulse. ASR and PPI were measured using the SR-LAB® (San Diego Instruments Inc., San Diego, CA) startle response measurement system, including software. In this system, a 3.9-cm (outside diameter) acrylic cylinder for holding the mouse is mounted on a platform (20.4 cm length X 12.7 cm width X 0.4 cm thick) with a piezoelectric accelerometer unit attached below the acrylic cylinder. The acrylic cylinder and platform are located in a sound attenuated chamber with a loudspeaker (28 cm above the cylinder) and house light. Following a modified version of standardized procedures (Paylor & Crawley, 1997), five acoustic stimulus types were presented, with four prepulse stimuli (0, 76, 80, or 84 dB; each 20 ms duration) and a startle stimulus (120 dB, 40 ms duration). The 17.39 min. test included a 5-min acclimation period followed by 10 repetitions of five different trial types and intertrial intervals (average 15 s) presented in pseudorandom order. Between-subjects variables included genotype, gender, and drug treatment (MPH, NIC, or VEH), and the within-subjects variable was prepulse trial type (76, 80, 84 dB). The dependent variables were the acoustic startle response (ASR), measured as the amplitude for prepulse 120 dB without a prepulse (0 dB), and prepulse inhibition (PPI) of the ASR, expressed as the percentage reduction of startle (120 dB) response amplitude when preceded by a prepulse: (Startle amplitude with prepulse – startle amplitude without prepulse / startle amplitude without prepulse) X 100. Note: Although mice were tested with 3 mg/kg/BW

NIC as indicated above, no effects of treatment were detected and therefore, only trials involving 6 mg/kg/BW are reported and discussed in the results and discussion.

Fluid intake in the home cage and fluid side dispenser bias. Total ad libitum fluid intake was measured as a control component for NIC self-administration. Mice were individually housed and provided with two fluid dispensers in the home-cage. Fluid dispensers consisted of 10 ml graduated pipettes that were fitted with stoppers from standard water bottles. One fluid dispenser was positioned in the typical home-cage (HC) location of water bottle placement during non-experimental conditions, and the other (novel) fluid dispenser was yoked to the HC pipette, such that it was positioned in the opposite side of the cage, relative to the HC fluid dispenser, in the same end of the cage. Saccharin vehicle (S-VEH), which consisted of deionized water sweetened with saccharin (0.4%), was freshly mixed each morning. S-VEH also served as the base fluid to which nicotine was added for NIC-self administration (details below). Fluid mixtures and measurements were confirmed and collected each morning by two experimenters, and pipettes were rinsed with 70% alcohol, followed by a rinse of deionized water prior to replacement of fluids each day. Total S-VEH (ml/kg/BW) and the proportion of S-VEH intake from each fluid dispenser were the variables of interest as measures of total fluid intake and fluid dispenser side bias, respectively.

Continuous self-administration with NIC. For assessment of NIC self-administration, fluid dispensers were provided as described above for fluid intake in the home cage. Fluids were saccharin vehicle (S-VEH) and nicotine (NIC), and mice were assigned to one of three conditions for fluid dispensers in the home-cage (position of

pipette novel/HC): S-VEH/NIC, NIC/S-VEH, S-VEH/S-VEH. For mice with NIC choice, fluid dispenser placement was counterbalanced within genotype. Two concentrations of NIC (30 mg/L and 60 mg/L dissolved in S-VEH) were presented for five consecutive days, with all mice in NIC and S-VEH choice condition receiving NIC 30 mg/L first, followed by NIC 60 mg/L. The concentrations of NIC (30 mg/L and 60 mg/L) were based on data reported in pilot studies and on reports that plasma levels of NIC in mice following chronic, oral, ad libitum, self-administration of NIC (60 mg/L) was found to be 34.4 ng/ml (Rowell et al., 1983), which is comparable to steady-state levels of about 40 ng/ml reported in chronic smokers (McNabb, 1984; Russell et al., 1980). Using both concentrations of NIC allowed mice to demonstrate that they titrated the NIC dose to establish optimal levels, since if they were titrating, a switch from the 30 mg/L to the 60 mg/L NIC solution should cause mice to compensate by reducing proportionately the volume of their intake in order to maintain the NIC dosage levels they had sustained when self-dosing with the 30 mg/L NIC solution. Only freshly mixed solutions of both S-VEH and NIC were used each day, and pipettes were rinsed with alcohol, followed by a rinse of deionized water, prior to replacement of fluids. NIC (ml/kg/BW) intake and S-VEH (ml/kg/BW) intake were calculated as the difference in fluid (ml/kg/BW) from pipettes, as measured and confirmed each day by two experimenters prior to refilling pipettes with fresh fluids.

The first measure of interest was NIC discrimination, which was operationally defined as a significant change in ratio of NIC/total fluids across and/or between concentrations. The second measure was the absolute volume of NIC fluid intake

(ml/kg/BW). The third measure was S-VEH intake (ml/g/BW) to assess changes in S-VEH across trials as mice titrated NIC. Finally, NIC self-administration was operationally defined as an increase in the proportion of NIC/total fluids compared to the expected ratio based on S-VEH/S-VEH control mice within trials of NIC at 30 and 60 mg/L concentrations.

Sociability and social recognition tests. The social recognition test measured the tendency of a subject mouse to approach another mouse (compared to a novel object) and engage in social investigation. The clear, glass test chamber, 30" long X 12" wide, contained three equal-sized zones, separated by two clear, Plexiglas divider walls. In Task 1 the subject mouse was placed in the center zone for 10 minutes to acclimate to the test chamber. Then, a stranger mouse (S1) of same sex and genotype was placed in either the left or right end zone in a clear holding cage (5.5" X 6.25" X 3.5"), which contained openings for ventilation. The opposite end zone contained an empty cage (EC), which was identical to the holding cage for the stranger mouse. Following acclimation, the Plexiglas walls were removed and the mouse was allowed to move freely within the test chamber for 10 min. Since *rl/rl* mice are characterized by motor impairment, and as such, could take longer to move from one end of the test chamber to the other, the proportion of time in each end zone was calculated relative to total time in end zones (versus total time in zones). The proportion of time in each end zone has been shown to reflect the proportion of time investigating each relative target (Crawley, 2004). Sociability was defined as spending similar or greater proportion of time with S1 compared to the EC.

In Task 2, which began immediately after completion of Task 1, the test subject remained in the test chamber, but S1 was removed and then repositioned in the same location when stranger mouse 2 (S2) was placed in the zone/position that was previously occupied by the EC. Preference for social novelty was defined as spending proportionately more time with S2 compared to S1. Crawley (2004) proposes that in a mouse model of impaired social interaction, mice will atypically show equal or less investigation of stranger 2 compared to stranger 1, reflecting a lack of normal preference for, or recognition of, social novelty.

Passive avoidance(PA). The PA test is designed to evaluate learning and memory, and to evaluate behavioral inhibition, which is the ability to inhibit responses. Behavioral inhibition is a key component of executive functioning (Barkley, 1997). In this study, each of two acquisition trial (s) began when the mouse was placed in the left chamber (start box) of the PA testing chamber (Coulbourne Instruments, Allentown PA), which consisted of a shuttle-box apparatus placed within an independent, sound-attenuating isolation box of wood construction. After 60 seconds, the guillotine door opened and, if the mouse entered the right side of the shuttle box (goal box), the guillotine door closed and a mild foot shock (1.0 mA, 2 s duration) was delivered. Mice were eliminated from the testing procedures if they failed to enter the right side (300 s maximum latency). The test trial, conducted on the following day, consisted of placing the mouse in the start box, as in Day 1, to measure latency to enter the goal box (maximum time 300 s).

Statistical analyses. All analyses were conducted initially as analysis of variance (ANOVA) with between subject factors of genotype (+/+, *+/rl, rl/rl*), treatment (NIC,

MPH, VEH) and gender. The within-subjects factor was repeated measures across multiple trials. Although hypotheses did not directly address gender effects, previous reports suggest that NIC behavioral assessments may be sensitive to gender effects in mice (Damaj, 2001), and thus, gender was included in the model. If main effects or interactions for gender were not significant, the gender term was removed from analyses. However, since the number of mice for each sex for each genotype was small, significant effects with gender should be interpreted with caution. In cases of significant interactions, additional tests of main effects were conducted either with LSQ (and effect screening) or repeated measures.

CHAPTER III

RESULTS

Acoustic Startle Response (ASR)

Analysis of the ASR for VEH mice with a three-way ANOVA with genotype (*+/+, +/rl, rl/rl*), gender, and treatment cohort (MPH, NIC) for startle amplitude revealed significant main effects of genotype, $F(2, 58) = 8.9113$, $p = .0004$, and gender, $F(1, 58) = 6.2870$, $p = .0150$. Consistent with previous findings for genotype, *+/rl* mice exhibited higher startle amplitude at 120dB compared to *+/+* and *rl/rl* mice, and male mice exhibited increased startle responses compared to female mice (see Figure 1). In addition, there was a significant main effect of group (MPH or NIC), $F(1, 58) = 5.5489$, $p = .0187$, and a trend for a Genotype X Gender X Group interaction $F(2, 58) = 2.6625$, $p = .0783$. Effect screening indicated that *+/rl* VEH mice differed between NIC and MPH treatment groups, $p = .0316$; therefore, to evaluate drug effects, data for *+/+* and *rl/rl* mice were analyzed separately from *+/rl* mice, and *+/rl* mice groups (MPH, NIC) were individually analyzed. However, there were no significant effects of treatment (MPH, NIC) on startle amplitude for any genotype.

Prepulse Inhibition (PPI)

Consistent with previous findings, PPI performance of VEH mice differed depending on genotype, $F(2, 54) = 16.1166$, $p < .001$, such that *rl/rl* mice exhibited

inferior PPI compared to +/+ and *+/rl* mice, $p < .0001$ (see Figure 2a). PPI for *+/rl* mice tended to be superior compared to +/+ mice, $p = .0551$ (see Figure 2b). In addition, PPI for VEH *+/rl* mice differed between MPH and NIC cohorts, $F(1, 24) = 6.0046$, $p = .0219$, while PPI for +/+ and *rl/rl* mice did not; therefore, analyses for treatment effects were conducted separately for *+/rl* mice.

For +/+ and *rl/rl* mice, gender did not contribute to a three-way ANOVA model, so gender was removed from the model. Two-way ANOVA with genotype (+/+, *rl/rl*) and treatment (MPH, NIC, VEH) as between-subjects factors and trial type (80, 84 dB) as repeated measures indicated a significant main effect of genotype, $F(1, 88) = 36.8544$, $p < .0001$, with PPI for +/+ mice superior to *rl/rl* mice, and a significant main effect of treatment, $F(2, 88) = 3.8853$, $p < .01$, in which PPI for NIC mice was superior to mice in VEH and MPH conditions, $p < .01$. The Genotype X Trial Type interaction, $F(1, 88) = 11.1791$, $p = .0012$, revealed that, regardless of treatment condition, PPI for +/+ mice increased with prepulse intensity, while PPI for *rl/rl* mice did not. The Genotype X Treatment interaction was not significant.

PPI for MPH *+/rl* mice did not significantly differ from VEH *+/rl* mice (data not shown), and mice in both conditions exhibited increased PPI with increased dB level, $F(1, 17) = 6.0719$, $p = .0247$. NIC *+/rl* mice tended to exhibit inferior PPI compared to VEH *+/rl* mice, $F(1, 32) = 4.1363$, $p = .0503$, and PPI increased as dB level increased, $F(1,32)$, $p < .0001$ (see Figure 2b).

Total Saccharin Vehicle (S-VEH) Intake

S-VEH intake for a control group (S-VEH/S-VEH) mice significantly increased across trials, $F(3, 21) = 4.2091$, $p = .0177$; however, inspection of Figure 3 indicated that increases in volume tended to be due to increased S-VEH by *+/rl* and *rl/rl* mice. In addition, total S-VEH volume tended to differ between genotypes, $F(2, 23) = 2.3590$, $p = .1170$, and effect screening indicated that S-VEH intake for *+/rl* mice was significantly greater compared to +/+ mice maintained constant levels of S-VEH intake of +/+ mice, $p < .05$.

Fluid Dispenser Side Bias

In mice presented with two S-VEH fluid dispensers (S-VEH/S-VEH), all S-VEH mice exhibited a larger proportion of fluid intake from dispensers positioned in the standard home-cage (HC) location compared to the novel position, $F(1, 23) = 28.0066$, $p < .0001$, indicating a bias for HC fluid dispensers. In addition, a significant main effect of genotype, $F(2, 23) = 4.4152$, $p = .0238$, indicated that genotypes differed in strength of fluid dispenser side bias for dispensers in the HC location versus the novel location throughout the 10 days of self-administration. Post hoc contrasts indicated that, compared to +/+ and *+/rl* mice, the strength of bias was significantly stronger for *rl/rl* mice, $F(1, 23) = 8.8305$, $p = .0068$, (see Figure 4).

Nicotine (NIC) Self-administration in the Home Cage (HC)

NIC discrimination. To determine if NIC mice differed in drinking patterns from S-VEH control mice, fluid intake for HC fluid dispenser was compared to fluid intake for the NOVEL fluid dispenser. ANOVA, with genotype, gender, and Treatment as between subjects factors and trial (days 6 & 10) detected no treatment effects for NOVEL dispensers; however, there was a Genotype X Treatment X Trial interaction, $F(2, 57) = 3.6854$, $p = .0312$, for HC fluid dispensers. Patterns of fluid intake for mice with NIC compared to S-VEH control mice differed between genotypes and treatment such that NIC +/+ mice increased NIC fluid intake, while S-VEH +/+ mice maintained S-VEH fluid intake (see Figure 5a), NIC *+/rl* mice decreased NIC fluid intake, while S-VEH *+/rl* mice increased S-VEH fluid intake (see Figure 5b), and NIC *rl/rl* mice increased NIC fluid intake, while S-VEH *rl/rl* mice increased S-VEH fluid intake (see Figure 5c).

Volume of NIC fluid intake. One-way ANOVA with genotype as the between-subjects factor across repeated measures (days 1-5) revealed that total NIC intake at 30 mg/L did not significantly differ across trials or between genotypes. However, when NIC concentration changed to 60 mg/L, a Genotype X Trial (days 6-10) interaction, $F(8, 92) = 2.3370$, $p = .0248$, revealed differences in the temporal pattern of self-administration such that +/+ mice did not significantly change NIC intake, *+/rl* mice decreased NIC intake, and *rl/rl* mice increased NIC intake (see Figure 6).

Volume of S-VEH fluid intake for NIC mice. In addition, as shown in Figure 7, S-VEH intake for mice with choice of NIC or S-VEH differed significantly between

genotypes, such that +/+ mice and *rl/rl* mice maintained consistent levels of S-VEH, while *+/rl* significantly increased S-VEH intake, $F(2, 36) = 3.6120$, $p = .0372$.

NIC/VEH ratio. The ratio of NIC to S-VEH differed across trials between genotypes when NIC concentration changed to 60 mg/L (trials 6-10), $F(2, 46) = 3.2834$, $p = .0465$, such that *+/rl* mice significantly decreased the ratio of NIC to VEH while +/+ and *rl/rl* mice exhibited non-significant increase (see Figure 8).

Social Behavior

Three way ANOVA with genotype , treatment (NIC, S-VEH), and gender as between-subjects variables and trial type (trials 1 and 2) as within-subject repeated measures indicated that time in the center zone differed between genotypes for both trials, $F(2, 112) = 33.3215$, $p < .0001$, with *rl/rl* mice spending more time in the center zone for each trial, $p < .0001$. Therefore, sociability and social recognition measures were analyzed by comparing proportion of time between the two end zones. Sociability was operationally defined as spending similar or greater proportion of time in $S1_1$ (zone with the stranger mouse) compared to EC (zone with empty chamber) during trial 1, using the formula $S1_1/(S1_1 + EC)$. Mice exhibited sociability if proportion of time in $S1_1$ was equal to or greater than .50. Social recognition, operationally defined as spending less time in the $S1_2$ zone compared to time in the S2 zone, was calculated using the formula $S1_2/ (S1_2 + S2)$. Mice exhibited social recognition if proportion of time in $S1_2$ was less than .50.

ANOVA with genotype (+/+, *+/rl*, *rl/rl*), treatment (NIC, VEH), and gender as between-subjects factors, across repeated measured (trial 1, trial 2), revealed a significant

Genotype X Treatment X Gender interaction, $F(2, 112) = 5.3131$, $p = .0062$, and a significant Genotype X Treatment X Trial Type interaction, $F(2, 112) = 6.4994$, $p = .0021$. Therefore, separate analyses by gender were conducted with genotype and treatment as between-subjects factors for each trial type. Post hoc contrasts were conducted for interactions.

Sociability and social recognition for female mice. As shown in Figure 9, sociability for female VEH mice did not differ between genotypes as measured by the proportion of time with S1 compared to EC. However, genotypes did differ on this measure depending on treatment condition, $F(2, 46) = 6.171$, $p = .0042$. Post hoc contrasts revealed that NIC female *+/rl* mice spent significantly more time with S1 compared to the EC, while NIC female *rl/rl* mice spent less time with S1 compared to the EC, $p = .0013$. Treatment differences for female +/+ mice were not reliably different on this measure of sociability.

For the measure of social recognition during trial 2, female mice differed by genotype in the proportion of time with $S1_2$ (versus S2), $F(2,46) = 3.4743$, $p = .0393$, regardless of treatment condition. As shown in Figure 9, female +/+ and female *+/rl* mice spent proportionately less time with $S1_2$ (M = .39) compared to EC, while proportion of time for female *rl/rl* mice was .52, $p = .017$. Notably, while female +/+ and female *+/rl* mice spent proportionately more time with $S1_1$ (stranger mouse in trial 1) compared to the $S1_2$ (same mouse, now familiar) in trial 2, female *rl/rl* mice spent a similar proportion of time with $S1_1$ as with $S1_2$.

Sociability and social recognition for male mice. In trial 1, male mice did not differ in the proportion of time spent with $S1_1$ versus EC. However, in trial 2, genotypes did differ on proportion of time with $S1_2$ in trial 2 depending on treatment condition, $F(2, 72) = 8.0958$, $p = .0007$. As shown in Figure 10, male NIC *+/rl* mice spent less time with $S1_2$ compared to male VEH *+/rl* mice, while male NIC *rl/rl* mice spent more time with $S1_2$ compared to male VEH *rl/rl* mice, $p = .0002$. Male +/+ mice did not reliably differ between treatment conditions.

Passive Avoidance (PA)

Latency for VEH mice did not differ between genotypes for the training trial, and latency for all genotypes increased significantly from the training trial (trial 1) to the test trial (trial 2), $F(1,48) = 46.6796$, $p < .0001$, which demonstrated that mice learned the association between entering the dark chamber and receiving a foot shock (data not shown). However, strength of learning for VEH mice differed between genotypes, $F(2,117) = 5.8475$, $p = .0038$, such that, compared to +/+ and *+/rl* mice, *rl/rl* mice exhibited a larger percent change in latency to enter the dark side of the test chamber during the test trial, $p < .05$ (see Figure 11). There was a significant interaction of Genotype X Treatment X Gender for percent change in latency, $F(4, 106) = 3.3135$, $p = .0134$. NIC +/+ mice tended to exhibit larger percent change in latency compared to VEH and MPH +/+ mice ($p = .08$), while NIC *+/rl* showed decreased percent change compared to VEH *+/rl* mice. Female MPH *+/rl* mice displayed significantly larger

percent change in latency compared to NIC and VEH female *+/rl* mice, $p = .0084$ (data not shown).

Treatment Validation

For a drug to be considered effective, at a minimum, there should be significant effects of treatment that are consistent with predictions based on the literature, and the treatment effects should be evident in a number of behavioral assays in a systematic manner. As shown in Table 4, treatment effects were evident for both NIC and MPH. In the PPI trials, NIC at 6 mg/kg/BW altered PPI such that NIC mice exhibited superior PPI compared to VEH mice, which is consistent with the literature. NIC at 3 mg/kg/BW did not reliably PPI (data not reported). In tests of social behavior, NIC (6 mg/kg/BW via gavage), mice (*+/rl*) exhibited increased social recognition, which is also consistent with the literature. In NIC self-administration trials, mice altered NIC intake when NIC concentration increased to 60 mg/L, indicating that mice could detect NIC. However, additional trials at each concentration may be required in future research to determine maximum level of self-administration. For MPH, Pilot trials indicated 30 mg/kg/BW to be an effective dose in several behavioral assays. However, for PPI trials in the present study, MPH was used as a psychostimulant comparison in +/+ mice, and, as predicted, there were no effects of MPH for +/+ mice. In addition, although MPH also did not alter learning for +/+ mice in PA, treatment effects were evident in reelin-compromised mice. Thus, based on the aforementioned results, doses of NIC or MPH that were administered via gavage appeared to be behaviorally effective.

CHAPTER IV

DISCUSSION

Recent reports of abnormal reelin-related neurobiology in individuals with schizophrenia, bipolar disorder, and autism have resulted in research involving reelin deficient mice as putative models across several domains associated with the aforementioned disorders. In particular, behavioral phenotypes of reelin deficient mice mirror several behaviors associated with schizophrenia, bipolar disorder, autism, and possibly ADHD, although disrupted reelin pathways have not been associated with ADHD. Additionally, reelin deficient mice exhibit abnormalities in cholinergic and dopaminergic neurotransmitter pathways, as do individuals in the aforementioned diagnostic categories. Thus, in the present study, behavioral phenotypes of reelin deficient mice were explored with the direct acetylcholine agonist nicotine (NIC), based in part on the observations that tobacco (and thus) nicotine is differentially used by individuals diagnosed with schizophrenia, bipolar disorder, autism, and ADHD. In addition, the effects of methylphenidate (MPH), which is a direct dopamine agonist prescribed for ADHD, were assessed for comparison of psychostimulant effects.

Behavioral Phenotypes of +/rl and rl/rl Mice

Table 3 summarizes behavioral phenotypes of VEH mice in the present study and in previous studies conducted in this lab (Salinger et al., 2003; Goode, 2006). Consistent

with earlier cohorts, VEH *+/rl* mice exhibited enhanced acoustic startle response and similar performance in emotional learning in the PA test compared to VEH +/+ mice. However, inconsistent with earlier cohorts, *+/rl* mice displayed elevated PPI compared to +/+ mice. Also, consistent with earlier cohorts, VEH *rl/rl* mice exhibited inferior PPI compared to VEH +/+ and *+/rl* mice. However, compared to +/+ and *+/rl* mice in PA, *rl/rl* mice displayed increased percent change in latency in the test trial, which is not consistent with normal latency reported in VEH RISP mice.

NIC-Related Improvement in PPI of +/+ and rl/rl Mice; Elevated PPI of +/rl Mice is Diminished by NIC

General behavioral phenotype for ASR and PPI. Consistent with previous work conducted in this lab, PPI for *rl/rl* mice was impaired compared to +/+ and *+/rl* mice, and VEH *+/rl* mice exhibited elevated acoustic startle response, indicating possible heightened responsiveness. In addition, *+/rl* mice displayed elevated PPI, which is not consistent with previous findings in this lab (Salinger et al., 2003), or with reports of impaired PPI (Tueting et al., 1999), although elevated PPI in *+/rl* mice has been reported by others (Barr, Fish, Markou, & Honer, 2007). Further, elevated PPI has been reported in individuals diagnosed with ADHD (compared to normal control subjects and subjects with ADHD plus Tic disorder) (Castellanos et al., 1996), while impaired PPI has been associated with reelin-compromised disorders of schizophrenia, bipolar disorder, and autism.

Pharmacological effects on PPI. ASR was not reliably altered by NIC; however, genotypes displayed differential sensitivity to NIC in PPI. As predicted, when NIC was administered via gavage at 6 mg/kg/BW, PPI for NIC +/+ mice was higher compared to VEH +/+ mice. For NIC *+/rl* mice, however, PPI was diminished, and since PPI for VEH *+/rl* mice was abnormally elevated, NIC-related reduction in PPI should be taken as a normalizing effect. Central to predictions in the present study is evidence of improved PPI for NIC *rl/rl* mice compared to VEH *rl/rl* mice, albeit a modest effect. Taken together, normalization of altered PPI by NIC administration is consistent with a therapeutic effect of NIC for humans diagnosed with schizophrenia. Furthermore, the observation that PPI and NIC effects on PPI differ in a non-linear manner (increase in PPI for *rl/rl* mice, and decrease in PPI for *+/rl* mice) suggest that level of reelin deficiency may result in altered ACh functioning. If so, differences in ACh pathways in reelin deficient mice may be useful for investigation of mechanisms that may be relevant to abnormal neurobiology and impaired sensorimotor gating associated with human neuropsychiatric disorders.

As expected, MPH did not alter PPI in +/+ mice, and MPH did not diminish impaired PPI of *rl/rl* mice, or reliably alter elevated PPI in *+/rl* mice. In addition, since MPH did alter PA learning in the present study and in pilot trials for other behavioral assays, the absence of an effect on PPI is not likely due to an inadequate dose—unless PPI is less sensitive to MPH than are other behaviors, in which case, higher doses could be required. However, results from this study are not inconsistent with the few reports in the literature. Thus, in the present study MPH did not reliably influence impaired PPI in

rl/rl mice—as had been predicted—assuming that PPI deficits in *rl/rl* mice model those in schizophrenia.

Abnormal S-VEH and NIC Consumption in Reelin Deficient Mice

General behavorial phenotype for fluid dispenser bias. While a bias for the HC pipette was expected in the NIC self-administration trials, and hence counterbalancing of HC and NOVEL fluid dispensers, the finding that *rl/rl* mice displayed an abnormally strong bias for the fluid dispenser in the HC position was not predicted. Nevertheless, this outcome may have implications for psychopathology. In particular, if the exaggerated side bias displayed by *rl/rl* mice reflects a preference for sameness or resistance to change, then *rl/rl* mice may model inflexible behavior (Crawley, 2007) that is associated with autism (American Psychiatric Association, 2000).

Although the fluid dispenser side bias does not solely provide evidence for resistance to change—which has been suggested as a measure of rigid behavior that is associated with autism (Crawley, 2007)—other outcomes reported in this lab may be useful to interpret the side bias of *rl/rl* mice. More specifically, in the light/dark (L/D) test, findings were previously reported in which, compared to +/+ and *+/rl* mice, *rl/rl* mice exhibited increased time in the light side of the test chamber and reduced exploratory transits into the enclosed dark side of the test chamber (Salinger et al, 2003; Goode, 2006). One possible interpretation of L/D behavior of *rl/rl* mice is that they have an abnormally high preference for the light side of the test chamber, which could reflect decreased anxiety-like behavior (Crawley, 2004). Contrarily, reduced transitions into the

dark side exhibited by *rl/rl* mice could reflect resistance to explore the novel, dark side of the chamber since mice were previously exposed to the test chamber without the dark enclosure. In support of the latter explanation, a pilot group of mice tested in L/D without prior experience to the chamber displayed increased number of transitions into the dark side (Salinger & Ladrow, not published) compared to mice previously exposed to the chamber.

Furthermore, in the present study, *rl/rl* mice in PA did not differ in latency to enter the dark test chamber during the training trial, which suggests that they exhibited normal exploratory behavior in an environment that was not familiar to them. In addition, recall that in the present study, *rl/rl* mice displayed normal sociability when presented with both a novel stranger mouse and a novel inanimate object; however, when they were subsequently presented with a now-familiar mouse (previously the stranger mouse) and a second, novel mouse, *rl/rl* mice failed to display normal preference for novel social stimuli. Although impaired attention to social cues has been proposed as one possible mechanism underlying diminished social preference (Manrique et al., 2005), it is possible, that abnormal social recognition or preference exhibited by *rl/rl* mice in the present study could reflect a preference for sameness or resistance to change.

Arguing against the explanation of preference for sameness of HC fluid dispensers is that in a novel object discrimination task—in which mice were habituated to a novel object and then presented with a choice of a new novel or now-familiar inanimate object—*rl/rl* mice did not exhibit resistance to explore the novel object (Salinger et al, 2003). Therefore, based on observations of an abnormally strong side bias for the HC

fluid dispenser, reduced exploratory behavior to a novel place in L/D, and preference for a familiar versus stranger mouse, additional studies are needed to determine if *rl/rl* mice exhibit a preference for sameness or resistance to change. If so, *rl/rl* mice may model rigid or inflexible behavior (Crawley, 2007) that is associated with autism (American Psychiatric Association, 2000).

General behavioral phenotype for fluid intake. No hypotheses were proposed for abnormalities in volume of fluid intake for S-VEH/S-VEH control mice. However, in the S-VEH/S-VEH condition, *+/rl* mice exhibited elevated levels of fluid intake, such that by the tenth and last measurement, *+/rl* mice drank significantly more S-VEH compared to +/+ mice. Similarly, S-VEH intake for *rl/rl* mice for the total 10-day trial was also elevated, although volume was less than that *+/rl* mice, and not significantly greater compared to +/+ mice. However, since mice were not provided a choice between tap water and S-VEH, it is not possible to determine if altered fluid intake for either genotype was a response to the presence of saccharin in VEH, or a reflection of impaired water balance regulation. Although further studies are needed, if confirmed, the excess fluid intake of reelin deficient mice may be of interest because of the possibility that reelin deficient mice may mirror abnormal fluid regulation, which has been well-documented in individuals with schizophrenia (de Leon, Verhgese, Tracy, Josiassen, & Simpson et al., 1994) and may also be relevant to ADHD (Antalis et al., 2006). Conversely, if elevated fluid intake reflects a preference for sweetened S-VEH, findings may also be of some interest insofar as it has been demonstrated that rodent strains selectively bred for

saccharin-water preference exhibit an increased propensity for drug self-administration (see review, Carroll, Morgan, Anker, Perry & Dess, 2008).

Behavioral phenotype for NIC self-administration. Based on abnormally high prevalence rates of smoking associated with schizophrenia (Vanable et al., 2003) (Poirier et al., 2002), bipolar disorder (Hughes et al., 1986), and ADHD (Pomerleau et al., 1995), it was predicted that reelin deficient mice would exhibit excessive NIC self-administration (ml/g/BW) compared to +/+ mice. Conversely, based on abnormally low prevalence rates of smoking associated with autism (Bejerot & Nylander, 2003), if mice exhibited lower NIC intake levels and/or decreased NIC intake over time compared to +/+ mice, then they would be viewed more appropriately as avoiding NIC as do individuals with autism.

In the present study, the observation that NIC self-administration for mice changed as NIC concentration increased from 30 mg/L to 60 mg/L provides evidence that mice detected and responded to the change in NIC concentration. In addition, although total NIC dosage in ml/kg/BW was not significantly different between genotypes, *rl/rl* mice increased NIC intake and maintained S-VEH intake across trials when NIC concentration increased to 60 mg/L, which suggests that *rl/rl* mice may self-administer NIC to a greater degree compared to +/+ and *+/rl* mice, given additional trials at 60 mg/L. Conversely, *+/rl* mice both decreased NIC 60 mg/L intake and increased S-VEH, which resulted in a significantly smaller NIC/S-VEH ratio compared to +/+ and *rl/rl* mice. If the decrease in the NIC/S-VEH ratio resulted from decreases in NIC alone, findings would suggest that mice titrated fluids to reduce NIC intake. If, however, the

ratio decreased due to increased S-VEH, mice could exhibit increased preference for S-VEH, as observed with S-VEH-only *+/rl* mice. However, the change in NIC self-administration among *+/rl* mice associated with the increase in NIC concentration from 30 mg/L to 60 mg/L suggests that, like *rl/rl* mice, *+/rl* mice are responsive to the presence of NIC and modulate their intake as NIC concentration increases, but in the opposite direction. Thus, it appears that *+/rl* and *rl/rl* titrate their NIC intake differently, such that increasing NIC concentration seems to induce *rl/rl* mice to increase NIC consumption as reflected in their intake expressed in ml/kg/BW (consistent with elevated NIC consumption among individuals with schizophrenia and ADHD) while apparently inducing *+/rl* mice to decrease their consumption of NIC as reflected in reductions in NIC dosage and in their NIC/S-VEH ratio (consistent with putatively depressed NIC consumption among individuals with autism). Interestingly, the observed titration patterns of NIC are consistent with a self-medication hypothesis in that NIC appeared to improve impaired PPI in *rl/rl* mice and diminish abnormally elevated PPI in *+/rl* mice in gavage trials. However, NIC effects on social behavior, such that NIC *+/rl* mice displayed increased social activity, while social activity of NIC *rl/rl* mice worsened, are not consistent with patterns of self-administration of NIC based on self-medication theories.

Impaired Social Interactions in rl/rl Mice; Genotypes Differ in Response to NIC

General behavioral phenotype for social activity. It was hypothesized that, if mice displayed a preference for an inanimate stimulus versus a social stimulus, they would

model decreased sociability, as do individuals with schizophrenia, bipolar (depressive episodes), and autism (American Psychiatric Association, 2000). In addition, if mice displayed a preference for a recently-familiar social stimulus versus a novel social stimulus, they would display a lack of novel preference and, as such, model social recognition/memory deficits associated with schizophrenia or autism (Crawley, 2004). All genotypes exhibited similar patterns of sociability as evidenced by slightly greater proportion of time in the zone with the novel social stimulus compared to the novel inanimate stimulus. Thus, from this perspective, mice do not appear to exhibit abnormal sociability. However, for the second social dimension of social recognition/memory, female VEH *rl/rl* mice spent a greater portion of time with the now-familiar stranger mouse compared to the new stranger mouse, which suggests that female *rl/rl* mice may model deficits in social recognition of socially relevant stimuli, which has been proposed to underlie social interactions associated with schizophrenia and autism; however, since gender was not included in the model, results should be interpreted with caution and additional trials conducted to confirm these findings.

Pharmacological outcomes for social activity. As predicted, social activity for NIC mice differed from VEH mice, which is consistent with previously described effects of NIC on attentiveness to socially relevant cues in normal rodents (Manrique et al., 2005), although NIC did not reliably alter social activity of +/+ mice. However, the observations that NIC *+/rl* mice exhibited increased social interactions, while NIC *rl/rl* counterparts demonstrated the reverse patterns of social behavior, suggests that for this behavior, degree of reelin deficiency may nonlinearly influence neurobiological

mechanisms that are sensitive to acetylcholine agonists. In addition, the aforementioned findings suggest that, while the social activity of *+/rl* mice does not appear to be different from that of wild type mice and not suitable for modeling abnormal social behavior in schizophrenia, bipolar disorder, or autism, NIC induced degradation of social behavior in *rl/rl* mice may reflect hypercholinergic responsiveness, which is consistent with suggestions concerning the status of individuals with autism (Lippiello, 2006).

Normal PA Learning in +/rl Mice and Elevated PA Learning in rl/rl Mice

General behavioral phenotype for PA learning. The fourth objective of this study was to assess response inhibition (executive functioning) in an assay that requires animals to learn an association between a response (moving from a light chamber into a dark chamber) and an aversive stimulus (mild foot shock delivered if animal enters dark chamber). It was hypothesized that if reelin-deficient mice model impaired response inhibition associated with executive functioning deficits observed in schizophrenia and ADHD, then the percent change in latency to enter the dark test chamber would be inferior to that observed in +/+ mice. In addition, since NIC has been shown to enhance performance in PA (King et al., 2003), if NIC enhanced performance of *+/rl* mice, then *+/rl* mice would model executive functioning deficits associated with schizophrenia and/or ADHD. If NIC exacerbated performance, mice would support theories related to autism (Lippiello, 2006). Additionally, since MPH also improves tasks that require response inhibition in individuals with ADHD (Aron et al., 2003), if reelin deficient mice exhibited inferior learning in PA, and if MPH improved passive avoidance performance,

then mice would model impaired inhibition associated with ADHD. If on the other hand, MPH exacerbated performance, mice would model behavior related to reported deleterious effects on cognitive performance in individuals with schizophrenia (Koreen et al., 1997).

The finding that, compared to +/+ mice, *+/rl* and *rl/rl* mice exhibited equivalent or elevated response inhibition in PA is not consistent with predictions of impaired response inhibition in reelin deficient mice as models of impaired response inhibition in individuals with schizophrenia and ADHD. Furthermore, the observation that male VEH *rl/rl* mice exhibited slightly stronger response inhibition relative to +/+ mice suggests an exaggerated effect of PA training. Thus, neither *+/rl* nor *rl/rl* mice displayed impaired response inhibition in the passive avoidance task, as had been predicted, assuming PA performance of *rl/rl* mice model response inhibition deficits in schizophrenia and/or ADHD.

Pharmacological outcomes for passive avoidance. Although NIC tended to enhance response inhibition for +/+ mice in the PA test, which is consistent with predicted enhancement of performance in PA test (King et al., 2003), it did not reliably increase response inhibition for any genotype. Observations of normal or elevated PA performance by reelin deficient mice, along with the absence of NIC effects on PA, suggest that *+/rl* and *rl/rl* mice do not model impaired response inhibition, as would be predicted if they modeled executive functioning deficits associated with schizophrenia and/or ADHD. In addition, although MPH gender effects were detected in the present study, the design of the study did not include MPH gender predictions, and as such

gender effects should not be considered to be interpretable. Furthermore, an intensified effect of MPH on PA would be predicted if mice modeled impaired response inhibition associated with ADHD; however, since the reelin deficient mice acquired PA as effectively or more effectively that +/+ mice, their responses to MPH in the PA procedure cannot be taken to support the view that reelin deficient mice exhibit deficits in executive function that model those seen in ADHD.

Reelin Deficiency in Mice is Associated with Impairments in Specific Behavioral Domains that Transcend Diagnostic Categories: Implications

Behavioral observations, taken together with the patterns of pharmacological influences on mouse behaviors from the present study, suggest that behavioral features of reelin deficient mice do not model any one disorder: schizophrenia, bipolar disorder, ADHD, and autism. Instead, the behavioral outcomes and responses of these behaviors to pharmacological manipulations in reelin deficient mice do suggest that degree of reelin disruption can result in non-linear changes in behavioral impairments. Findings of the present study also suggest that reelin deficiency in mice is associated with impairments in specific behavioral domains that transcend diagnostic categories (see Table 5).

Reelin deficiency and altered sensorimotor gating. Deficits in PPI and differences in the nature of reelin disruption have been documented in schizophrenia, bipolar disorder, and autism. However, the relationship, if any, between differences in reelin disruption and sensorimotor functioning in the aforementioned pathologies has not, to my knowledge, been reported. However, it seems plausible that altered neurobiology as a

consequence of differences in reelin production could result in subtle differences in sensorimotor functioning. If so, reelin deficient mice may be useful to decipher altered underlying neurobiological mechanisms involved in sensorimotor gating across pathologies.

Reelin deficiency and impaired social behavior. Impaired social interactions are frequently observed in autism, as well as schizophrenia, bipolar disorder, and ADHD. However, the nature of social impairment varies depending on the disorder and subtype or phase of disorder. More specifically, social withdrawal is typically associated with autism spectrum disorders (American Psychiatric Association, 2000), schizophrenia (American Psychiatric Association, 2000; see review, Mueser & McGurk, 2004), and bipolar disorder during the depressive episodes (Hirschfeld, 2004). However, intrusive or inappropriate behavior, which has been associated with subtypes of schizophrenia (American Psychiatric Association, 2000), manic phases of bipolar disorder (National Institute of Mental Health, 2009), and hyperactive and combination subtypes of ADHD (American Psychiatric Association, 2000), can result in social isolation.

Findings of impaired social recognition or diminished preference for social novelty in *rl/rl* mice suggest that disruptions in reelin-related processes may be involved in underlying mechanisms associated with impaired social behavior. A putative underlying component of social withdrawal and isolation is impaired or altered attention to socially relevant cues (Sasson et al., 2007). However, as previously discussed, it may also be the case that *rl/rl* mice exhibit a preference for sameness or rigid, inflexible behavior, which results in diminished social interactions. Thus, additional studies are

needed to confirm social impairments in *rl/rl* mice and to determine underlying behavioral components associated with impaired social recognition. Based on the present observations, *rl/rl* mice may be useful as models of social withdrawal associated with neuropsychiatric disorders that include cerebral reelin deficiency as a part of their phenotypes.

Reelin deficiency and impaired executive functioning. Executive functioning is a construct referring to the ability to adjust and regulate behavior in response to environmental cues (Barkley, 1997). While schizophrenia, bipolar disorder, autism, and ADHD have been characterized by abnormal executive functioning, the nature of impairment and putative component deficits associated with impairment appear to vary across diagnostic categories. For example, in schizophrenia, impairments involving at least two constructs have been identified and proposed as underlying components in impaired executive functioning: (1) rule generation and selection, and (2) dynamic adjustments in control (Kerns, Nuechterlein, Braver, & Barch, 2008), both of which presumably result, in part, in impaired response inhibition. Similarly, individuals with ADHD, depending on subtype, have also been characterized by impaired response inhibition (Barkley, 1997). Executive functioning in bipolar disorder seems to be less defined (Balanza-Martinez et al., 2008). However, in autism, impairments in cognitive flexibility have been characterized as a resistance to change, rather than inability to inhibit responding (Russo et al., 2007).

In the present study, reelin deficient mice did not exhibit executive functioning deficits of abnormal response inhibition in the PA test. However, other behaviors of *rl/rl*

mice could reflect inflexible, rigid behavior associated with psychopathologies. More specifically, observations of an abnormally intense bias for the HC fluid dispenser, and lack of preference for a novel social stimulus versus a familiar mouse in the present study, together with observations of abnormally diminished exploratory transitions to the novel, dark side in light/dark test in previous research (Salinger et al., 2003) could reflect resistance to change. Thus, additional studies are needed in which *rl/rl* mice are first permitted to establish a stable behavior, followed by a task in which they are "asked" to make a change in the established behavior. If *rl/rl* mice exhibit a preference for sameness, or resistance to change, they may model rigid, inflexible behavior, which is a core deficit of autism (American Psychiatric Association, 2000) and a proposed animal model for autism (Crawley, 2007).

Reelin deficiency and nicotine abuse. Nicotine abuse has been well-documented in individuals with schizophrenia (Poirier et al., 2002; Vanable et al., 2003), bipolar disorder (Hughes et al., 1986), and ADHD (Pomerleau et al., 1995), but not in individuals with autism (Bejerot & Nylander, 2003). Furthermore, excessive use of nicotine by populations diagnosed with schizophrenia, bipolar disorder, and ADHD, and evidence of a therapeutic effect of nicotine on those individuals has led to self medication theories (Khantzian, 1997). Thus, in the present study, *rl/rl* mice exhibited impaired PPI and NIC related improvement in PPI, so that both the impaired PPI and its improvement under the influence of NIC would parallel and model impaired PPI and improvement in response to NIC that has been noted among individuals diagnosed with schizophrenia. If so, the measure of NIC-related improvement in PPI would provide both face and predictive

validity for the use of impaired PPI in reelin deficient mice to model that aspect of pathology in humans diagnosed with schizophrenia, etc. Furthermore, if *rl/rl* mice exhibit a propensity to self-administer NIC, they may also provide a model for self medication theories related to psychopathologies, or they may provide a model for reelin deficiency based models for addictive behaviors in general.

Implications of Drug Effects on Behaviors of Reelin Deficient Mice: Their Potential as Models of Psychopathology

In the present study, NIC-related improvements in PPI and PA in +/+ mice, and in social behaviors in *+/rl* mice that are consistent with the literature provide treatment validation for NIC. Thus, observed NIC-related changes in PPI in *rl/rl* mice suggest that *rl/rl* mice are responsive to cholingeric agonist treatment. However, modest improvement of PPI in NIC *rl/rl* mice compared to that of +/+ mice suggest that altered neurobiology in *rl/rl* mice may involve altered metabolism of NIC, reduced availability of cholinergic targets in PPI circuitry, or an altered dose-response relationship in which *rl/rl* mice have a shallower slope than that for +/+ mice. Moreover, indications that *rl/rl* mice may self-administer NIC, based on increased responding at 60 mg/L compared to 30 mg/L, could also suggest that *rl/rl* mice are less sensitive to NIC effects compared to *+/rl* and +/+ mice, or that they require higher concentrations of NIC. Additional trials of NIC self-administration are needed to determine if *rl/rl* mice continue to self-administer NIC at 60 mg/L, and if so, post self-administration trials of PPI could be conducted to determine what levels of NIC intake, if any, are optimal for PPI.

Conversely, in tests of social behavior, decreased social interactions were observed in NIC *rl/rl* mice, and increased social activity in NIC *+/rl* mice, which suggests that the reelin-related alterations in the neurobiology underlying social interactions are differentially influenced by NIC compared to alterations in neurobiology related to PPI, since the direction of NIC effects in PPI was reverse of effects on social behavior. It could be the case that, if NIC effects have a general ∩-shaped function (Picciotto, 2003), a smaller dose might have produced opposite effects on social behavior. However, if cholinergic neurobiology underlying social behavior in *rl/rl* mice is such that NIC degrades function, the present findings would be consistent with proposed theories related to cholingeric hyper-responsiveness in autism (Lippiello, 2006). In addition, the finding of enhanced social activity of NIC *+/rl* mice, compared to diminished effects on social activity of NIC *rl/rl* mice, suggests that differences in the level of reelin deficiency results in differential effects of NIC.

MPH was tested as a psychostimulant comparison for NIC, and also based on reports that MPH exacerbates behavior in individuals with schizophrenia. As predicted, MPH did not significantly improve PPI in +/+ mice. However, MPH did not further diminish PPI in *rl/rl* mice, as would be predicted if weak PPI in *rl/rl* mice modeled weak PPI associated with schizophrenia. It is possible, however, that since PPI is virtually absent in *rl/rl* mice, further degradation by MPH would be difficult to detect.

Conclusion

Observations from the present study suggest that behavioral abnormalities of reelin deficient mice and the responses of these abnormalities to NIC and/or MPH do not model those of any one disorder: schizophrenia, bipolar disorder, autism, or ADHD. However, behavioral outcomes of reelin deficient mice may be relevant to one or more of the disorders. More specifically, *rl/rl* mice displayed impaired PPI and diminished social behavior, both of which are associated with schizophrenia, bipolar disorder, and autism, and all three disorders are associated with disrupted reelin pathways. In addition, when taken together with earlier assays, behaviors exhibited by *rl/rl* mice may reflect resistant to change, which could model rigid, inflexible behavior associated with autism. Furthermore, observations of impaired PPI of *rl/rl* mice that is improved with NIC administration, as observed in individuals with schizophrenia, and a propensity by *rl/rl* mice to self administer NIC, may be relevant as a model of self-medication proposed in neuropsychiatric disorders such as schizophrenia, bipolar disorder, and ADHD (Khantzian, 1997). However, observations of enhanced social activity of NIC *+/rl* mice, compared to diminished effects on social activity of NIC *rl/rl* mice, suggests that differences in the level of reelin deficiency result in ACh pathways that have been altered in qualitatively different fashion by the quantitative differences in reelin levels and thus, differences in responsiveness to NIC. Finally, elevated fluid intake exhibited by reelin deficient mice may be model polydipsia associated with schizophrenia or ADHD or it may reflect a preference for saccharin water (versus unsweetened), in which case, the

elevated fluid intake exhibited by reelin deficient mice may be useful as models for analyzing neural processes underlying substance abuse in humans (Carroll et al., 2008).

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APPENDIX A. TABLES

Table 1

Behavioral and Pharmacological Effects Profiles Associated with Diagnostic Categories

Behavior		Schizophrenia			Bipolar Disorder			Autism			ADHD		
Human	Animal	Model	NIC	MPH	Model	NIC	MPH	Model	NIC	MPH	Model	NIC	MPH
Sensorimotor gating	PPI	↓	↑	↓	↓	↑	NP	↓	↓	NP	↔	↔ or ↑	↔ or ↓
Nicotine abuse	NIC Self-admin	↑	NA	NA	↑	NA	NP	↓	NA	NA	↑	NA	NA
Social interactions	Sociability	↓	NP	NA	↓ or ↑	NP	NP	↓	NP	NA	↔	NA	NA
Attention - social cues	Social recognition	↓	↑	NA	↓	↑	NP	↓	↓	NA	↓ or ↑	NA	NA
Executive functioning: Response inhibition	Passive avoidance	↓	↑	↓	↓	↑	NP	NP	NP	NP	↓	↑	↑

Note. ↑ – Elevated behavior; ↓ – Diminished behavior; ↔ – Normal behavior; NA– Not applicable; NP – No prediction

Table 2

Subject Numbers by Genotype, Treatment (TX), Sex, and Behavioral Assay

Behavioral assay		+/+		*+/rl*		*rl/rl*	
		MPH	NIC	MPH	NIC	MPH	NIC
		(F/M)	(F/M)	(F/M)	(F/M)	(F/M)	(F/M)
Acoustic startle & prepulse inhibition	VEH	5/6	4/8	5/5	9/10	4/6	6/5
	TX	5/4	6/11	5/5	8/13	4/6	7/8
Social behavior	VEH	NA	4/8	NA	9/10	NA	6/5
	TX	NA	4/11	NA	8/14	NA	8/7
Passive avoidance	VEH	4/5	2/3	5/5	7/5	3/4	6/5
	TX	4/5	3/6	5/5	7/8	4/6	8/7
NIC self-administration	VEH	NA	2/3	NA	7/9	NA	6/3
	TX	NA	3/6	NA	7/5	NA	8/7

Note. Numerals to the left of slashes represent the number of female (F) mice; numerals to the right of slashes represent the number of male (M) mice. NA – Not applicable

Table 3

Comparison of VEH Mice in the Present Study with Other Cohorts

	+/rl			*rl/rl*		
Test Measure	2003	2006	Current	2003	2006	Current
Acoustic startle response	↑	↔	↑	↓	↔	↔
PPI % Startle Reduction	↔	↔	↑	↓	↓	↓
PA Change in latency	NA	↔	↔	NA	↔	↑
Total fluid (VEH) intake	NA	NA	↑	NA	NA	↑
Fluid dispenser bias	NA	NA	↔	NA	NA	↑
Sociability	NA	NA	↔	NA	NA	↔
Social recognition	NA	NA	↔	NA	NA	↓

Note. Significance set at $p < 0.05$. Arrows indicate significantly less (↓), significantly greater (↑), or no change (↔) compared to +/+ mice. NA indicates that either the measure was not reported or was not analyzed in the 2003 and 2006 studies. Note that VEH mice in the current and RISP cohorts were administered VEH treatments with gavage in PPI, SR, and PA trials, while all mice in trials conducted by Salinger et al. (2003) received no VEH/gavage treatment.

Table 4

Summary of Treatment Effects

	Compared to +/+		TX effect compared to respective genotype VEH					
	VEH		NIC			MPH		
Behavior	*+/rl*	*rl/rl*	+/+	*+/rl*	*rl/rl*	+/+	*+/rl*	*rl/rl*
Acoustic startle	↑	↔	↔	↔	↔	↔	↔	↔
PPI	↑	↓	↑	↓	↑	↔	↔	↔
NIC Self-admin								
30 mg/L	NA	NA	↔	↔	↔	NA	NA	NA
60 mg/L	NA	NA	↔	↓	↑	NA	NA	NA
Total S-VEH intake	↑	↔	NA	NA	NA	NA	NA	NA
Side bias	↔	↑	NA	NA	NA	NA	NA	NA
Sociability	↔	↔	↔	↑	↓	NA	NA	NA
Social recognition	↔	↓	↔	↑	↓	NA	NA	NA
Passive avoidance	↔	↑	*↑	↓	↔	↔	↑	↑

Note. Significance set at $p < 0.05$. Arrows indicate significantly less (↓), significantly greater (↑), or no change (↔) compared to +/+ mice. * Indicates non-significant trend. NA – Not applicable; NP – No prediction

Table 5

Human Behaviors and Animal Behavior Assays; Behavioral Outcomes and Treatment Effects for Reelin Deficient Mice

Altered human behaviors shared among psychopathologies			Related behavior in reelin-deficient mice (VEH)			Effects of TX
Human behavior	Reduced ↔ Elevated		Animal behavior	Reduced ↔ Elevated		
Startle			Acoustic startle		*+/rl*	NIC ↓ *+/rl*
PPI	SZ, BPM, Autism	ADHD	PPI	*rl/rl*	*+/rl*	NIC ↑ +/+ ↑ *rl/rl* NIC ↓ *+/rl*
Nicotine abuse	Autism	SZ, BP, ADHD	NIC Self-admin	*+/rl*	*rl/rl*	
Polydipsia /elevated thirst		SZ, ADHD	Total fluid intake (S-VEH)		*rl/rl*, *+/rl*	
Social interactions	SZ, BPD, Autism ADHD-I	SZA , BPM ADHD-HC	Sociability			NIC ↑*+/rl* ↓ *rl/rl*
Social cognition (attn to cues)	SZ, Autism ADHD		Social recognition	*rl/rl*		NIC ↑ *+/rl* ↓ *rl/rl*
EF: Response inhibition	SZ, ADHD		Passive avoidance		*rl/rl*	MPH ↑ *+/rl* ↑ *rl/rl*
EF: Inflexible behavior		Autism	Fluid dispenser side bias		*rl/rl*	

Note. Significance set at $p < 0.05$. Arrows indicate reduced (↓), elevated (↑), or no change (↔). EF – Executive function; SZ – Schizophrenia; SZA – Schizophrenia affective subtype; BP – Bipolar disorder; BPD – Bipolar depressive; BPM – Bipolar manic; ADHD-HC – Attention deficit hyperactivity disorder hyperactive and combined subtypes; ADHD-I – Attention deficit hyperactivity disorder inattentive subtype.

APPENDIX B. FIGURES

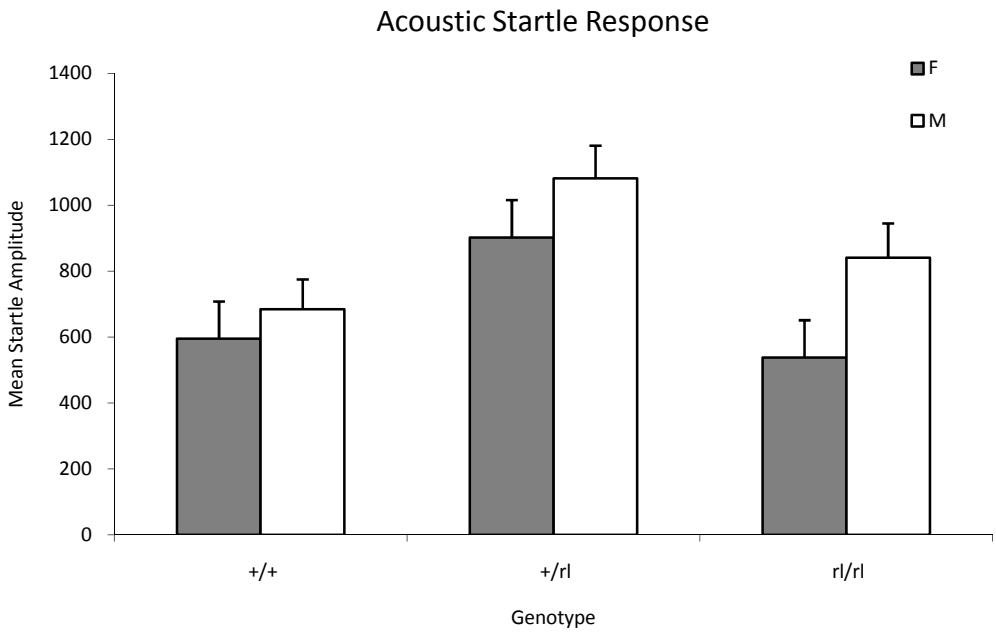
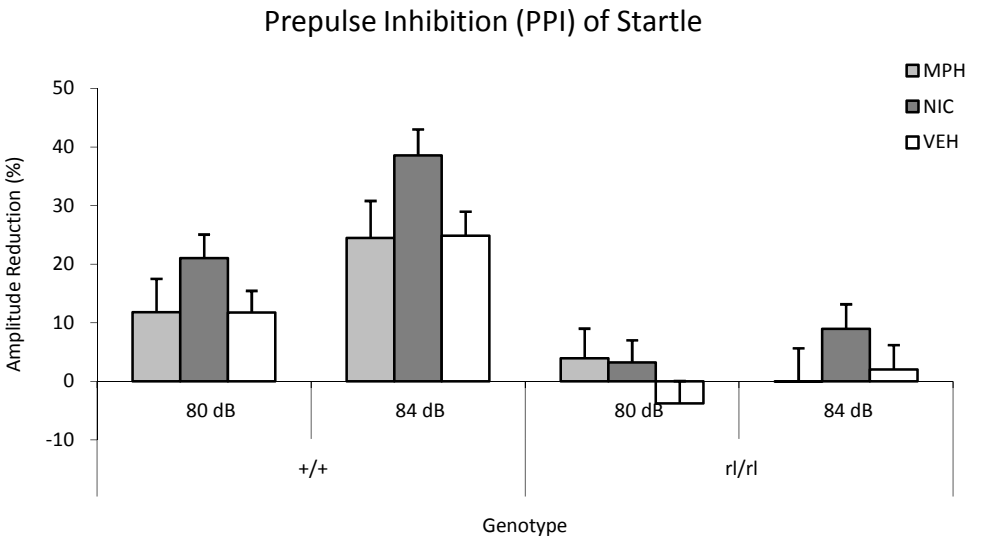


Figure 1. Acoustic startle response *(+SE*) for VEH mice. Compared to +/+ and *rl/rl* mice, *+/rl* mice exhibited increased startle amplitude, $p < .01$; Male mice exhibited increased startle amplitude compared to female mice, $p = .0150$.

a



b

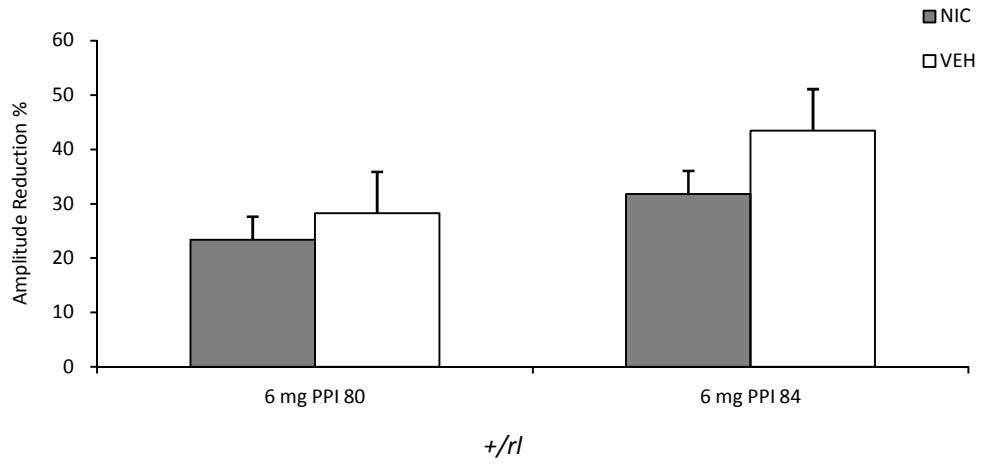


Figure 2. Prepulse inhibition (PPI) of startle. Mean amplitude reduction in acoustic startle response *(+SE*). Figure 2a shows PPI for *rl/rl* mice was inferior to +/+ mice, $p < .0001$, and NIC +/+ and NIC *rl/rl* mice exhibited increased PPI compared to MPH and VEH mice, $p < .01$. In Figure 2b, NIC *+/rl* mice exhibited decreased % PPI compared to VEH *+/rl* mice, $p = .05$.

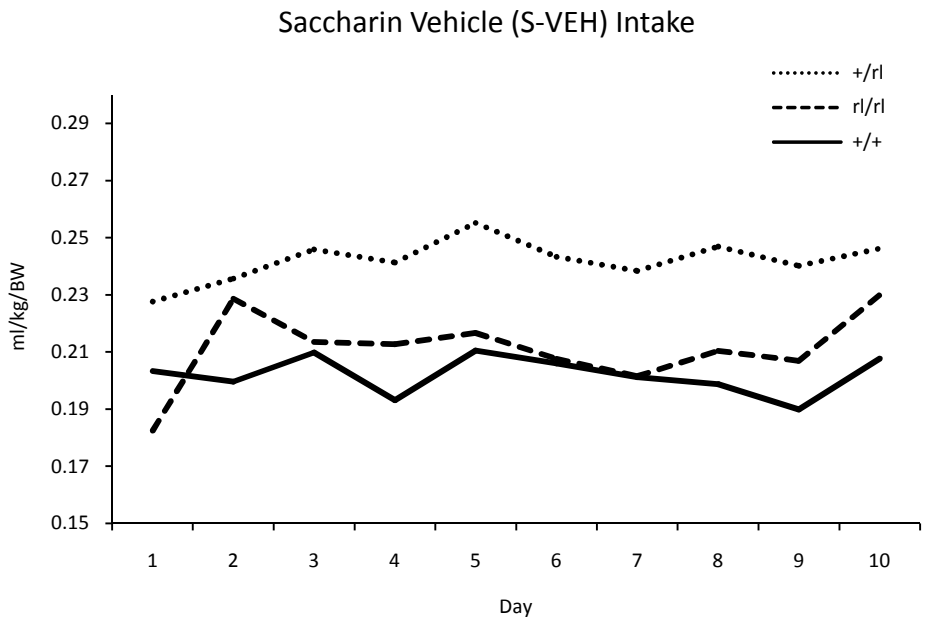


Figure 3. Saccharin vehicle (S-VEH) intake. In a control group of mice in which two fluid dispensers containing S-VEH (S-VEH/S-VEH) were presented, total S-VEH levels tended to differ between genotypes, $p = .1170$, with higher S-VEH intake for *+/rl* mice, $p < .05$, compared to +/+, and levels that increased over time for *+/rl* and *rl/rl* mice.

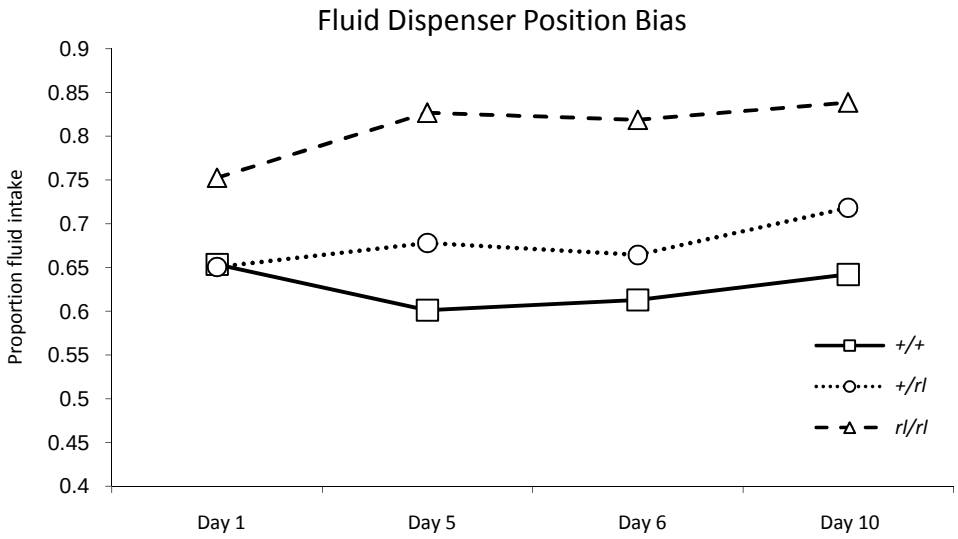


Figure 4. Fluid dispenser position bias. Genotypes differed in the proportion of fluid intake from HC dispensers compared to fluid dispensers in the novel position, $p < .05$. While all mice exhibited a bias for HC fluid dispensers, strength of bias was significantly greater for *rl/rl* mice compared to *+/rl* and +/+ mice, $p = .0068$.

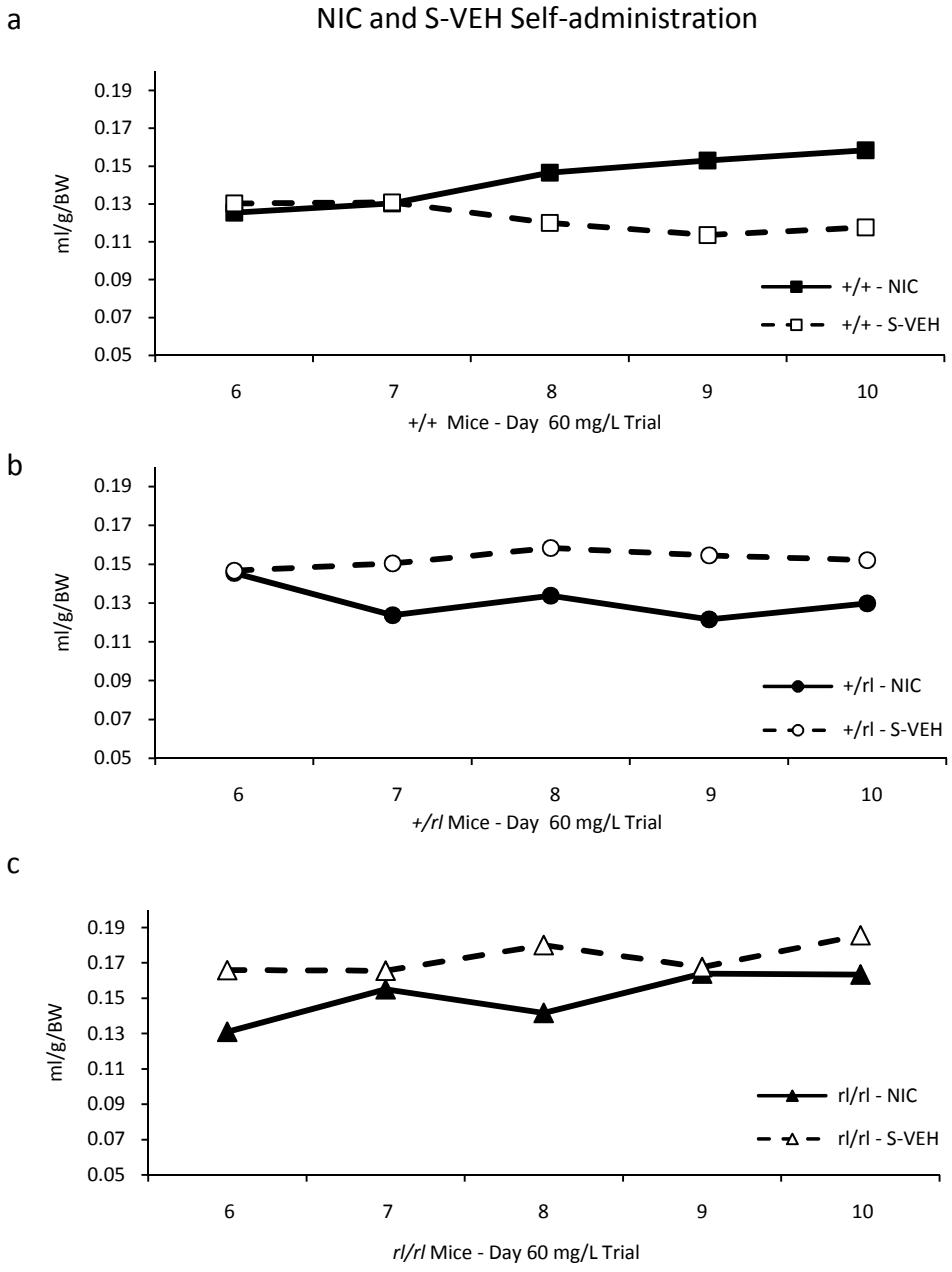


Figure 5. NIC and S-VEH self-administration. Changes in NIC 60 mg/L intake for fluid dispensers in HC position indicate that mice could discriminate change in concentration from 30 mg/L to 60 mg/L. (a) +/+ mice increased NIC and maintained S-VEH fluid intake. (b) *+/rl* mice decreased NIC and increased S-VEH. (c) *rl/rl* mice increased both NIC and S-VEH.

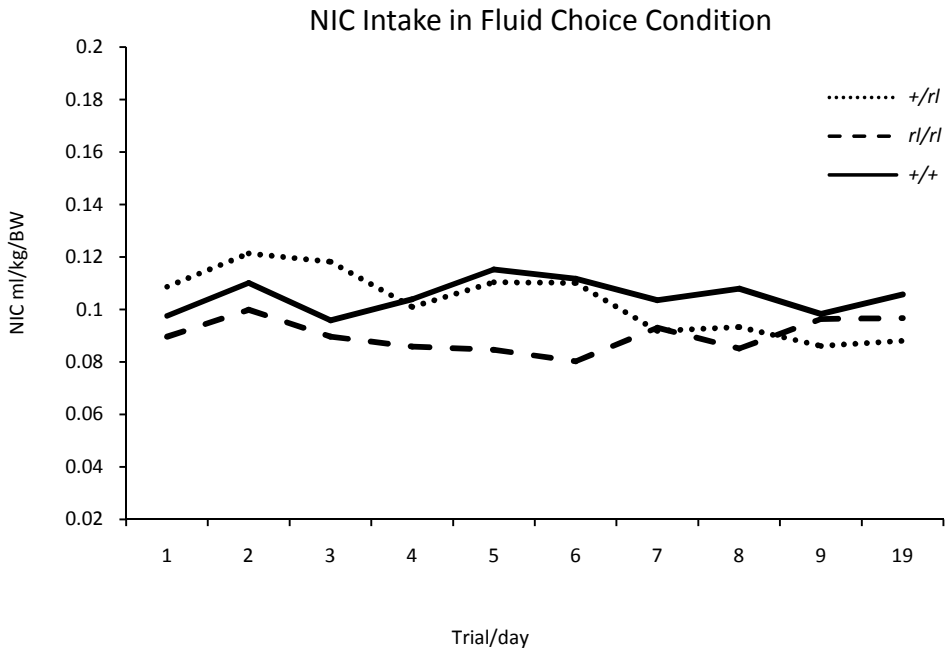


Figure 6. NIC intake in fluid choice condition. Genotypes did not differ in total ml/kg/BW of self-administration NIC. However, at 60 mg/L, *+/rl* mice decreased NIC intake compared to intake when NIC was available at 30 mg/L, while *rl/rl* mice increased NIC intake, $p < .05$.

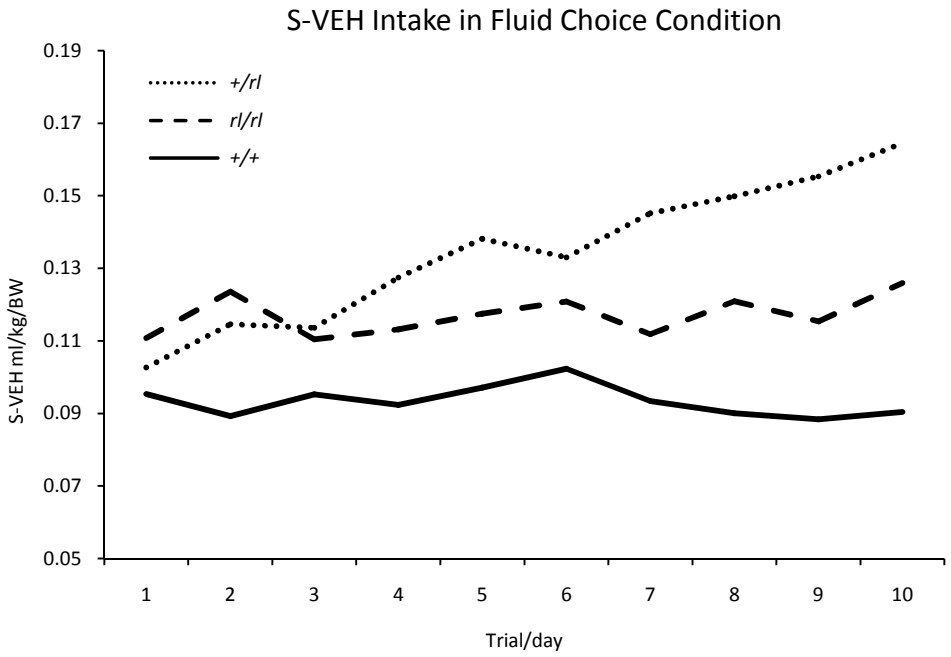


Figure 7. S-VEH intake in fluid choice condition. *+/rl* mice with choice of NIC and S-VEH significantly increased intake across trials, $p < .05$, while +/+ and *rl/rl* mice maintained level of S-VEH intake across trials.

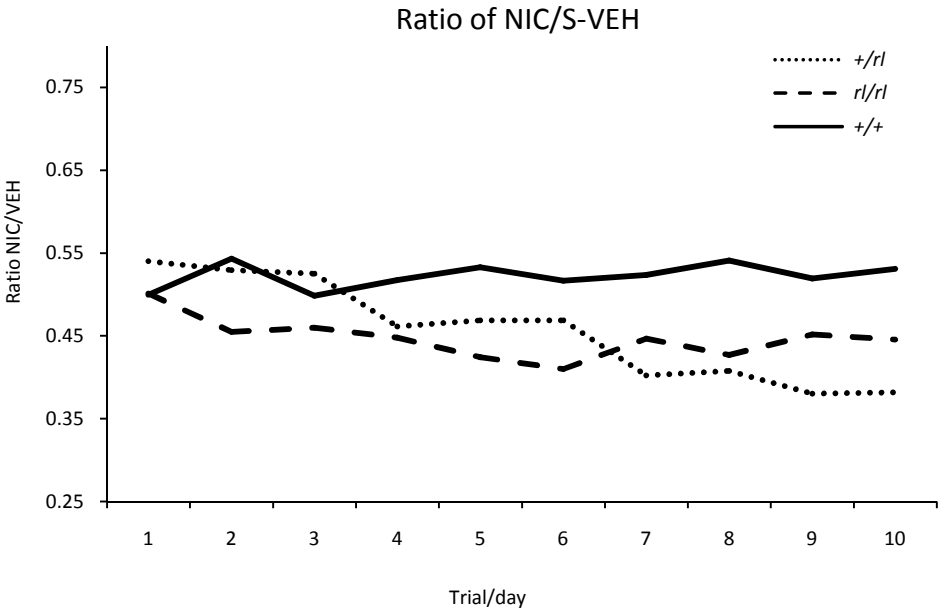


Figure 8. Ratio of NIC/S-VEH. *+/rl* mice decreased NIC/S-VEH ratio from trial days 6-10, while +/+ and *rl/rl* mice maintained NIC/S-VEH ratio across trials.

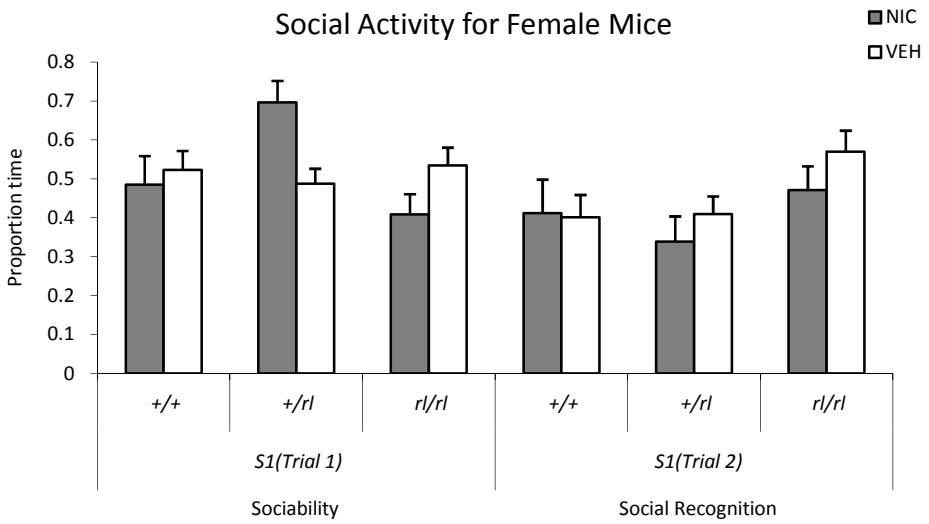


Figure 9. Social activity for female mice. In Trial 1, *sociability* was defined as the proportion of time with stranger mouse (S1) versus empty chamber (EC) calculated as S1/(EC+S), where higher proportion time reflected greater sociability. Female NIC *+/rl* mice spent a larger proportion of time with S1 compared to VEH *+/rl* mice, while female NIC *rl/rl* mice tended to spend proportionately less time with S1 compared to female VEH *rl/rl* mice, $p = .0013$. In Trial 2, *social recognition* was defined as the proportion of time with now-familiar mouse ($S1_2$) versus new stranger mouse (S2) calculated as $S1_2$/($S1_2$ + S2), where lower proportion time with $S1_2$ reflected higher degree of social recognition or memory. Female *rl/rl* mice spent proportionately more time with $S1_2$ compared to female *+/rl* and +/+ mice, $p = .017$.

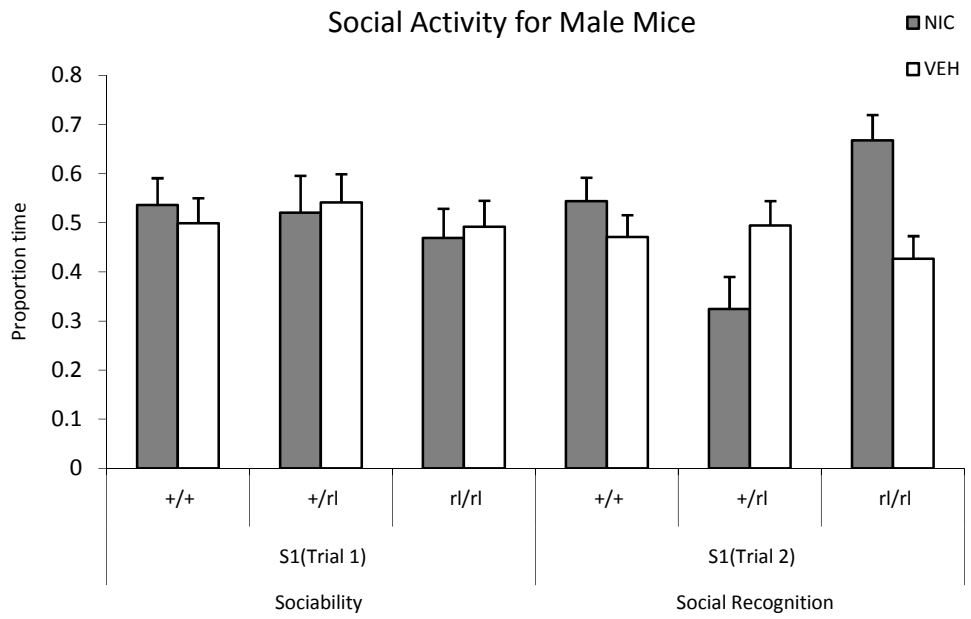


Figure 10. Social activity for male mice. In Trial 1, *sociability* was defined as the proportion of time with stranger mouse (S1) versus empty chamber (EC) calculated as S1/(EC+S), where higher proportion time reflected greater sociability. Male genotypes did not differ by genotype or treatment. In Trial 2, *social recognition* was defined as the proportion of time with now-familiar mouse ($S1_2$) versus new stranger mouse (S2) calculated as $S1_2$/ ($S1_2$ + S2), where lower proportion time with $S1_2$ reflected higher degree of social recognition or memory. Male NIC *+/rl* mice spent less time in $S1_2$ zone compared to Male VEH *+/rl* mice, while male NIC *rl/rl* mice spent more time in $S1_2$ compared to male VEH *rl/rl* mice, $p = .0002$.

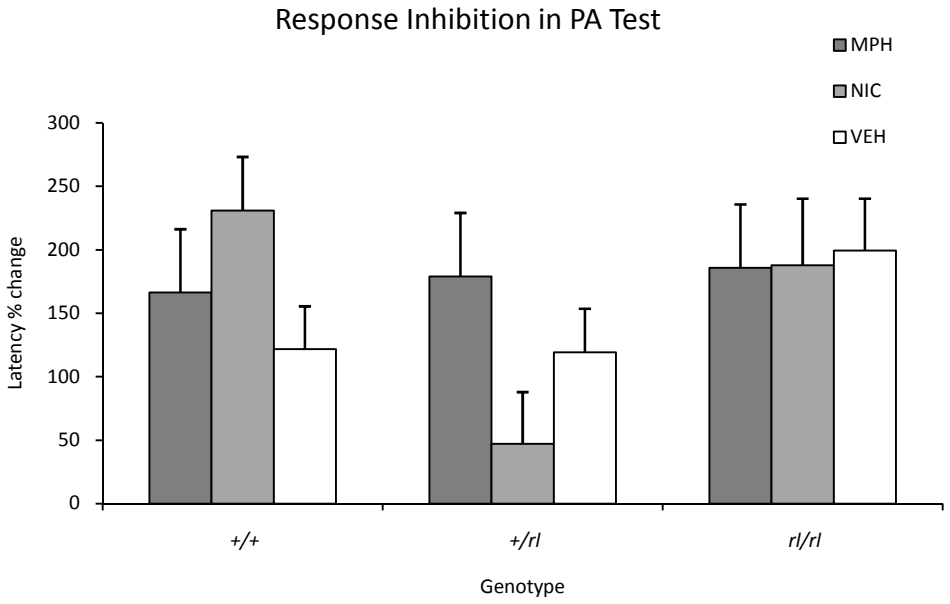


Figure 11. Response inhibition in PA test. Latency to enter the test chamber significantly increased for all VEH genotypes from trial 1 to trial 2 (expressed as % change), although the strength of % change was greater for VEH *rl/rl* mice compared to +/+ and *+/rl* mice, $p = .03$. NIC *+/rl* mice exhibited smaller % change compared to MPH and VEH mice, $p = .0314$, while NIC +/+ mice exhibited greater % change compared to MPH and VEH mice, $p = .05$.