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The reeler mutant mouse (*rl/rl*) has been proposed to be a behavioral model of schizophrenia. The purpose of the current research was to study the effects of the antipsychotic, risperidone, on the *rl/rl*, because if the *rl/rl* models schizophrenia, then risperidone should improve its cognitive, emotional, and sensorimotor gating deficits. Wildtype (+/+), *rl/rl,* and hybrid (+/*rl)* mice were tested in open field, light-dark, PPI, nose poke, and passive avoidance after receiving 1.0 mg/kg of risperidone or vehicle via oral gavage for 3 weeks, and dosing continued throughout testing. Overall, there were few significant effects of the drug, but when it did have an effect on the *rl/rl*, it worsened its performance, and so the results could not support the hypothesis. Future research should re-examine the *rl/rl* in a wider battery of behavioral tests in order to determine if the *rl/rl* models another disorder, such as autism.

THE EFFECTS OF RISPERIDONE, AN ATYPICAL ANTIPSYCHOTIC, ON THE REELER MUTANT MOUSE, A POTENTIAL MODEL OF SCHIZOPHRENIA

by

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Approved by

_____
Committee Chair

To my family, for their support;

to my friends, for their encouragement;

to my stepchildren, for their wonderful questions;

and to my husband,

who always greeted me with a hug,

even when I came home smelling of mice…

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

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CHAPTER I

INTRODUCTION

Given the complexity of human psychiatric disorders, it is unlikely that any one animal could ever model all of the symptoms associated with such a disorder (Joober, Boska, Benkelfat, & Rouleau, 2002; Crawley, 2000; Lipska & Weinberger, 2000; van den Buuse, Garner, Gogos, & Kusljic, 2005). However, animals are nonetheless useful in the study of human diseases, because they allow for more controlled study of specific aspects of a disorder than can typically be obtained in studies with humans (Crawley, 2000). The goals of using animals in studying human conditions are to test hypotheses about the mechanisms of the disease and to predict human responses to treatment (Crawley, 2000). In that regard, then, animals can represent a disorder on different levels, depending on whether one is interested in studying phenomenology, etiology (such as a genetic predisposition to a disorder), or treatment response (Lipska & Weinberger, 2000).

According to Crawley (2000), there are several criteria that a potential animal model of a disorder must satisfy in order to be considered a good model (Table 1). As can be seen from the table, an animal's validity as a model of a disorder increases with the number of ways it replicates aspects of a disease. However, it must be remembered that psychological disorders involve neural circuitry and emotions that are most likely unique to humans, and so an animal's utility as a model must not be overstated (Crawley, 2000).

Table 1
Criteria for Good Animal Models of Human Psychiatric Diseases

1. Replicates at least one symptom of the human disease
2. Responds to treatments that are effective in the human disease
3. Is unaffected by treatments that are ineffective in the human disease
4. Conceptual analogy to the etiology of the human disease is desirable but not necessary
5. Conceptual analogy to multiple components of the human disease is desirable:
    a. Behavioral symptoms
    b. Neuroanatomical abnormalities
    c. Neurochemical abnormalities
    d. Temporal progression
    e. Precipitating event

Source: Crawley (2000).

Schizophrenia is a complex psychological disorder comprised of a broad constellation of abnormalities. Although much has been learned about the behavioral and biological abnormalities of schizophrenia, what still remain largely unclear are its origins and development over time (Wong & Van Tol, 2003). Given the overwhelming personal and societal costs and burdens of the illness, it seems prudent to further scientific understanding of the disease in order to increase the effectiveness of treatment, to increase prediction of who is at risk to develop the disorder, and to improve prevention in those found to be at risk. Animals that meet any of the criteria above for schizophrenia could be useful in advancing our knowledge of this disease.

The positive symptoms listed in the American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders,* 4th edition, text revised (*DSM-IV-TR*; 2000) are the most recognizable symptoms of the disease, and include

hallucinations, delusions, and disordered thought. However, it is difficult to imagine how one could induce observable hallucinations or delusions in an animal (Lipska & Weinberger, 2000; Crawley, 2000), and so animal models of schizophrenia have tended to focus on schizophrenia's other symptoms, such as its negative symptoms and cognitive deficits, which are also more common and chronic among those with schizophrenia than are the positive symptoms (Le Pen, Grottick, Higgins, & Moreau, 2003). Negative symptoms include flattened affect/abnormal emotionality, alogia, and anhedonia (*DSM-IV-TR*). Cognitive deficits include executive functioning deficits, impaired verbal and nonverbal working memory, and inattention (Conklin, Curtis, Calkins, & Iacono, 2005; Fitzgerald, Lucas, Redoblado, Winter, et al., 2004; Gooding & Tallent, 2004; Silver, Feldman, Bilker, & Gur, 2003).

Impaired sensorimotor gating is another common feature of schizophrenia that is easily measured in animals (e.g., van den Buuse, Garner, & Koch, 2003). Sensorimotor gating refers to the brain's filtering out of irrelevant stimuli in order to prevent sensory over-stimulation of higher brain functions (Oranje, van Oel, Gispen-de Wied, Verbaten, & Kahn, 2002). The most common way to measure sensorimotor gating is by examining the prepulse inhibition of the acoustic startle response (PPI) (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001). PPI measures the individual's unlearned, reflexive suppression of a response to a startling stimulus when that stimulus is preceded by a non-startling one (e.g., Geyer et al., 2001; Kumari & Sharma, 2002; Braff, Geyer, & Swerdlow, 2001). Schizophrenia patients were first reported to have deficits in PPI compared to controls by Braff, Stone, Callaway, Geyer, et al. in 1978, and since then,

numerous researchers have found deficits in PPI in schizophrenia patients (Parwani, Duncan, Bartlett, Madonick, et al., 2000; Meincke, Mörth, Voβ, Thelen, et al., 2004; Ludewig, Geyer, & Vollenweider, 2003; Mackeprang, Kristiansen, & Glenthoj, 2002).

In the past decade, the results of a post-mortem study indicated that schizophrenia patients have about a 50% decreased expression of both protein and mRNA reelin in the brain compared to non-schizophrenic controls (Impagnatiello, Guidotti, Pesold, Dwivedi, et al., 1998). Reelin is a key signaling protein that plays a crucial role in prenatal corticogenesis (Teuting, Costa, Dwivedi, Guidotti, et al., 1999). In the absence of reelin, new neurons are positioned below layers of older neurons instead of developing in the "inside-out" manner characteristic of normative cortical development (Teuting et al., 1999; Luque, Morante-Oria, & Fairén, 2003). Because of the reelin deficiency found in schizophrenia patients, mice with similar reelin deficiencies have been hypothesized to be models of schizophrenia (Teuting et al., 1999; Qiu, Korwek, Pratt-Davis, Peters, et al., 2006).

The heterozygous reeler mouse (+/*rl*), which has one copy of the reelin gene and therefore produces about 50% of normal reelin levels compared to wildtype (+/+) controls, was first hypothesized to model schizophrenia by Teuting et al. (1999). The +/*rl* was reported to have deficient PPI compared to +/+, and displayed greater anxiety on an elevated plus maze (although there were data from four male mice only on this test). Because such emotional and cognitive deficits are also found in people who have schizophrenia, they concluded that the +/*rl* was a model of schizophrenia. Most recently, Qiu et al. (2006) also reported that the +/*rl* is a model of schizophrenia. They arrived at

their conclusion based on the results of one test of contextual fear conditioning, and on a significant difference in PPI (for which they did not report their methods) at only one prepulse intensity. In the same report, however, they found no differences between the two genotypes in overall activity, thermal nociception, startle responding, anxiety-like behaviors, cued freezing, and spatial learning.

However, the results of three separate studies fail to confirm those findings of schizophrenia-relevant abnormalities in the behavioral phenotype of +/rl mice (Salinger, Ladrow, & Wheeler, 2003; Groves, O'Meara, Handford, Smith, et al., 2003; Podhorna & Didriksen, 2004). Salinger et al. (2003) reported that +/*rl* and +/+ mice were indistinguishable from each other on a wide variety of behavioral tests, including PPI, open field, light/dark, and nose-poke (a test of cognitive functioning). Groves et al. (2003) studied male +/*rl* and +/+ mice and found no differences between them in PPI or locomotor activity. In 2004, Podhorna and Didriksen also reported the results of an experiment designed to investigate phenotypic differences between +/*rl* and +/+ mice. In accord with Salinger et al. (2003) and Groves et al. (2003), they concluded that +/*rl* mice were not distinguishable from +/+, and further stated that the +/*rl* may not be a good model of schizophrenia.

Unlike Teuting et al. (1999), Groves et al. (2003), and Podhorna and Didriksen (2004), however, Salinger et al. (2003) also studied the homozygous reeler mutant mouse (*rl/rl*) in addition to the +/*rl* and +/+ and reported that the *rl/rl* displayed significant behavioral abnormalities compared to the other two genotypes. Furthermore, the abnormalities displayed by this mouse suggested that it and not the +/*rl* might be a more

appropriate model of schizophrenia. The *rl/rl* was found to have significant deficits in PPI, consistent with findings from the human schizophrenia literature (Braff et al., 1978; Parwani, et al., 2000; Meincke, et al., 2004; Ludewig, et al., 2003; Mackeprang, et al., 2002). The *rl/rl* was also shown to exhibit abnormal emotional behaviors, including a long latency to enter the dark half of the test chamber in the light/dark task, fewer boli in the open field, and an abnormal lack of preference to remain on perimeter areas of the open field during exploration (see Crawley, 2000 for a more detailed description of tests used to measure emotionality in mice). The *rl/rl* displayed significant cognitive deficits compared to +/*rl* and +/+ mice as well, including deficits in working memory. Because of the shared behavioral deficits and deficiencies in reelin production between *rl/rl* mice and humans with schizophrenia, the *rl/rl* was hypothesized to model schizophrenia, and is the genotype under investigation here. Because of the conflicting findings reported in the literature on the +/*rl*, however, +/*rl* mice were included in this experiment in order to determine if their behavioral phenotype would confirm Salinger et al.'s (2003) previous findings.

The focus on reelin-deficient mice in this research is not meant to imply that other models of schizophrenia do not exist. However, the *rl/rl* mouse is unique in that, not only are its genetic and behavioral abnormalities naturally occurring instead of experimentally induced, but also that it shows behavioral abnormalities across multiple domains similar to those found in schizophrenia, instead of showing deficits only in one area, such as PPI or hyperactivity. Other models of schizophrenia include (but are in no way limited to) rats that have received neonatal ventral hippocampal lesions and show deficits in

cognition and social interactions in adulthood (e.g., Lipska, 2004); rodents that have drug-induced hyperactivity or drug-disrupted PPI (for a review, see van den Buuse et al., 2005); and mice with experimentally-induced genetic mutations, such as the chakragati mouse, which shows abnormal social interactions (e.g., Torres, Hallas, Vernace, Jones, et al., 2004).Because the *rl/rl* has naturally-occurring deficits, it may more closely model the development of the disorder in humans, which would satisfy Crawley's (2000) etiology criterion, shown in Table 1.

Table 2 shows the ways in which *rl/rl* mice have already been found to meet several of Crawley's (2000) requirements as a good animal model of schizophrenia. As mentioned above, the *rl/rl* has been shown to have deficits in PPI, emotionality, and cognition by Salinger et al. (2003), and also has been found by other research to have neurochemical (Curran & D'Arcangelo, 1998) and neuroanatomical (D'Arcangelo & Curran, 1998) abnormalities, similar to those found in people with schizophrenia.

However, some of these requirements have not been met by the *rl/rl* because they have not yet been studied. For example, treatment responses in the *rl/rl* are still unknown. As Crawley (2000) made apparent, an animal model must respond appropriately to a treatment effectively used in humans with the disorder, but the animal also must *not* respond positively to treatments that do not improve symptoms in humans with the disorder. However, a search of the literature revealed no published studies of *rl/rl* mice being treated with any drug, much less an antipsychotic drug such as those used to treat schizophrenia. It should be noted that +/*rl* but not *rl/rl* mice have been tested in various behavioral assays following the administration of various drugs, including

benzodiazepines (e.g., Costa, Davis, Pesold, Teuting, & Guidotti, 2002; Tremolizzo, Carboni, Ruzicka, Mitchell, et al., 2002; Carboni, Teuting, Tremolizzo, Sugaya, et al., 2004). However, these experiments did not include *rl/rl* mice, which is the genotype proposed here to be a behavioral model of schizophrenia, and the drugs used in the experiments by Costa's group were not drugs that have been approved to treat schizophrenia in humans. Accordingly, research into treatment effects on the *rl/rl* will begin by examining the effects of an antipsychotic drug, risperidone, used to treat schizophrenia in humans.

Table 2
The Reeler Mouse as a Model of Schizophrenia

Criterion	Description and Sources
Symptomatology	PPI, emotional, and cognitive deficits (Salinger et al., 2003)
Treatment responsive	Unknown because unstudied
Etiology	Reelin deficiencies (Curran & D'Arcangelo, 1998)
Behavioral symptoms	PPI, emotional, and cognitive deficits (Salinger et al., 2003)
Neuroanatomy	Abnormal lamination (D'Arcangelo & Curran, 1998)
Neurochemical symptoms	Reelin deficiencies (Curran & D'Arcangelo, 1998)
Temporal progression	Unknown because unstudied
Precipitating event	Unknown because unstudied

Risperidone has been found to be more effective than haloperidol (a first-generation, typical antipsychotic) at treating positive and negative symptoms of schizophrenia (Marder & Meibach, 1994) and cognitive deficits associated with the disease (Bilder, Goldman, Volavka, Czobor, et al., 2002). Because of its superiority over haloperidol, risperidone was predicted to be more likely to produce observable effects on

the *rl/rl* and so was the drug chosen to be used in this experiment.

Previous studies investigating the effects of antipsychotics on animal models of schizophrenia have tended to focus on animals with disrupted PPI and have often limited or even avoided the use of measures of other schizophrenia-relevant behaviors. Whether deficits in PPI are induced or are naturally occurring in an animal, researchers use those animals to test new medications that could potentially treat schizophrenia patients (for a review, see Geyer et al., 2001), since disrupted PPI is a common feature in patients with schizophrenia that can be attenuated by antipsychotic medication (Kumari, Soni, & Sharma, 2002; Oranje et al., 2002; Duncan, Szilagyi, Schwartz, Kunzova, et al., 2003). Risperidone has been found to be effective at improving PPI both in mice naturally displaying deficient PPI (Ouagazzal, Jenck, & Moreau, 2001; Browman, Komater, Curzon, Rueter, et al., 2004) and in mice with experimentally-induced deficits in PPI (Le Pen & Moreau, 2002).

In order to test further the hypothesis that *rl/rl* mice model schizophrenia, a pharmacological study was conducted in which risperidone was administered to the animals in order to determine if *rl/rl* mice's cognitive, emotional, and sensorimotor gating deficits would improve in response to the drug. The specific prediction tested in this study was that the administration of risperidone to *rl/rl* mice would normalize their cognitive, emotional, and sensorimotor gating deficits, thereby causing *rl/rl* mice treated with risperidone to behave more like +/+ and +/*rl* mice not treated with the drug. If risperidone did significantly improve the deficits found in the *rl/rl* mouse, then this animal would gain further validation as an appropriate model for schizophrenia.

CHAPTER II

METHOD

Subjects

Fifty-four experimentally naïve mice (background strain, B6C3Fe a/a-, Jackson Laboratories, Bar Harbor, ME) bred in our animal colony served as subjects, divided as shown in Table 3. Crawley (1999) stated that there should be 10 animals of each genotype per condition for standardized experimental designs and appropriate statistical tests. Thus, 60 animals were needed for this experiment; however, due to difficulties in breeding and an unexpectedly high neonatal mortality rate in our colony, we were able to obtain only 54 animals for experimentation.

Table 3
Distribution of Subjects by Sex, Genotype, and Drug Condition

	+/+	+/*rl*	*rl/rl*
Vehicle	5 Males / 6 Females	3M / 4F	5M / 4F
Risperidone	5M / 5F	4M / 4F	5M / 4F

Breeding difficulties also required three separate cohorts of mice. The first cohort contained 28 mice, which consisted of 14 +/+ (six males), four +/*rl* (all female), and 10 *rl/rl* (seven males). The second cohort contained 18 mice, which consisted of six +/+

(three males), six +/*rl* (four males), and six *rl/rl* (two males). The last cohort contained only eight mice – one male +/+, three male +/*rl*, two female +/*rl*, one male *rl/rl,* and one female *rl/rl*. All animals were at least 70 days of age when behavioral trials began. Animals were housed in cages of 2-4 same-sex littermates, and food and water were available *ad libitum* at all times, except during nose-poke testing, as described below. All behavioral testing occurred between 1100 and 1700h. Testing protocols were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Greensboro.

Drug Treatment

Each day, animals in the drug condition received 1.0 mg of risperidone per kg of body weight via oral gavage. This dose was chosen because it was consistently found to be effective in behavioral assays on rodents reported in the literature (Ouagazzal et al., 2001; Le Pen & Moreau, 2002). We also pilot tested 11 male animals (five *rl/rl* and six +/+) by giving them either risperidone or vehicle for three weeks and testing them in PPI at the end of each week. Results showed that there was a significant effect of the drug after the third test, which suggested that a three-week exposure to 1.0 mg/kg per day of risperidone by gavage should be sufficient to produce differential genotype responses to the drug, if those differences truly existed.

Thus, three weeks prior to experimental testing for each cohort, all animals in the drug group began receiving risperidone, in water, via oral gavage at the rate of 1.0 mg/kg of body weight. One-milligram tablets of risperidone (obtained through a generous donation from a local psychiatrist, Dr. Raouf Badawi) were dissolved in 2.0 mL of tap

water. The animals in the vehicle control group began receiving comparable oral gavage administrations of water.

Drug/vehicle administration continued until the end of behavioral testing. Each day, animals received the drug or vehicle beginning at 1700h (+/- 30min) and all animals in the then currently-running cohort were dosed. Studies suggest that blood and brain levels remain within therapeutic limits for risperidone and its active metabolite, 9-hydroxyrisperidone, for 24 hours in both rodents (Aravagiri & Marder, 2002) and humans (Möller, 2005; Megens, Awouters, Schotte, Meert, et al., 1994). Dosing in such a fashion also gave the animals an entire night to recover from any stress associated with gavage dosing before being tested.

Materials and Procedures

Behavioral data were collected in five behavioral assays - open field, light-dark, PPI, nose-poke, and passive avoidance, in that order - which measure emotionality, sensorimotor gating, and cognitive functioning (Crawley, 2000). The order in which individual animals in a cohort were run through each test was determined by random assignment of cages.

Tests of Emotionality

Assessments of open field and light-dark performance used the TruScan apparatus and TruScan 2.01 software (Coulbourn Instruments, Allentown, PA). The TruScan arena is a clear acrylic enclosure, open on top, 26 cm square and 40 cm high. Two sensor beams arrays that detect animal movement are adjusted in height for each assay, and accessory equipment is added as needed to modify the TruScan arena for each individual

test. Two independent sets of TruScan apparatus permit the testing of two individual mice concurrently.

Open field. The open field (OF) test occurred in the empty TruScan arena. The only accessory added was a plain brown floorplate or dropping pan. One sensor beam, at floor level, measured the animal's movement in the *X, Y* plane, while a second beam, six centimeters above the floor, measured the animal's rearing movements.

The open field test is designed to measure the level of an animal's anxiety and its level of exploratory behavior when it is exposed to a novel environment (Crawley, 2000). Each animal was placed for one hour into the TruScan arena as described above. Dependent variables for this test are time spent at rest, time spent on the perimeter (defined as the area no more than 3.25 cm away from the walls) of the chamber as opposed to time spent in the middle (any area that is not in the margin), rearing, stereotypic movements (grooming, head bobbing and/or extensions, and short body extensions that displace the center of the animal no more than 1.52 centimeters and then return the animal to its starting point within two seconds), and defecation.

In this test, normal animals initially exhibit behaviors that are consistent with anxiety; for example, most produce boli and remain in perimeter areas while exploring the arena. Throughout the hour-long trial, animals with normal executive functioning habituate to the new environment and their activity levels decrease dramatically. However, *rl/rl* mice produce significantly fewer boli and spend more time exploring non-perimeter areas than do +/+ or +/*rl* mice, and they also produce many stereotyped movements. They also remain active longer than do control mice (Salinger et al., 2003).

It was predicted that the administration of risperidone to the *rl/rl* would cause the animals to respond to the open field environment in a manner more consistent with the behavior found in +/+ and +/*rl* mice; in other words, they would, for example, produce more boli and remain in perimeter areas more often.

Light-dark. The light-dark (LD) test also occurred in the TruScan arena, with the same arrangement of sensor beams as used in the open field and with the same floorplate. Additionally, however, a four-sided, dark acrylic insert (25.4 cm long x 13.34 cm wide x 38.74 cm high) with a small opening (4.14 cm x 4.14 cm) facing the center of the arena was placed into the arena, thereby dividing the arena into lighted and darkened halves. The insert is opaque to visible light but transmits the near-infrared illumination used by the sensor beams.

The light-dark test provides another measure of anxiety (Crawley, 2000) and occurred for each animal the day after the animal was in the open field. The animal was placed into the TruScan arena, modified as described above, into the lighted half and allowed to explore for five minutes. Dependent variables are the latency to enter the darkened half of the chamber for the first time and the total number of transitions between the lighted and darkened halves. Most rodents prefer to spend more time in the dark, although mice like to explore novel environments as well (Crawley, 2000), so after the initial entry into the darkened half of the chamber, mice should transition fairly frequently. *Rl/rl* mice display a longer latency to enter the dark half of the chamber compared to +/+ and +/*rl* mice and therefore have fewer transitions (Salinger, et al., 2003), which may mean that they are less anxious about being in the open than are

control mice (because they spend more time in the light half the chamber). These findings are again consistent with the idea that the *rl/rl* mice are displaying a schizophrenia-like flattened affect. It was predicted that administration of risperidone would decrease the latency of the *rl/rl* to enter the darkened half and increase the number of transitions the *rl/rl* made (i.e., it would cause the *rl/rl* to behave more like +/+ and +/*rl* mice).

Test of Sensorimotor Gating

Prepulse inhibition of the startle response (PPI) was measured in the San Diego Instruments SR-LAB chamber with SR-LAB software (San Diego Instruments, San Diego, CA). The chamber is sound-attenuated and houses a platform containing a force transducer to capture a mouse's body movements and convert them into a signal sent to a computer, which then converts the signal of the mouse's movement after the beginning of a trial into a startle response amplitude.

Mice were placed into a holding container that was placed onto the platform, which in turn was situated directly beneath a speaker mounted into the top of the startle chamber such that the speaker was directly above the mouse. White noise and white noise pulses were produced over the speaker.

The PPI test was approximately 20 minutes and consisted of a five-minute habituation period, during which the animal was allowed to habituate to the environment and to the background-level white noise (65 dB). There were also 50 trials of five different trial types, resulting in ten trials for each trial type. The trial types were as follows: 1) background noise only; 2) startling, 120-dB stimulus only (no prepulse); 3) startling stimulus preceded by a prepulse of 76 dB; 4) startling stimulus preceded by a

prepulse of 80 dB; and 5) startling stimulus preceded by a prepulse of 84 dB. Each animal experienced the same five trial types in the same order as every other mouse; however, the order of presentation for each trial type when the program was written was randomly determined. On trials on which they were used, prepulses were produced for 20 milliseconds and there were 80 milliseconds from the offset of the prepulse to the onset of the startling stimulus, which was produced for 40 milliseconds. On startle-only trials, the startling stimulus was not preceded by a prepulse and was produced for 40 milliseconds. On background only trials, no audible stimuli other than background-level white noise were produced. The interval between trials was randomly assigned, ranging between nine and 25 seconds.

Normal animals are able to inhibit a startle response to the startling stimulus if the startle is preceded by a non-startling prepulse. *Rl/rl* mice, however, are significantly less able to reduce the amplitude of their startle response when the startle stimulus is preceded by a prepulse (Salinger et al., 2003); that is, *rl/rl* mice have a weak PPI. It was predicted that risperidone would increase the *rl/rl* mouse's PPI to levels more consistent with +/*rl* and +/+ animals.

Tests of Learning and Memory

Nose-poke. Nose-poke (NP) testing occurred in the TruScan arena with several important changes to assess reference and working memory. An aluminum floorplate was inserted into the TruScan arena. This floorplate consists of 16 evenly spaced holes, and 0.95 cm under the floorplate is a sub-floor with 16 wells, one directly beneath each hole. One sensor beam was placed at the level of the sub-floor, thus recording each time the

animal pokes its nose into the well, and one sensor beam was placed at the level of the aluminum floorplate, thus measuring each movement of the animal in the *X, Y* plane as well.

Because this test used food rewards in order to measure reference and working memory, before assessment could begin each animal had its weight reduced to between ~ 85% to ~ 90% of free-feeding body weight. Fragments (~0.006 g) of Nestle's® chocolate mini-morsels were placed into four of the sixteen wells of the sub-floor; the same four were the only ones baited throughout testing. Animals were habituated to the task for two days to learn that the wells contain a food reward and to learn to eat the chocolate. (Different holes were used in the habituation task than those used during testing.) Testing occurred over a period of seven days following the two days of habituation. On the testing days, animals were allowed up to five minutes to recover all four morsels, and testing ended when the animal visited the fourth baited hole for the first time. Variables of interest for this task include reference and working memory ratios each day, and the number of errors and repeat entries made each day. Reference memory is defined as the total number of entries and re-entries into baited holes divided by the total number of entries into all holes; that is, this measure defines how well the animal has learned which holes contain rewards based on the animal's preferential entries into those holes. Ratios for spatial working memory are determined by dividing the number of novel entries into baited holes by the total number of entries and re-entries into baited holes; in other words, this measure examines how well the animal remembers which of the baited holes it has already visited. Errors are defined as the total number of entries into unbaited holes from

the beginning of the task until the end of the task, and repeat entries are the total number of re-entries into any previously visited hole, whether baited or not.

Rl/rl mice may have deficits in spatial working memory (Salinger et al., 2003). It was predicted that *rl/rl* mice on vehicle would make more errors during this task and have lower spatial working memory and reference memory ratios, and that the administration of risperidone would significantly decrease the number of errors and repeats that *rl/rl* make in this task, thereby improving their working and reference memory scores and causing them to appear more like +/+ and +/*rl* mice.

Passive avoidance. Passive avoidance (PA) testing took place in the Coulbourn Instruments Habitest passive avoidance chamber with Graphic State 2.0 software (Coulbourn Instruments, Allentown, PA). The apparatus consists of a test chamber (7" x 15" x 13") that is divided into equally-sized halves. The right half of the chamber is covered with opaque black film, thus making this half of the chamber dark, while the left half has a house-light in the ceiling. A guillotine door separates the two halves. A dropping plate is placed beneath a shock-floor grid, which serves as the floor of the apparatus. The whole test chamber is placed inside a larger sound-attenuated chamber. The doors of the outer chamber have peep-holes that allow the experimenter to monitor the animals inside the apparatus.

Similar to the light-dark assessment, this test relies on a rodent's preference to be in the dark versus being in a well-lighted, open area. On the training day, an animal was placed into the left, lighted half of the test apparatus and allowed to explore for 60 seconds, after which time the guillotine door between the halves opened. One second

after the mouse entered the darkened half, however, the door between the halves was closed and then the animal received a startling but non-painful 1.0 mA footshock that was two seconds in duration. After 30 seconds, the animal was returned to its home cage. The next day, the procedure was identical, except that the animal was not shocked upon entering the darkened chamber. The variable of interest in this task is the difference in latencies to enter the darkened chamber the first versus the second day.

The protocol was set up such that an animal must have been entirely on the right side of the chamber (the darkened half) for one second before the door closed, and only after the door closed did the animal receive the shock. Although animals could explore around the door between halves and even partially enter the right side, thus breaking the sensor beam between halves, latencies to enter the right side of the chamber were defined as the entries that caused the door between halves to close. Animals that did not enter the right side of the chamber were assigned a latency of 300 seconds, because they were allowed up to five minutes to enter the right side before the test ended.

This was the first time *rl/rl* mice had been tested in PA, and so their typical behaviors in this test were unknown. However, because previous research suggested that *rl/rl* mice may have deficits in response inhibition (Salinger et al., 2003), it was predicted that the *rl/rl*, compared to control mice, would be less able to suppress the tendency to enter the darkened chamber the second day, despite receipt of a shock upon doing so the previous day. Thus, it was predicted on the second day, after having received a footshock in the darkened chamber on the first day, that all mice would increase the latency of their entry into the darkened chamber. Moreover, it was predicted *rl/rl* mice would increase

their latency less than would the other genotypes, expressed as a percentage of the latency on day 1. Finally, regarding risperidone effects, it was predicted that the administration of risperidone to *rl/rl* mice would cause the *rl/rl* to have longer latencies on the second day than they would if they had only received the vehicle solution, thereby causing them to behave more like +/+ and +/*rl* mice.

However, after the first cohort of 28 animals had been tested in PA and it looked as though there were no genotype differences emerging, the methods were slightly altered for the next two cohorts (N=26) in such a way that experimental data could be collected in an uncontaminated fashion but would allow for a different method to be piloted. The first day of testing (the training day) was unchanged, but on the second day, animals were run through two trials instead of one. The first trial on the second day was another training trial, in which the animal received a shock upon entering the dark half of the chamber, as it had on the previous day's trial. The second trial on the second day became the test trial and thus no shock was administered. The goal of this pilot testing was to determine if two training trials, instead of just one, would be sufficient to elicit genotype differences. Because the difference between the original method and the pilot method occurred only after the animal had already entered the dark half of the chamber on the first trial of the second day, the latency for which was the behavior of interest, that response was uncontaminated and was therefore commensurate with the latency data from the first 28 animals, and so the experimental data were uncorrupted.

Startle response habituation (SRH). SRH data were obtained during the PPI test based on the maximum startle amplitude exhibited by animals on the 10 startle-only

trials. *Rl/rl* mice were found to show less habituation to the startle stimulus than +/*rl* and +/+ mice (Salinger et al., 2003), and it was predicted that the administration of risperidone would cause them to habituate to the startle stimuli similarly to +/+ mice.

Statistical Analyses

A three-way analysis of variance (ANOVA) with genotype, treatment condition, and sex as between-subject factors was used to analyze the number of fecal boli produced in OF testing and the number of transitions between the light and dark halves and the initial latency to enter the dark in LD testing. Three-way ANOVAs with repeated measures, either across time, within a trial, or across multiple trials, with genotype, treatment condition, and sex as between-subject factors, were used to analyze all other measures. When a repeated-measures ANOVA revealed time as a significant factor in open field and nose-poke tests, polynomial extractions were used to account for its non-random nature (Salinger et al., 2003). Main effects and interactions were analyzed with contrasts. Sex was included in analyses in order to avoid treating it as an error component; however, because too few of each sex were included in each condition, a reasonable interpretation of sex differences was not possible. Significance for all analyses was set at $p < 0.05$.

CHAPTER III

RESULTS

Analysis of Vehicle-Treated Animals

The hypotheses in this experiment were based on observed differences between the *rl/rl* and the other two genotypes that were evident when none of the animals had received a drug manipulation (i.e., Salinger et al., 2003). Therefore, in order to ensure that unexpected genotype differences did not emerge as a result of stress related to the gavage procedure, or some other difference in experimentation that could confound the results and their interpretation, analyses were run separately for vehicle-treated animals only.

Tests of Emotionality

Open field. A two-way analysis of variance with sex and genotype as between-subjects variables for rest time during the first five minutes of open field exposure was non-significant overall, $F(5, 21) = 1.8447$, $p= 0.1476$, although the analysis for genotype was significant, $F(2, 21) = 4.1137$, $p= 0.0311$, with *rl/rl* mice spending less time at rest.

A two-way, repeated-measures analysis of variance with sex and genotype as between-subjects variables and with time as the within-subjects variable was used to analyze rest time over the 60-minute trial. The between-subjects analysis was significant overall, $F(5, 21)= 11.8497$, $p< 0.0001$. This was due to genotype: $F(2, 21) = 28.9588$, $p< 0.0001$, in which the *rl/rl* spent significantly less time at rest than the +/*rl* and

+/+, F(1, 21) = 56.0877, p< 0.0001 (Figure 1). The other variables were not significant: sex, F(1, 21) = 2.2228, p= 0.1509, and sex x genotype, F(2, 21) = 0.3218, p= 0.7283. The within-subjects analysis did not reveal any significant results: time x sex, F(11, 11) = 0.4381, p= 0.9066; time x genotype, F(22, 22) = 2.0277, p< 0.0523; and time x sex x genotype, F(22, 22) = 1.0462, p< 0.4583.

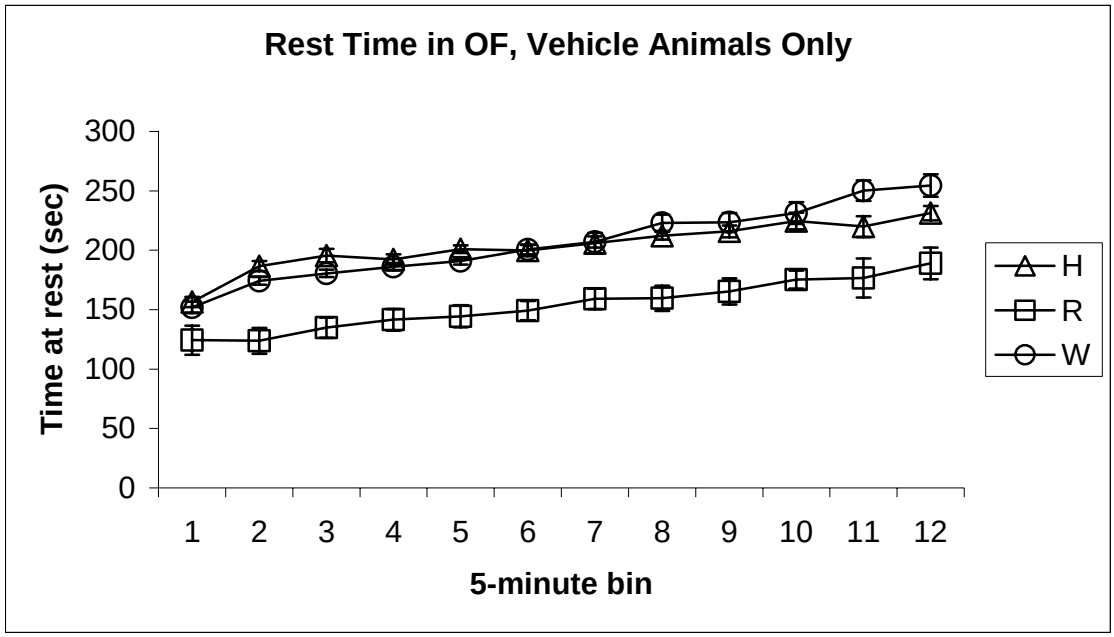

Figure 1. Rest Time in OF, Vehicle Animals Only. Time at rest in the open field for vehicle-treated animals only. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+.

The number of boli produced was analyzed with a two-way analysis of variance with sex and genotype as between-subjects variables. The ANOVA was significant overall, F(5, 21) = 3.1368, p= 0.0287. There was a significant main effect of genotype, F(2, 21) = 4.7863, p= 0.0194, but not for sex, F(1, 21) = 0.8308, p= 0.3724, nor was the interaction of sex and genotype significant, F(2, 21) = 2.7491, p= 0.0870. Contrasts

showed that the *rl/rl* produced significantly more boli in the open field than did the +/*rl* and +/+, F(1, 21) = 8.4342, p= 0.0085 (Figure 2).

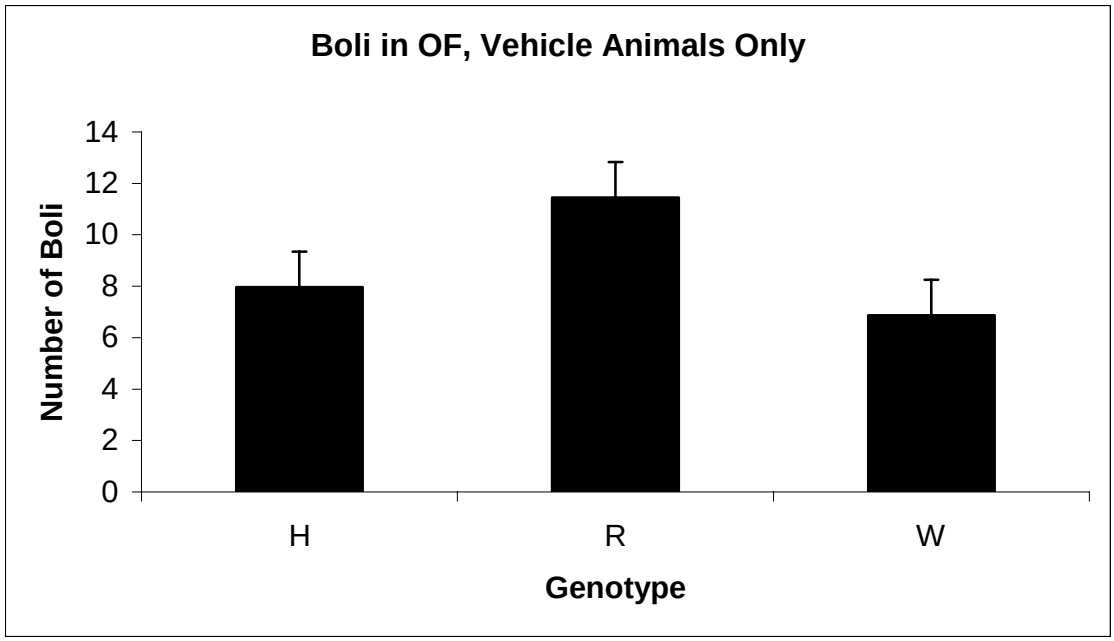

Figure 2. Boli in OF, Vehicle Animals Only. Number of boli produced in the open field by vehicle-treated animals only. H= +/*rl*, R= *rl/rl*, and W= +/+.

The percentage of time spent in the margins of the open field was analyzed with a two-way repeated-measures ANOVA with sex and genotype as between-subjects variables and with time as the within-subjects variable. The between-subjects analysis was significant overall, F(5, 21) = 3.8292, p= 0.0127. There was not a significant main effect of sex, F(1, 21) = 0.0908, p= 0.7661, nor was the interaction of sex and genotype significant, F(2, 21) = 0.3804, p= 0.6882. There was a significant main effect of genotype, F(2, 21) = 8.7288, p= 0.0017. A contrast showed that the *rl/rl* spent a significantly smaller percentage of time in the margins compared to +/*rl* and +/+, F(1, 21)

= 16.7069, p= 0.0005 (Figure 3). The within-subjects analysis revealed a significant time x sex interaction, F(11, 11) = 2.9956, p= 0.0411, in which females showed a more consistent increase over time in the percentage of time spent in the margins compared to males. There was not a significant time x genotype interaction, F(22, 22) = 1.8256, p= 0.0830, nor was time x sex x genotype significant, F(22, 22) = 1.4282, p= 0.2049.

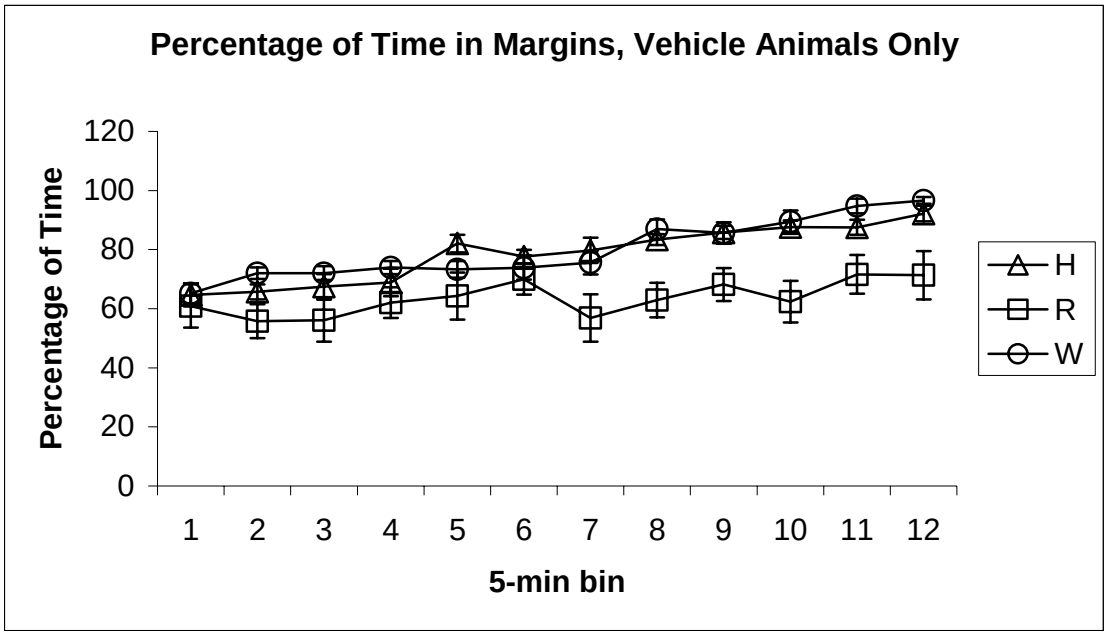

Figure 3. Percentage of Time in Margins, Vehicle Animals Only. The percentage of time spent in the margins of the open field for vehicle-treated animals only. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+.

The number of stereotypic movements was also analyzed with a two-way repeated-measures ANOVA. The between-subjects analysis was significant overall, F(5, 21) = 6.7199, p= 0.0007. There was not a main effect of sex, F(1, 21) = 0.4149, p= 0.5264, nor was there a significant interaction of sex and genotype, F(2, 21) = 1.5216, p= 0.2415. There was a significant main effect of genotype, F(2, 21) = 15.0265, p< 0.0001, and a

contrast showed that this was due to the *rl/rl*, who made more stereotypic movements compared to +/*rl* and +/+ mice, $F(1, 21) = 30.0471$, $p < 0.0001$ (Figure 4).

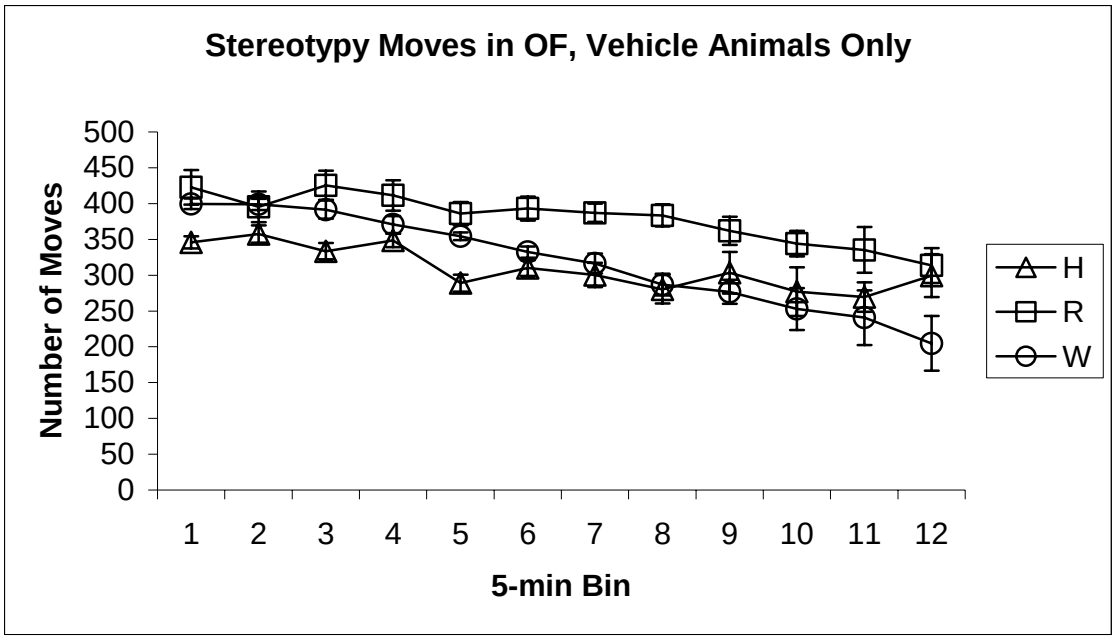

Figure 4. Stereotypy Moves in OF, Vehicle Animals Only. The number of stereotypy moves made by vehicle-treated animals only. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+.

The within-subjects analysis did not reveal a significant time x sex interaction, $F(11, 11) = 1.5304$, $p = 0.2459$, nor a significant time x sex x genotype interaction, $F(22, 22) = 1.6383$, $p = 0.1274$. There was a significant time x genotype interaction, $F(22, 22) = 2.1235$, $p = 0.0421$. A contrast showed that the number of stereotypies produced by *rl/rl* remained fairly constant across time compared to +/+, who showed a steady decline in the number of stereotypic moves produced, $F(11, 11) = 3.1101$, $p = 0.0364$ (Figure 4). Finally, the amount of rearing produced in the open field was also analyzed with a two-way, repeated-measures ANOVA. The between-subjects analysis was not significant, $F(5, 21) = 1.0352$, $p = 0.4230$. The within-subjects analysis did not show a significant

time x sex interaction, F(11, 11) = 0.8469, p= 0.6061, nor was there a significant time x sex x genotype interaction, F(22, 22) = 1.8843, p= 0.0725. There was a significant time x genotype interaction, F(22, 22) = 2.4557, p= 0.0202, in which both the +/*rl*, F(11, 11) = 3.5945, p= 0.0222, and the *rl/rl*, F(11, 11) = 3.7813, p= 0.0185, differed from the +/+ (Figure 5). The +/+ showed a steady decline in the amount of rearing over time, but the +/*rl* showed a less obvious decline and reared more in the last half of the trial than did the +/+. The *rl/rl* displayed a more complex pattern of results, displaying very little rearing at the beginning of the trial and then showing more rearing as the trial progressed, but still reared less than did the +/+.

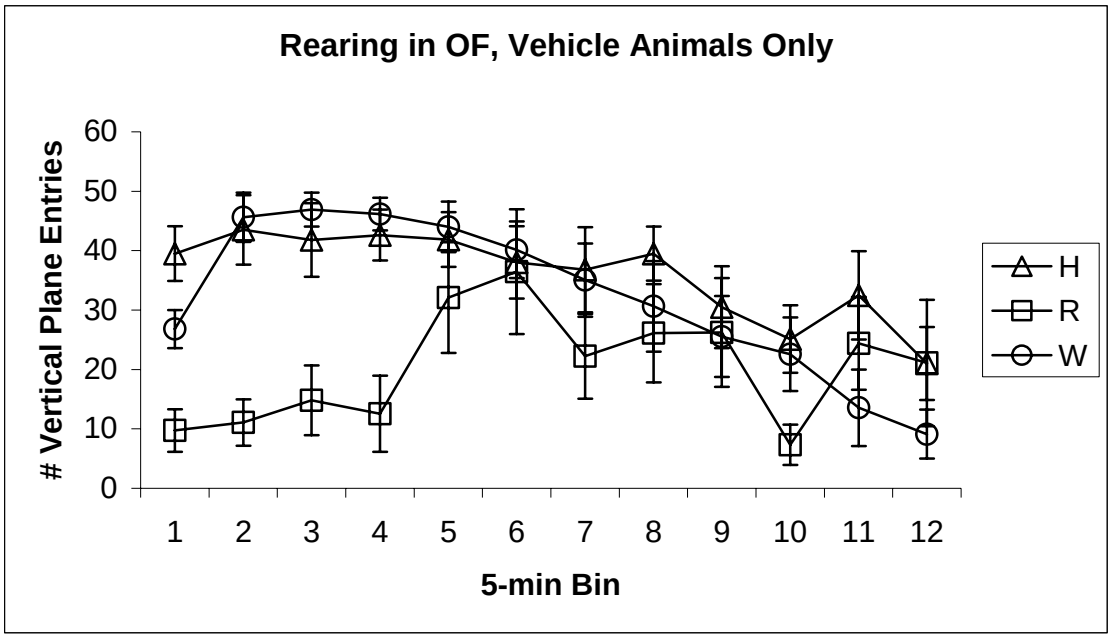

Figure 5. Rearing in OF, Vehicle Animals Only. Amount of rearing (number of vertical plane entries) in the open field for vehicle-treated animals only. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+.

Light-dark. The latency of the initial entry to the dark half of the chamber was analyzed with a two-way analysis of variance, with sex and genotype as the between-subjects factors. The ANOVA was not significant overall, $F(5, 21) = 1.6148$, $p= 0.1997$, although the analysis of genotype was significant, $F(2, 21) = 3.7783$, $p= 0.0397$, with *rl/rl* showing a much longer latency (Figure 6).

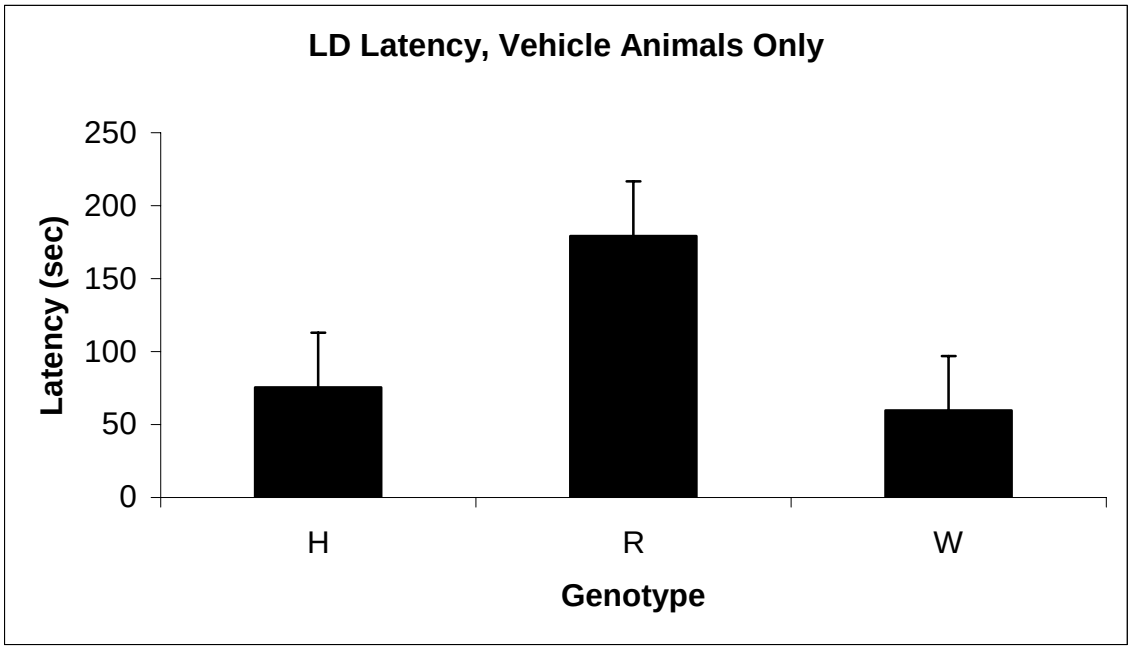

Figure 6. LD Latency, Vehicle Animals Only. The latency to enter the dark half of the chamber in the light/dark test for vehicle-treated animals only. H= +/*rl*, R= *rl/rl*, and W= +/+.

The number of transitions between halves was also analyzed with a two-way ANOVA. This analysis was also non-significant, $F(5, 21) = 2.5389$, $p= 0.0601$, although genotype was significant, $F(2, 21) = 3.6130$, $p= 0.0448$, with *rl/rl* mice showing fewer transitions (Figure 7).

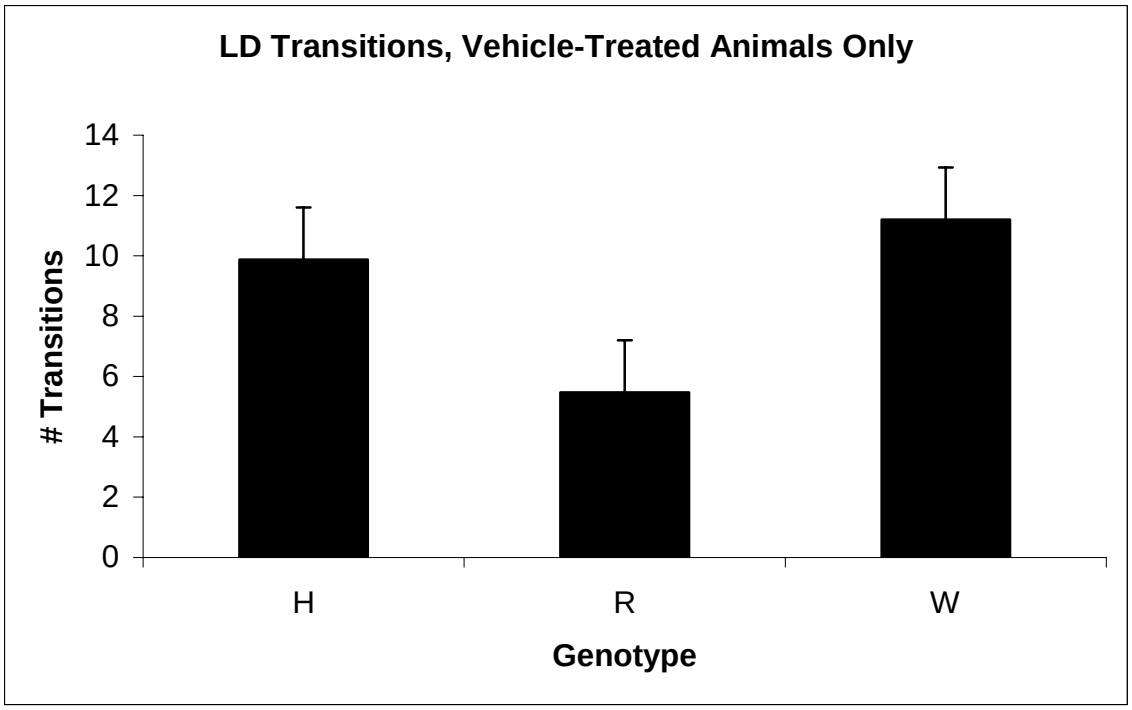

Figure 7. LD Transitions, Vehicle-Treated Animals Only. The number of transitions between halves in the light-dark test for vehicle-treated animals only. H= +/*rl*, R= *rl/rl*, and W= +/+.

Test of Sensorimotor Gating

The amplitude of startle response on trials in which there was a prepulse was compared to the amplitude when only a startle stimulus occurred, for each prepulse intensity and each animal. Prepulse inhibition of startle (PPI) was the percent reduction of the startle response amplitude when both a prepulse and startle stimulus are presented relative the startle response amplitude when only a startle stimulus occurs. First, means were obtained for each of the five trial types (background only, startle only, and the three prepulse trial types - 76 dB, 80 dB, and 84 dB). The mean startle response for background-only trials was subtracted from the mean startle responses for each of the other trial types in order to correct for baseline movement. To obtain the measure of PPI

for each of the three prepulse intensities, the background-corrected means were used in the following formula for each animal: (mean startle on startle-only trials - mean startle on prepulse trials)/(mean startle on startle-only trials). The fraction that was obtained in this formula was then multiplied by 100 to obtain a percentage.

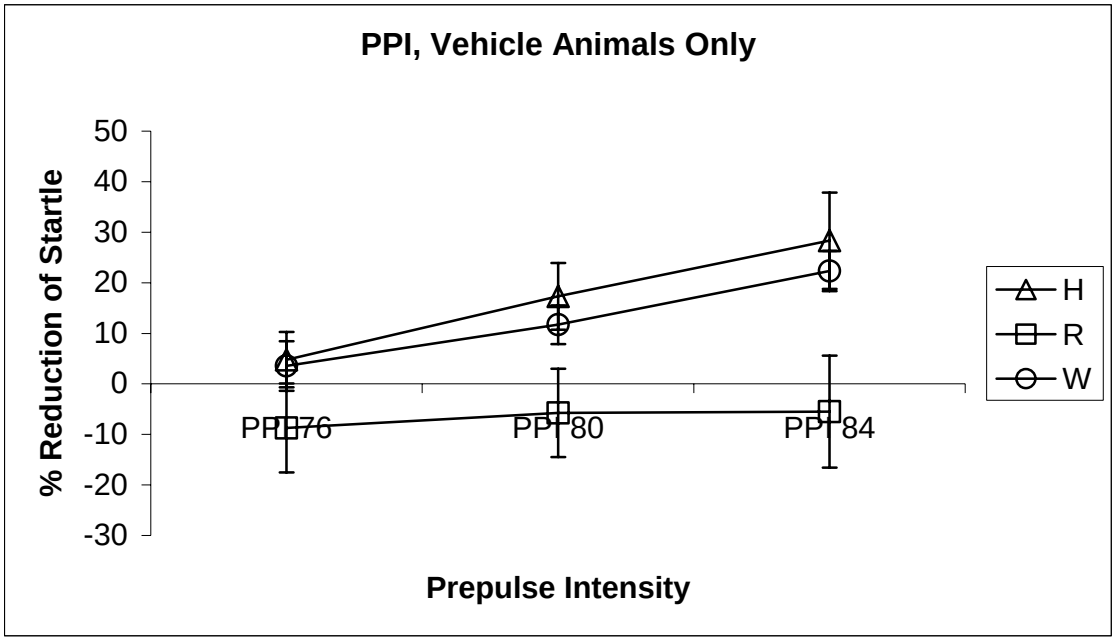

Figure 8. PPI, Vehicle Animals Only. Percent reduction of startle for vehicle-treated animals only. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+.

The percent decrease in startle response on prepulse trials was analyzed with a two-way repeated-measures ANOVA with sex and genotype as between-subjects factors and with prepulse intensity as the within-subjects factor. The between-subjects analysis was not significant overall, $F(5, 21) = 1.7351$, $p= 0.1704$, although the analysis of genotype was significant, $F(2, 21) = 3.5610$, $p= 0.0466$, with *rl/rl* mice showing less PPI than the other two genotypes (Figure 8). The within-subjects analysis did not reveal any

significant results: prepulse intensity x sex, F(2, 20) = 1.5551, p= 0.2356; prepulse intensity x genotype, F(4, 40) = 1.0180, p< 0.4097; and prepulse intensity x sex x genotype, F(4, 40) = 0.2363, p< 0.9162.

Tests of Learning and Memory

Nose-poke. Reference memory was analyzed with a two-way repeated-measures analysis of variance, with sex and genotype as between-subjects variables and with time as the within-subjects variable. The between-subjects ANOVA was not significant, F(5, 21) = 2.2994, p= 0.0816. The within-subjects analysis also did not reveal any significant results: time x sex, F(6, 16) = 2.1113, p= 0.1089; time x genotype, F(12, 32) = 0.6245, p= 0.8056; and time x sex x genotype, F(12, 32) = 1.2221, p= 0.3111.

Working memory was analyzed as well, with a two-way, repeated-measures ANOVA. The between-subjects analysis was not significant, F(5, 21) = 2.6419, p= 0.0528. The within-subjects analysis did not reveal any significant results: time x sex, F(6, 16) = 1.1072, p= 0.4006; time x genotype, F(12, 32) = 1.4730, p= 0.1857; and time x sex x genotype, F(12, 32) = 0.7765, p= 0.6695.

The number of errors (defined above) made was analyzed with a two-way, repeated-measures analysis of variance. The between-subjects analysis was significant overall, F(5, 21) = 6.2260, p= 0.0011. There was not a significant main effect for genotype, F(2, 21) = 2.8728, p= 0.0789, but there was a main effect of sex, F(1, 21) = 9.9652, p= 0.0048, and sex x genotype was also significant, F(2, 21) = 7.7789, p= 0.0030. A contrast showed that female +/*rl* mice made significantly more errors compared to female *rl/rl* and +/+ mice, F(1, 21) = 21.2625, p= 0.0002 (Figure 9). The within-subjects

analysis did not reveal any significant results: time x sex, F(6, 16) = 1.4994, p= 0.2407; time x genotype, F(12, 32) = 1.1346, p< 0.3685; and time x sex x genotype, F(12, 32) = 0.6176, p< 0.8114.

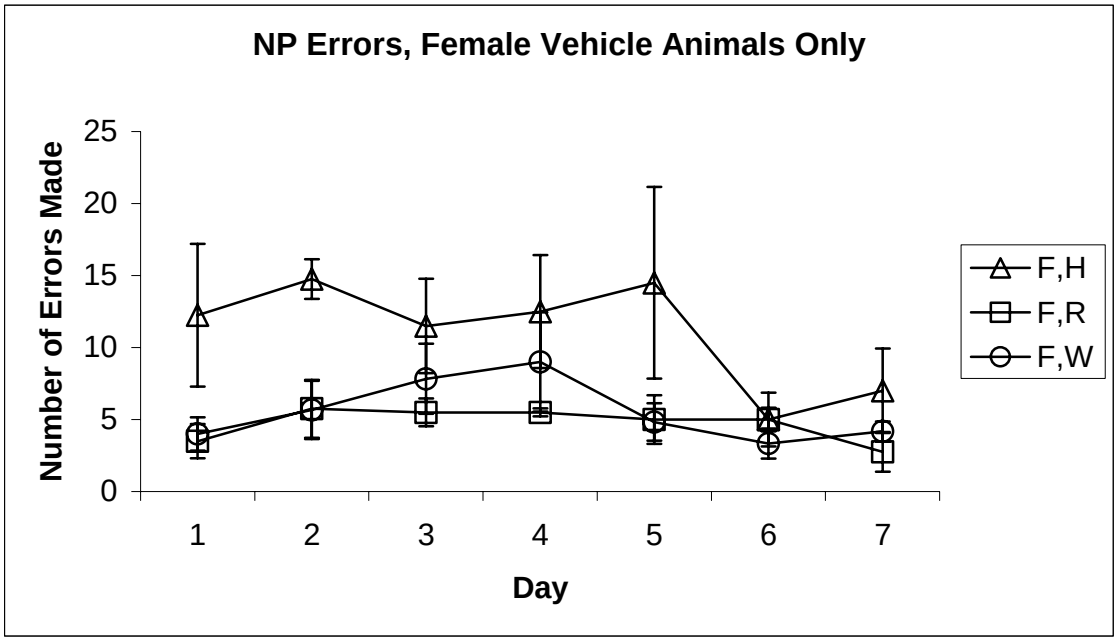

Figure 9. NP Errors, Female Vehicle Animals Only. Errors made in NP by female, vehicle-treated animals only. Triangles represent female +/*rl*, squares represent female *rl/rl*, and circles represent female +/+.

Finally, the number of repeat entries, defined as the total numbers of re-entries to any previously visited hole on a given day, was analyzed with a two-way, repeated-measures ANOVA. The between-subjects ANOVA was significant overall, F(5, 21) = 3.4894, p= 0.0188. There was not a main effect either of sex, F(1, 21) = 2.7590, p= 0.1116, or of genotype, F(2, 21) = 0.7918, p= 0.4661, but there was a significant interaction of sex and genotype, F(2, 21) = 6.9166, p= 0.0049. A contrast showed that the male *rl/rl* differed significantly from the male +/*rl* and +/+, F(1, 21) = 8.2234, p= 0.0092,

in that the male *rl/rl* made significantly more repeat entries than did the other two male genotypes (Figure 10). The within-subjects analysis did not reveal any significant results: time x sex, $F(6, 16) = 2.6264$, $p= 0.0574$; time x genotype, $F(12, 32) = 1.2657$, $p< 0.2852$; and time x sex x genotype, $F(12, 32) = 0.6501$, $p< 0.7838$.

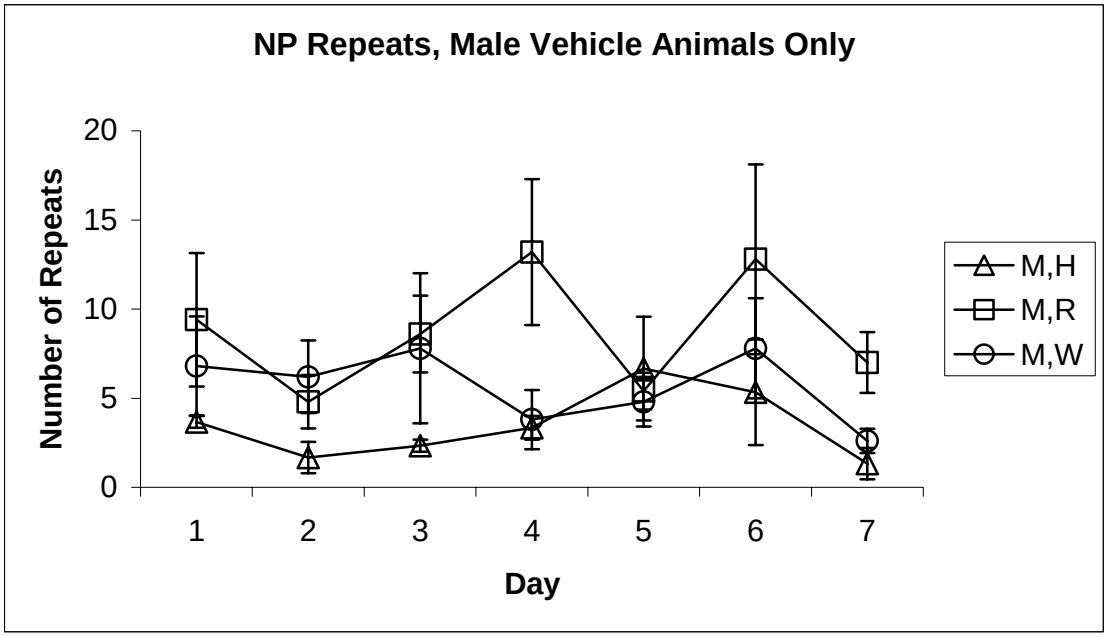

Figure 10. NP Repeats, Male Vehicle Animals Only. Repeat entries made in NP by male, vehicle-treated animals only. Triangles represent male +/*rl*, squares represent male *rl/rl*, and circles represent male +/+.

Passive avoidance. Percent change in latencies for entry into the darkened half of the chamber were analyzed with a two-way, repeated-measures analysis of variance with sex and genotype as between-subjects variables and day as the within-subjects variable. The between-subjects analysis was non-significant, $F(5, 21) = 1.1222$, $p= 0.3790$, and the within-subjects analysis did not reveal any significant interactions: day x sex, $F(1, 21) = 0.5811$, $p= 0.4544$; time x genotype, $F(2, 21) = 1.1380$, $p= 0.3394$; and time x sex x genotype, $F(2, 21) = 0.1130$, $p= 0.8937$.

Startle response habituation. Startle response habituation was analyzed by comparing the mean startle response amplitude for the first five startle-only trials to the mean startle response amplitude for the last five startle-only trials. The two means were compared with a two-way, repeated measures ANOVA with sex and genotype as the between-subjects variables and with block of trials as the within-subjects variable. The between-subjects analysis was not significant, $F(5, 21) = 1.1319$, $p= 0.3744$. The within-subjects analysis also did not reveal any significant effects: block x sex, $F(1, 21) = 0.7499$, $p= 0.3963$; block x genotype, $F(2, 21) = 2.5492$, $p= 0.1021$; and block x sex x genotype, $F(2, 21) = 0.1554$, $p= 0.8571$.

Analysis of the Effects of Risperidone

Tests of Emotionality

Open field. A three-way, between-subjects analysis of variance of rest time during the first five minutes of open field exposure with sex, genotype, and treatment condition as between-subjects variables was significant overall, $F(11, 42) = 4.0387$, $p= 0.0005$. There was a main effect of genotype, $F(2, 42) = 18.7788$, $p< 0.0001$ (Figure 11). A least-squares means contrast showed that this was due to the *rl/rl*, who spent significantly less time at rest compared to the other two genotypes, $F(1, 42) = 37.5384$, $p<0.0001$. There were no other significant main effects or interactions: sex, $F(1, 42) = 1.3169$, $p= 0.2576$; sex x genotype, $F(2, 42) = 0.3108$, $p= 0.7346$; treatment condition, $F(1, 42) = 0.0069$, $p= 0.9340$; sex x treatment condition, $F(1, 42) = 0.2758$, $p= 0.6022$; genotype x treatment condition, $F(2,42) = 1.3334$, $p= 0.2745$; and sex x genotype x treatment condition, $F(2, 42) = 0.5747$, $p= 0.5672$.

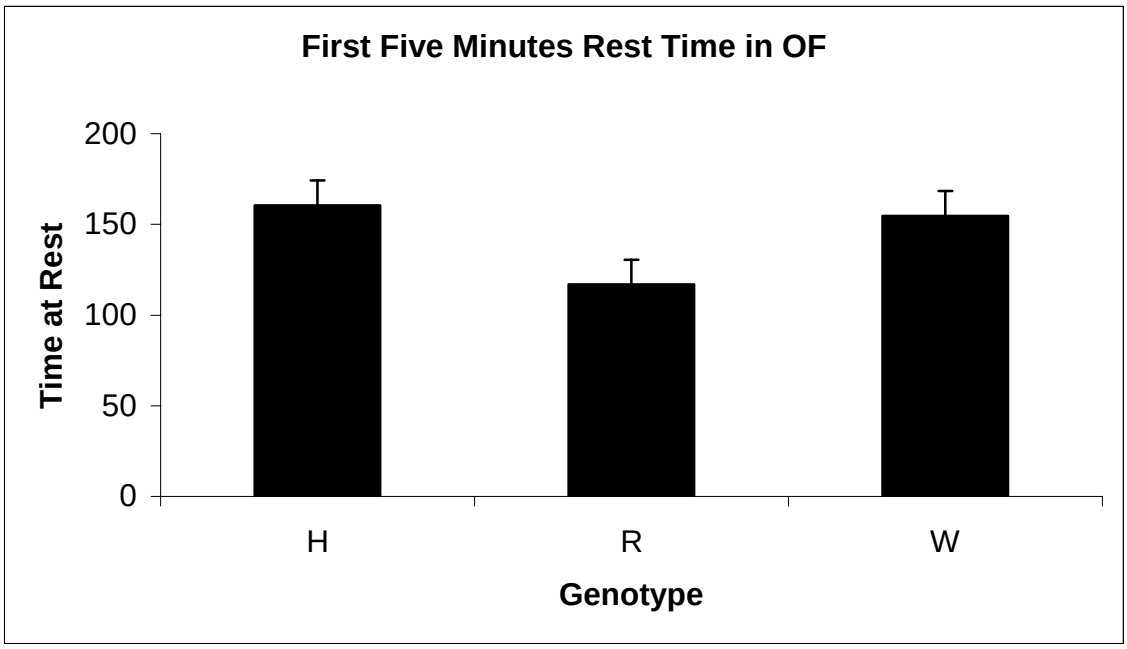

Figure 11. First Five Minutes Rest Time in OF. Rest time in the first five minutes of open field exposure. H= +/*rl*, R= *rl/rl*, and W= +/+.

As animals habituate to the open field over the hour-long trial, they tend to spend less time exploring and thus spend more time at rest. The between-subjects ANOVA for rest time during the 60-minute trial was significant overall, $F(11, 42) = 10.7456$, $p < 0.0001$, and there was a significant main effect both for sex, $F(1, 42) = 4.7957$, $p = 0.0341$, in which males spent more time at rest than females, and for genotype, $F(2, 42) = 56.3270$, $p < 0.0001$ (Figure 12). Contrasts revealed that the genotype effect was due to the *rl/rl*, who spent less time at rest overall compared to +/+ and +/*rl* mice, $F(1, 42) = 108.6606$, $p < 0.0001$. There were no other significant results revealed by the between-subjects ANOVA: sex x genotype, $F(2, 42) = 0.7666$, $p = 0.4710$; treatment condition, $F(1, 42) = 0.3646$, $p = 0.5492$; sex x treatment condition, $F(1, 42) = 0.1133$, $p = 0.7381$;

genotype x treatment condition, F(2, 42) = 0.8769, p= 0.4235; and sex x genotype x treatment condition, F(2, 42) = 0.0544, p= 0.9471.

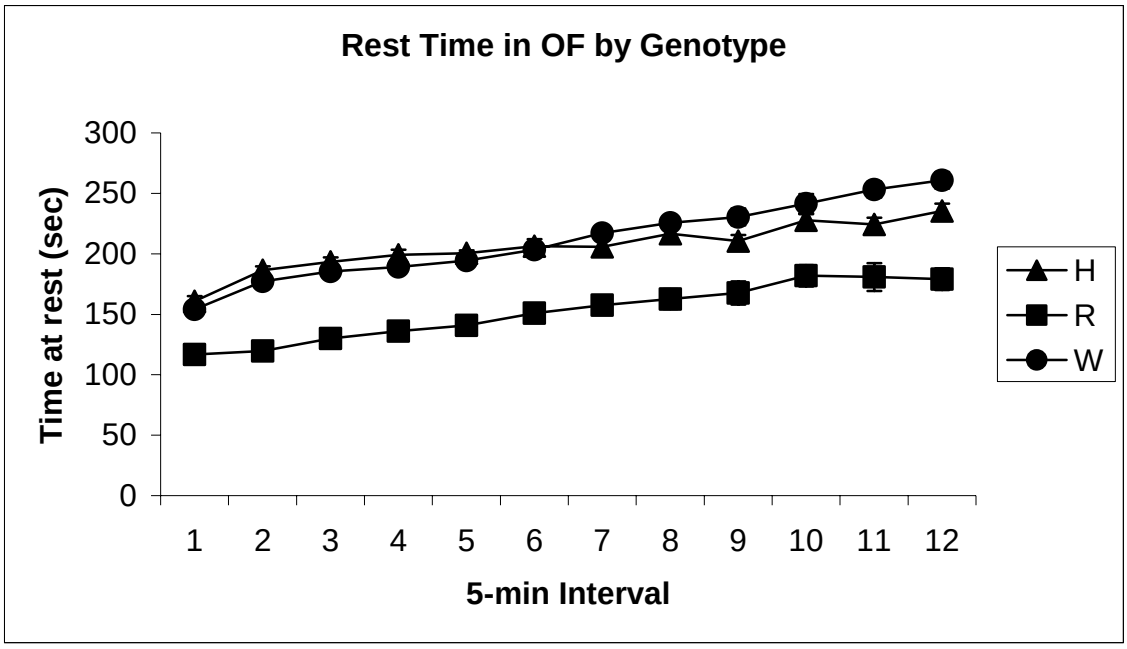

Figure 12. Rest Time in OF by Genotype. The mean amount of time spent at rest over the 60-minute trial. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+.

The repeated-measures, within-subjects ANOVA for rest time revealed a significant time x genotype interaction, F(22, 64) = 1.8861, p< 0.0259. Time x sex x genotype was also significant, F(22, 64) = 2.2444, p< 0.0064. Contrasts revealed that male *rl/rl* spent significantly less time at rest than did male +/*rl* and +/+ mice, F(11, 32) = 2.3450, p= 0.0298 (Figure 13). The other results were not significant: time x sex, F(11, 32) = 1.2790, p= 0.2803; time x treatment condition, F(11, 32) = 0.5775, p= 0.8325; time x sex x treatment condition, F(11, 32) = 1.0850, p= 0.4032; time x genotype x treatment condition, F(22, 64) = 1.0660, p< 0.4052; and time x sex x genotype x treatment

condition, F(22, 64) = 0.4369, p< 0.9834.

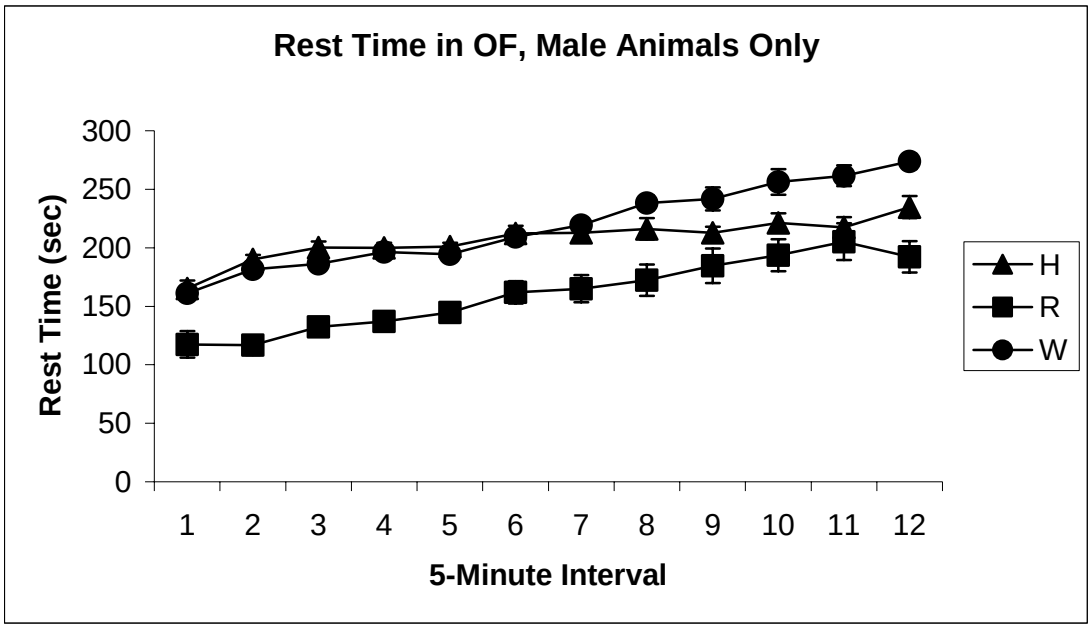

Figure 13. Rest Time in OF, Male Vehicle Animals Only. Time at rest during open field for male animals. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+.

Polynomial contrasts revealed a significant genotype x linear interaction, F(2, 42) = 7.8993, p= 0.0012, in which the +/*rl* showed a different pattern of increasing time at rest compared to both the *rl/rl* and the +/+, F(1, 42) = 6.4586, p= 0.0148, by starting off performing similarly to the +/+ but then leveling off in the last half of the trial compared to the +/+. There was also a significant genotype x cubic component, F(2, 42) = 3.2542, p= 0.0485, in which the *rl/rl* compared to the +/*rl* and +/+ steadily spent more time at rest as the first 45 minutes of the trial progressed before increasing time at rest markedly over the next five minutes and then leveling off for the last 10 minutes of the trial, F(1, 42) = 5.5168, p= 0.0236 (Figure 12).

The number of fecal boli produced by each animal during its trial was counted in order to obtain an autonomic measure of anxiety-related arousal that is not dependent on normal locomotor coordination (Salinger et al., 2003). A three-way ANOVA with sex, genotype, and treatment condition as between-subjects factors revealed a significant main effect of genotype, $F(2, 42) = 7.3297$, $p= 0.0019$, which appeared to be part of the larger interaction of sex and genotype, $F(2, 42) = 4.2197$, $p= 0.0214$. A least-squares means contrast showed that the male +/*rl* produced significantly more boli than did the female +/*rl*, $F(1, 42) = 4.6054$, $p= 0.0377$. All other results were non-significant; sex, $F(1, 42) = 0.0623$, $p= 0.8042$; treatment condition, $F(1, 42) = 1.5852$, $p= 0.2150$; sex x treatment condition, $F(1, 42) = 0.9626$, $p= 0.3321$; genotype x treatment condition, $F(2, 42) = 0.3982$, $p= 0.6740$; and sex x genotype x treatment condition, $F(2, 42) = 0.1340$, $p= 0.8750$.

The proportion of time spent in the marginal areas of the open field compared to central areas is also a measure of anxiety; high levels of anxiety are associated with a higher proportion of time spent in the marginal areas (Salinger et al., 2003). The three-way between-subjects ANOVA was significant overall, $F(11, 42) = 5.4106$, $p< 0.0001$, and there was a significant main effect of genotype, $F(2, 42) = 26.6808$, $p< 0.0001$. Contrasts showed that this effect was also due to the *rl/rl*, who spent a significantly smaller proportion of time overall in the margins compared to +/*rl* and +/+ mice, $F(1, 42) = 50.4557$, $p< 0.0001$ (Figure 14). There were no other significant main effects or interactions: sex, $F(1, 42) = 0.8601$, $p= 0.3590$; sex x genotype, $F(2, 42) = 0.7978$, $p= 0.4570$; treatment condition, $F(1, 42) = 0.0229$, $p= 0.8804$; sex x treatment condition, F(1,

42) = 0.2068, p= 0.6516; genotype x treatment condition, F(2, 42) = 0.4233, p= 0.6576; and sex x genotype x treatment condition, F(2, 42) = 0.1614, p= 0.8515.

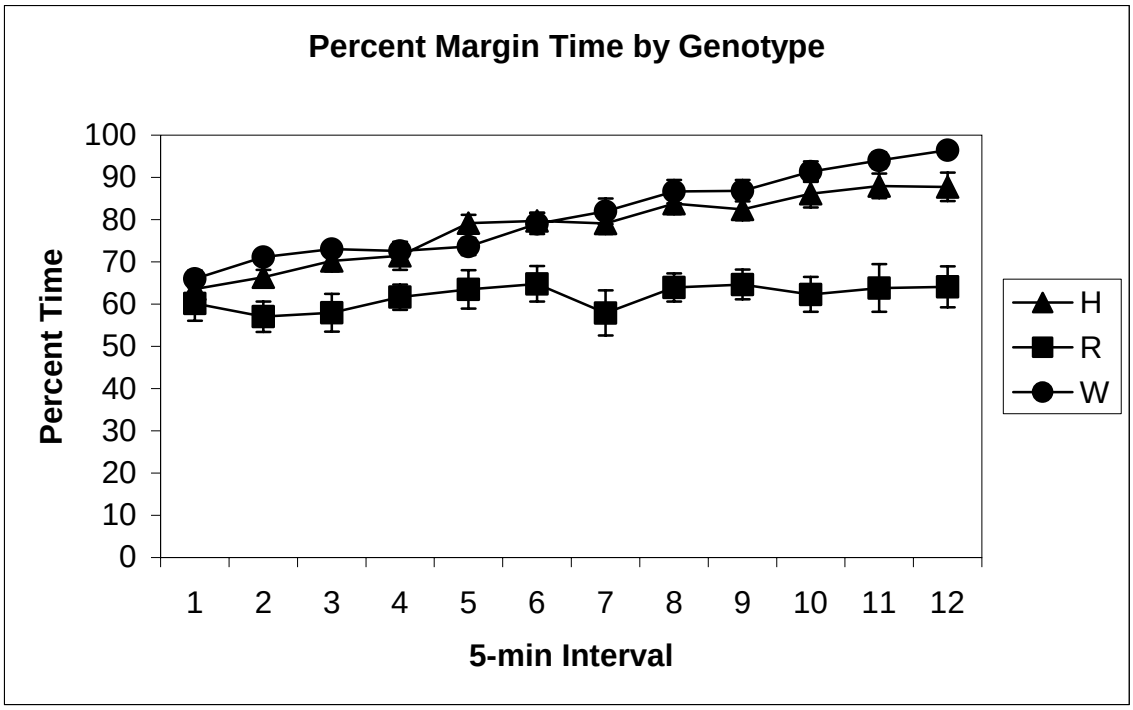

Figure 14. Percent Margin Time by Genotype. The mean percentage of time spent in the margins of the open field by genotype over the 60-minute trial. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+.

The repeated-measures, within-subjects ANOVA revealed no significant results, although time x genotype approached significance, F(22, 64) = 1.6934, p< 0.0533. The results for time x sex were F(11, 32) = 1.0348, p= 0.4405; time x sex x genotype, F(22, 64) = 1.2270, p< 0.2585; time x treatment condition, F(11, 32) = 1.5451, p= 0.1639; time x sex x treatment condition, F(11, 32) = 1.1605, p= 0.3513; time x genotype x treatment condition, F(22, 64) = 0.7455, p= 0.7755; and time x sex x genotype x treatment condition, F(22, 64) = 1.6868, p< 0.0546.

However, there was a main effect of time, $F(11, 32) = 6.6934$, $p < 0.0001$, and so polynomial extractions were used. These extractions revealed a significant genotype x linear interaction, $F(2, 42) = 7.5599$, $p = 0.0016$. Contrasts showed that *rl/rl* mice spent a significantly smaller proportion of time in the margins than did the other two genotypes, $F(1, 42) = 13.6322$, $p = 0.0006$ (Figure 14).

The number of small, non-locomotor, repetitive movements (stereotypies) made by each animal during the hour-long session was also recorded. A between-subjects ANOVA was significant overall, $F(11, 42) = 5.6439$, $p < 0.0001$, and there was also a significant main effect of genotype, $F(2, 42) = 25.4304$, $p < 0.0001$. Contrasts showed that this main effect was due to the *rl/rl*, who made significantly more stereotypic movements when compared to the +/*rl* and +/+, $F(1, 42) = 48.4438$, $p < 0.0001$ (Figure 15). There were no other significant main effects or interactions: sex, $F(1, 42) = 0.7314$, $p = 0.3973$; sex x genotype, $F(2, 42) = 1.9682$, $p = 0.1524$; treatment condition, $F(1, 42) = 3.4192$, $p = 0.0715$; sex x treatment condition, $F(1, 42) = 0.0185$, $p = 0.8925$; genotype x treatment condition, $F(2, 42) = 1.7953$, $p = 0.1786$; and sex x genotype x treatment condition, $F(2, 42) = 0.0196$, $p = 0.9806$.

A repeated-measures, within-subjects ANOVA with sex, genotype, and treatment condition as between-subjects factors and time as the within-subjects factor revealed a significant time x genotype interaction, $F(22, 64) = 2.5717$, $p < 0.0018$. Contrasts showed that *rl/rl* mice were significantly different from +/*rl* and +/+ mice, $F(11, 32) = 2.1234$, $p = 0.0479$, in that they produced more of these movements than the other two genotypes. Also, +/*rl* mice were significantly different from +/+ mice, $F(11, 32) = 3.0394$, $p =$

0.0069; while +/+ mice showed a steady decline in the number of such movements made over time, the number of these stereotypic movements made by the +/*rl* remained much more constant (Figure 15).

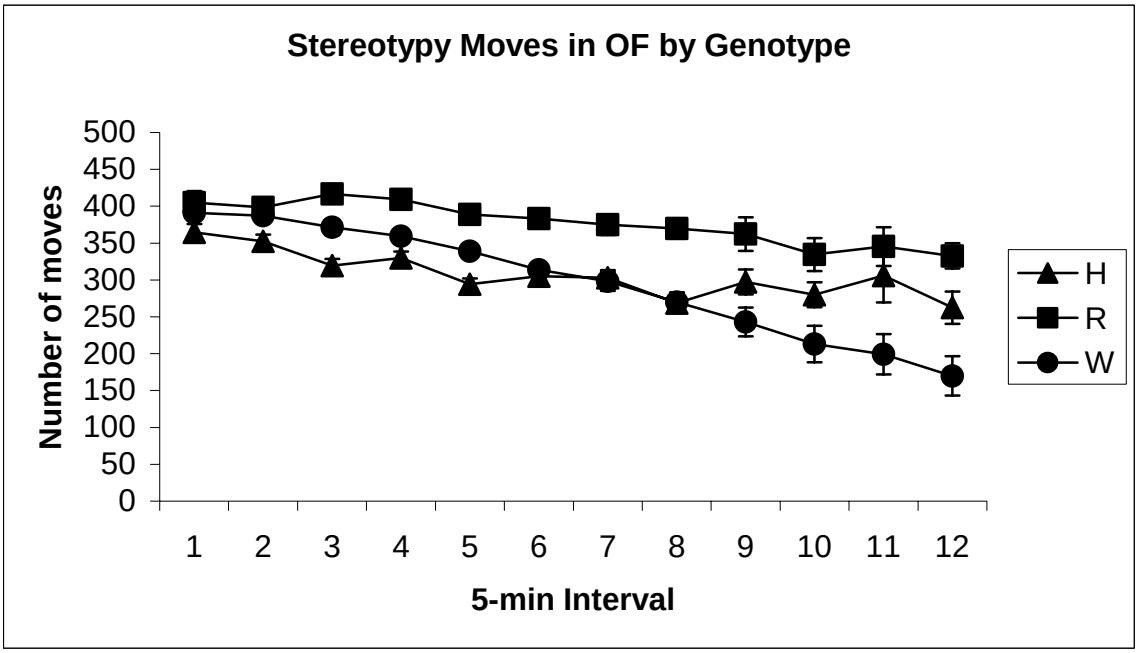

Figure 15. Stereotypy Moves in OF by Genotype. Mean number of repetitive, non-locomotor movements made by each genotype. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+.

The analyses did not show any other significant results: time x sex, $F(11, 32) = 0.7615$, $p= 0.6739$; time x sex x genotype, $F(22, 64) = 0.9566$, $p< 0.5273$; time x treatment condition, $F(11, 32) = 0.9170$, $p= 0.5362$; time x sex x treatment condition, $F(11, 32) = 1.3936$, $p= 0.2234$; time x genotype x treatment condition, $F(22, 64) = 1.1086$, $p< 0.3622$; and time x sex x genotype x treatment condition, $F(22, 64) = 0.8347$, $p< 0.6732$.

Polynomial extractions for stereotypic movements revealed a significant genotype x linear component, $F(2, 42) = 14.7056$, $p< 0.0001$. The polynomial extractions also

revealed a significant sex x genotype x linear component, $F(2, 42) = 3.3324$, $p= 0.0454$, in which the male and female *rl/rl* differed significantly from one another, $F(1, 42) = 5.0504$, $p= 0.0229$; female *rl/rl* mice were fairly stable in the number of stereotypic movements they made over the trial, while male *rl/rl* mice showed a sharp decline in the last half of the trial compared to the first half, when they performed similarly to the females. There was also a significant sex x genotype x cubic component, $F(2, 42) = 3.4860$, $p= 0.0397$, in which male and female +/*rl* mice differed significantly from one another, $F(1, 42) = 4.3613$, $p= 0.0429$; males and females showed a similar decreasing trend during the first two-thirds of the trial, and then males increased stereotypic movements relative to females. Additionally, there was a significant genotype x quadratic component, $F(2, 42) = 4.7030$, $p= 0.0144$, in which the +/*rl* significantly differed from both the *rl/rl* and the +/+, $F(1, 42) = 8.6726$, $p= 0.0052$. Finally, there was a significant sex x treatment condition x quadratic interaction, $F(1, 42) = 5.7392$, $p= 0.0211$, in which males and females in the vehicle condition differed from one another, $F(1, 42) = 4.3099$, $p= 0.0440$; males and females showed similar decreasing trends until the last 10 minutes of the trial, in which males continued to decrease but females increased (Figure 16). This difference was not found for the risperidone-treated animals (Figure 17).

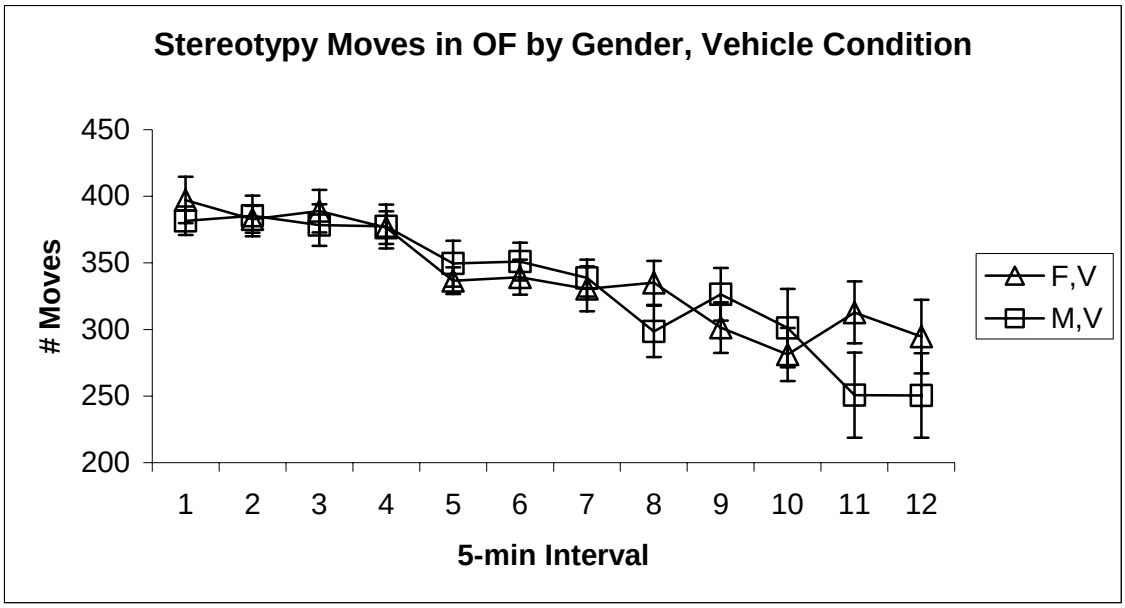

Figure 16. Stereotypy Moves in OF by Gender, Vehicle Condition. Mean number of stereotypic movements made by animals in the vehicle condition during the open field test. Females are represented by triangles and males by squares.

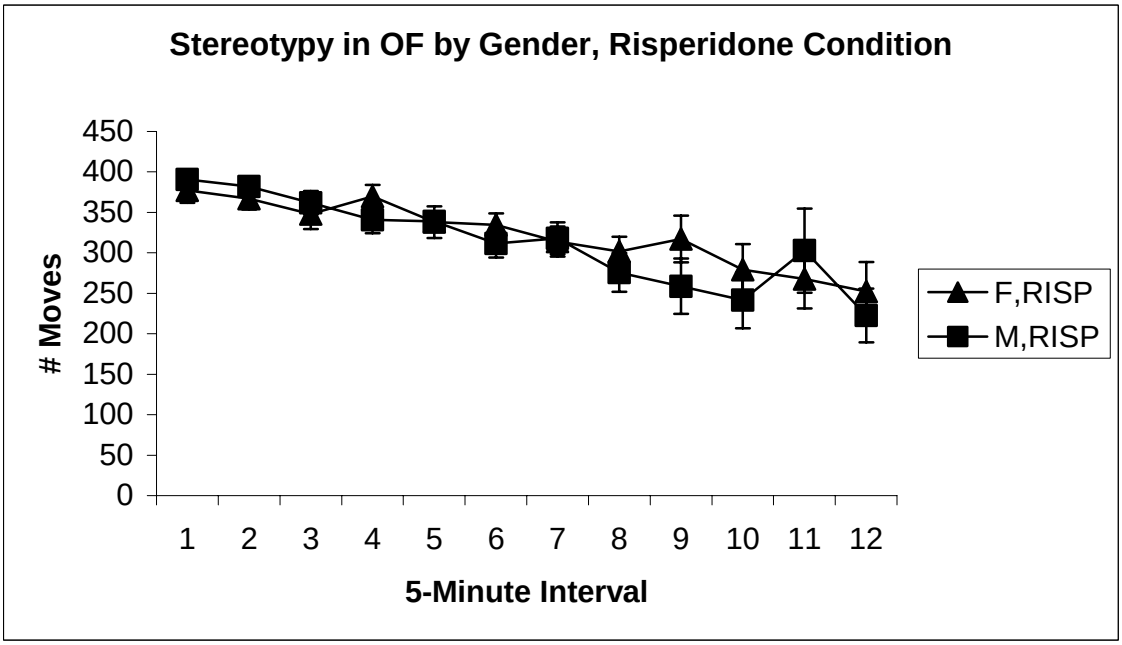

Figure 17. Stereotypy Moves in OF by Gender, Risperidone Condition. Mean number of stereotypic movements made by animals in the risperidone condition during the open field test. Females are represented by triangles and males are represented by squares.

Rearing (i.e., vertical plane entries) during the trial was measured as well. A three-way, between-subjects ANOVA of rearing activity was not significant, $F(11, 42) = 1.0800$, $p= 0.3997$. A repeated-measures, within-subjects ANOVA revealed a significant time x genotype interaction, $F(22, 64) = 1.9809$, $p< 0.0180$. Contrasts showed that the *rl/rl* differed significantly from the +/+ and +/*rl* by rearing more as the trial progressed instead of rearing less, as did the +/*rl* and +/+, $F(11, 32) = 3.1456$, $p= 0.0055$, while +/*rl* and +/+ did not significantly differ from one another, $F(11, 32) = 1.0797$, $p= 0.4070$ (Figure 18). There were no other significant results for the within-subjects analysis: Time x sex, $F(11, 32) = 0.6431$, $p= 0.7786$; time x sex x genotype, $F(22, 64) = 1.0902$, $p< 0.3804$; time x treatment condition, $F(11, 32) = 0.8250$, $p= 0.6167$; time x sex x treatment condition, $F(11, 32) = 0.9404$, $p= 0.5164$; time x genotype x treatment condition, $F(22, 64) = 0.8171$, $p< 0.6941$; time x sex x genotype x treatment condition, $F(22, 64) = 0.5911$, $p< 0.9147$.

Polynomial extractions revealed a significant genotype x linear component for rearing, $F(2, 42) = 16.1311$, $p< 0.0001$. A contrast showed that *rl/rl* mice significantly differed from both +/*rl* and +/+ mice, $F(1, 42) = 24.2057$, $p< 0.0001$. *Rl/rl* mice had more erratic behavior than the other two genotypes, but had fewer rearing incidents overall until the last third of the trial, when the +/+ decreased to less than the *rl/rl*. There was also a significant genotype x cubic component, $F(2, 42) = 3.3629$, $p= 0.0442$. A contrast showed that +/+ differed significantly from +/*rl* and *rl/rl*, $F(1, 42) = 6.6524$, $p= 0.0135$. Unlike +/*rl* and *rl/rl* mice, who remained somewhat stable across time in the

number of vertical entries made (although, again, *rl/rl* mice displayed a more complex pattern), +/+ mice showed a significant decreasing trend over the duration of the trial (Figure 18).

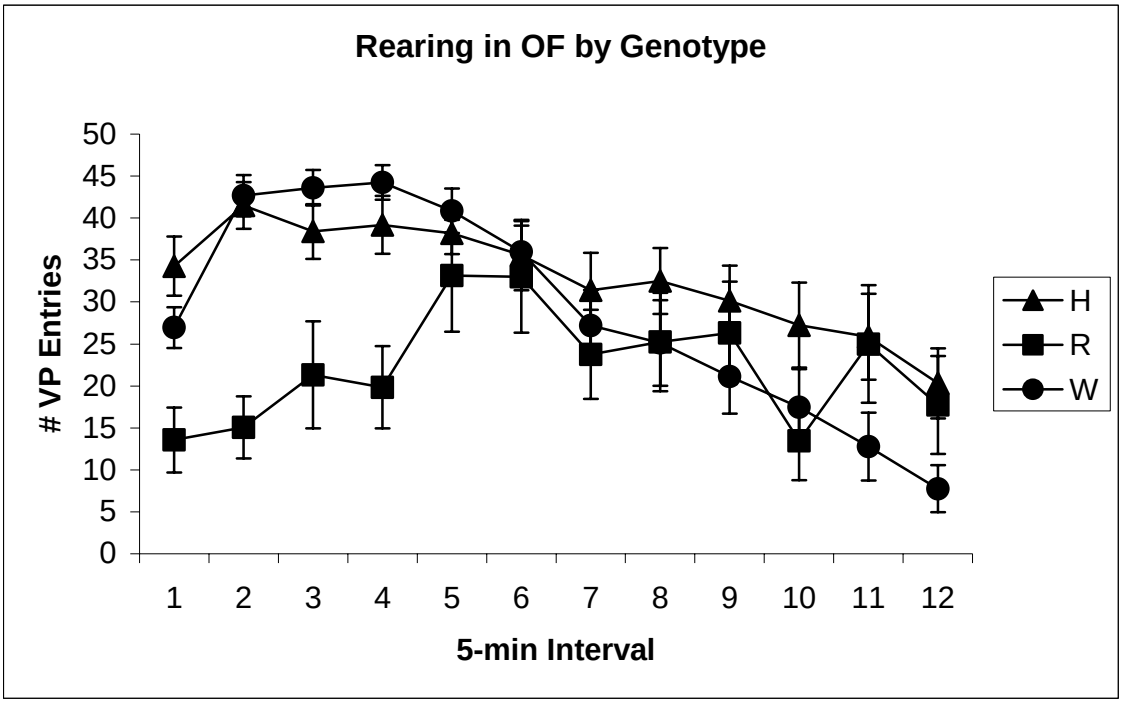

Figure 18. Rearing in OF by Genotype. Mean number of vertical plane entries (i.e., rearing) in the open field by genotype. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+.

Light-dark. The latency of each animal to make its initial crossing into the darkened half of the chamber was measured. A three-way ANOVA with sex, genotype, and treatment condition as between-subjects factors was significant overall, $F(11, 42) = 2.3977$, $p < 0.0206$. The ANOVA also revealed a main effect of genotype, $F(2, 42) = 12.0644$, $p < 0.0001$ (Figure 19). A least-squares means contrast revealed that this difference was due to the *rl/rl*, $F(1, 42) = 23.0149$, $p < 0.0001$, who showed a much longer latency to enter the dark than both the +/+ and +/*rl*. There were no other

significant main effects or interactions: sex, F(1, 42) = 0.0132, p= 0.8606; sex x genotype, F(2, 42) = 0.3004, p= 0.7421; treatment condition, F(1, 42) = 0.0155, p= 0.9015; sex x treatment condition, F(1, 42) = 0.0736, p= 0.7875; genotype x treatment condition, F(2, 42) = 0.1910, p= 0.8268; and sex x genotype x treatment condition, F(2, 42) = 0.6108, p= 0.5477.

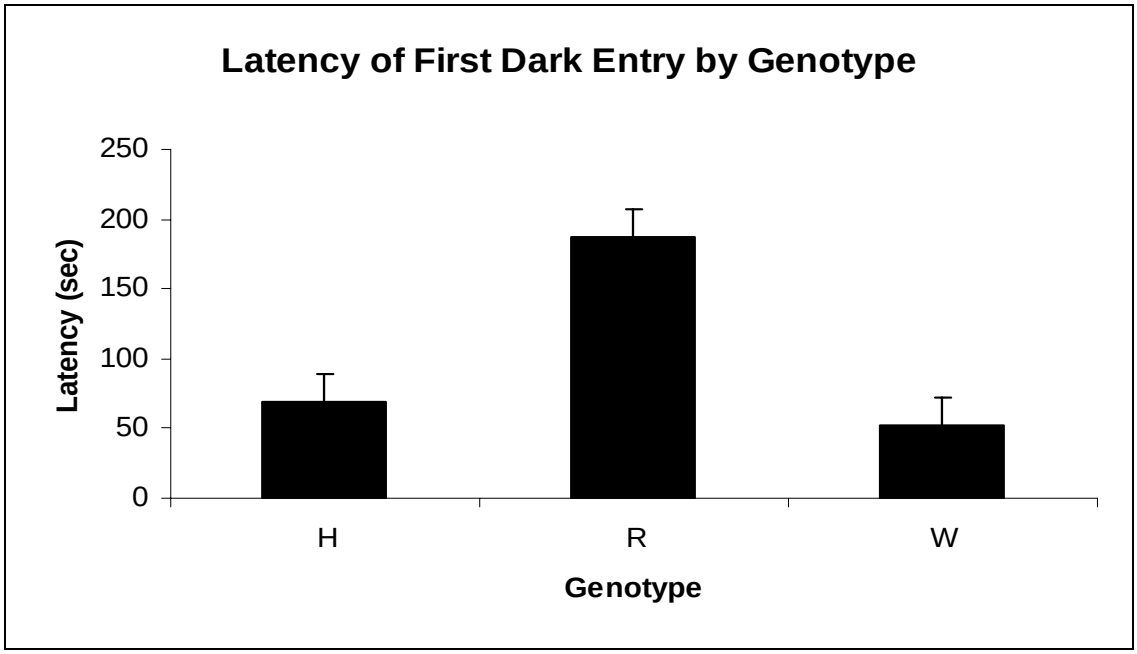

Figure 19. Latency of First Dark Entry by Genotype. Mean latency to first entry to darkened half of light-dark chamber by genotype. H=+/*rl*, R=*rl/rl*, and W=+/+.

A three-way ANOVA of the total number of transitions between the light and dark halves with sex, genotype, and treatment condition as between-subjects factors was also significant, F(11, 42) = 2.6796, p= 0.0106. There was again a main effect of genotype, F(2, 42) = 10.1286, p= 0.0003 (Figure 20). Least-squares means contrasts also revealed that this was due to the *rl/rl*, F(1, 42) = 20.2284, p< 0.0001, with *rl/rl* mice passing between the halves significantly less than the other two genotypes. There were no

other significant main effects or interactions: sex, $F(1, 42) = 1.1964$, $p= 0.2803$; sex x genotype, $F(2, 42) = 1.3327$, $p= 0.2747$; treatment condition, $F(1, 42) = 0.2691$, $p= 0.6066$; sex x treatment condition, $F(1, 42) = 0.0047$, $p= 0.9455$; genotype x treatment condition, $F(2, 42) = 1.3064$, $p= 0.2816$; and sex x genotype x treatment condition, $F(2, 42) = 1.5325$, $p= 0.2278$.

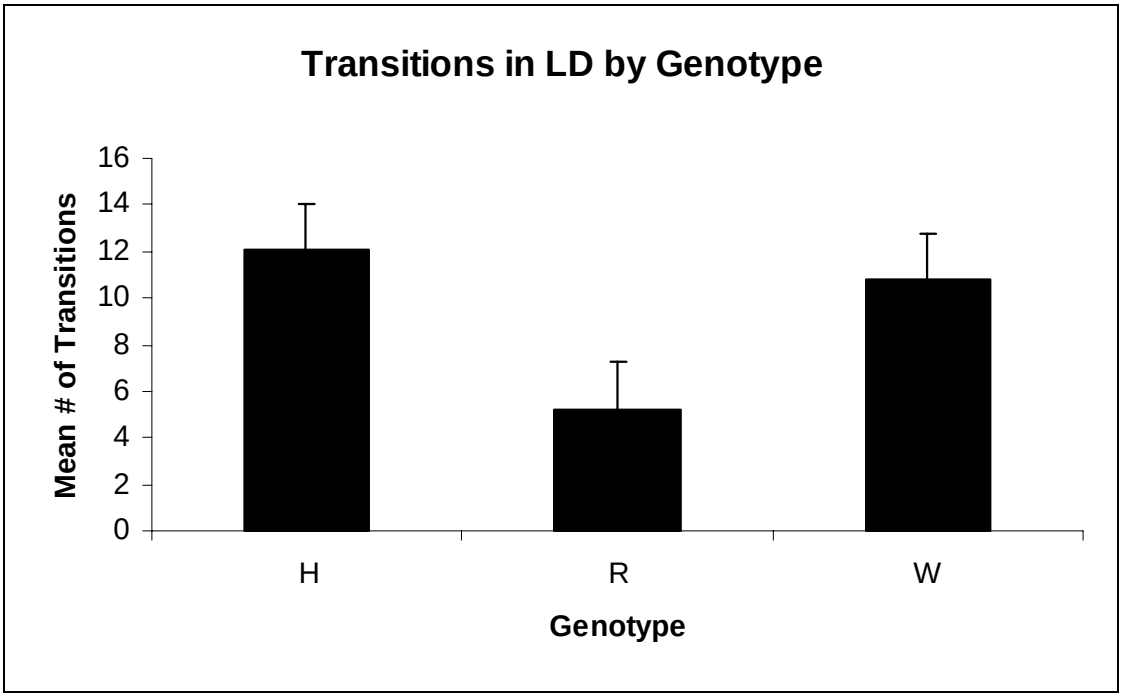

Figure 20. Transitions in LD by Genotype. Mean number of transitions between light and dark halves in light-dark test by genotype. H=+/*rl*, R=*rl/rl*, and W=+/+.

Test of Sensorimotor Gating

A repeated-measures, within-subjects ANOVA for percent reduction in startle response with sex, genotype, and treatment condition as between-subjects factors and prepulse intensity as the within-subjects factor, revealed a significant main effect of prepulse intensity, $F(2, 41) = 18.1440$, $p< 0.0001$, in which animals showed greater PPI

as the intensity of the prepulse increased. There was also a significant interaction of prepulse intensity and genotype, $F(4, 82) = 5.6546$, $p < 0.0005$. Contrasts showed that this result was due to the *rl/rl*, who failed to increase prepulse inhibition as prepulse intensity increased, compared to the +/+ and +/*rl*, $F(2, 41) = 12.6155$, $p < 0.0001$ (Figure 21).

There were no other significant main effects or interactions; prepulse intensity x sex, $F(2, 41) = 1.2042$, $p = 0.3103$; prepulse intensity x sex x genotype, $F(4, 82) = 0.5766$, $p < 0.6804$; prepulse intensity x treatment condition, $F(2, 41) = 0.1921$, $p = 0.8259$; prepulse intensity x sex x treatment condition, $F(2, 41) = 1.2783$, $p = 0.2894$; prepulse intensity x genotype x treatment condition, $F(4, 82) = 1.0659$, $p < 0.3787$; and prepulse intensity x sex x genotype x treatment condition, $F(4, 82) = 1.1366$, $p < 0.3451$.

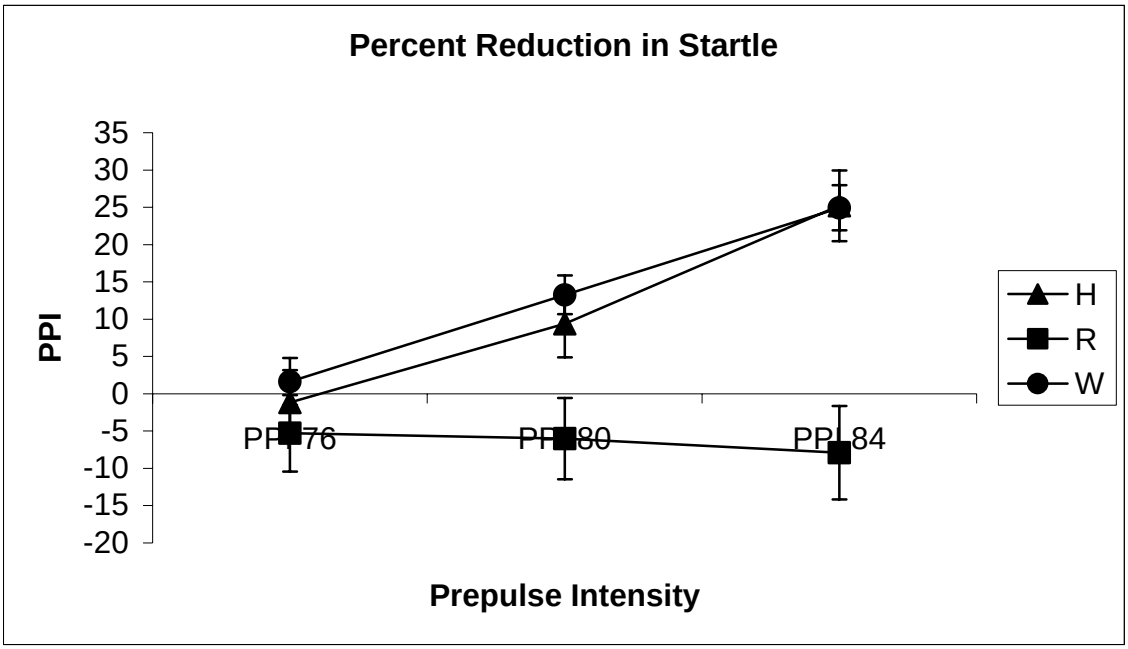

Figure 21. Percent Reduction in Startle. The mean percent reduction in startle response by genotype. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+.

Tests of Learning and Memory

Nose-poke. Reference memory ratios were determined each day during nose-poke testing, using the formula described above in the methods section. A between-subjects ANOVA was not significant, $F(11, 42) = 1.2337$, $p= 0.2959$. A repeated-measures, within-subjects ANOVA for reference memory with sex, genotype, and treatment condition as between-subjects factors and time as a within-subjects factor revealed no significant main effects or interactions; time x sex, $F(6, 37) = 0.6798$, $p= 0.6668$; time x genotype, $F(12, 74) = 1.1111$, $p< 0.3644$; time x sex x genotype, $F(12, 74) = 1.1259$, $p< 0.3530$; time x treatment condition, $F(6, 37) = 2.2352$, $p= 0.0612$; time x sex x treatment condition, $F(6, 37) = 2.2185$, $p= 0.0629$; time x genotype x treatment condition, $F(12, 74) = 0.5683$, $p< 0.8606$; and time x sex x genotype x treatment condition, $F(12, 74) = 1.0108$, $p< 0.4480$.

There was a significant main effect of time, $F(11, 42) = 3.9002$, $p= 0.0006$, and polynomial extractions revealed a significant treatment condition x quadratic interaction, $F(1, 42) = 8.8497$, $p= 0.0048$. Vehicle-treated animals had higher reference memory at the beginning of testing than did risperidone-treated animals, but then these ratios decreased for vehicle-treated animals while increasing for risperidone-treated animals, with risperidone-treated animals averaging slightly higher ratios until the end of testing, when both groups performed the same (Figure 22).

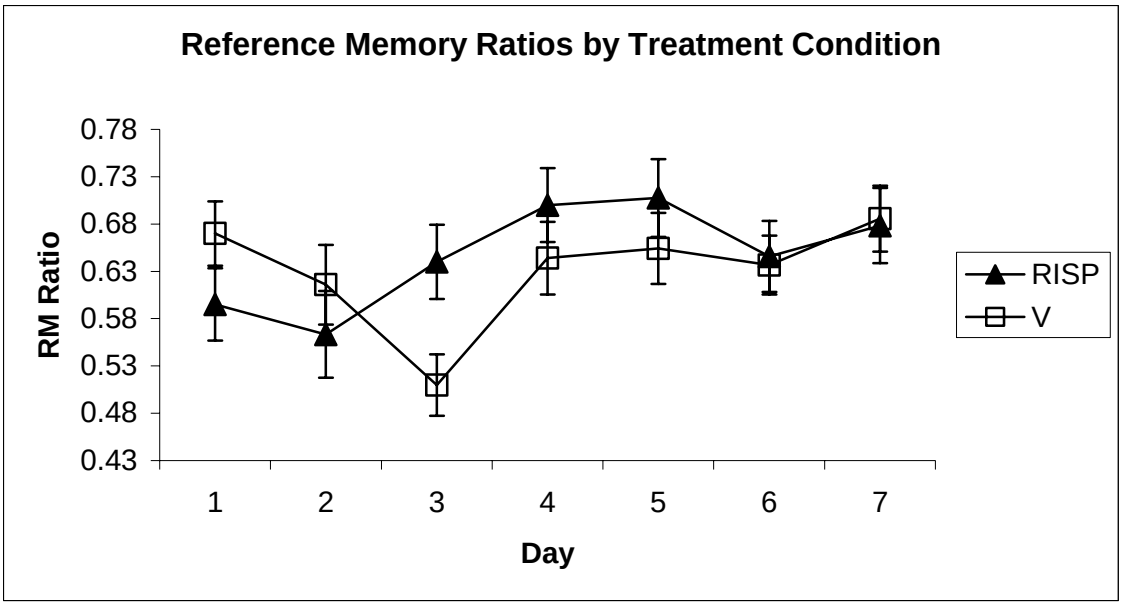

Figure 22. Reference Memory Ratios by Treatment Condition. Mean reference memory ratios in nose-poke testing over seven days by treatment condition. Closed triangles represent risperidone-treated mice and open squares represent vehicle-treated animals.

Working memory ratios were also determined each day for each animal, using the formula described above in the methods section. A between-subjects ANOVA was significant overall, $F(11, 42) = 2.7799$, $p= 0.0083$. There was a significant main effect of genotype, $F(2, 42) = 7.8605$, $p= 0.0013$ as well as a significant interaction of sex and genotype, $F(2, 42) = 3.4258$, $p= 0.0419$. Contrasts showed that male and female *rl/rl* mice were significantly different from one another, $F(1, 42) = 4.5312$, $p= 0.0392$. Across the seven-day testing period, there were times when there were not any differences in working memory scores for male and female *rl/rl*, but overall the male *rl/rl* had lower working memory ratios than did the female *rl/rl* (Figure 23). There were not any other significant main effects or interactions: sex, $F(1, 42) = 1.2124$, $p= 0.2771$; treatment condition, $F(1, 42) = 0.0893$, $p= 0.7665$; sex x treatment condition, $F(1, 42) = 0.2798$, $p=$

0.5996; genotype x treatment condition, F(2, 42) = 1.1517, p= 0.3259; and sex x genotype x treatment condition, F(2, 42) = 1.5125, p= 0.2321.

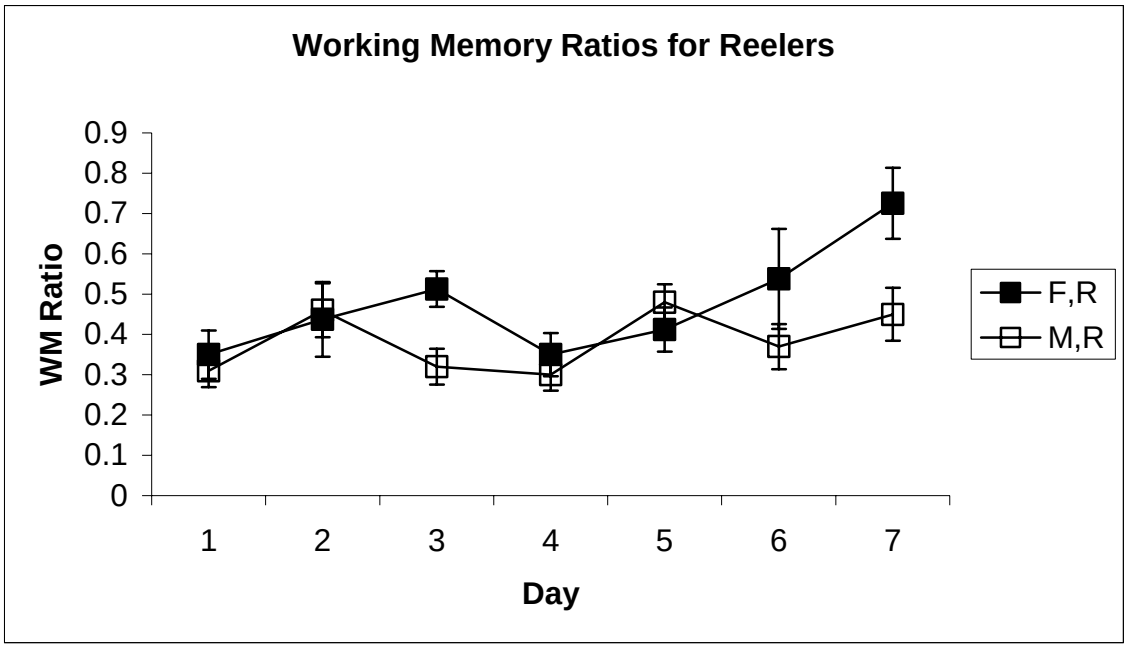

Figure 23. Working Memory Ratios for Reelers. Working memory ratios for male and female *rl/rl*. Closed squares represent female *rl/rl* and open squares represent male *rl/rl*.

A repeated-measures, within-subjects ANOVA revealed no significant effect of time x sex, F(6, 37) = 1.5223, p= 0.1980, or of time x genotype, F(12, 74) = 1.5034, p< 0.1422. There was a significant time x sex x genotype interaction, F(12, 74) = 2.0664, p< 0.0297. A contrast revealed that male +/*rl* mice were significantly different from female +/*rl* mice, F(6, 37) = 2.6964, p= 0.0284, in that the female +/*rl* had lower working memory ratios than the male +/*rl* on days 2 and 4 but then had a higher working memory ratio on day 6 (Figure 24). There were no other significant effects; for time x treatment condition, F(6, 37) = 0.5514, p= 0.7656; time x sex x treatment condition, F(6, 37) =

0.6839, p= 0.6636; time x genotype x treatment condition, F(12, 74) = 0.7479, p<0.7004; time x sex x genotype x treatment condition, F(12, 74) = 0.3347, p<0.9802.

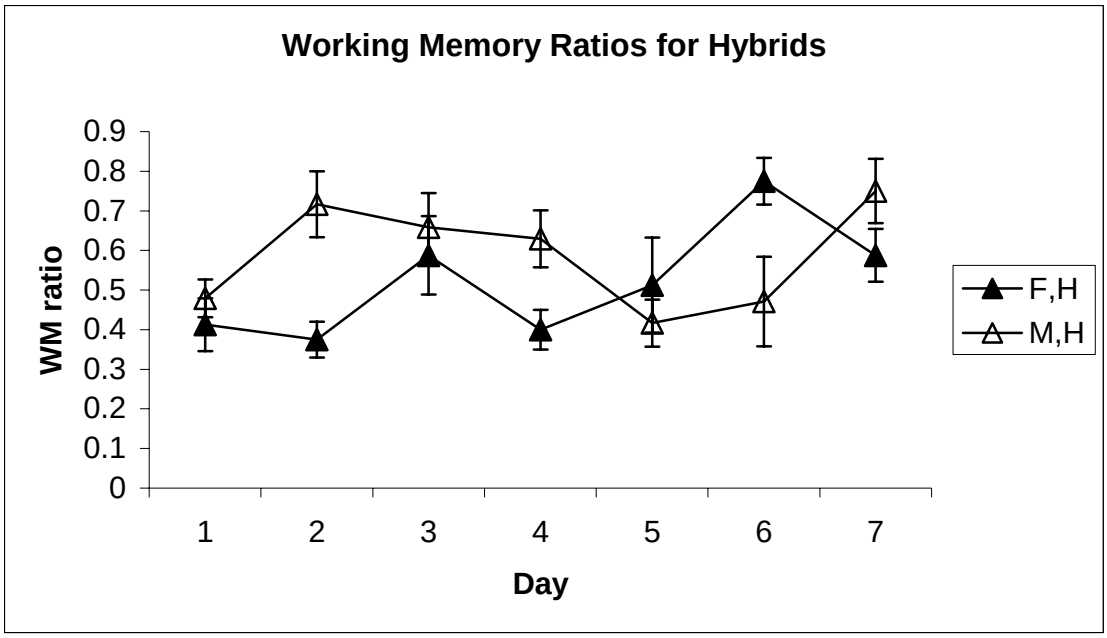

Figure 24. Working Memory Ratios for Hybrids. Working memory ratios for male and female +/*rl*. Closed triangles represent female +/*rl* and open triangles represent male +/*rl*.

The number of errors, defined as the total number of visits to never-baited holes during each trial, was also analyzed. A between-subjects ANOVA was significant overall, F(11, 42) = 2.1863, p= 0.0341, with a significant main effect of genotype, F(2, 42) = 3.4380, p= 0.0414, and a significant interaction of genotype and treatment condition, F(2, 42) = 3.2690, p= 0.0479. Contrasts showed that the *rl/rl* on risperidone made more errors for the first four days than all other genotype and treatment condition groups, F(1, 42) = 7.1557, p= 0.0106 (Figure 25). There were no other main effects or interactions in the between-subjects analysis: sex, F(1, 42) = 0.6584, p= 0.4217; sex x genotype, F(2, 42) = 1.0838, p= 0.3476; treatment condition, F(1, 42) = 0.0000, p=

0.9997; sex x treatment condition, F(1, 42) = 3.9393, p= 0.0537; and sex x genotype x treatment condition, F(2, 42) = 2.1744, p= 0.1263.

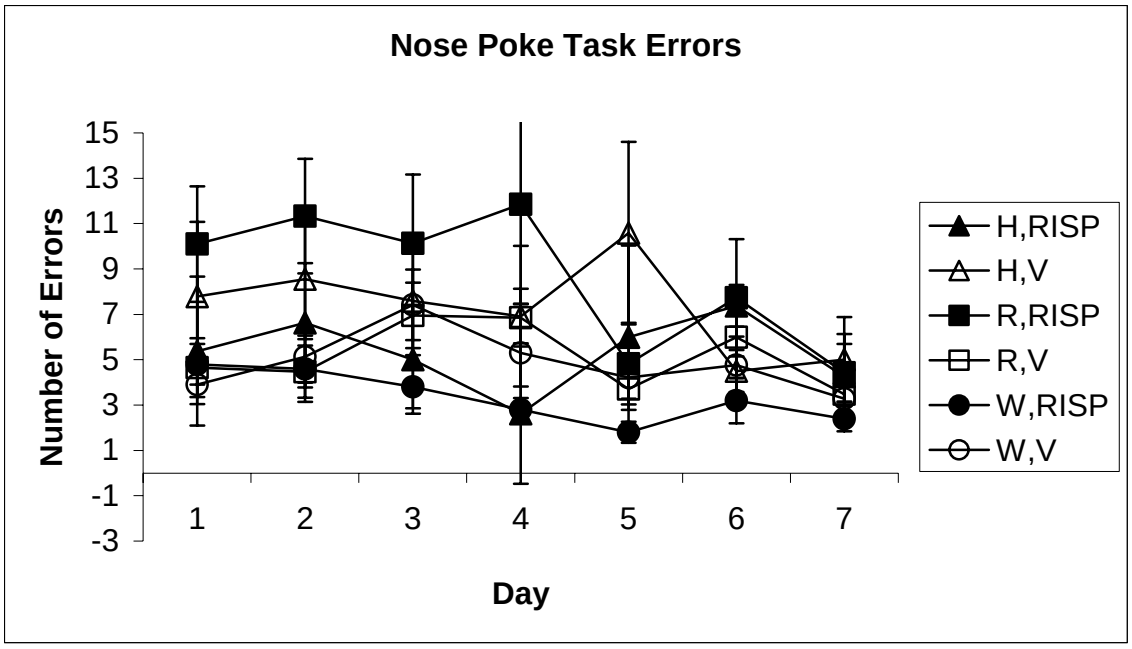

Figure 25. Nose Poke Task Errors. The number of errors made. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+. Closed figures represent risperidone-treated animals and open figures represent vehicle treated animals.

A repeated-measures, within-subjects ANOVA for task errors did not reveal any significant results; time x sex, F(6, 37) = 2.3235, p= 0.0528; time x genotype, F(12, 74) = 1.7020, p< 0.0835; time x sex x genotype, F(12, 74) = 1.0695, p< 0.3979; time x treatment condition, F(6, 37) = 0.6555, p= 0.6856; time x sex x treatment condition, F(6, 37) = 0.7682, p= 0.5997; time x genotype x treatment condition, F(12, 74) = 1.0940, p< 0.3779; and time x sex x genotype x treatment condition, F(12, 74) = 0.5388, p< 0.8823.

The number of times an animal went back to any previously visited hole, regardless of its status as a baited or non-baited hole, ("repeats") was measured and

analyzed. A between-subjects ANOVA was significant overall, $F(11, 42) = 3.4522$, $p= 0.0017$. There was a main effect of genotype, $F(2, 42) = 8.2638$, $p= 0.0009$, and a significant genotype x treatment condition interaction, $F(2, 42) = 3.8387$, $p= 0.0294$, which were part of the larger sex x genotype x treatment condition interaction, $F(2, 42) = 3.6364$, $p= 0.0350$ (interpreted below). There were no other significant main effects or interactions revealed by the between-subjects analysis: sex, $F(1, 42) = 0.0321$, $p= 0.8587$; sex x genotype, $F(2, 42) = 1.7950$, $p= 0.1786$; treatment condition, $F(1, 42) = 0.2075$, $p= 0.6511$; and sex x treatment condition, $F(1, 42) = 3.3875$, $p= 0.0728$.

In order to examine the three-way interaction above, a separate ANOVA was run for males and females with genotype and treatment as between-subjects variables. The ANOVA for males was not significant, $F(5, 21) = 2.5274$, $p= 0.0610$, but was significant for females, $F(5, 21) = 7.9562$, $p= 0.0002$. Genotype x treatment condition was also significant for females, $F(2, 21) = 13.6553$, $p= 0.0002$. A contrast showed that the female *rl/rl* on risperidone was significantly different from the female *rl/rl* treated with vehicle and from all female +/*rl* mice, $F(1, 21) = 9.9206$, $p= 0.0048$ (Figure 26). It is also obvious from plotting female *rl/rl* and female +/*rl* data separately that risperidone had opposite effects on these two genotypes; risperidone worsened female *rl/rl* performance (Figure 27) but improved performance for female +/*rl* (Figure 28).

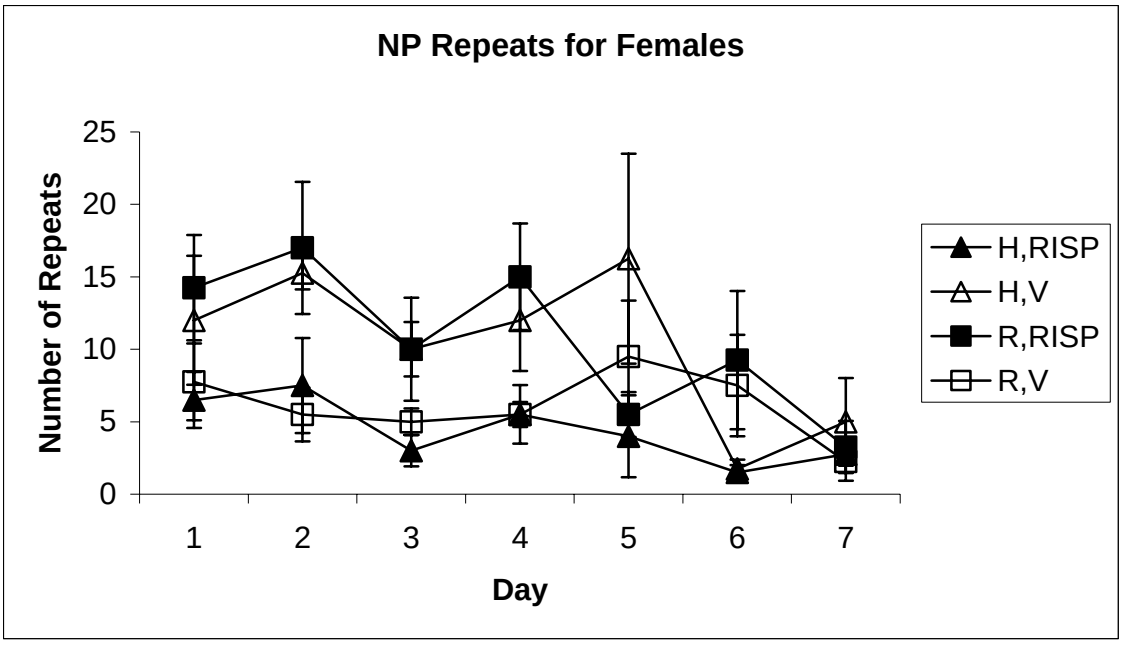

Figure 26. NP Repeats for Females. Mean numbers of repeats each day in the nose-poke test for female *rl/rl* and +/*rl*. Triangles represent +/*rl* and squares represent *rl/rl*. Closed figures represent animals treated with risperidone and open figures represent animals given vehicle.

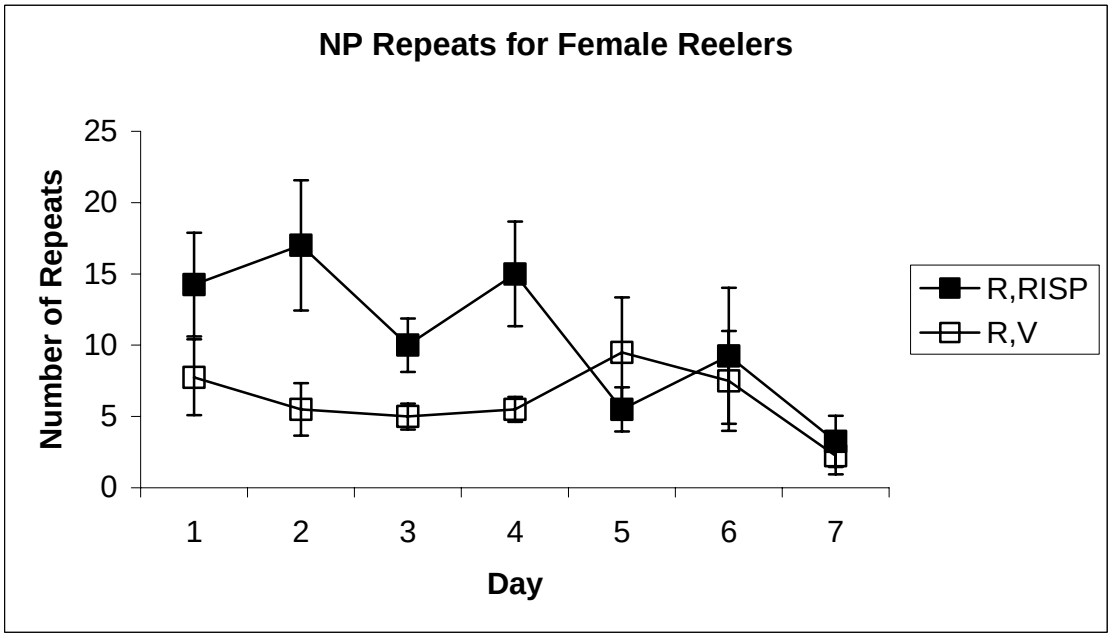

Figure 27. NP Repeats for Female Reelers. Mean number of repeats made each day by female *rl/rl*. Open squares represent female *rl/rl* given vehicle and closed squares represent female *rl/rl* given vehicle.

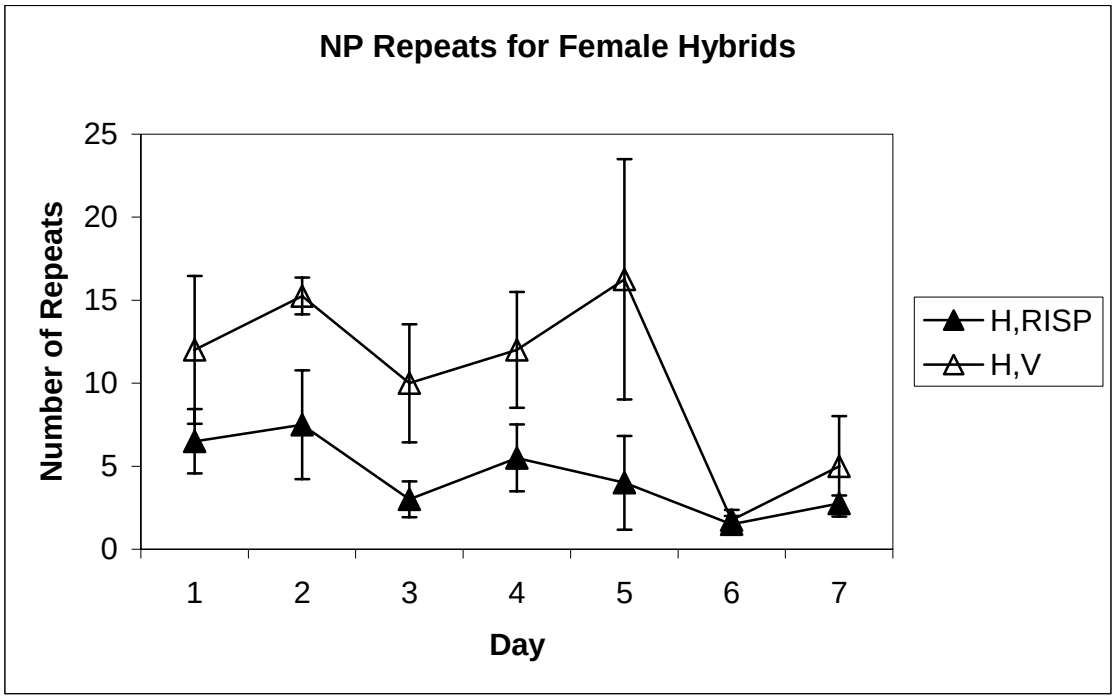

Figure 28. NP Repeats for Female Hybrids. Mean number of repeats made by female +/*rl* in nose poke each day. Closed triangles represent risperidone-treated +/*rl* and open triangles represent vehicle-treated +/*rl*.

A repeated-measures, within-subjects ANOVA for repeat entries revealed a significant time x sex interaction, $F(6, 37) = 4.3770$, $p= 0.0020$, in which males compared to females made fewer repeats on day 2 but more on day 6. There were no other significant results. For time x genotype, $F(12, 74) = 1.8610$, $p< 0.0536$; for time x sex x genotype, $F(12, 74) = 1.6899$, $p<0.0864$; for time x treatment condition, $F(6, 37) = 1.0778$, $p= 0.3934$; time x sex x treatment condition, $F(6, 37) = 0.7020$, $p= 0.6498$; time x genotype x treatment condition, $F(12, 74) = 1.3569$, $p< 0.2063$; and time x sex x genotype x treatment condition, $F(12, 74) = 0.6114$, $p< 0.8260$.

Because time was a significant factor for this analysis, however, $F(6, 37) = 10.1061$, $p< 0.0001$, polynomial extractions were run. There was a significant sex x linear

component, F(1, 42) = 7.4300, p= 0.0093 (similar to that reported above), and a significant genotype x treatment condition x linear component was also revealed, F(2, 42) = 4.2231, p= 0.0213. *Rl/rl* mice on risperidone were found to have made significantly more repeat entries compared to all other genotype x treatment condition groups during the first four days of testing before dropping to within the range of the other groups for the remaining days of the test (Figure 29), F(1, 42) = 6.7581, p= 0.0128. The significant genotype x treatment condition x cubic component verified these results, F(2, 42) = 4.4128; *rl/rl* mice on risperidone compared to all other groups, F(1, 42) = 0.0159.

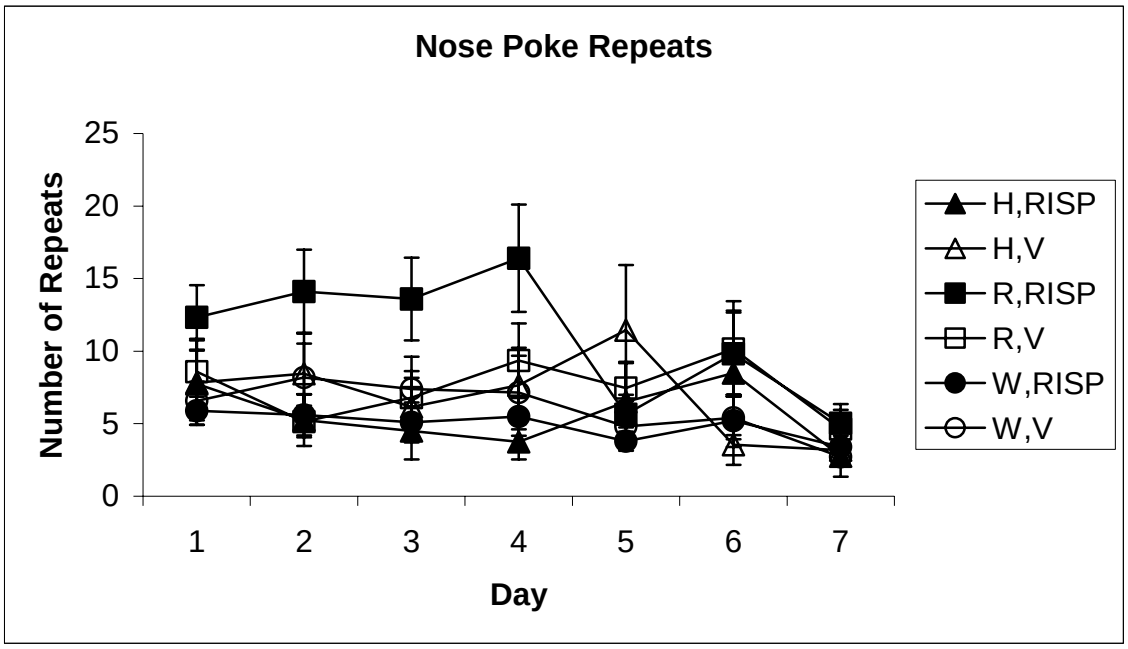

Figure 29. Nose Poke Repeats. Mean number of visits to previously visited holes in nose poke testing each day. Triangles represent +/*rl*, squares represent *rl/rl*, and circles represent +/+. Closed shapes represent risperidone-treated animals and open shapes represent vehicle-treated animals.

Passive avoidance. Latencies to enter the dark side after the opening of the door dividing it from the lighted start chamber were analyzed with a three-way repeated-

measures ANOVA with sex, genotype, and treatment condition as between-subjects variables and with day as the within-subjects variable. The between-subjects analysis was not significant, $F(11, 42) = 1.3205$, $p= 0.2473$. The within-subjects analysis returned a significant main effect of day, $F(1, 42) = 69.7560$, $p< 0.0001$, in which all animals significantly increased their latency to enter the right side on day 2 compared to day 1 (Figure 30).

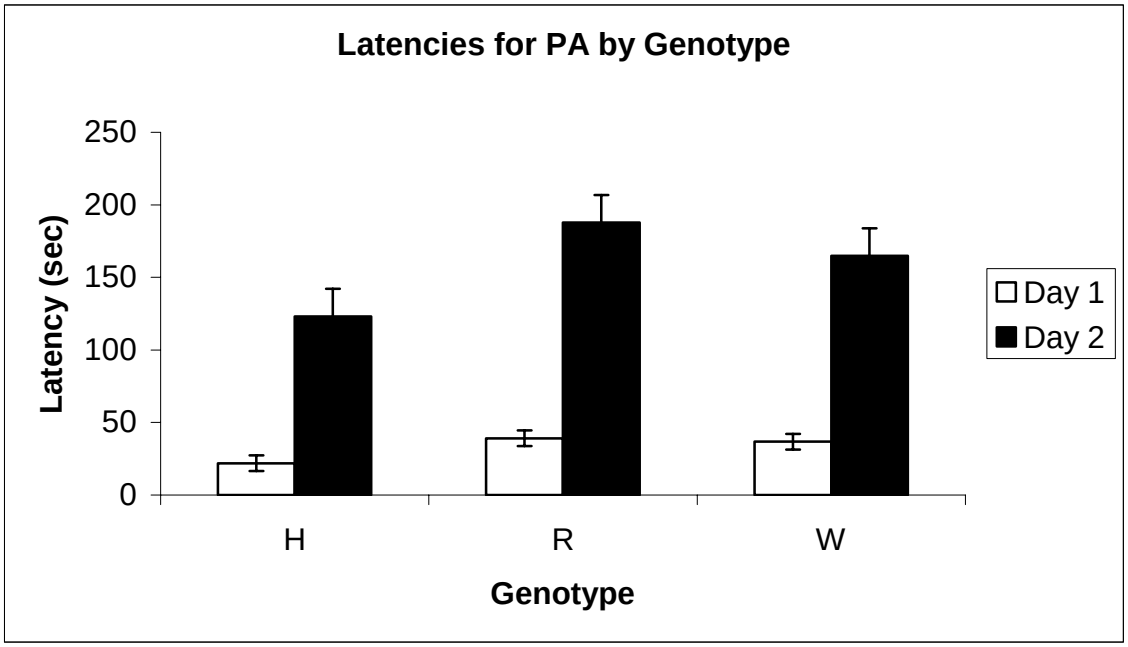

Figure 30. Latencies for PA by Genotype. Mean latencies in PA for days 1 and 2. H= +/*rl*, R= *rl/rl*, and W= +/+.

There were no other significant interactions: day x sex, $F(1, 42) = 0.6229$, $p= 0.4344$; day x genotype, $F(2, 42) = 1.0025$, $p= 0.3756$; day x sex x genotype, $F(2, 42) = 0.7463$, $p= 0.4803$; day x treatment condition, $F(1, 42) = 0.0025$, $p= 0.9600$; day x sex x treatment condition, $F(1, 42) = 0.1023$, $p= 0.7506$; day x genotype x treatment condition,

F(2, 42) = 0.3249, p= 0.7244; and day x sex x genotype x treatment condition, F(2, 42) = 0.9148, p= 0.4084. The percent change in latency was also figured for each animal using the following formula: (Latency day 2 - Latency day 1)/Latency day 1. The percent change in latency was analyzed with a three-way ANOVA, and it was non-significant overall, F(11, 42) = 0.4805, p= 0.9049.

Startle response habituation. Startle response habituation was also measured, using the maximum startle amplitude on each of the 10 startle-only trials in the PPI test. The mean startle amplitude was obtained for startle trials one through five and for startle trials six through 10, and the two blocks of trials were compared to determine if the animals were habituating to the startle stimulus. The two means were analyzed with a repeated-measures ANOVA with sex, genotype, and treatment condition as between-subject variables and with block of trials as a within-subjects variable.

The between-subjects ANOVA was not significant, F(11, 42) = 0.8682, p= 0.5768. The repeated-measures, within-subjects ANOVA also did not return any significant results: block x sex, F(1, 42) = 0.1176, p= 0.7333; block x genotype, F(2, 42) = 0.2099, p= 0.8115; block x sex x genotype, F(2, 42) = 0.0393, p= 0.9615; block x treatment condition, F(1, 42) = 0.1407, p= 0.7095; block x sex x treatment condition, F(1, 42) = 0.5323, p= 0.4697; block x genotype x treatment condition, F(2, 42) = 2.6145, p= 0.0851; and block x sex x genotype x treatment condition, F(2, 42) = 0.3713, p= 0.6921.

Piloting Alternative Passive Avoidance Methods

There were 26 animals that underwent the modified PA testing. Latency to the right side for each trial was analyzed with a three-way, repeated-measures ANOVA with

sex, genotype, and treatment condition as the between-subjects variable and with day as the within-subjects variable.

The between-subjects analysis was not significant, $F(11, 14) = 0.4740$, $p= 0.8905$, nor did the within-subjects analysis reveal any significant results: time x sex, $F(2, 13) = 0.3356$, $p= 0.7209$; time x genotype, $F(4, 26) = 1.7814$, $p< 0.1629$; time x sex x genotype, $F(4, 26) = 1.6717$, $p< 0.1867$; time x treatment condition, $F(2, 13) = 0.3974$, $p= 0.6799$; time x sex x treatment condition, $F(2, 13) = 1.3403$, $p= 0.2956$; time x genotype x treatment condition, $F(4, 26) = 0.2057$, $p< 0.9329$; and time x sex x genotype x treatment condition, $F(4, 26) = 0.5942$, $p< 0.6700$. However, it appears that significant genotype differences may have been revealed with a larger sample size (Figure 31).

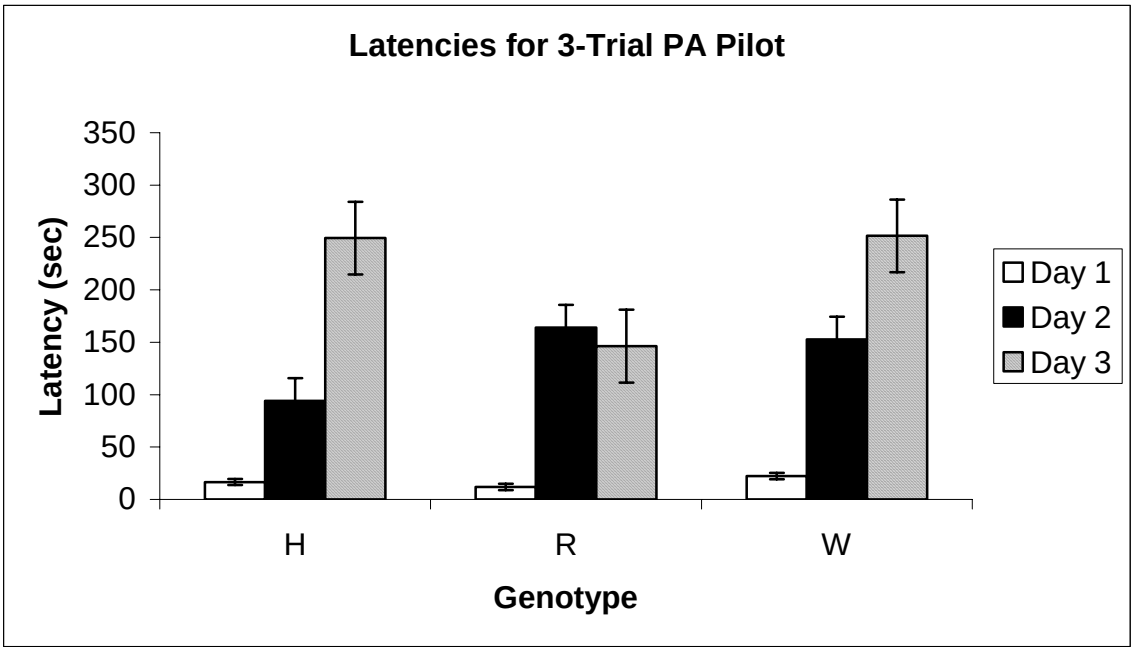

Figure 31. Latencies for 3-Trial PA Pilot. Mean latencies for three trials of passive avoidance by genotype. H= +/*rl*, R= *rl/rl*, and W= +/+.

CHAPTER IV

DISCUSSION

The purpose of this research was to test the hypothesis that the *rl/rl* mutant mouse is an appropriate animal model of schizophrenia, based on its deficits in sensorimotor gating, cognitive functioning, and emotionality (Salinger et al., 2003). It was predicted that if the *rl/rl* were an appropriate model of schizophrenia, then the atypical antipsychotic, risperidone, should reduce those deficits, because the drug has been shown to be effective in treating those symptoms in humans with schizophrenia (Kumari et al., 2002; Oranje et al., 2002; Duncan et al., 2003; Bilder et al., 2002; Marder & Meibach, 1994). This prediction was tested by administering the drug to the *rl/rl* and to two other genotypes, the +/+ (control) and the +/*rl*, with another group of these animals receiving the vehicle, tap water, and evaluating the performance of these groups on assays of cognitive functioning, emotionality, and sensorimotor gating.

The hypotheses tested in this experiment were based on the assumption that the animals receiving vehicle instead of the drug would show genotype differences consistent with those differences found in the previous research by Salinger et al (2003). It is possible, however, that the daily gavage procedures used to administer vehicle and risperidone doses, or some other unintended difference in experimental conditions, might have made data from the current experiment incommensurate with those from Salinger et al (2003). If those expected genotype differences were not found in this research for

animals in the vehicle condition, then these results could not be interpreted under the hypothesis that reelers are models of schizophrenia, because the risperidone would have been acting on animals displaying a modified phenotype. Therefore, genotype differences among animals in the vehicle condition had to be compared to previous research before interpretation of any possible risperidone effects could begin.

Moreover, there was one test (passive avoidance) used in the present experiment that was not used in Salinger et al. (2003), and two tests that used somewhat different methods (nose-poke) or parameters (open field). Because it was unknown how the different genotypes would typically respond on the PA test and under the current NP methods, the vehicle analysis was also important in determining whether or not these methods were adequate to test the hypotheses. If predicted genotype differences did not emerge in the vehicle analysis for these tests, then the tests would not be useful for confirming or denying the prediction that risperidone would improve deficits in the *rl/rl*.

Analysis of Vehicle-Treated Animals

Main effects of genotype from the current vehicle-only analysis are summarized in Table 4, as well as the genotype results reported in Salinger et al. (2003). As can be seen, in most respects the gavage procedures did not interfere with the replication of findings from the three tests for which the current methods were the same as those used in the earlier study, suggesting that the methods employed for these tests are adequate for testing the current hypothesis that performance deficits in *rl/rl* mice should be diminished by risperidone treatment. The present vehicle analysis also confirms the observations by

Salinger et al. (2003), Groves et al. (2003), and Podhorna and Didriksen (2004) that the +/*rl* mice show no systematic behavioral differences from the +/+ mice.

Table 4
Hybrids and Reelers Compared to Wildtypes in Salinger et al., 2003, and in the Current Study (Vehicle Animals Only)

		+/rl		*rl/rl*	
Test	Measure	2003	Current	2003	Current
OF	5-min rest time	↔	↔	↓	↓*
	60-min rest time	↔	↔	↓	↓
	Boli	↔	↔	↓	↑
	Margin Time	↔	↔	↓	↓
	Stereotypy Moves	↔	↔	↑	↑
	Rearing	↔	↑	↓	↓
LD	Latency to Dark	↔	↔	↑	↑*
	Transitions	↔	↔	↓	↓*
NP	Reference Memory	↔	↔	↓	↔
	Working Memory	↔	↔	↓	↔
	Errors†	NA	↔	NA	↔
	Repeats†	NA	↔	NA	↔
PPI	% Startle Reduction	↔	↔	↓	↓
	Startle habituation	↔	↔	↓	↔
PA	Change in latency	NA	↔	NA	↔

Note: Horizontal arrows (↔) indicate values that are not significantly different from +/+ animals. Up arrows (↑) indicate values that are significantly greater than the values for +/+s. Down arrows (↓) indicate values that are significantly less than the values for +/+s. Significance was set at $p < 0.05$. NA indicates that either the measure was not reported or was not analyzed in the 2003 study.
*The results for genotype were significant, but the overall ANOVA was not.
†There was no main effect of genotype in the current study, although there was a sex x genotype interaction.

The open field test used in the current experiment was only slightly modified from Salinger et al's previous (2003) research. In this study, the upper sensor beam, used to detect and measure rearing, was lowered by one centimeter to account for *rl/rl* mice's ataxia and smaller body size, because visual observations of *rl/rl* mice in the open field

suggested that their body height when rearing was not adequate to break the sensor beam when it was positioned at seven centimeters above the ground. Though *rl/rl* mice showed significantly less rearing compared to +/+ mice both in the present study and in Salinger et al. (2003), visually comparing the rearing curves reported in Salinger et al. (2003) to the present figures for rearing suggests that the lower placement of the upper sensor beam in the current study did detect more rearing among *rl/rl* mice than was found in the earlier study, especially in the latter portion of the 60-minute trial. Conversely, although +/*rl* mice reared more than +/+ mice in the present study, comparing the two curves for rearing from Salinger et al. (2003) and the present study suggest that this difference was due to noise in the present study, because the two curves are very similar between studies.

The nose-poke task was simplified in the current experiment compared to that used previously. In the 2003 study, spatial working memory was initially assessed with all 16 holes baited, and mice were tested in the 4-hole task only after they had already learned and been tested in the 16-hole task. In those experiments, when transferred from the 16-hole to the 4-hole version of the task, *rl/rl* mice initially showed inferior reference memory ratios compared to +/*rl* and +/+ mice, but after four days on the 4-hole version of the task, there were no genotype differences found for reference memory. The 4-hole version of the nose-poke task was used here because it was believed that genotype differences would still be found but would require fewer days of testing and fewer materials. However, for animals in the vehicle condition of the present research, not only were there no genotype differences in reference memory, but there were also no genotype

differences found in any of the other nose-poke measures.

The discrepancy between the 2003 results for the nose-poke task, in which *rl/rl* mice showed deficits compared to other mice, and the current results, in which they did not, may be due to the fact that animals had to "unlearn" a harder, 16-hole nose-poke task before learning the simpler 4-hole version in the 2003 study, whereas animals in the current study did not have to do so. Thus, the more complex sequence used in the 2003 task may have revealed genotype differences that are not seen in simpler tasks. In any case, the current nose-poke procedures failed to distinguish between the genotypes in the vehicle condition and hence the current nose-poke results render uninterpretable the results of nose-poke experiments during risperidone treatment.

Passive avoidance was used in the current study but not in Salinger et al. (2003). Because this was the first experiment to our knowledge to test *rl/rl* mice in passive avoidance, it was unknown whether they would behave differently from other mice. It was predicted, based on Salinger et al.'s (2003) previous research in which *rl/rl* mice displayed behaviors that could be interpreted as stemming from deficient response inhibition, that *rl/rl* mice would perform more poorly on passive avoidance (i.e., show a smaller difference between days 1 and 2 in latency to enter the right half of the chamber) than the other genotypes. However, the analyses of PA data under vehicle treatment did not show any genotype differences in passive avoidance.

It is possible that vehicle-treated *rl/rl* mice do not display deficiencies in response inhibition compared to vehicle-treated +/+ mice after a single training session because more PA training was required than was provided in these experiments. The pilot data

from experiments in which PA training and testing extended to three trials suggest that genotype differences may emerge with the addition of a second training session.

A review of the literature showed that, although the set-up of the test chamber may differ from experiment to experiment (e.g., a step-down versus a step-through test), and although the duration and intensity of the footshock may vary, the method of PA used here is a method commonly used by other researchers (i.e., having one training trial separated by 24 hours from the test trial) (Lukawski, Nieradko, & Sielklucka-Dziuba, 2005; Taniguchi, Doe, Matsuyama, Kitamura, et al., 2005; Rasmussen, Fink-Jensen, Sauerberg, Swedberg, et al., 2001; Picciotto, Zoli, Léna, Bessis, et al., 1995; Ciamei, Aversano, Cestari, & Castellano, 2001). However, there are also researchers who train animals until they learn not to cross over into the dark half before testing the learned association (Acevedo, Pfankuch, Ohtsu, & Raber, 2006; Shimamura, Sato, Waguri, Uchiyama, et al., 2006), and so the use of more training trials would not be inconsistent with other methods used in the literature.

Alternatively, it is possible that vehicle-treated mice do not display deficiencies in PA because the intensity of the footshock used in this experiment created such a strong passive avoidance response that it obscured possible differences between the genotypes in their ability to withhold responding. This is not to say that *rl/rl* mice do not have deficits in response inhibition, but rather to suggest that the parameters used in the current PA test, while effective in producing PA, may not have been effective for detecting genotype differences among vehicle-treated animals.

Overall, there are three conclusions that can be drawn from the analysis of vehicle-treated animals in this experiment. First, genotype differences emerged from OF, LD, and PPI testing that are consistent with previous findings by Salinger et al. (2003), Groves et al. (2003), and Podhorna and Didriksen (2004). Second, *rl/rl* mice displayed abnormal behaviors on those tests, which make it reasonable to hypothesize that they model a human psychopathology such as schizophrenia. Third, current data from NP and PA cannot be used to test the hypothesis that *rl/rl* mice are models of schizophrenia.

Treatment Validation

It was also necessary to determine whether risperidone treatment in the present experiment was effective enough to test the current hypothesis that the behavioral abnormalities of the *rl/rl* would be improved with the administration of risperidone. At a minimum, this means that there should be significant effects of risperidone treatment on the behavioral phenotypes of the mice in the present experiments. These effects should be evident in a number of behavioral assays and they should be systematic and mutually consistent. Table 5 shows a summary of the measures for which there was either a main effect of treatment condition or for which treatment condition was part of a significant interaction. As can be seen, there were few measures for which there was a significant effect of treatment, either alone or in an interaction; in fact, risperidone had a significant effect on fewer than one-third of the measures reported here.

If the dose of risperidone used in this test was adequate, then multiple performance measures in the domains of cognition, emotionality, and sensorimotor gating should have been significantly affected by risperidone administration. However, only

measures related to cognition (habituation of stereotypic movements in OF; task errors, repeats, and reference memory in NP) were affected by the drug in this experiment.

Table 5
Summary of Treatment Condition Results

Test	Measure	Effect
Open Field	Stereotypy Moves	Risperidone eliminated existing sex differences
Nose Poke	Reference Memory	Some improvement overall with risperidone
Nose Poke	Errors	*rl/rl* on risperidone made more errors
Nose Poke	Repeats	*rl/rl* on risperidone made more repeats; female *rl/rl* on risperidone made more repeats than female *rl/rl* on vehicle; female +/*rl* on risperidone made fewer repeats than female +/*rl* on vehicle

The small number drug treatment effects seen here could have been Type I errors. If these risperidone effects were not merely Type I errors, then the few significant effects ought at least to be consistent across measures. Thus, the critical question regarding the adequacy of the risperidone dosage becomes: Are the effects of risperidone on habituation and in the nose-poke task mutually consistent? In other words, when genotype was affected by the drug, was the same genotype always affected, and did the drug consistently improve or worsen performance of that genotype? Similarly, when sex was differentially affected by the drug, was it always the same sex, and were the effects of the drug on that sex consistent across measures?

The limitations of the current nose-poke test for testing this experiment's overall hypothesis that *rl/rl* are models of schizophrenia and that risperidone would improve their

deficits have been discussed previously, but those data can still be useful for determining the consistency of drug effects, especially since most of the significant drug effects for this experiment appeared in this test. There were three NP measures for which there was a significant effect of risperidone: reference memory, errors, and repeats.

Risperidone appears to have had a mildly positive effect on reference memory overall. Although animals treated with both risperidone and vehicle showed improvements in reference memory over time with both groups performing the same at the end of testing, risperidone caused those improvements to begin earlier in training. For nose-poke errors, however, *rl/rl* mice treated with risperidone made more errors for the first four days of testing than did all other animals. This effect is consistent with the effect of risperidone on *rl/rl* mice for task repeats; *rl/rl* mice, especially females, treated with risperidone made more repeat entries on the first four days of testing than did all other animals. Conversely, female +/*rl* mice treated with risperidone made fewer repeat entries than did female +/*rl* mice treated with vehicle.

Risperidone eliminated existing sex differences for habituation of stereotypies in the open field. Throughout the hour-long trial, males and females in both treatment conditions showed a decline in the number of stereotypic movements made over time; in other words, they both showed habituation to the open field environment. However, in the vehicle condition, females showed a relative increase in the number of stereotypies made in the last part of the trial (i.e., they showed relatively decreased habituation). In the risperidone condition, females did not show this decrease in habituation; they continued showing a decline in stereotypies, consistent with the males. For this measure, then,

risperidone increased late-stage habituation for females; in other words, it subtly improved this measure of primitive learning for female animals.

The question posed above was, are the effects of risperidone on habituation in the open field and in the nose-poke task consistent? That is, when genotype was affected by the drug, was the same genotype always affected, and did the drug consistently improve or worsen performance of that genotype? Both *rl/rl* and +/*rl* mice were affected by the drug in some measures. When +/*rl* mice were affected by risperidone, their behaviors improved. When *rl/rl* mice were affected by the drug, their behaviors worsened. The second element of the above question was, when a sex was differentially affected by the drug, was it always the same sex, and were the effects of the drug on that sex consistent across measures? Females appeared to be more sensitive to effects of risperidone than were males. Hence, when risperidone affected the sexes differentially, it was always the females who were affected. Whether the females' behaviors improved or worsened depended on their genotype, consistent with the genotype-specific effects of the drug. When female +/*rl* mice were affected, their behaviors improved, but when female *rl/rl* mice were affected, their behaviors worsened. Thus, it would appear that the significant treatment results, though few in number, were at least mutually consistent, and so it seems likely that they were not the result of a Type I error but represented a risperidone treatment effect instead.

A review of the literature supports the interpretation that these few significant treatment results represent a true finding and not a Type I error. In most studies, 1.0 mg/kg was the highest dosage of risperidone used, and was effective at altering various

behaviors, such as freezing in contextual fear conditioning (Miyamoto, Tsuji, Takeda, Ohzeki, et al., 2004); vacuous chewing (Carvalho, Silva, Abilio, Barbosa, et al., 2003); PPI (Olivier, Leahy, Mullen, Paylor, et al., 2001); and marble burying in anxiety studies (Li, Morrow, & Witkin, 2006; Matsushita, Egashira, Harada, Okuno, et al., 2005). In the study by Carvalho et al. (2003), even doses as low as 0.1 mg/kg affected vacuous chewing movements. In the other studies listed above, no dose higher than 1.0 mg/kg was used and yet all detected effects of risperidone treatment on behaviors of interest here. Thus, from the literature it seems that the dose used in the current study should have been adequate to affect a range of behavioral characteristics in the cognitive, emotional, and sensorimotor gating domains and to do so in a systematic, consistent fashion. Hence, the literature suggests that the dose of risperidone used here is the maximal dose that would be experimentally appropriate. Moreover, in this literature, behaviors other than those reported here are sensitive to the dose of risperidone used here; in the case of some behaviors, doses only 10% of those used here were effective at altering behavior. This is consistent with the view that the sparse behavioral effects of risperidone treatment reported here are not the result of type I errors occurring in a background of insufficient risperidone dosing. At the same time the literature indicates that the dose of risperidone used here should not only be effective but also should have produced a wider array of behavioral effects than those observed in the present study. Perhaps, however, the strain used here (C6B3), which a search of the literature indicates has never been tested with risperidone, is less sensitive to risperidone or metabolizes it more rapidly than did the mice used in the studies cited. However, the studies by Costa and colleagues (Costa et al.,

2002; Tremolizzo et al., 2002; Carboni et al., 2004) examined drug effects on +/*rl* mice from the same background strain as those used here and found significant effects of the drugs. If those results are true, then it suggests that +/*rl* mice, at least, are not hyporesponsive to drugs, and so, unless a complete lack of reelin reduces drug efficacy, *rl/rl* mice also should not be hyporesponsive. Thus, it does seem that the sparse drug effects are a true finding and not a Type I error.

Implications and Directions for Future Research

So what do these results suggest? First, the genotype differences found in Salinger et al. (2003) were supported, both for the *rl/rl* and for the +/*rl*, and supported the findings by Groves et al. (2003), Salinger et al. (2003), and Podhorna and Didriksen (2004) that the +/*rl* is behaviorally indistinguishable from the +/+. Second, because risperidone produced very few effects on the *rl/rl*, and because the few existing effects worsened the *rl/rl*'s performance instead of improving it as predicted, the conclusion to be drawn from this experiment is that *rl/rl* mice do not model schizophrenia. However, it is possible that *rl/rl* mice model another human disorder, such as one that is not consistently responsive to risperidone. The behavioral abnormalities found in *rl/rl* mice, including deficits in PPI, cognition, and emotionality, are not unique to schizophrenia and are found in other pathologies as well, especially those related to executive functioning.

The *rl/rl* mice display hyperactivity in the open field as well as other behaviors that could be explained as executive functioning deficits, such as impulsivity (for example, their apparent inability to cease movement toward a more dominant mouse in the social dominance task and their faster approach to novel objects than other genotypes,

reported in Salinger et al., 2003). Therefore, one disorder that *rl/rl* mice could model is Attention Deficit/Hyperactivity Disorder (AD/HD), as hyperactivity and impulsivity are two of the core symptoms for a diagnosis of AD/HD (*DSM-IV-TR*). The hypothesis that *rl/rl* mice may model AD/HD has been tested in our laboratory by using four different doses of methylphenidate on *rl/rl*, +/+, and +/*rl* mice, and the data from that experiment are currently being analyzed and interpreted. To date, it is unknown whether these results support or refute this hypothesis.

Another possible disorder that the *rl/rl* could model is autism. Some of the symptoms of autism include impairment in social interaction, impairments in communication, and the presence of repetitive, stereotyped behavior (*DSM-IV-TR*). Interestingly, autism was at one time often confused with childhood-onset schizophrenia (Asarnow & Asarnow, 2003), and also shares many overlapping symptoms with AD/HD; in fact, there commonly is such a high frequency of overlap between symptomatology in AD/HD and autism that clinicians are directed to generate a primary diagnosis of autism rather than of AD/HD or of autism with comorbid AD/HD when symptoms consistent with both are present (King & Bostic, 2006). Recently, a study has suggested that sensorimotor gating deficiencies may be present in adults with autism as well (Perry, Minassian, Lopez, Maron, & Lincoln, 2006), although there is very little published literature on the subject. Thus, many of the behavioral abnormalities of the *rl/rl* that have been hypothesized to model schizophrenia and/or AD/HD might actually model autism instead, since deficient executive function, emotionality, cognitive dysfunction, and stereotypy (all of which are found in the *rl/rl*) are also found in people with autism

(Klinger, Dawson, & Renner, 2003).

There is also evidence that reelin irregularities are associated with autism and autism spectrum disorders, not just with schizophrenia. A post-mortem study found that, compared to control brains, brains of autistic individuals were found to have decreased levels of both processed and unprocessed reelin in the cerebellum (Fatemi, Stary, Halt, & Realmuto, 2001), and genetic studies have found an association between autism and several reelin gene variants in humans (Persico, D'Agruma, Maiorano, Totaro, et al., 2001; Serajee, Zhong, & Mahbubul Huq, 2006; Skaar, Shao, Haines, Stenger, et al., 2005). There are also several shared brain abnormalities common to people with autism (Bauman, 1991; Courchesne, 1997) and *rl/rl* mice (Goffinet, 1984), including decreased Purkinje cell numbers as well as cytoarchitectonic abnormalities in the hippocampus, amygdala, and entorhinal cortex, although humans with autism do not display the inverted laminae of the cortex associated with the *rl/rl* brain (Persico et al., 2001).

Medicinal treatment of autism is fairly difficult, and medications are typically prescribed in order to control symptoms instead of to treat the disease itself (Tuchman, 2004). According to King and Bostic (2006, p. 163), "virtually every psychotropic medication available has been examined in patients who have [autism spectrum disorders]," but none of them specifically treats autism or has FDA approval to do so. Thus, if *rl/rl* mice modeled autism rather than schizophrenia, risperidone would not be expected to produce reliable, large effects, consistent with the findings reported here. Accordingly, it would be difficult to test the hypothesis that *rl/rl* mice are models of autism by using an antipsychotic or another type of drug.

Crawley (2004), however, has hypothesized that there are many behavioral tests that can measure autistic-like behaviors in mice that might address the possibility that *rl/rl* mice model autism or autism spectrum disorder, rather than schizophrenia. For example, the social recognition task measures a mouse's preference (or lack thereof) to investigate a stranger conspecific versus a novel, inanimate object. Normal animals would spend more time sniffing and otherwise investigating another mouse than they would spend investigating a novel inanimate object. An animal thought to model autism, however, would not be expected to show this preference because of the autistic symptom of inappropriate social interactions. Similarly, social communication can be measured in mice through responses to social olfactory cues and parental responses to pup ultrasonic vocalizations.

Therefore, one way to begin testing the hypothesis that *rl/rl* model autism would be to observe their behaviors on a battery of social interaction measures, for which there are currently no published data; to date, the only such test in which *rl/rl* have been analyzed was the social dominance task in Salinger et al (2003). Currently, our lab is investigating differential genotype responding in the social recognition task, but those results have not yet been analyzed. Thus, there is a need for a more extensive social phenotype of the *rl/rl* to be determined in order to conclude that the *rl/rl* models autism.

Conclusions

Risperidone, in the dosage used here, produced an unexpectedly limited number of statistically significant effects a variety of behavioral assays. Moreover, assuming those significant effects were not the result of type I errors, they influenced the behavior

of *rl/rl* mice in a direction that was the opposite of that needed to confirm the prediction that, as good models for schizophrenia, *rl/rl* mice would show improvements in cognition, emotionality, and sensorimotor gating due to risperidone. Although the *rl/rl* exhibits several behavioral abnormalities consistent with a schizophrenia-like pathology, those same deficits can be found in other disorders as well. Future research should examine the hypothesis that *rl/rl* mice model autism by analyzing their behavior on a wider variety of social measures.

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