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# Anxiety-like Behavior in C57BL/6J and DBA/2J Mice: Pharmacological Characterization of the Elevated Zero Maze and the Influence of Age-related Differences on Behavior

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# ANXIETY-LIKE BEHAVIOR IN C57BL/6J AND DBA/2J MICE: PHARMACOLOGICAL CHARACTERIZATION OF THE ELEVATED ZERO MAZE AND THE INFLUENCE OF AGE-RELATED DIFFERENCES ON BEHAVIOR

By

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## Abstract

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Anxiety disorders affect a significant proportion of the population and can be debilitating in some circumstances. The exact etiology of these disorders remains to be determined and animal models are an important part of that effort. The elevated zero maze, a behavioral measure of anxiety, was introduced as an alternative to the popular elevated plus maze. While the elevated zero maze has been pharmacologically validated in rats, the available data in mice is more limited. Similarly, the data available on anxiety-like behavior in adolescent mice lacks breadth despite considerable evidence suggesting that developmental processes during this period play a role in the etiology of anxiety disorders. In order to extend the available pharmacological data on the elevated zero maze and clarify age-related differences in anxiety-like behavior, three experiments using C57BL/6J and DBA/2J mice were performed. Experiments 1 and 2 examined the effects of chlordiazepoxide and a serotonin norepinephrine reuptake inhibitor on anxiety-like behavior in these strains. Experiment 3 examined age-related differences between these strains in anxiety like behavior during periadolescence, adolescence, and late adolescence. Anxiety-like behavior was found to vary with strain, task, drug, and age. These data extend our knowledge of baseline behavior in these stains and extend the foundation upon which to understand the etiology and treatment of anxiety disorders.

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# Anxiety-Like Behavior in C57BL/6J and DBA/2J Mice: Pharmacological Characterization of the Elevated Zero Maze and the Influence of Age-Related Differences on Behavior.

It is estimated that each year 40 million adults in the United States suffer from anxiety disorders, presenting a significant burden to both patients and society as a whole (NIMH, 2007). Included in the class of anxiety disorders are: panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, social phobia, specific phobias, and generalized anxiety disorder (NIMH, 2007). While the symptoms of these disorders vary widely both between and within disorders, they all share the common attribute of being characterized by excessive, irrational fear and worry (NIMH, 2007).Though the exact causes of these disorders are not clear, overarching estimates based on twin and adoption studies reveal that approximately 30% of the variability in anxiety traits can be explained by genetic factors, with estimates of heritability between .3-.5 (Clement et al., 2002). This moderate heritability suggests that these disorders are complex traits involving multiple, interacting genetic factors and experiential factors. To date, a diathesis-stress model has largely been used to explain their etiology. Briefly, this model suggests that some individuals have genetic/ biological traits rendering them vulnerable to the negative effects of stress, and if a sufficient stressor or series of stressors are experienced, a pathological state may then ensue. A large body of literature has documented the effects of various stressors and biological factors on the development of anxiety disorders in humans and anxiety-like behavior in animal models (for reviews see: Lesch, 2001; Clement et al., 2002; Finn et al., 2003; Gordon and Hen, 2004; Murray et al., 2009). However, our knowledge of the biology and experiential components of anxiety disorders

remains incomplete, and animal models continue to be an important element in furthering our understanding.

Numerous animal models of anxiety-like behavior are available, and have been useful in the study of the biology and pharmacology of anxiety-like traits. The vast majority of these models were developed using rats. However, the use of mice in neuroscience research has become more widespread as they offer a greater opportunity to exploit genetic models. While many of the behavioral models developed for rats have been successfully 'shrunken' down for use with mice, more pharmacological data on these models is available from rats than mice. As has been pointed out many times in the literature, mice are not simply little rats and considerable ethological and biological differences exist. Further, pharmacological research using mice has largely been performed using outbred strains, thereby reducing the range of conclusions that might be drawn from these investigations and limiting the ability to make assertions about the role of genetics in the phenomena under study (Taft et al., 2006). Despite their limited employment, inbred strains of mice provide a convenient tool for genetic research, and they have been shown to vary on a number of important traits including baseline behavior (e.g., Cook et al., 2001; Liu and Gershenfeld, 2003), response to antidepressant drugs (Crowley et al., 2005), monoamine concentrations in the brain (Jones et al., 1996), and structure of proteins involved in serotonin neurotransmission (Hackler et al., 2006). Additionally, the screening of inbred mouse strains is critical for the detection of background effects that often influence the interpretation of studies using transgenic mice (Crawley et al., 1997). Thus, a basic but necessary step towards fully utilizing the wealth

of murine genetic models in anxiety research is to more fully characterize these behavioral models using inbred strains of mice.

Towards this aim, three experiments were performed using C57BL/6J (B6) and DBA/2J (D2) mice. Since its introduction in 1994 (Shepherd et al., 1994), the elevated zero maze (EZM) has garnered considerable use as a behavioral model of anxiety, and while it has been pharmacologically validated using rats, the extent of available pharmacological data on mice is limited. Therefore, the acute effects of a classical benzodiazepine, chlordiazepoxide (CDZ) on behavior in the EZM were examined. Additionally, very few antidepressants have been examined in the EZM despite their frequent use in the treatment of many anxiety disorders. Thus, the behavioral effects of a common serotonin norepinephrine reuptake inhibitor (SNRI) were examined as well. (The drug used was acquired from the pharmaceutical company holding rights to this drug, and a contractual agreement requires the pharmaceutical company to be allowed to review all presentations and publications of experiments using the drug provided. Unfortunately, this company collapsed during the recent economic crisis, and we were unable to meet our obligations. Therefore, this drug will be referred to as 'SNRIX' in order to maintain confidentiality until said review or release from the agreement can be obtained.) Lastly, there is considerable evidence that anxiety traits and the propensity towards the development of anxiety disorders is shaped in early development and adolescence (vide infra), but the data on anxiety-like behavior in young mice is lacking as compared to that available in rats. Hence, the behavior of young mice in a number of anxiety-related models including the EZM was examined.

**Pharmacological Characterization of the Elevated Zero Maze**

All currently available rodent models of anxiety examine an animal's behavior in response to an aversive stimulus or situation. The conditioned models of anxiety normally use some noxious stimulus such as electric shock, while the unconditioned models of anxiety examine rodents' natural tendency to explore a novel environment while avoiding situations that might be potentially dangerous (e.g., open/unprotected spaces, unfamiliar foods, etc.) Of the available unconditioned models, the elevated plus-maze (EPM) is one of the most commonly used. The EPM consists of two alley-ways arranged perpendicularly at their center creating four arms and a central square. Two of these arms are enclosed by walls while the other two remain open. Generally rodents will explore both enclosed and open arms of the maze but spend a great deal more time in the enclosed arms, and time spent in the open versus closed arms and entries into the open arms have been pharmacologically validated as measures of anxiety-like behavior. However, there is ambiguity in interpreting time spent in the center intersection of the maze. To address this problem, Shepherd and colleagues (1994) developed the EZM.

The EZM consists of a circular runway with alternating open and closed quadrants. The circular design eliminates the ambiguity of the central square in the EPM and allows for uninterrupted ambulation, which might provide a more sensitive measure of anxiety-like behavior (Shepherd et al., 1994). It was initially shown that diazepam and CDZ decreased anxiety-like behavior in rats, while the anxiogenic compound 1-(m-chloro-phenyl)piperazine (mCPP) increased anxiety-like behavior (Shepherd et al., 1994). Since that time, a number of benzodiazepines at various doses have been shown to have an anxiolytic effect in rats (see Table 1). On the other hand, the initial adaptation of

the EZM for mice was used to examine induced mutations (e.g., Heisler et al., 1998), and only later were the effects of classical anxiolytic (Bilkei-Gorzo et al., 2002) and anxiogenic (Bilkei-Gorzo et al., 2004) compounds demonstrated. Still, the range of drugs, doses, and mouse strains examined remains constricted (see Table 2). Further, only a few reports are available on the effects of antidepressant medications in the EZM in rats or mice (see Tables 1 and 2) despite these medications being the most common treatment for many anxiety disorders (Dulawa et al., 2004).

**Table 1. Effects of acute administration of standard anxiolytic and anxiogenic drugs in rats on the elevated zero-maze**

| Drug | Strain | Dosages Tested | Open Time | Activity | LAT | Head Dip | Stretch Attend | Reference |
|---|---|---|---|---|---|---|---|---|
| Anxiolytics | | | | | | | | |
| alprazolam | Sprague-Dawley | .005, .05, .5 mg/kg IP | .005↓; .05, .5 NE | .005 ↓; .05, .5 NE | | NE | | Bentué-Ferrer et al., 2001 |
| CDZ | Sprague-Dawley | .5, 1, 2 mg/kg SC | 1, 2 ↑ | | | .5, 2 ↑ | .5, 2 ↓ | Shepherd et al., 1994 |
| | | 2.5, 5, 10 mg/kg IP | 5, 10 ↑ | 2.5, 5 ↑ | | | | Steckler et al., 2005 |
| | | 6 mg/kg SC | ↑ | NE | | NE | ↓ | Weiss et al., 1998 |
| | Wistar | 10 mg/kg PO | ↑ | ↑ | | ↑ | ↓ | Cryan et al., 2004 |
| diazepam | Charles Foster | .25, .5, 1 mg/kg IP | .5, 1 ↑ | | | .5, 1 ↑ | NE | Ramanathan et al., 1998 |
| | Sprague-Dawley | .125, .25, .5 mg/kg SC | .5 ↑ | | | .25, .5 ↑ | .5 ↓ | Shepherd et al., 1994 |
| | | .5 mg/kg SC | ↑ | | | ↑ | ↓ | Shepherd et al., 1996 |
| | Wistar | 1, 2 mg/kg | ↑ | NE | | 2 ↑ | NE | Frankowska et al., 2007 |
| | | .25, .5, 1, 2 mg/kg SC | ≥ .5 ↑ | | NE | NE | ↓ | Matto et al., 1997 |
| lorazepam | Sprague-Dawley | .015 mg/kg IP | ↓ | ↓ | | ↓ | | Bentué-Ferrer et al., 2001 |

(Table Continues)

**Table 1. Effects of acute administration of standard anxiolytic and anxiogenic drugs in rats on the elevated zero-maze**

| Drug | Strain | Dosages Tested | Open Time | Activity | LAT | Head Dip | Stretch Attend | Reference |
|---|---|---|---|---|---|---|---|---|
| buspirone | Wistar | .04, .2, 1, 5 mg/kg SC | NE | | NE | NE | NE | Matto et al., 1997 |
| desipramine | Wistar | 10, 20 mg/kg IP | NE | ↓ | NE | NE | NE | Pähkla et al., 2000 |
| fluoxetine | Wistar | 5, 10 mg/kg IP | NE | 10 ↓ | NE | 10 ↓ | NE | Pähkla et al., 2000 |
| Anxiogenics | | | | | | | | |
| DMCM | Sprague-Dawley | .3 mg/kg IP | ↓ (NS) | ↓ (NS) | | ↓ | | Bentué-Ferrer et al., 2001 |
| | Wistar | .1, .5, 1, 1.5 mg/kg IP | NE | | 1.5 ↓ | ↑ | NE | Matto et al., 1997 |
| | | .5, 1.5 mg/kg IP | ↓ | ↓ | ↑ | ↓ | | Pähkla et al., 2000 |
| mCPP | Sprague-Dawley | .25, .5, 1 mg/kg SC | .5, 1 ↓ | | | 1 ↓ | .5, 1 ↑ | Shepherd et al., 1994 |

For the behavioral measures (open time, activity, latency [LAT], head dip, and stretch attend), doses producing observed effect are reported where the effect was dose dependent. Blank cells indicate that the measure was not reported by the authors. Open time refers to time spent in open quadrants of the maze (percentage or actual as reported by authors). Activity refers to locomotor activity in the maze as reported by authors. Latency (LAT) refers to latency to first enter an open quadrant of the maze. Head dip refers to observations of the animal dipping its head over the edge of the open quadrants of the maze. Stretch attend refers to observations of animals adopting a stretched-attend posture, typically defined as an elongated posture with the snout stretched forward. CDZ = chlordiazepoxide, DMCM = methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate, mCPP = m-chlorophenyl-piperazine, IP = intraperitoneal injection, SC = subcutaneous injection, PO = *per os* administration, ↑ = increase in measure, ↓ = decrease in measure, NE = no effect, (NS) = large observed effect but did not reach statistical significance.

**Table 2. Effects of standard anxiolytic and anxiogenic drugs in mice on the elevated zero-maze**

| Drug | Strain | Dosages Tested | Open Time | ACT | LAT | Head Dip | Stretch Attend | Reference |
|---|---|---|---|---|---|---|---|---|
| Anxiolytics | | | | | | | | |
| alprazolam | BALB/c | 1 µg ICV | ↑ | | | | | Ring et al., 2006 |
| CDZ | BALB/c | 30, 56 µg ICV | 56 ↑ | NE | | | | Leonard et al., 2008 |
| | | 3, 10, 30 µg ICV | 30 ↑ | | | | | Malberg et al., 2007 |
| | | 10 mg/kg PO | ↑ | ↑ | ↓ | ↑ | ↓ | Mombereau et al., 2004 |
| | C57BL/6J | 5, 10, 20 mg/kg IP | 20 ↓ | | NE | NE | 20 ↓ | Mathiasen et al., 2008 |
| | DBA/2J | 5, 10, 20 mg/kg IP | NE | | NE | NE | 20 ↓ | Mathiasen et al., 2008 |
| | NMRI | 5, 10, 20 mg/kg IP | 5 ↑ | | 5 ↓ | 5, 10 ↑ | 10, 20 ↓ | Mathiasen et al., 2008 |
| | | 5, 10, 20 mg/kg IP | 10, 20 ↑ | | | | | Troelsen et al., 2005 |
| | OF1 | 10 mg/kg PO | ↑ | ↑ | ↓ | ↑ | ↓ | Cryan et al., 2004 |
| | | 10 mg/kg PO | ↑ | ↑ | ↓ (NS) | NE | ↓ | Jacobson and Cryan, 2008 |
| diazepam | C57BL/6J | 1 mg/kg IP | | ↑ | | | ↓ | Bilkei-Gorzo et al., 2002 |
| | | 2 mg/kg IP | ↑ | ↑ | | | | Bilkei-Gorzo et al., 2004 |
| | DBA/2J | 2 mg/kg IP | ↑ | NE | | | | Bilkei-Gorzo et al., 2004 |
| buspirone | C57BL/6J | 1 mg/kg IP | | NE | | | ↓ | Bilkei-Gorzo et al., 2002 |

(Table Continues)

**Table 2. Effects of standard anxiolytic and anxiogenic drugs in mice on the elevated zero-maze**

| Drug | Strain | Dosages Tested | Open Time | ACT | LAT | Head Dip | Stretch Attend | Reference |
|---|---|---|---|---|---|---|---|---|
| amitriptyline | NMRI | .3, 1, 3, 10 IP | NE | NE | NE | NE | NE | Troelsen et al., 2005 |
| | | 10 mg/kg PO 21 days | NE | NE | NE | NE | NE | |
| desipramine | 129SvEv X C57BL/6 | 12.5 mg/kg IP | NE | | NE | NE | NE | Gur et al., 2007 |
| citalopram | NMRI | 5, 10, 20, 40 mg/kg IP | NE | NE | NE | NE | NE | Troelsen et al., 2005 |
| | | 10 mg/kg PO 21 days | NE | NE | NE | NE | NE | |
| fluoxetine | NMRI | 1, 3, 10, 30 mg/kg IP | NE | NE | NE | NE | NE | Troelsen et al., 2005 |
| paroxetine | NMRI | 1, 3, 10, 30 mg/kg IP | NE | NE | NE | NE | NE | Troelsen et al., 2005 |
| duloxetine | NMRI | 1, 3, 10, 30 mg/kg IP | NE | NE | 10 ↑ | 10 ↑ | NE | Troelsen et al., 2005 |
| | | 10 mg/kg PO 21 days | ↑ | ↑ | NE | ↑ | ↓ | |
| venlafaxine | NMRI | 3, 10, 30, 60 mg/kg IP | NE | NE | NE | NE | NE | Troelsen et al., 2005 |
| reboxetine | NMRI | 1, 3, 10, 30 mg/kg IP | 3, 10 ↓ | 1, 3 ↓ | NE | NE | NE | Troelsen et al., 2005 |
| | | 10 mg/kg PO 21 days | NE | NE | NE | NE | NE | |

(Table Continues)

Table 2. Effects of standard anxiolytic and anxiogenic drugs in mice on the elevated zero-maze.

| Drug | Strain | Dosages Tested | Open Time | ACT | LAT | Head Dip | Stretch Attend | Reference |
|---|---|---|---|---|---|---|---|---|
| Anxiogenics | | | | | | | | |
| mCPP | C57BL/6J | .5 mg/kg IP | NE | NE | ↑ | | | Bilkei-Gorzo et al., 2004 |
| | DBA/2J | .5 mg/kg IP | ↓ | ↓ | NE | | | Bilkei-Gorzo et al., 2004 |

All effects refer to acute administration unless otherwise noted. For the behavioral measures (open time, activity [ACT], latency [LAT], head dip, and stretch attend), doses producing observed effect are reported where the effect was dose dependent. Blank cells indicate that the measure was not reported by the authors. Open time refers to time spent in open quadrants of the maze (percentage or actual as reported by authors). Activity (ACT) refers to locomotor activity in the maze as reported by authors. Latency (LAT) refers to latency to first enter an open quadrant of the maze. Head dip refers to observations of the animal dipping its head over the edge of the open quadrants of the maze. Stretch attend refers to observations of animals adopting a stretched-attend posture, typically defined as an elongated posture with the snout stretched forward. CDZ = cholrdiazepoxide, mCPP = m-chlorophenyl-piperazine, ICV = intracerebroventricular injection, IP = intraperitoneal injection, PO = *per os* administration, ↑ = increase in measure, ↓ = decrease in measure, NE = no effect, (NS) = large observed effect but did not reach statistical significance.

Noting these limitations, two experiments were performed using B6 and D2 mice. The B6 and D2 strains were selected as they are among the most widely available and thoroughly phenotyped, making them excellent reference populations. Additionally, these strains have been shown to differ in baseline behavior on measures thought to reflect aspects of depression (Alcaro et al., 2002; Crowley et al., 2005) and anxiety (Cook et al., 2001; Wahlsten et al.; 2003), as well as in response to treatment with antidepressant agents (Liu et al., 2001; Lucki et al., 2001; Crowley et al., 2005).

*Experiment 1.* At the conception of this experiment, the effects of CDZ in mice on the EZM had only been reported in the outbred mouse strains, OF1 (Cryan et al., 2004) and NMRI (Troelsen et al., 2005), and the inbred strain, BALB/c (Mombereau et al., 2004; Malberg et al., 2007). Thus, we thought that it would be beneficial to examine the effects of CDZ in B6 and D2 mice. Since that time, Mathiasen et al. (2008) have reported on the effects of CDZ in these strains on the EZM. However, there are some peculiarities to their observations. First, it is notable that, at baseline, B6 mice displayed greater anxiety-like behavior than D2 mice, which is contrary to strain differences reported in most studies (e.g., Tarantino et al., 2000; Tang et al., 2002; Milner and Crabbe, 2008). Second, the only effect of CDZ on anxiety behavior observed was an anxiogenic effect in B6 mice at the 20 mg/kg dose. Therefore, to clarify and extend the available data, the acute effects of three doses of CDZ (2.5, 5, and 7.5 mg/kg) were examined in the EZM. Considering the report of Mathiasen (2008), it was difficult to predict the direction of the effect, if any, that CDZ would have had here. However, in view of the typical effects of the benzodiazepines, it was expected to elicit an anxiolytic response.

*Experiment 2.* In addition to the limited characterization of the effects of antidepressants in the EZM, many of such studies have excluded key aspects of the clinical manifestations of these disorders. For example, the prevalence of anxiety and depressive disorders is far greater in women than in men (Anisman and Matheson, 2005; Toufexis et al., 2006), there is evidence of sex differences in serotonin transmission (Bagdy, 1998; Jones and Lucki, 2005), and differences in therapeutic efficacy of antidepressants (Jones and Lucki, 2005; Duman et al., 2006). However, the majority of animal research on the behavioral effects of antidepressants has not included females (Palanza, 2001; Cryan and Mombereau, 2004; Dulawa and Hen, 2005). While female subjects have been generally left out due to increased complexity and cost associated with their inclusion, those studies that have made use of female animals have reported sex differences in baseline behavior (Cryan and Mombereau, 2004), serotonin transmission (Jones et al., 1996), and behavioral responses to treatment with antidepressant agents (David et al., 2001; Monleón et al., 2002; Caldarone et al., 2003; Leuner et al., 2004; Lifschytz et al., 2006) suggesting that the inclusion of females may provide insight into gender disparities in anxiety-related disorders.

Similarly, the majority of patients experience a 2 – 3 week lag between the onset of antidepressant use and any therapeutic effects. Yet, most preclinical studies of antidepressant drugs have only examined the effects of acute administration, and relatively few behavioral models of depression or anxiety have been responsive to chronic administration of antidepressants (Brocco et al., 2002; Dulawa and Hen, 2005; Malberg and Blendy, 2005). This fact presents a number of problems. Foremost, the validity of these models must be drawn into question, as they fail to incorporate what

appears to be a central feature of these drugs in clinical populations (i.e., efficacy only after prolonged administration [Dulawa et al., 2004; Mitchell and Redfern, 2005]). While an animal model is not expected to incorporate every feature of complex disorders such as anxiety and depression, extending the use of commonly employed models to include simple, but commonly neglected, variables could increase our understanding of these disorders and their treatment.

During the early 1990's the 'third generation' antidepressants were approved for the treatment of depression in the U.S., and within a decade, these medications accounted for a considerable proportion of antidepressant drugs prescribed (Hansen et al., 2010; Vlahiotis et al., 2011). Among these are the serotonin norepinephrine reuptake inhibitors (SNRIs), which act similarly to the tricyclic antidepressants in that they inhibit both serotonin and norepinephrine reuptake but have a more favorable side effect profile due to their greater specificity (Olver et al., 2001). SNRIX was one of the first SNRIs approved for use and its clinical efficacy in the treatment of depression and anxiety is well established (Kent, 2000). However, behavioral studies of SNRIX in mice are relatively limited, and only the acute effects of SNRIX have been examined in the EZM (Troelsen et al., 2005).

Seeking to address this, we treated male and female B6 and D2 mice with SNRIX or vehicle for 29 days and then measured behavior on a battery of seven tests. Our primary interest was the effects of chronic administration of SNRIX on behavior in the EZM. Therefore, behavior on the EZM was measured on the 30$^{th}$ day following the initiation of drug treatment. Additionally, noting the general lack of behavioral data on the effects of SNRIX in mice, animals were put through a testing battery for an additional

three days. The testing battery used has previously been shown to be an effective high throughput behavioral screen in a large scale mutagenesis project (Cook et al., 2007) and allows for a timely survey of a broad range of behaviors. Thus, we hypothesized that this experimental design would allow us to efficiently detect strain and/ or sex dependent effects of chronic antidepressant administration if they did exist, while enhancing the range of available data on the behavioral effects of SNRIX.

**Behavioral Characterization of Adolescent Mice**

With the major physiological, cognitive, and social changes characteristic of adolescence, comes an increased occurrence of impulsivity, risk-taking behavior, and psychopathology. For example, in the U.S., 50% of adolescents have consumed alcohol and over 30% have used an illicit drug by about age 15 (Johnston et al., 2008). Additionally, the initial onset of generalized anxiety disorder, panic disorder, and obsessive compulsive disorder frequently occurs during this time (Lesch, 2001; Grover et al., 2005; Gregory et al., 2008). In fact, symptoms of depression and/ or anxiety are the leading reasons for mental health treatment among adolescents, and suicide is the third leading cause of death among children 15 – 19 years of age (HRSA, 2004).

While there is evidence that the common emergence of mood disorders during adolescence is related to the physical maturation of the brain (for example see: Casey et al., 2010), it is also clear that these developmental processes are taking place within the larger gene X environment interactions that shape both healthy and pathological outcomes (Leonardo and Hen, 2008; Casey et al., 2010). Hence, it has been proposed that the gene X environment interactions thought to underlie the etiology of the mood disorders would be better conceptualized as gene X environment X development

interactions (Leonardo and Hen, 2008). In attempting to understand these three-way interactions, murine models provide convenient means to examine the effects of genetic and environmental factors; however, the available data on baseline behavioral differences among juvenile mice is relatively sparse. Further, the data that is available primarily comes from pharmacological studies examining a wide range of ages, and often baseline age-related differences are not reported or age groups are analyzed separately.

Noting the limited research in this area, Hefner and Holmes (2007) examined behavior of male B6 mice at 4, 6, and 8 weeks of age which map onto what has been termed periadolescence (weaning to approximately 1 week prior to puberty), mid-adolescence, and late-adolescence, respectively (Adriani et al., 2004). They used three measures of anxiety-related behavior (fear conditioning, EPM, and open field) and a measure of depression-like behavior (the forced swim test). They found age-related differences in fear conditioning, anxiety-like behavior in the open field, and depression-like behavior in the forced swim test but no differences in anxiety-like behavior in the EPM. Considering that they found age-related differences in some, but not all, anxiety-related measures and the possibility that age-related differences could vary with genotype, we sought to extend this inquiry to include additional measures and an additional inbred strain. Therefore, the behavior of male B6 and D2 mice beginning at PND 28, 42, and 56 was examined in a modified version of the testing battery used in Experiment 2 above.

*Experiment* 3. The choice of B6 and D2 mice was based on the characteristics noted previously (Vide Supra). The behavioral battery used was the same as that in Experiment 2 except startle/prepulse inhibition was excluded in order to allow all animals

to be tested in a single week. Given that Hefner and Holmes (2007) found the effects of age to be task specific, we expected that this battery would help clarify the nature of these differences as it provides two additional tests of anxiety-like behavior and a different test of depressive-like behavior.

## Method

### Subjects

B6 and D2 mice (35 to 40 days of age for Experiments 1 and 2; 21, 35, and 49 days of age for Experiment 3) were purchased from the Jackson Laboratory (Bar Harbor, MN, USA), and housed five per cage in the University of Memphis vivarium. All animals had access to food and water *ad libitum* and were maintained on a 12:12 light/dark cycle with lights on at 06:30 and lights off at 18:30. The average temperature in the vivarium, to date, is 23° C with humidity varying between 30 and 70%. The number of animals used in Experiments 1 and 2 are reported in Tables 3 and 4, respectively. A total of 60 mice (10 per experimental cell) were used in Experiment 3.

**Table 3. Number of animals used (*n*) by strain and CDZ dose in Experiment 1**

| Strain | Saline | 2.5 mg/kg | 5 mg/kg | 7.5 mg/kg |
|---|---|---|---|---|
| C57BL/6J | 15 | 10 | 10 | 10 |
| DBA/2J | 10 | 10 | 10 | 10 |

Only males were examined.

**Table 4. Number of animals used (*n*) by strain, sex, and drug dose in Experiment 2**

| | B6 | | D2 | |
|---|---|---|---|---|
| Drug Treatment | Male | Female | Male | Female |
| Saline | 9 | 11 | 12 | 10 |
| 10 mg/kg | 12 | 11 | 11 | 12 |
| 30 mg/kg | 12 | 12 | 11 | 12 |

B6 = C57Bl/6J, D2 = DBA/2J.

**Procedure**

*Experiment 1.* At PND 60, mice were weighed and placed in a darkened holding area for at least 30 minutes. After this habituation period mice received an intraperitoneal (IP) injection of either CDZ (2.5, 5, or 7.5 mg/kg) or saline. Thirty minutes later they were tested on the EZM.

*Experiment 2.* At PND 60, animals were weighed and received an injection of either SNRIX (10 or 30 mg/kg IP) or saline, once daily for 29 days. At PND 90, animals began four days of behavioral testing. Behavioral testing was conducted in the following order: Day 1: elevated zero-maze; Day 2: open field and hotplate; Day 3: light/dark, startle, and fear conditioning training; Day 4: testing of contextual and cued conditioning, and tail suspension.

*Experiment 3.* Animals were allowed one week to habituate to the vivarium before behavioral testing. At PND 28, 42, or 56 (4wk, 6wk, 8wk, respectively) animals began a four day battery of testing conducted in the following order: Day 1: elevated zero-maze; Day 2: open field and hotplate; Day 3: light/dark and fear conditioning training; Day 4: testing of contextual and cued conditioning, and tail suspension.

For all experiments, testing was conducted so that animals belonging to each experimental cell were represented throughout each testing period. All apparatus were cleaned with 70% isopropanol between mice and allowed to dry. For Experiments 2 and 3 all animals were tested using each measure in the same order and allowed a minimum habituation period of 30 min before each test.

**Drugs**

CDZ was purchased from Sigma Chemicals (St. Louis, MO, USA). The chosen doses are largely based on those previously shown to have an anxiolytic effect in mice on the EPM (Lister et al., 1987; Raupp et al., 2008; Clément et al., 2009; Paterson et al., 2010) but below those producing sedation (Fielding and Hoffman, 1979). SNRIX was provided by the pharmaceutical company holding rights to this drug. Dosages of 10 and 30 mg/kg were chosen as approximations of both those reported in the literature to be behaviorally effective in mice and those shown to produce serum levels within the therapeutic range when administered chronically (Ahern et al., 2006.) Dosages were calculated as the weight of the salt. Both drugs were dissolved in .9% saline (w/v) and administered in an injection volume of 10 ml/kg. Control animals were injected with an equivalent volume of .9% saline alone.

**Behavioral Testing and Apparatus**

**Elevated Zero Maze.** Elevated zero mazes were manufactured by AccuScan Instruments (Columbus, OH, USA). The apparatus is an elevated black circular platform consisting of open and closed quadrants. Because we are interested in the avoidance of open versus closed areas and to minimize differences in light intensity between the open and closed quadrants, the closed quadrants are enclosed by clear acrylic walls 28.5 cm in

height (Martínez et al., 2002). The closed quadrants are each equipped with infrared light beams allowing the amount of time spent and activity in the closed quadrants to be monitored. The open quadrants have a slightly raised Plexiglas lip to prevent the mice from falling off of the maze. The zero-maze has been described in detail elsewhere (Cook et al., 2001).

Mazes were separated from one another by solid partitions such that each maze is equidistant from three extra-maze walls. A greater range of behavior is generally displayed when testing is performed under dim and/or red light (Kalinichev et al., 2002; Tang et al., 2002). Therefore, like others (Parfitt et al., 2007), each maze was dimly lit by a 15W red light bulb suspended approximately 125 cm above the maze, providing an average illumination of 14 lx at the level of each quadrant.

On the day of testing, animals were acclimated to a darkened holding area prior to testing. Test duration was five minutes. Animals were placed in a closed quadrant to begin the test period. Latency to enter an open quadrant, total time spent in open and closed quadrants, and activity in the closed quadrants was recorded. Activity levels can vary greatly because their measurement is a function of time spent in the closed quadrants; therefore, we evaluate activity as beam breaks per second spent in the closed quadrants.

**Open Field.** The open field arenas consist of a Plexiglas open field insert (24.13 cm x 45.72 cm) in a HamiltonKinder SmartFrame™ system (HamiltonKinder, Poway,CA, USA). The system uses two 4x8 photobeam arrays, one to detect horizontal movements and one to detect vertical movements. The average illumination of the arena is 60 lx.

Animals were placed in the center of the open field to begin the 20 min test period. Based on the position and sequence of beam breaks during the test period the following measures were evaluated: distance traveled , rears, and percentage of time spent in the center of the arena (defined as the 9 x 10 cm area located 15 cm from front and back walls and 4.5 cm from the left and right walls of the arena), percentage of time spent in the corners of the arena, percentage of total distance traveled occurring in the center of the arena, and habituation ratio (i.e. the ratio of distance traveled during the last five minutes of testing to the sum of the distance traveled during the first and last five minutes of testing.) At the end of the testing period animals were returned to their home cage until the next test.

**Hotplate.** A hotplate algesia meter (Model 39) manufactured by IITC Inc. (Woodland Hills, CA, USA) was used. The unit has an anodized aluminum plate measuring 27.94 X 26.67 X 1.91 cm. A heat sink compound (Radio Shack® Cat no. 273-1372) was used between the hotplate surface and the aluminum plate to facilitate an even distribution of heat. The aluminum plate was held in position with binder clips. A small, bottomless translucent grey Plexiglas enclosure (8.5 X 6.5 X 8.5 cm) was used to confine animals to the center of the aluminum plate.

All overhead lights were turned off 30 minutes prior to testing. A small desk lamp directed away from the algesia meter and a mirror placed behind the hotplate was used to facilitate observations of the animal. The hotplate was heated to 52° C, and a surface thermometer was used to verify the temperature throughout the test. The mouse was placed in the Plexiglas enclosure in the center of the hotplate and observed for pain responses associated with the hind paws indicated by licking, shaking, or jumping. Once

a pain response was displayed, the animal was immediately removed from the hotplate and the latency recorded. If the animal did not show a pain response within 20 seconds, it was immediately removed and the test discontinued. For those animals that did not respond, the maximum latency of 20 seconds was assigned.

**Light-Dark.** The light dark apparatus is identical to the apparatus used for open field only different inserts are used. The light/dark inserts are of the same overall dimensions, except that one half is made of clear Plexiglas and the other half is made black Plexiglas. The two halves are separated by a manual guillotine door that allows the animal to move freely between the two compartments when removed. The light half of the light/dark enclosure is illuminated by a 15 W light bulb approximately 48 cm above the chamber providing an average illumination of 33 lx in the light half and 1 lx in the dark half of the apparatus.

Overhead lights were turned off thirty minutes prior to testing. To begin the 10 minute test period, animals were placed in the light half of the box and the guillotine door was then removed. The distance traveled in the either side of the box, percentage of time spent in the light side of the apparatus, and the percentage of the total distance traveled that occurred in the light side were measured. At the end of the test period animals were returned to their home cages.

**Acoustic Startle/Prepulse Inhibition.** A HamiltonKinder SM100 Startle Monitor was used. The system consists of base plate, mouse sensing plate, mouse restrainer, and Newton impulse Calibrator (calibrated in newtons) enclosed in a sound attenuating cabinet. The cabinet measures 35.56 X 27.62 X 49.53 cm and has 35 db of attenuation.

The system is designed to provide +/- 1 db accuracy on a scale from 57-120 db and to minimize variability between test chambers (<1 db; www.hamiltonkinder.com.)

Animals were placed in the startle chamber with a 65 db background white noise and allowed to habituate for two minutes. The two minute period was followed by 55 pseudo-random trials separated by 15 second intertrial intervals. A 120 db white noise burst was used as the acoustic startle stimulus. Pre-pulses were 70, 80, and 85 db white noise bursts lasting 20 ms which precede the startle stimulus by 100 ms. Startle response to the startle stimulus and to each of the pre-pulse db levels was measured. Pre-pulse inhibition was calculated using the following formula [100- (pre-pulse startle/acoustic startle) x 100]. Animals were returned to their home cage following testing.

**Fear Conditioning.** The Hamilton-Kindler SmartFrame system was used in conjunction with fear conditioning inserts (24.13 cm x 22.86 cm). The inserts have a grid floor connected to a shock generator and the top of the box includes a speaker attached to a sound generator.

*Training.* Animals were placed in the fear conditioning chambers and allowed to habituate for 2.5 minutes. Animals were then presented with three pairings of an 85 db tone and a 0.36 mA foot shock separated by a 2.5 minute intertrial interval. The tone was presented for 30 seconds and the shock was administered during the last 2 seconds of the tone. Because this test is automated, beam breaks were measured in 30 second intervals. The average number of beam breaks per 30 second interval for the first two minutes of training was used as an indication of baseline activity. The ratio of baseline activity to the number of beam breaks during the 30 second interval following the final tone + shock

pairing (suppression of activity by training) was used as an indicator of the degree to which the training procedure suppressed activity.

*Contextual Conditioning*. On the day following the training session, animals were placed back into the same chambers where they underwent training. During this 6 minute session, activity (beam breaks) per 30 second bin was measured and compared to activity during the habituation period on the training day. The ratio of baseline activity to activity during re-exposure to the training context was used as a measure of conditioning to the context.

*Cued Conditioning.* Approximately 2 hr later, behavior was tested in an altered context. The fear conditioning chambers were altered by placing a plastic grey tile over the grid floor, placing a black Plexiglas insert over the walls of the chambers, and attaching a small cup containing orange oil diluted in water in the upper corner of the box. Animals were allowed to explore the altered environment for 2.5 minutes, after which time, the conditioned stimulus (tone) was presented for 2.5 minutes. Activity (beam breaks) was measured in 30 second bins. Activity suppression during presentation of the tone was evaluated relative to activity during the habituation period in the altered context. Animals were returned to their home cage following each session.

**Tail suspension.** A tail suspension apparatus manufactured by MedAssociates (St. Albans, VT, USA) was used. Each unit consists of a linear load cell with an amplifier and filter connected to a transducer. The units are each enclosed by an open faced cubicle.

All animals were weighed to the nearest .1 g prior to tail suspension testing. The body weights were entered into the tail suspension program, which automatically

calculates a threshold for force of movement for each animal. At the start of the session each animal's tail was taped to the transducer. Force of movement was recorded for six minutes. The session was divided into twelve 30 second intervals, and time spent above threshold was examined for the entire session and each interval. Animals were observed during testing and tail climbing recorded. Those animals that climbed their tails were not included in the analysis.

**Analysis**

*Experiment 1.* Data for the dependent variables were examined using analysis of variance (ANOVA) with strain and CDZ dose as between subjects variables.

*Experiment 2.* Data from the light/ dark box and tail suspension test were lost during the experiment for some animals due to a computer error. The number of animals missing and the treatment groups to which they belonged is reported along with the results from these tests. Prior to the analysis of data from these tests, all observations were coded dichotomously as missing or not. This was then compared to all other variables in the study via bivariate correlation in order to determine the nature of the missing data. Significant correlations between missing observations and other variables are reported along with the results.

Three-way ANOVA using strain, sex, and drug dosage as the between subjects variables for each independent measure were used to analyze the data. The only exceptions to this were the analyses of body weight, interval data from the tail suspension test, and tail climbing behavior. For body weight and tail suspension intervals repeated measures ANOVA were performed using strain, sex, and drug dosage as between subject factors and weight or interval as the respective within subjects variable. Data for tail

climbing during the tail suspension test were coded dichotomously and analyzed using logistic regression analysis with the independent variables strain, sex, and drug dosage as predictors.

*Experiment 3.* Data were analyzed using separate two-way ANOVAs for each dependent measure with strain and age as between subject factors. Again here, tail suspension interval data were analyzed using a repeated measures ANOVA.

For all analyses, where significant effects were found ($p < .05$), *post hoc* comparisons were made using Tukey's HSD method or analysis of simple effects was employed, as appropriate. Effect sizes are presented for select variables in the discussion to aid in the interpretation of the results. All analyses and calculations were performed using SPSS 11.5 except for calculations of effect size, which was performed using GPower 3.0.5.

## Results

### Experiment 1

Upon inspection of the data, we found that some animals' measures on latency to enter an open quadrant and percentage of time in the open quadrants of the maze were extreme. Two animals' latency measures were more than three standard deviations from the mean (one B6 mouse in the 2.5 mg/kg group and one D2 mouse in the saline group), and four animals scored more than three standard deviations from the mean on the percentage of time spent in the open quadrants (one B6 mouse given 2.5 mg/kg, one D2 mouse given 2.5 mg/kg, and two D2 mice in the 7.5 mg/kg group). Therefore, these animals were considered to be outliers and were not included in the respective analyses. Data are presented in Table 5.

**Activity in the closed quadrants.** ANOVA revealed a significant effect of strain on activity, $F$ (1, 77) = 3.928, $p$ = .05, with B6 mice being slightly more active than D2 mice (M = 2.54, SD = .41 and M = 2.36, SD = .42, respectively). ANOVA failed to detect an effect of CDZ or an interaction between strain and CDZ administration, $F$ (3, 77) = 2.033, $p$ = .116, and $F$ (3, 77) = .919, $p$ = .436, respectively.

**Table 5. Means and standard errors of measures in the elevated zero maze for Experiment 1 by strain and drug treatment**

| | Saline | 2.5 mg/kg | 5 mg/kg | 7.5 mg/kg |
|---|---|---|---|---|
| Strain | Activity | | | |
| B6 | 2.59 (.11) | 2.28 (.13) | 2.77 (.13) | 2.53 (.13) |
| D2 | 2.32 (.13) | 2.27 (.13) | 2.40 (.13) | 2.47 (.13) |
| | Latency (s) to Enter an Open Quadrant | | | |
| B6 | 5.97 (2.77) | 10.46 (3.57) | 4.64 (3.39) | 3.05 (3.39) |
| D2 | 12.30 (3.57) | 15.78 (3.39) | 13.50 (3.39) | 20.90 (3.39) |
| | Percentage of Time Spent in the Open Quadrants | | | |
| B6 | 24.91 (2.68) | 20.12 (3.46) | 18.12 (3.28) | 29.47 (3.66) |
| D2 | 12.10 (3.28) | 15.40 (3.46) | 10.18 (3.28) | 24.11 (3.66) |

Animals were administered saline, 2.5, 5, or 7.5 mg/kg chlordiazepoxide thirty minutes prior to testing. Numbers are means and numbers in parentheses are standard errors of the means. Activity is measured as beam breaks per second spent in the closed quadrants of the maze. B6 = C57BL/6J, D2 = DBA/2J

**Latency to first enter an open quadrant.** ANOVA detected a significant effect of strain, $F$ (1, 75) = 15.55, $p$ < .001, with B6 mice exhibiting a shorter latency than D2 mice. However, there was no effect of CDZ and no strain by drug treatment interaction, $F$ (3, 75) = .678, $p$ = .568, and $F$ (3, 75) = 1.233, $p$ = .304, respectively.

**Percentage of time spent in the open quadrants.** ANOVA revealed a significant effect of strain, $F$ (1, 73) = 10.88, $p$ = .002, with B6 mice spending more time in the open

quadrants than D2 mice. Likewise, a significant effect of CDZ was found, $F$ (3, 73) = 4.911, $p$ = .004. See Figure 1, panel A. Post hoc testing indicated that none of the doses tested differed from saline alone, but animals administered 7.5 mg/kg CDZ spent more time in the open quadrants of the maze than those given either 2.5 or 5 mg/kg. Examining the means by strain suggests that the effect of CDZ is largely driven by an effect of 7.5 mg/kg increasing time spent in the open in D2 but not B6 mice. See Figure 1, panel B. However, the strain by drug treatment interaction was not significant, $F$ (3, 73) = .677, $p$ = .569. None the less, if the data are analyzed separately by strain an effect of CDZ is found in D2 mice but not B6 mice (D2 mice: $F$ (3, 33) = 3.44, $p$ = .028; B6 mice: $F$ (3, 40) = 2.207, $p$ = .102).

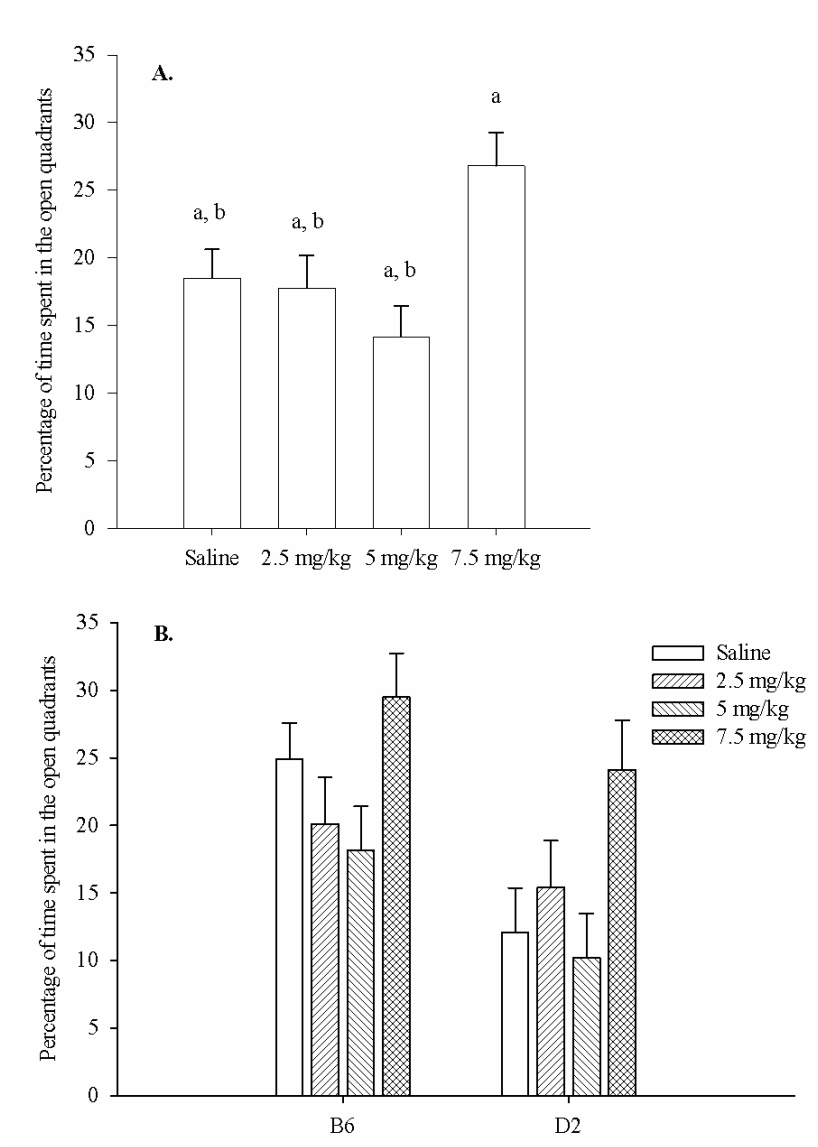


**Figure 1.** Effects of CDZ on the percentage of time spent in the open quadrants of the elevated zero maze. Data are presented as means ± SEM. **A.)** Main effect of drug dose. Groups that do not share a common lower case letter are significantly different at the level $p < .05$. **B.)** The effect of CDZ by dose and strain. The interaction was non-significant ($F$ (3, 73) = .677, $p$ = .569).

## Experiment 2

Body weight

To determine if drug administration affected animals' body weight, weights during drug treatment at PND 60, 70, 80, and 89, as well as weight prior to tail

suspension testing on the final day of testing (PND 93) were selected for analysis. Repeated measures ANOVA did not detect any effects of the drug treatment on body weight ($p > .2$ for all; Data not presented)

Elevated Zero Maze

**Activity in the closed quadrants.** ANOVA revealed a significant main effect of strain, $F$ (1, 123) = 6.117, $p < .05$, with B6 mice being more active than D2 mice. ANOVA failed to detect significant main effects of sex or drug treatment: $F$ (1, 123) = .045, $p = .83$, and $F$ (2, 123) = .653, $p = .52$, respectively. Likewise, ANOVA failed to detect significant strain x sex, strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (1, 123) = 1.173, $p = .281$; $F$ (2, 123) = .829, $p = .44$; $F$ (2, 123) = 1.292, $p = .279$; and $F$ (2, 123) = .393, $p = .68$; respectively. (See Table 6 for data.)

**Latency to enter an open quadrant.** ANOVA revealed a significant main effect of strain, $F$ (1, 123) = 10.016, $p = .002$, with B6 mice entering an open quadrant sooner than D2 mice (M = 4.84, SEM = 1.31, and M = 10.69, SEM = 1.30, respectively). However, ANOVA failed to detect an effect of sex or drug treatment: $F$ (1, 123) = 050, $p = .82$, and $F$ (2, 123) = 1.832, $p = .16$, respectively. ANOVA revealed a significant strain x sex interaction, $F$ (1, 123) = 3.953, $p = .049$. Analysis of simple effects found no difference between D2 males and females, but B6 females displayed a shorter latency than B6 males. B6 and D2 males did not differ, but B6 females displayed a shorter latency than did D2 females. ANOVA did not detect significant strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (2, 123) = 2.099, $p =$

.127; $F$ (2, 123) = .202, $p$ = .82; and $F$ (2, 123) = .422, $p$ = .66; respectively. (See Table 6 for data.)

**Table 6. Means and standard errors of measures in the elevated zero maze for Experiment 2 by strain, sex, and drug treatment**

| | B6 | | D2 | |
|---|---|---|---|---|
| Drug Treatment | Male | Female | Male | Female |
| | Activity | | | |
| Saline | 2.22 (.17) | 2.22 (.16) | 1.99 (.15) | 2.01 (.16) |
| 10 mg/kg | 2.04 (.15) | 2.08 (.16) | 2.14 (.16) | 1.81 (.15) |
| 30 mg/kg | 2.16 (.15) | 2.46 (.15) | 1.91 (.16) | 2.00 (.15) |
| | Latency (s) to Enter an Open Quadrant | | | |
| Saline | 6.66 (3.57) | 2.72 (3.23) | 11.84 (3.09) | 19.93 (3.38) |
| 10 mg/kg | 6.68 (3.09) | 4.64 (3.23) | 7.03 (3.23) | 8.77 (3.09) |
| 30 mg/kg | 6.07 (3.09) | 2.26 (3.09) | 7.06 (3.23) | 9.50 (3.39) |
| | Percentage of Time Spent in the Open Quadrants | | | |
| Saline | 16.80 (3.14) | 15.88 (2.84) | 11.62 (2.72) | 15.09 (2.98) |
| 10 mg/kg | 19.57 (2.72) | 19.22 (2.84) | 16.68 (2.84) | 16.24 (2.72) |
| 30 mg/kg | 16.13 (2.72) | 23.09 (2.72) | 9.11 (2.84) | 9.06 (2.72) |

Activity is measured as beam breaks per second spent in the closed quadrants. Numbers in parentheses are standard errors. B6 = C57Bl/6J, D2 = DBA/2J.

**Percentage of time spent in an open quadrant.** ANOVA revealed a main effect of strain, $F$ (1, 123) = 11.335, $p$ = .001, with B6 animals spending a greater percentage of time in the open quadrants of the maze. ANOVA failed to detect significant effects of sex or drug treatment, $F$ (1, 123) = .786, $p$ = .38, and $F$ (2, 123) = 1.935, $p$ = .149, respectively. Likewise, none of the interactions were found to be significant: strain x sex, $F$ (1, 123) = .076, $p$ = .78; strain x drug treatment, $F$ (2, 123) = 2.460, $p$ = .090; sex x

drug treatment, $F$ (2, 123) = .490, $p$ = .61; strain x sex x drug treatment, $F$ (2, 123) = 1.35, $p$ = .36. (See Table 6 for data.)

Open Field

**Total distance traveled.** ANOVA revealed a significant main effect of strain, $F$ (1, 123) = 30.006, $p$ < .001, with B6 mice traveling a greater distance than D2 mice. Likewise, ANOVA revealed a significant effect of sex, $F$ (1, 123) = 6.411, $p$ = .013, with males traveling a greater distance than females. ANOVA failed to detect a significant main effect of drug treatment, $F$ (2, 123) = .704, $p$ = .497. However, a significant strain x sex interaction was found, $F$ (1, 123) = 18.059, $p$ < .001. Analysis of simple effects revealed that B6 males and females did not differ, but D2 males traveled a greater distance than D2 females. Further, while B6 females traveled a greater distance than D2 females, B6 and D2 males did not differ. ANOVA failed to detect significant strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (2, 123) = .413, $p$ = .66; $F$ (2, 123) = .596, $p$ = .55; and $F$ (2, 123) = .623, $p$ = .54; respectively. (See Table 7 for data.)

**Rears.** ANOVA failed to detect significant main effects of strain, sex, or drug treatment: $F$ (1, 123) = 1.537, $p$ = .212; $F$ (1, 123) = .206, $p$ = .65; and $F$ (2, 123) = .653, $p$ = .52; respectively. However, a significant strain x sex interaction was found, $F$ (1, 123) = 11.756, $p$ = .001. Analysis of simple effects revealed that B6 females reared more frequently than B6 males, but D2 males reared more frequently than D2 females. Likewise, D2 males reared more frequently than B6 males, and B6 females reared more frequently than D2 females. ANOVA failed to detect significant strain x drug treatment,

sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (2, 123) = .204, $p$ = .82; $F$ (2, 123) = .156, $p$ = .86; and $F$ (2, 123) = 1.557, $p$ = .22; respectively. (See Table 7 for data.)

**Table 7. Means and standard errors for measures in the open field in Experiment 2 by strain, sex, and drug treatment**

| | B6 | | D2 | |
|---|---|---|---|---|
| Drug Treatment | Male | Female | Male | Female |
| | Total Distance (cm) Traveled | | | |
| Saline | 2031.1 (207.7) | 2155.0 (187.9) | 2010.8 (179.9) | 1012.9 (197.0) |
| 10 mg/kg | 1839.0 (179.9) | 2204.4 (187.9) | 1648.8 (187.9) | 971.8 (179.9) |
| 30 mg/kg | 1868.5 (179.9) | 1934.0 (179.9) | 1682.4 (187.9) | 1166.8 (179.9) |
| | Number of rears | | | |
| Saline | 106.67 (13.90) | 124.00 (12.58) | 125.42 (12.04) | 100.40 (13.19) |
| 10 mg/kg | 101.50 (12.04) | 144.82 (12.58) | 130.91 (12.58) | 91.25 (12.04) |
| 30 mg/kg | 128.42 (12.04) | 132.08 (12.04) | 127.27 (12.58) | 108.00 (12.04) |
| | Habituation Ratio | | | |
| Saline | .442 (.029) | .378 (.026) | .417 (.025) | .379 (.028) |
| 10 mg/kg | .458 (.025) | .362 (.026) | .451 (.026) | .358 (.025) |
| 30 mg/kg | .409 (.025) | .367 (.025) | .421 (.026) | .369 (.025) |
| | Percentage of Time Spent in Corners | | | |
| Saline | 42.09 (1.97) | 44.89 (1.78) | 38.68 (1.71) | 29.22 (1.87) |
| 10 mg/kg | 45.70 (1.71) | 41.80 (1.78) | 37.36 (1.78) | 26.79 (1.71) |
| 30 mg/kg | 39.90 (1.71) | 43.49 (1.71) | 40.09 (1.78) | 26.20 (1.71) |
| | Percentage of Distance Traveled Occurring in the Center | | | |
| Saline | 39.12 (2.25) | 37.77 (2.04) | 26.26 (1.95) | 16.95 (2.13) |
| 10 mg/kg | 37.41 (1.95) | 38.24 (2.04) | 25.98 (2.04) | 13.65 (1.95) |
| 30 mg/kg | 42.08 (1.95) | 34.54 (1.95) | 27.90 (2.04) | 19.45 (1.95) |

Numbers are means. Numbers in parentheses are standard errors. Habituation ratio is the distance traveled during the last five minutes of testing over the sum of the distance traveled during the first and last five minutes of testing. B6 = C57Bl/6J, D2 = DBA/2J.

**Habituation Ratio.** ANOVA failed to detect a main effect of strain, $F$ (1, 123) = .054, $p$ = .82. However, a significant main effect of sex was found, $F$ (1, 123) = 18.097, $p$ < .001, with males demonstrating a greater degree of habituation to the arena than females. ANOVA failed to detect a main effect of drug treatment, $F$ (2, 123) = .420, $p$ = .66. Likewise, no significant strain x sex, strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions were found: $F$ (1, 123) = .043, $p$ = .84; $F$ (2, 123) = .127, $p$ = .88; $F$ (2, 123) = 1.032, $p$ = .36; and $F$ (2, 123) = .114, $p$ = .89; respectively. (See Table 7 for data.)

**Percentage of time spent in the corners of the arena.** ANOVA revealed a main effect of strain, $F$ (1, 123) = 94.476, $p$ < .001, with B6 mice spending a greater percentage of time in the corners than D2 mice. A main effect of sex was also found, $F$ (1, 123) = 26.328, $p$ < .001, with males spending a greater percentage of time in the corners than females. ANOVA failed to detect a significant main effect of drug treatment, $F$ (1, 123) = .584, $p$ = .58. However, a significant strain x sex interaction was found, $F$ (1, 123) = 35.356, $p$ < .001. Analysis of simple effects revealed that while B6 males and females did not differ, D2 males spent a greater percentage of time in the corners than did D2 females. Further, while B6 and D2 males did not differ, B6 females spent a greater percentage of time in the corners than did D2 females. ANOVA failed to detect significant strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (2, 123) = .843, $p$ = .43; $F$ (2, 123) = 1.197, $p$ = .306; and $F$ (2, 123) = 2.430, $p$ = .092; respectively. (See Table 7 for data.)

**Percentage time spent in the center of the arena.** ANOVA revealed a main effect of strain, $F$ (1, 123) = 297.257, $p$ < .001, with B6 mice spending a greater

percentage of time in the center of the arena than D2 mice. Likewise, a main effect of sex was detected, $F$ (1, 123) = 15.924, $p < .001$, with males spending more time in the center than females. ANOVA failed to detect a main effect of drug treatment, $F$ (2, 123) = .217, $p = .81$. A significant strain x sex interaction was found, $F$ (1, 123) = 4.584, $p = .034$. Analysis of simple effects confirmed that B6 mice of both sexes spent more time in the center of the arena than did D2 mice of both sexes. Further, while there was no difference between B6 males and females, D2 females spent more time in the center than D2 males. ANOVA did not detect a significant strain x drug treatment interaction, $F$ (2, 123) = .048, $p = .953$, but the sex x drug treatment interaction was found to be significant, $F$ (2, 123) = 3.475, $p = .034$. Analysis of simple effects found that among females the drug treatment had no effect. However, in males, 30 mg/kg increased the amount of time spent in the center of arena, but 10 mg/kg of the drug had no effect. Further, males and females given SAL or 10 mg/kg of the drug did not differ, but males given 30 mg/kg spent more time in the center than did females given the same dose. Additionally, the strain x sex x drug treatment interaction was found to be significant, $F$ (2, 123) = 4.701, $p = .011$, see Figure 2. Analysis of simple effects showed that under all treatment conditions B6 mice spent more time in the center than D2 mice. Among D2 mice, males and females did not differ, and there was no effect of drug treatment. Among B6 mice administered either saline or 10 mg/kg of the drug, there were no differences between drug treatments or sexes. However, B6 males administered 30 mg/kg of the drug spent more time in the center than B6 males administered SAL or 10 mg/kg of the drug, but an opposite relation was seen among B6 females. Those given SAL or 10 mg/kg of the drug did not differ, but those given 30 mg/kg spent less time in the center than those given 10 mg/kg.

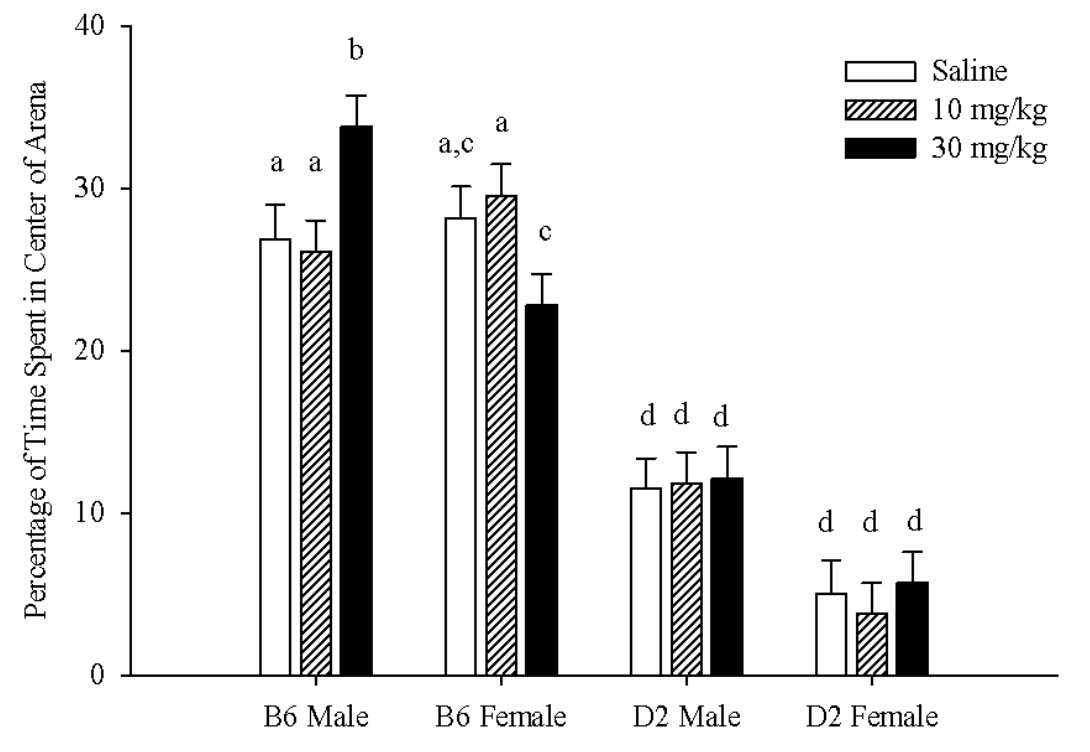


**Figure 2.** The effect of SNRIX on percentage of time spent in the center of the open field arena by strain, sex, and dose. Data are presented as means ± SEM. Groups that do not share a common lower case letter are different at the level, $p < .05$. B6 = C57BL/6J, D2 = DBA/2J.

**Percentage of total distance traveled occurring in the center of the arena.**

ANOVA revealed a main effect of strain, $F$ (1, 123) = 200.182, $p < .001$, with B6 mice traveling a greater distance in the center than D2 mice. Additionally, the main effect of sex was significant, $F$ (1, 123) = 29.729, $p < .001$, with males traveling a greater distance in the center of the arena than females. ANOVA failed to detect a main effect of drug treatment, $F$ (2, 123) = 1.203, $p = .304$. A significant strain by sex interaction was found, $F$ (1, 123) = 9.916, $p = .002$. Analysis of simple effects revealed that both B6 males and females traveled a greater distance in the center than did their respective D2 counterparts. Further, B6 males and females did not differ, but D2 males traveled a greater distance in

the center than did D2 females. ANOVA failed to detect significant strain x drug treatment or sex x drug treatment interactions: $F$ (2, 123) = .750, $p$ = .47, and $F$ (2, 123) = .510, $p$ = .60, respectively. Likewise, the strain x sex x drug treatment interaction failed to reach significance, $F$ (2, 123) = 2.411, $p$ = .094. (See Table 7 for data.)

Hotplate

**Latency to display hindpaw pain response.** ANOVA revealed a main effect of strain, $F$ (1, 123) = 16.980, $p$ < .001, with B6 mice showing a pain response sooner than D2 mice. ANOVA failed to detect a main effect of sex or drug treatment: $F$ (1, 123) = 1.306, $p$ = .255, and $F$ (2, 123) = 1.068, $p$ = .347, respectively. Likewise, ANOVA failed to detect a significant strain x sex, strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interaction: $F$ (1, 123) = .261, $p$ = .611; $F$ (2, 123) = .292, $p$ = .747; $F$ (2, 123) = 1.152, $p$ = .320; and $F$ (2, 123) = 1.158, $p$ = .318; respectively. (See Table 8 for data.)

**Table 8. Mean latency (s) to display a pain response in the hotplate algesia test in Experiment 2 by strain, sex, and drug treatment**

| | B6 | | D2 | |
|---|---|---|---|---|
| Drug Treatment | Male | Female | Male | Female |
| Saline | 15.28 (1.58) | 15.50 (1.43) | 17.67 (1.37) | 19.73 (1.50) |
| 10 mg/kg | 12.29 (1.37) | 14.70 (1.43) | 16.54 (1.43) | 18.75 (1.37) |
| 30 mg/kg | 14.36 (1.37) | 15.78 (1.37) | 19.08 (1.43) | 16.36 (1.37) |

Numbers in parentheses are standard errors. B6 = C57Bl/6J, D2 = DBA/2J.

Light-Dark

Although all animals were tested in the light/ dark apparatus, a computer file was lost resulting in data being lost for four B6 males: two received saline, one treated with 10 mg/kg SNRIX, and 1 treated with 30 mg/kg SNRIX. To determine the nature of the missing data, we examined the correlations of the missing data with all other variables examined in the experiment. The largest correlation was with activity in the EZM, $r$ = .229, $p$ = .039. Considering that the significant correlations found were relatively weak, the decision was made to proceed with the analysis despite the data not being missing at random.

**Percentage of time spent in the light.** ANOVA revealed a main effect of strain, $F$ (1, 119) = 54.108, $p$ < .001, with B6 mice spending a greater percentage of time in the light side of the apparatus. Additionally, a main effect of sex was found, $F$ (1, 119) = 18.595, $p$ < .001, with males spending more time in the light than females. ANOVA failed to detect a significant main effect of drug treatment, $F$ (2, 119) = 1.223, $p$ = .298. However a significant strain x sex interaction was found, $F$ (1, 119) = 4.678, $p$ = .033. Analysis of simple effects revealed that B6 males and females did not differ while D2 males spent more time in the light half of the apparatus than did D2 females. Further, B6 males spent more time in the light than D2 males, and, likewise, B6 females spent more time in the light than did D2 females. ANOVA failed to detect a significant strain x drug treatment interaction, $F$ (2, 119) = .831, $p$ = .438. ANOVA failed to detect a significant sex x drug treatment interaction despite a trend towards significance, $F$ (2, 119) = 2.855, $p$ = .061. Likewise, although not significant, ANOVA revealed a trend towards a significant strain x sex x drug treatment interaction, $F$ (2, 119) = 2.798, $p$ = .065. See

Figure 3. Further inspection suggested that 10 and 30 mg/kg SNRIX resulted in B6 males spending a decreased amount of time in the light side of the apparatus as compared to saline treated B6 males while neither dose had an effect on any other strain and sex combination as compared to their respective control. Further, of those animals in the saline condition, B6 males spent the greatest percentage of time in the light followed by B6 females and D2 males, which did not differ, and then D2 females. B6 males, B6 females, and D2 males administered 10 mg/kg SNRIX did not differ whereas D2 females administered the same dose spent less time in the light than all three. Likewise, the same pattern of response was seen for those animals treated with 30 mg/kg SNRIX.

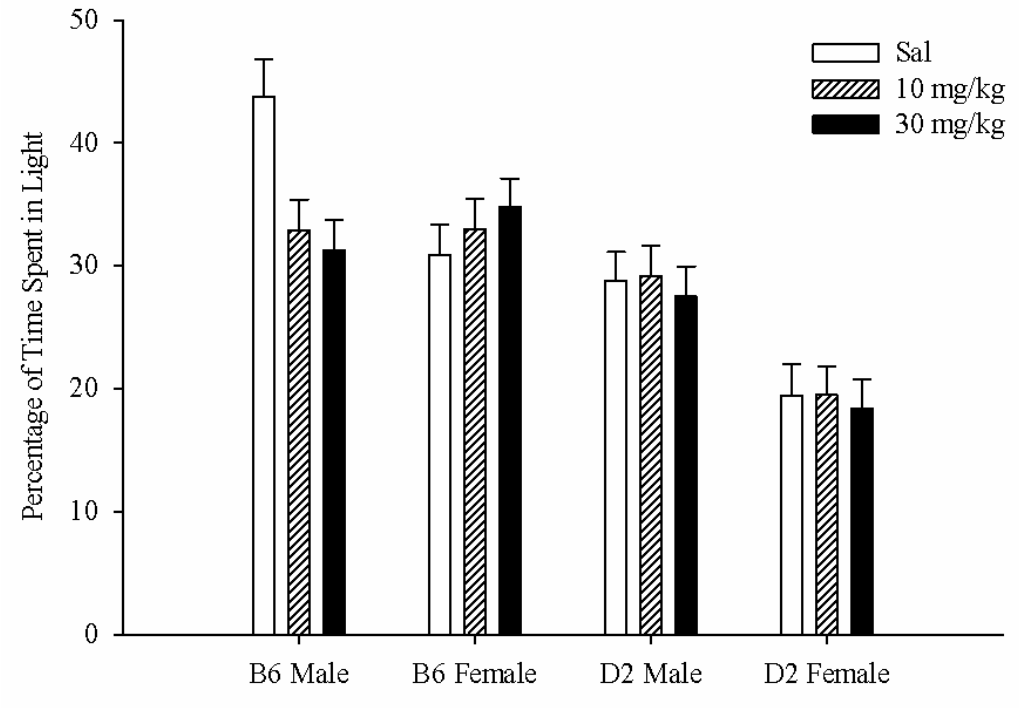


**Figure 3.** The effect of SNRIX on percentage of time spent in the light side of the light-dark apparatus by drug, sex, and dosage. Data are presented as means ± SEM. The strain by sex by drug treatment interaction did not quite reach significance ($F$ (2, 119) = 2.798, $p$ = .065, ES: $f$ = .217). B6 = C57BL/6J, D2 = DBA/2J.

**Total distance traveled in the light/ dark apparatus.** ANOVA revealed a significant main effect of strain, $F$ (1, 119) = 4.718, $p$ = .032, with B6 mice traveling a greater distance than D2 mice. However, the main effects of sex and drug treatment were not found to be significant, $F$ (1, 119) = 1.911, $p$ = .17, and $F$ (2, 119) = .188, $p$ = .83, respectively. ANOVA did find a significant strain x sex interaction, $F$ (1, 119) = 19.280, $p$ < .001. Analysis of simple effects revealed that male B6 and D2 animals did not differ, but female B6 animals traveled a greater distance than female D2 animals. When comparing male and female B6 mice, it was found that females traveled further than males. On the other hand, male D2 mice traveled further than female D2 mice. No other significant interactions were found: strain x drug treatment, $F$ (2, 119) = .236, $p$ = .79; sex x drug treatment, $F$ (2, 119) = 1.245, $p$ = .292; and strain x sex x drug treatment, $F$ (2, 119) = .669, $p$ = .52. (See Table 9 for data.)

**Percentage of total distance traveled occurring in the light side of the apparatus.** ANOVA revealed a significant main effect of strain, $F$ (1, 119) = 144.405, $p$ < .001, with the percentage of distance traveled occurring in the light by B6 mice being greater than that of D2 mice. A significant main effect of sex was also found, $F$ (1, 119) = 12.714, $p$ = .001, with the percentage of total distance traveled in the light being greater for males than females. ANOVA failed to detect a main effect of drug treatment, $F$ (2, 119) = .004, $p$ = .996. Likewise, ANOVA did not detect significant, strain x sex, strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (1, 119) = .845, $p$ = .36; $F$ (2, 119) = .857, $p$ = .427; $F$ (2, 119) = 1.619, $p$ = .203; and $F$ (2, 119) = 1.721, $p$ = .183; respectively. (See Table 9 for data.)

**Table 9. Mean distance (cm) traveled and mean percentage of that distance traveled occurring in the light side of the light-dark apparatus by strain, sex, and drug treatment in Experiment 2**

| | B6 | | D2 | |
|---|---|---|---|---|
| Drug Treatment | Male | Female | Male | Female |
| | Total Distance Traveled | | | |
| Saline | 1006.9 (93.0) | 1142.6 (74.2) | 1191.3 (71.1) | 813.7 (77.8) |
| 10 mg/kg | 1008.4 (74.2) | 1097.8 (74.2) | 1116.7 (74.2) | 839.4 (71.1) |
| 30 mg/kg | 990.6 (74.2) | 1157.6 (71.1) | 987.1 (74.2) | 889.4 (71.1) |
| | Percentage of Total Distance Occurring in Light | | | |
| Saline | 40.26 (2.30) | 32.13 (1.84) | 23.40 (1.76) | 19.05 (1.93) |
| 10 mg/kg | 35.06 (1.84) | 33.99 (1.84) | 25.93 (1.84) | 19.38 (1.76) |
| 30 mg/kg | 34.26 (1.84) | 34.91 (1.76) | 24.46 (1.84) | 20.90 (1.76) |

Numbers in parentheses are standard errors. B6 = C57Bl/6J, D2 = DBA/2J.

Acoustic Startle/ Prepulse Inhibition

**Response to 120 db startle stimulus.** ANOVA revealed a significant main effect of strain, $F$ (1, 123) = 156.750, $p < .001$, with B6 mice displaying a greater response than D2 mice. A significant main effect of sex was found as well, $F$ (1, 123) = 14.926, $p < .001$, with males responding more forcefully than females. ANOVA failed to detect a significant main effect of drug treatment, $F$ (2, 123) = .232, $p = .794$. A significant strain x sex interaction was found, $F$ (1, 123) = 6.883, $p = .01$. Analysis of simple effects revealed that B6 males displayed a greater startle response than did B6 females, but D2 males and females did not differ. Additionally, the response of B6 males was greater than D2 males, and B6 females displayed a greater response than D2 females. ANOVA failed to detect significant strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (2, 123) = .545, $p = .581$; $F$ (2, 123) = .031, $p = .969$; and $F$ (2, 123) = .001, $p = .999$; respectively.

**Percentage of prepulse inhibition of the startle response at 70 db.** ANOVA revealed a significant main effect of strain, $F$ (1, 123) = 49.846, $p < .001$**,** with B6 mice displaying a greater degree of inhibition than D2 mice. ANOVA failed to detect a significant main effect of sex, $F$ (1, 123) = .810, $p = .37$. There was a non-significant trend towards an effect of drug treatment, $F$ (2, 123) = 2.832, $p = .063$, see Figure 4. Further inspection showed that while 10 mg/kg SNRIX was no different than SAL, animals administered 30 mg/kg SNRIX displayed a greater degree of inhibition than did those administered SAL. No significant interactions were found: strain x sex, $F$ (1, 123) = 1.780, $p = .185$; strain x drug treatment, $F$ (2, 123) = .142, $p = .867$; sex x drug treatment, $F$ (2, 123) = .013, $p = .849$; or strain x sex x drug treatment, $F$ (2, 123) = .823, $p = .442$.

**Percentage of prepulse inhibition of the startle response at 80 db.** ANOVA revealed a significant main effect of strain, $F$ (1, 123) = 158.469, $p < .001$, with B6 mice displaying a greater degree of inhibition than D2 mice. A significant main effect of sex was also found, $F$ (1, 123) = 9.426, $p = .003$, with males showing more inhibition than females. Additionally, ANOVA revealed a significant main effect of drug treatment, $F$ (2, 123) = 4.291, $p = .016$. See Figure 4. *Post hoc* analysis indicated that animals administered 10 mg/kg SNRIX did not differ from control animals. However, animals treated with 30 mg/kg SNRIX displayed a greater degree of inhibition than either those treated with 10 mg/kg SNRIX or saline. ANOVA failed to detect significant strain x sex, strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (1, 123) = 2.143, $p = .146$; $F$ (2, 123) = 1.929, $p = .150$; $F$ (2, 123) = .820, $p = .443$; and $F$ (2, 123) = 1.652, $p = .196$; respectively.

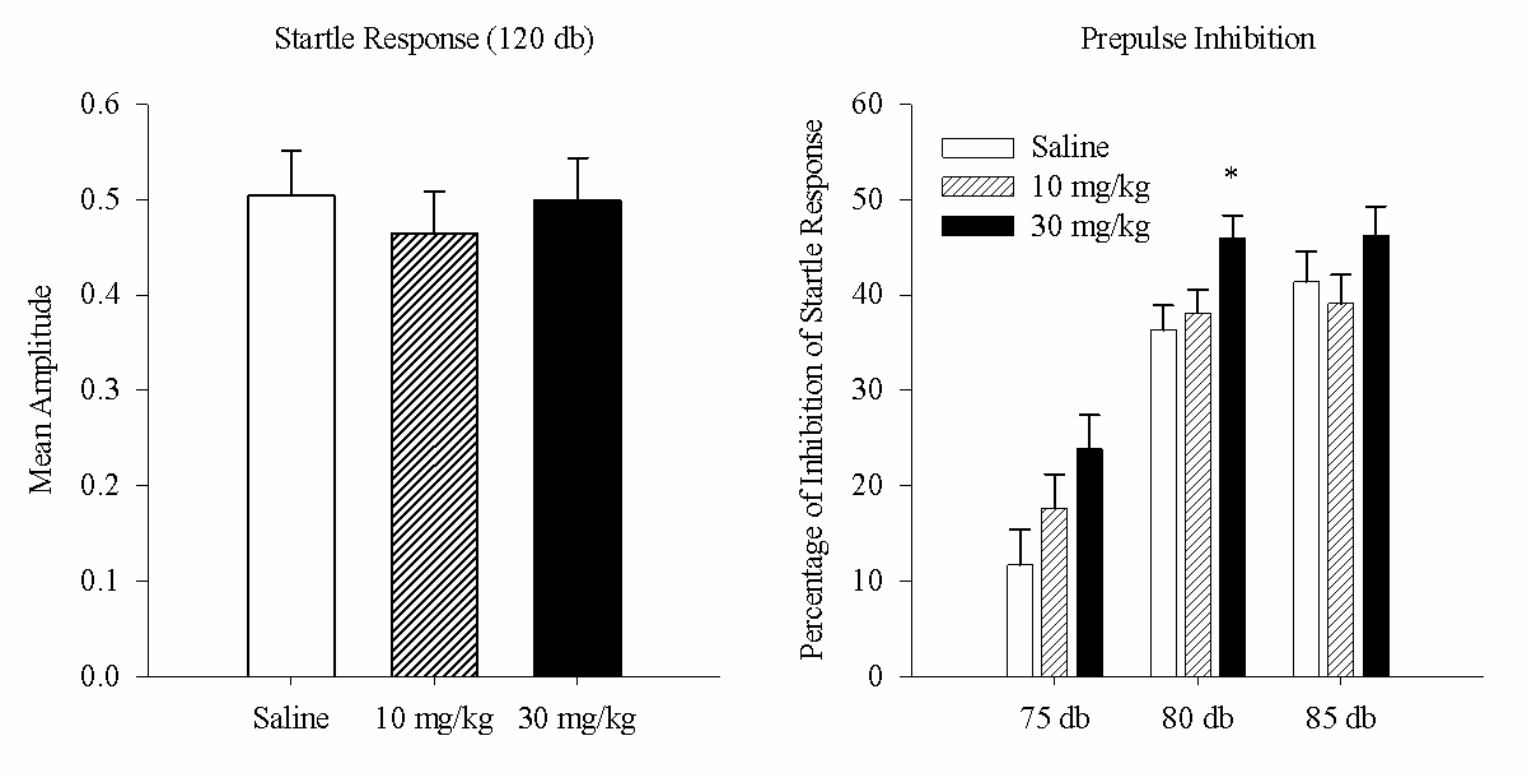


**Figure 4.** The effect of SNRIX on startle response and prepulse inhibition of the startle response. Data are presented as means ± SEM. SNRIX had no effect on the startle response to a 120 db white noise burst. 30 mg/kg, but not 10 mg/kg SNRIX increased inhibition of the startle response with the 80 db prepulse (* = $p < .02$ compared to saline). A similar effect was seen with the 75 db prepulse, but this did not quite reach significance ($F$ (2, 123) = 2.832, $p$ = .063, ES: $f$ = .215) SNRIX had no effect on prepulse inhibition at 85 db.

**Percentage of prepulse inhibition of the startle response at 85 db.** ANOVA detected a significant main effect of strain, $F$ (1, 123) = 113.078, $p < .001$, with B6 mice displaying a greater degree of inhibition than D2 mice. ANOVA failed to detect significant main effects of sex or drug treatment: $F$ (1, 123) = 2.869, $p = .093$, and $F$ (2, 123) = 1.492, $p = .229$, respectively. Likewise, ANOVA failed to detect significant strain x sex, strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (1, 123) = .469, $p = .495$; $F$ (2, 123) = 2.369, $p = .098$; $F$ (2, 123) = .448, $p = .64$; and $F$ (2, 123) = .034, $p = .967$; respectively.

Fear Conditioning

**Training:** *Baseline activity.* ANOVA revealed a significant main effect of strain, $F$ (1, 123) = 163.795, $p < .001$, with B6 mice being more active than D2 mice. ANOVA failed to detect significant main effects of sex or drug treatment: $F$ (1, 123) = .722, $p = .397$, and $F$ (2, 123) = .047, $p = .954$, respectively. ANOVA did uncover a significant strain x sex interaction, $F$ (1, 123) = 5.614, $p = .019$. Analysis of simple effects revealed that B6 females were more active than B6 males, but D2 males and females did not differ. Further, B6 males were more active than D2 males, and B6 females were more active than D2 females. ANOVA failed to detect significant strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (2, 123) = .009, $p = .991$; $F$ (2, 123) = .651, $p = .523$; and $F$ (2, 123) = .061, $p = .941$; respectively.

*Suppression of activity by training.* ANOVA revealed a non-significant trend towards an effect of strain, $F$ (1, 123) = 3.706, $p = .057$, with B6 mice showing a greater suppression of activity than D2 mice. No other effects approached significance: sex, $F$ (1, 123) = .981, $p = .324$; drug treatment, $F$ (2, 123) = .748, $p = .476$; strain x sex, $F$ (1, 123)

= .900, $p$ = .345; strain x drug treatment, $F$ (2, 123) = 1.760, $p$ = .176; sex x drug treatment, $F$ (2, 123) = 1.021, $p$ = .363; and strain x sex x drug treatment, $F$ (2, 123) = .314, $p$ = .731. (See Table 10 for data.)

**Table 10. Means and standard errors of fear conditioning measures in Experiment 2 by strain, age, and sex**

| | B6 | | D2 | |
|---|---|---|---|---|
| Drug Treatment | Male | Female | Male | Female |
| | Suppression of Activity by Training | | | |
| Saline | .336 (.125) | .282 (.113) | .344 (.108) | .407 (.118) |
| 10 mg/kg | .311 (.108) | .304 (.113) | .325 (.113) | .321 (.108) |
| 30 mg/kg | .228 (.108) | .297 (.108) | .395 (.113) | .712 (.108) |
| | Suppression of Activity by Training Context | | | |
| Saline | .336 (.060) | .323 (.054) | .702 (.052) | .633 (.057) |
| 10 mg/kg | .313 (.052) | .361 (.054) | .795 (.054) | .543 (.052) |
| 30 mg/kg | .352 (.052) | .347 (.052) | .707 (.054) | .645 (.052) |
| | Suppression of Activity by Cue | | | |
| Saline | .238 (.050) | .192 (.045) | .258 (.043) | .223 (.047) |
| 10 mg/kg | .220 (.043) | .190 (.045) | .342 (.045) | .288 (.043) |
| 30 mg/kg | .222 (.043) | .153 (.043) | .217 (.045) | .269 (.043) |

Numbers are means, and numbers in parentheses are standard errors. Suppression of activity by training is calculated as beam breaks during the 30 seconds following the final tone-shock pairing divided by the average number of beam breaks per 30 second bin during the first two minutes of the training session. Suppression of activity by the training context is calculated as the average number of beam breaks per 30 second bin during exposure to the training context on the second day divided by the average number of beam breaks per 30 second bin during the first two minutes of the training session. Suppression of activity by the cue is calculated as the average number of beam breaks per 30 second bin during the first three minutes of exposure to the altered context on the second day divided by the average number of beam breaks per 30 second bin during the presentation of the tone in the altered context. (Smaller numbers indicate a greater suppression of activity.) B6 = C57Bl/6J, D2 = DBA/2J

**Contextual conditioning:** *Suppression of baseline activity by training context.* ANOVA detected a significant main effect of strain, $F$ (1, 123) = 114.865, $p < .001$, with B6 mice displaying less activity in the training context than D2 mice relative to their baseline activity during training. ANOVA failed to detect main effects of sex or drug treatment: $F$ (1, 123) = 3.598, $p = .06$, and $F$ (2, 123) = .070, $p = .933$, respectively. However, ANOVA revealed a significant strain x sex interaction, $F$ (1, 123) = 4.929, $p = .028$. Analysis of simple effects revealed that B6 males and females did not differ, but D2 males displayed a lesser suppression of activity in response to the training context than did D2 females. Further, D2 mice of both sexes were more active during exposure to the training context as compared to their baseline activity than B6 mice of the respective sex. ANOVA failed to detect significant strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (2, 123) = .012, $p = .988$; $F$ (2, 123) = .504, $p = .605$; and $F$ (2, 123) = 1.746, $p = .179$, respectively. (See Table 10 for data.)

**Cued conditioning:** *Activity in the altered context.* ANOVA failed to detect a significant main effect of strain, $F$ (1, 123) = 2.091, $p = .151$. However, a significant main effect of sex was found, $F$ (1, 123) = 3.993, $p = .048$, with females showing a lower level of activity in the altered context than males. ANOVA failed to detect a significant effect of drug treatment, $F$ (2, 123) = .758, $p = .471$. The strain x sex interaction was significant, $F$ (1, 123) = 6.690, $p = .011$. Analysis of simple effects revealed that B6 males and females did not differ, but D2 females were less active than D2 males. Additionally, B6 and D2 males did not differ, but D2 females were less active than B6 females. ANOVA failed to detect significant strain x drug treatment, sex x drug

treatment, or strain x sex x drug treatment interactions: $F$ (2, 123) = .422, $p$ = .657; $F$ (2, 123) = .484, $p$ = .618; and $F$ (2, 123) = 1.487, $p$ = .230, respectively.

*Cue suppression of activity in the altered context.* ANOVA revealed a significant main effect of strain, $F$ (1, 123) = 6.093, $p$ = .015, with B6 mice displaying a greater reduction in activity during the presentation of the tone than D2 mice. ANOVA failed to detect significant main effects of sex or drug treatment: $F$ (1, 123) = 1.385, $p$ = .242, and $F$ (2, 123) = 1.092, $p$ = .339, respectively. Likewise, ANOVA failed to detect significant strain x sex, strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (1, 123) = .488, $p$ = .486; $F$ (2, 123) = .917, $p$ = .402; $F$ (2, 123) = .193, $p$ = .825; and $F$ (2, 123) = .750, $p$ = .475; respectively. (See Table 10 for data.)

Tail Suspension

All animals were tested on tail suspension, but data from 10 mice were lost due to a computer error. Of those animals seven were B6 (3 males receiving 10 mg/kg SNRIX, 1 female receiving saline, 1 female receiving 10 mg/kg SNRIX, and 2 females receiving 30 mg/kg SNRIX) and three were D2 (1 male receiving 10 mg/kg SNRIX and 2 females receiving 30 mg/kg SNRIX). We examined the correlations of the missing data with all other variables included in the experiment to determine if the data was missing at random. The missing data was significantly correlated with three variables: activity during the final 30 s of training for fear conditioning, $r$ = .230, $p$ = .007; activity in the altered context of fear conditioning, $r$ = .205, $p$ = .017; and tail climbing during tail suspension testing, $r$ = - .180, $p$ = .036. Although the data can not be said to be missing at random, the correlations are relatively small, and the analysis was carried out on available data.

**Tail climbing.** We observed a large number of animals that climbed their tails during the tail suspension procedure, 31.2 %. Data for tail climbing was recorded (coded dichotomously) in the experimenter's notes, and therefore no data was lost for this variable. Logistic regression analysis indicated that strain did not predict tail climbing, *OR*: .701, $p = .375$, *95% CI*: .320 – 1.536. However, sex did predict tail climbing, *OR*: 3.766, $p = .001$, *95% CI*: 1.668 – 8.500, with males being over three times more likely to climb their tails than females. Drug treatment was not associated with tail climbing, $p = .586$ (10 mg/kg SNRIX versus SAL: *OR*: 1.104, $p = .837$, *95% CI*: .429 – 2.838; 30 mg/kg SNRIX versus SAL: *OR*: .680, $p = .442$, *95% CI*: .255 – 1.818). Animals that climbed their tail were omitted from further analysis of tail suspension data.

**Time below threshold (immobility):** *Time below threshold per 30 s block of the six minute session.* The six minute session was divided into 30 s intervals and analyzed via a repeated measures ANOVA using interval as a within subjects variable. Repeated measures ANOVA revealed a significant effect of interval, $F(11, 814) = 66.132$, $p < .001$. Further, inspection of the data showed that time spent below threshold did not change during the first two intervals. Immobility increased between the third and eighth interval at which point it reached a plateau. Repeated measures ANOVA revealed a significant interval by strain interaction, $F(11, 814) = 8.466$, $p < .001$. Inspection of the data showed that B6 mice initially exhibited less immobility than D2 mice. However, B6 mice demonstrated increasing immobility at a rate greater than D2 mice resulting in B6 mice reaching a greater level of immobility than D2 mice from interval six through ten. B6 and D2 mice did not differ between intervals 10 and 12. Repeated measures ANOVA failed to detect any further significant interactions: interval x sex, $F(11, 814) = .986$, $p =$

.457; interval x drug treatment, $F$ (22, 814) = .953, $p$ = .524; interval x strain x sex, $F$ (11, 814) = .597, $p$ = .832; interval x strain x drug treatment, $F$ (22, 814) = .876, $p$ = .627; interval x sex x drug treatment, $F$ (22, 814) = .975, $p$ = .495; or interval x strain x sex x drug treatment, $F$ (22, 814) = 1.036, $p$ = .416.

*Overall time below threshold.* ANOVA failed to detect a significant main effect of strain, $F$ (1, 74) = 2.729, $p$ = .103. A significant main effect of sex was found, $F$ (1, 74) = 4.089, $p$ = .047, with females spending a greater amount of time immobile than males. ANOVA failed to detect a significant main effect of drug treatment, $F$ (2, 74) = .361, $p$ = .698. Likewise, ANOVA failed to detect significant strain x sex, strain x drug treatment, sex x drug treatment, or strain x sex x drug treatment interactions: $F$ (1, 74) = .164, $p$ = .687; $F$ (2, 74) = 1.143, $p$ = .324, $F$ (2, 74) = .648, $p$ = .526; and $F$ (2, 74) = .615, $p$ = .544; respectively. (See Table 11 for data.)

**Table 11. Mean time (s) spent below threshold (immobility) during the tail suspension test in Experiment 2 by strain, sex, and drug treatment**

| | B6 | | D2 | |
|---|---|---|---|---|
| Drug Treatment | Male | Female | Male | Female |
| Saline | 177.00 (23.45) | 210.56 (15.64) | 152.00 (17.73) | 172.38 (16.58) |
| 10 mg/kg | 209.00 (23.45) | 192.10 (14.83) | 162.80 (20.98) | 188.57 (17.73) |
| 30 mg/kg | 159.17 (19.15) | 194.00 (14.83) | 164.89 (15.64) | 196.00 (17.73) |

Animals that climbed their tails were not included in the analysis. Numbers in parentheses are standard errors. B6 = C57Bl/6J, D2 = DBA/2J.

## Experiment 3

Elevated Zero Maze

**Activity in the closed quadrants.** ANOVA found a significant effect of strain, $F$ (1, 54) = 18.004, $p < .001$, with B6 mice being more active than D2 mice. Likewise, the effect of age was significant, $F$ (1, 54) = 3.287, $p = .045$. (See Figure 5.) 6wk mice were the most active followed by 8wk and 4wk mice. Post hoc testing showed that the 8wk mice were the same as 4wk or 6wk, but the 6wk mice were more active than their 4wk counterparts. The strain by age interaction failed to reach significance, $F$ (2, 54) = .763, $p = .471$. (Data are presented in Table 12.)

**Latency to enter an open quadrant.** ANOVA indicated a significant effect of strain, $F$ (1, 54) = 12.849, $p = .001$, with D2 mice showing a longer latency than B6 mice. However, there was no effect of age, $F$ (2, 54) = 1.903, $p = .159$, and the strain by age interaction was non-significant, $F$ (2, 54) = .459, $p = .634$. (Data are presented in Table 12.)

**Percentage of time spent in an open quadrant.** ANOVA revealed a significant effect of strain, $F$ (1, 54) = 7.067, $p = .01$, with B6 mice spending more time in the open than D2 mice. There was a trend towards a main effect of age, but this did not quite reach significance, $F$ (2, 54) = 2.971, $p = .060$. (See Figure 5.) 8wk mice spent the least amount of time in the open followed by 4wk and 6wk mice, which spent the greatest amount of time in the open. The strain by age interaction was found to be non-significant, $F$ (2, 54) = .975, $p = .384$. (Data are presented in Table 12.)

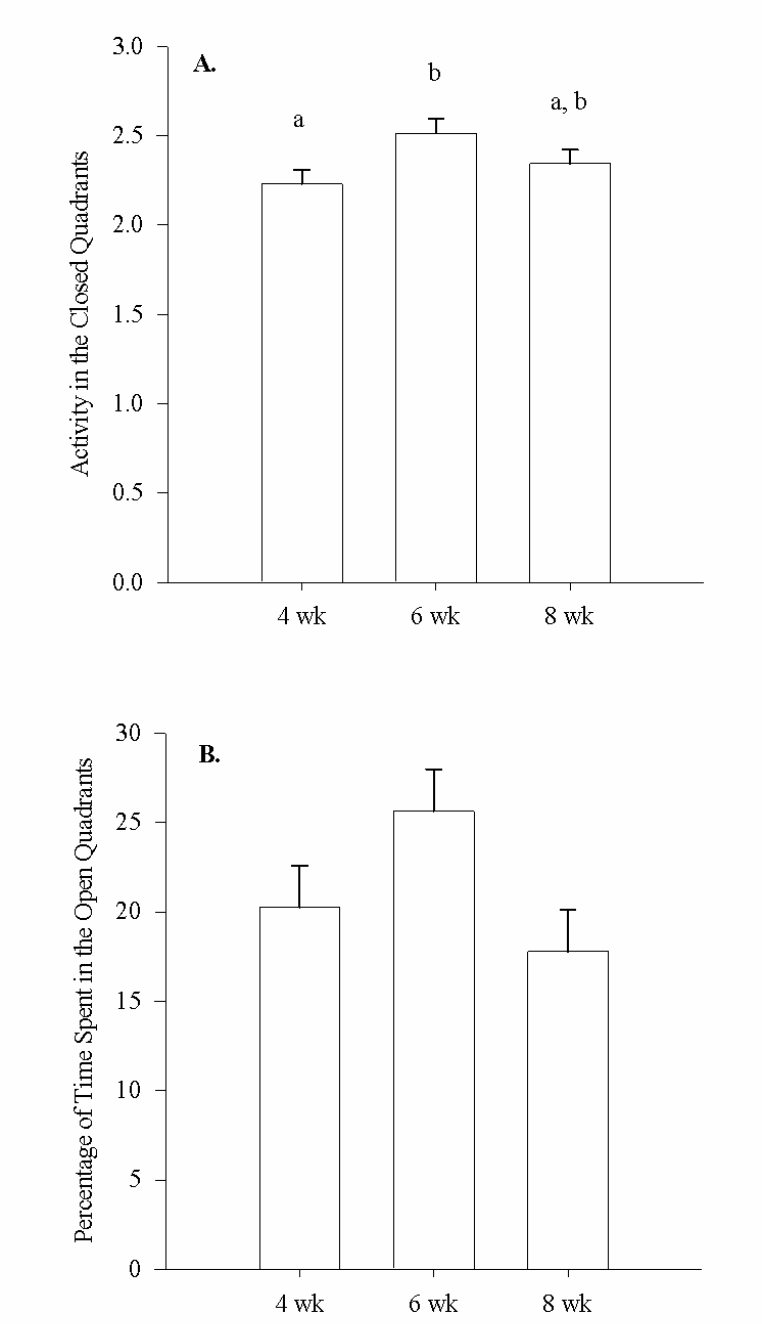


**Figure 5.** Age-related differences in behavior in the elevated zero maze. Data are presented as Means ± SEM **A.)** Activity in the closed quadrants of the maze. Activity is measured as beam breaks per second spent in the closed quadrants. Groups that do not share a common lowercase letter are different at the level, $p < .05$. **B.)** Percentage of time spent in the open quadrants of the maze. The main effect of age did not quite reach significance ($F$ (2, 54) = 2.971, $p$ = .060, ES: $f$ = .331). 4 wk = four weeks of age, 6 wk = six weeks of age, 8 wk = eight weeks of age.

**Table 12. Means and standard errors of measures in the elevated zero maze by age and strain**

| | 4 weeks | 6 weeks | 8 weeks |
|---|---|---|---|
| Strain | Percentage of Time in the Open Quadrants | | |
| B6 | 23.28 (3.29) | 27.23 (3.29) | 23.88 (3.29) |
| D2 | 17.23 (3.29) | 24.03 (3.29) | 11.68 (3.29) |
| | Activity in the Closed Quadrants | | |
| B6 | 2.49 (.11) | 2.63 (.11) | 2.55 (.11) |
| D2 | 1.97 (.11) | 2.40 (.11) | 2.14 (.11) |
| | Latency (s) to Enter an Open Quadrant | | |
| B6 | 4.09 (3.22) | 5.89 (3.22) | 7.29 (3.22) |
| D2 | 10.52 (3.22) | 15.15 (3.22) | 19.89 (3.22) |

Numbers are means, and numbers in parentheses are standard errors. Activity is measured as beam breaks per second in the closed quadrants. B6 = C57Bl/6J, D2 = DBA/2J.

Open Field

**Total distance traveled.** ANOVA revealed a significant effect of strain on the distance traveled in the open field, $F$ (1, 54) = 22.701, $p < .001$, with B6 mice traveling further than D2 mice. The main effect of age and the strain by age interaction failed to reach significance, $F$ (2, 54) = .497, $p = .611$, and $F$ (2, 54) = 1.813, $p = .173$, respectively. (Data are presented in Figure 6.)

**Rears.** ANOVA found no effects of the independent variables on the number of rears in the open field: strain, $F$ (1, 54) = .019, $p = .892$; age, $F$ (2, 54) = .144, $p = .866$; and strain by age, $F$ (2, 54) = 1.501, $p = .232$. (Data are presented in Table 13.)

**Habituation Ratio.** ANOVA indicated that the independent variables were without effect on the habituation to the open field: strain, $F$ (1, 54) = .671, $p = .416$; age,

$F$ (2, 54) = 1.538, $p$ = .224; and strain by age, $F$ (2, 54) = 2.014, $p$ = .143. (Data are presented in Table 13.)

**Percentage of time spent in the corners of the arena.** ANOVA revealed a significant effect of strain on time spent in the corners, $F$ (1, 54) = 47.051, $p$ < .001, with B6 mice spending more time in the corners than D2 mice. However, there was no effect of age, $F$ (2, 54) = .909, $p$ = .409, and the strain by age interaction was not significant, $F$ (2, 54) = .143, $p$ = .867. (Data are presented in Table 13.)

**Table 13. Means and standard errors of number of rears, habituation ratio, and percentage of time spent in the corners of the open field by age and strain**

| | 4 weeks | 6 weeks | 8 weeks |
|---|---|---|---|
| Strain | Rears | | |
| B6 | 128.2 (15.53) | 141.2 (15.53) | 147.5 (15.53) |
| D2 | 157.1 (15.53) | 142.4 (15.53) | 122.6 (15.53) |
| | Habituation Ratio | | |
| B6 | .378 (.036) | .403 (.036) | .377 (.036) |
| D2 | .486 (.036) | .382 (.036) | .362 (.036) |
| | Percentage of Time Spent in Corners of the Arena | | |
| B6 | 45.99 (2.08) | 44.00 (2.08) | 43.51 (2.08) |
| D2 | 34.06 (2.08) | 33.60 (2.08) | 30.96 (2.08) |

Numbers are means, and numbers in parentheses are standard errors. Habituation ratio is the distance traveled during the last five minutes of testing over the sum of the distance traveled during the first and last five minutes of testing. B6 = C57Bl/6J, D2 = DBA/2J.

**Percentage time spent in the center of the arena.** ANOVA found a significant effect of strain, $F$ (1, 54) = 74.792, $p$ < .001, with B6 mice spending more time in the center of the arena than D2 mice. The effect of age did not reach significance, $F$ (2, 54) = 1.223, $p$ = .302, but there was a significant strain by age interaction, $F$ (2, 54) = 5.625, $p$

= .006. (See Figure 6.) Analysis of simple effects showed that at all ages B6 mice spent more time in the center than D2 mice. Among B6 mice, 4wk and 6wk did not differ, but 8wk mice spent more time in the center than either of the younger age groups. On the other hand, there was no difference between the age groups for D2 mice.

**Percentage of total distance traveled occurring in the center of the arena.** ANOVA revealed a significant main effect of strain, $F$ (1, 54) = 49.635, $p < .001$, with B6 mice traveling a greater distance in the center of the arena than D2 mice. The effect of age failed to reach significance, $F$ (2, 54) = .114, $p = .893$, but there was a significant strain by age interaction, $F$ (2, 54) = 3.729, $p = .030$. (See Figure 6.) Analysis of simple effects was not able to detect any differences by age for either strain, but confirmed that at all ages B6 mice traveled a greater distance than D2 mice.

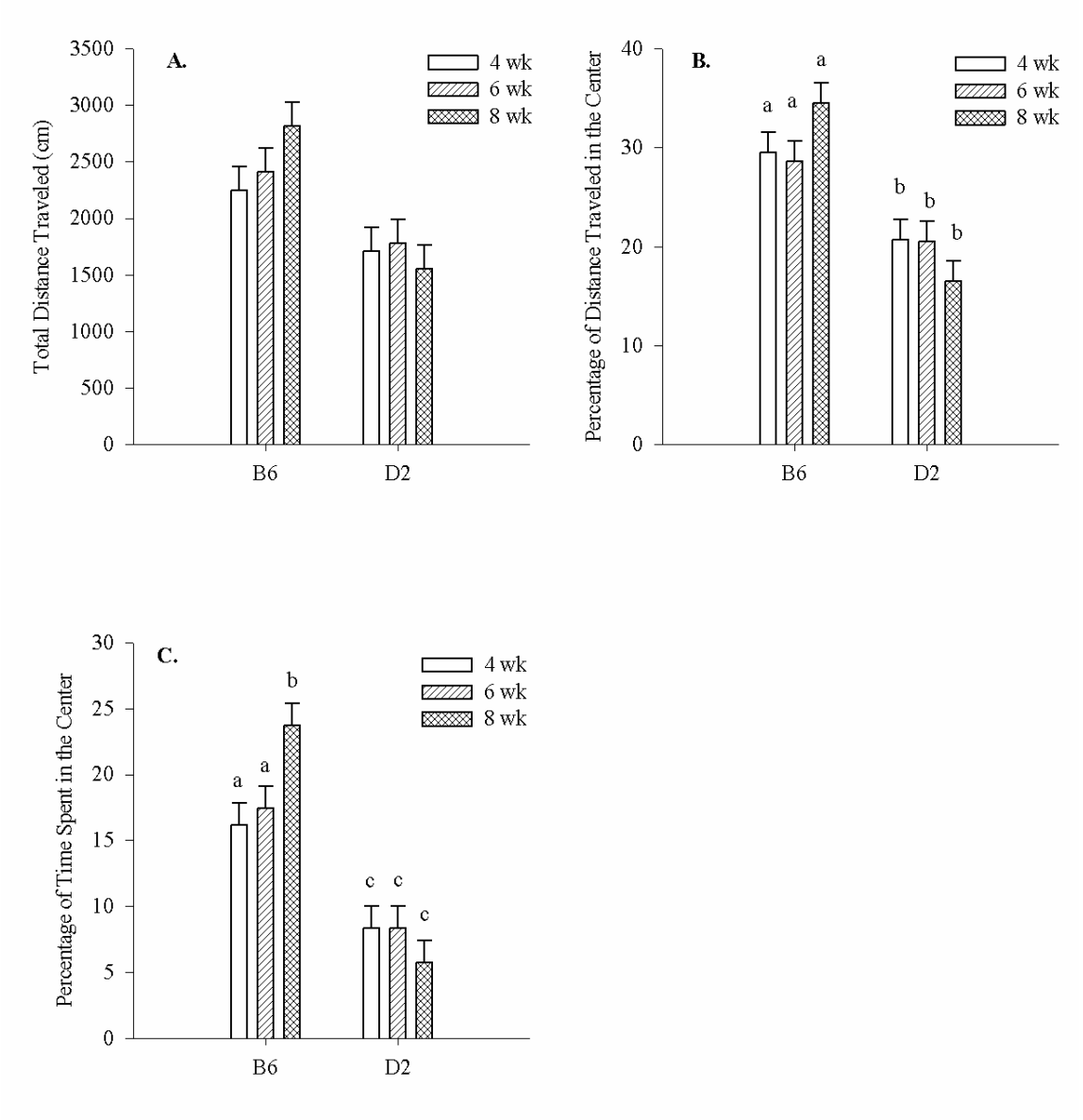


**Figure 6.** Effect of age and strain on measures in the open field. Data are presented as means ± SEM. **A.)** Total distance traveled (cm). The strain by age interaction was not significant and is presented only for the purpose of comparison. **B.)** The percentage of the total distance traveled that occurred in the center of the open field. **C.)** Percentage of time spent in the center of the open field. Groups that do not share a common lowercase letter are different at the level, $p < .05$. 4 wk = four weeks of age, 6 wk = six weeks of age, 8 wk = eight weeks of age, B6 = C57Bl/6J, D2 = DBA/2J.

Hotplate

**Latency to display hindpaw pain response.** ANOVA did not find any effects of the independent variables on latency to display a pain response: strain, $F$ (1, 54) = .005, $p$ = .944; age, $F$ (2, 54) = .011, $p$ = .989; strain by age, $F$ (2, 54) = 1.164, $p$ = .320. (Data are presented in Table 14.)

**Table 14. Mean latency (s) to display a pain response in the hotplate algesia test by age and strain**

| Strain | 4 weeks | 6 weeks | 8 weeks |
|---|---|---|---|
| B6 | 17.75 (2.16) | 17.65 (2.16) | 14.70 (2.16) |
| D2 | 16.16 (2.16) | 15.70 (2.16) | 18.63 (2.16) |

Numbers in parentheses are standard errors. B6 = C57Bl/6J, D2 = DBA/2J.

Light-Dark

**Percentage of time spent in the light.** ANOVA revealed a significant effect of strain, $F$ (1, 54) = 13.688, $p$ = .001, with B6 mice spending more time in the light side of the apparatus. However, the effect of age and the strain by age interaction were not significant, $F$ (2, 54) = 1.852, $p$ = .167, and $F$ (2, 54) = 1.435, $p$ = .247, respectively. (Data are presented in table 15.)

**Total distance traveled in the light-dark apparatus.** ANOVA showed that there was a significant effect of strain, $F$ (1, 54) = 7.267, $p$ = .009, with B6 mice traveling a greater distance than D2 mice. The effect of age and the age by strain interaction were not significant, $F$ (2, 54) = 1.254, $p$ = .294, and $F$ (2, 54) = 1.705, $p$ = .191, respectively. (Data are presented in table 15.)

**Table 15. Means and standard errors of measures in the light-dark apparatus by age and strain**

| | 4 weeks | 6 weeks | 8 weeks |
|---|---|---|---|
| Strain | Percentage of Time Spent in the Light Side | | |
| B6 | 39.86 (2.69) | 37.15 (2.69) | 37.63 (2.69) |
| D2 | 32.15 (2.69) | 33.35 (2.69) | 24.73 (2.69) |
| | Distance (cm) Traveled | | |
| B6 | 1066.2 (76.45) | 1057.7 (76.45) | 1236.4 (76.45) |
| D2 | 1044.2 (76.45) | 878.6 (76.45) | 932.7 (76.45) |
| | Percentage of Distance Traveled in the Light Side | | |
| B6 | 42.49 (1.9) | 37.77 (1.9) | 39.34 (1.9) |
| D2 | 31.35 (1.9) | 27.61 (1.9) | 22.88 (1.9) |

Numbers are means, and numbers in parentheses are standard errors. B6 = C57Bl/6J, D2 = DBA/2J.

**Percentage of total distance traveled occurring in the light side of the apparatus.** ANOVA found a significant effect of strain, $F$ (1, 54) = 65.754, $p < .001$, with B6 mice traveling a greater distance in the light than D2 mice. Likewise, the effect of age was significant, $F$ (2, 54) = 5.001, $p = .010$. (See Figure 7.) Post hoc testing indicated that 6wk mice, which traveled an intermediate distance in the light, did not differ from those 8wk or 4wk. On the other hand, the 4wk mice traveled a greater distance in the light than did the 8wk mice. ANOVA indicated that the stain by age interaction was not significant, $F$ (2, 54) = 1.593, $p = .213$. (Data are presented in table 15.)

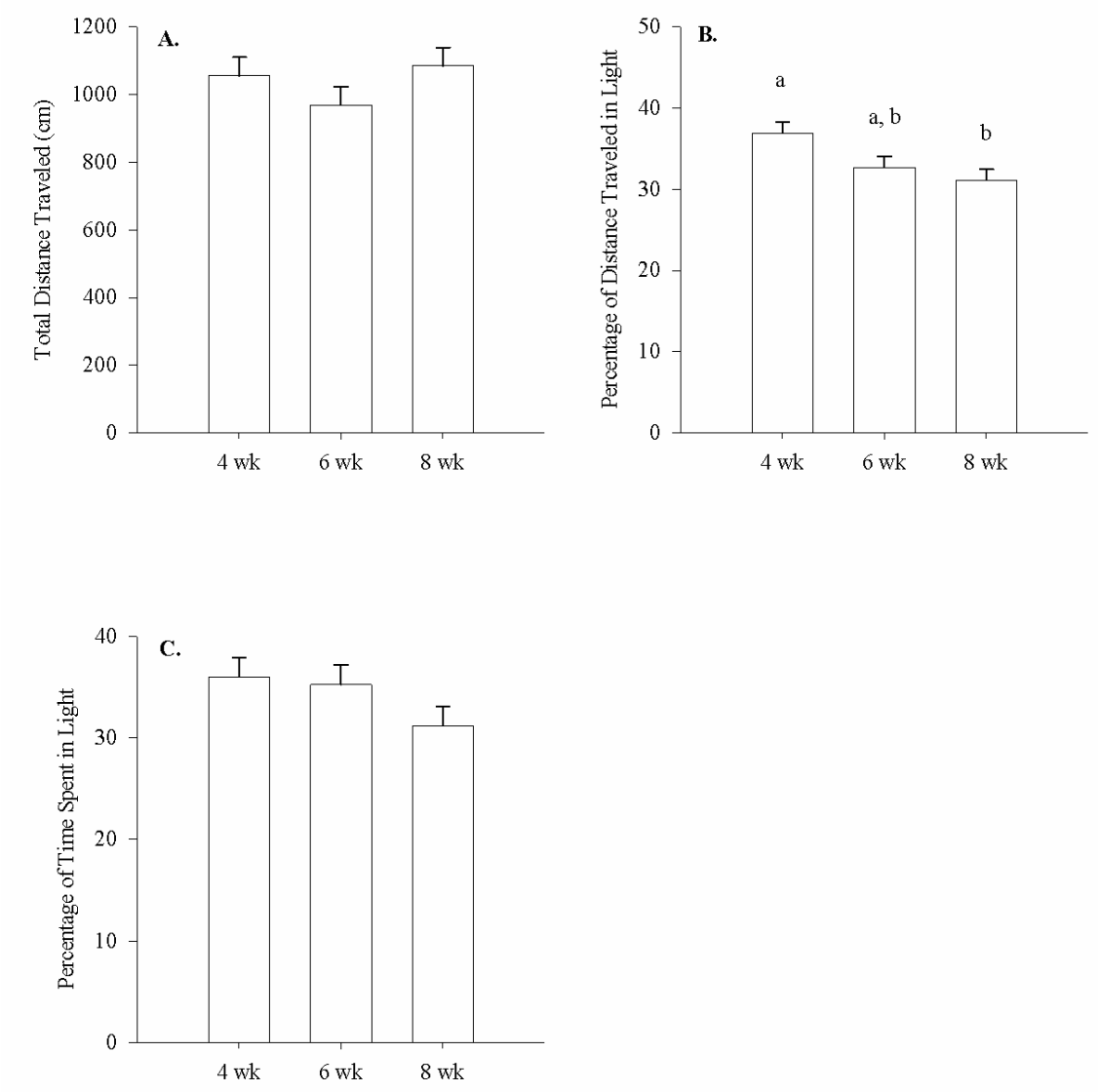


**Figure 7.** Age-related differences in measures in the light-dark apparatus. Data are presented as means ± SEM. **A.)** Total distance traveled in the light-dark apparatus. The effect of age was not significant and is presented for the purpose of comparison. **B.)** Percentage of the total distance traveled occurring in the light side of the apparatus. Groups that do not share a common letter are different at the level, $p < .05$. **C.)** Percentage of time spent in the light side of the apparatus. The effect of age was not significant and is presented for the purpose of comparison. 4 wk = four weeks of age, 6 wk = six weeks of age, 8 wk = eight weeks of age.

Fear Conditioning

**Training:** *Baseline activity.* ANOVA found a significant effect of strain on baseline activity, $F$ (1, 54) = 34.488, $p < .001$, with B6 mice being more active than D2 mice. However, the main effect of age and the strain by age interaction were not significant, $F$ (2, 54) = .205, $p = .815$, and $F$ (2, 54) = .916, $p = .406$, respectively. *Suppression of activity by training.* ANOVA did not find the independent variables to have an effect on the suppression of activity by training: strain, $F$ (1, 54) = .236, $p = .629$; age, $F$ (2, 54) = .447, $p = .642$; and age by strain, $F$ (2, 54) = 1.012, $p = .370$. However, the data did indicate that the training was successful with all animals displaying a reduction in activity following the final shock-tone pairing (M = .378, SEM = .035).

**Contextual conditioning:** *Suppression of baseline activity by training context.* ANOVA indicated that B6 mice showed a greater reduction of activity upon exposure to the training context the following day than did D2 mice, $F$ (1, 54) = 14.400, $p < .001$. However, the main effect of age and the strain by age interaction were not significant, $F$ (1, 54) = .540, $p = .586$, and $F$ (2, 54) = .906, $p = .410$, respectively.

**Cued conditioning:** *Activity in the altered context.* ANOVA found that B6 mice were more active at baseline in the altered context than D2 mice, $F$ (1, 54) = 7.245, $p = .009$, but there was no effect of age, $F$ (2, 54) = .227, $p = .798$. Likewise, the strain by age interaction was not significant, $F$ (2, 54) = .180, $p = .836$. *Cue suppression of activity in the altered context.* ANOVA revealed that D2 mice showed a greater suppression of activity in the altered context in response to the tone than did B6 mice, $F$ (1, 54) = 20.869, $p < .001$. However, the main effect of age and the strain by age interaction were

not significant, $F$ (2, 54) = 2.055, $p$ = .138, and $F$ (2, 54) = .425, $p$ = .656, respectively.

(Data are presented in Table 16.)

**Table 16. Means and standard errors of fear conditioning measures by age and strain.**

| | 4 weeks | 6 weeks | 8 weeks |
|---|---|---|---|
| Strain | Suppression of Activity by Training | | |
| B6 | .44 (.09) | .41 (.09) | .34 (.09) |
| D2 | .39 (.09) | .26 (.09) | .43 (.09) |
| | Suppression of Activity by Training Context | | |
| B6 | .55 (.07) | .56 (.07) | .43 (.07) |
| D2 | .74 (.07) | .67 (.07) | .73 (.07) |
| | Suppression of Activity by Cue | | |
| B6 | .38 (.05) | .30 (.05) | .40 (.05) |
| D2 | .14 (.05) | .14 (.05) | .24 (.05) |

Numbers are means, and numbers in parentheses are standard errors. Suppression of activity by training is calculated as beam breaks during the 30 seconds following the final tone-shock pairing divided by the average number of beam breaks per 30 second bin during the first two minutes of the training session. Suppression of activity by the training context is calculated as the average number of beam breaks per 30 second bin during exposure to the training context on the second day divided by the average number of beam breaks per 30 second bin during the first two minutes of the training session. Suppression of activity by the cue is calculated as the average number of beam breaks per 30 second bin during the first three minutes of exposure to the altered context on the second day divided by the average number of beam breaks per 30 second bin during the presentation of the tone in the altered context. (Smaller numbers indicate a greater suppression of activity.) B6 = C57Bl/6J, D2 = DBA/2J.

Tail Suspension

Three animals fell off the tail suspension apparatus during testing (one mouse from each of the following groups: B6, 6wk; D2, 4wk; and D2 6wk). These animals were therefore not included in the analysis. We did not observe any animals that climbed their tails.

**Time below threshold (immobility):** *Time below threshold per 30 s block of the six minute session.* Repeated measures ANOVA indicated that the within-subjects variable, interval, was significant, $F$ (11, 561) = 58.144, $p < .001$, with immobility increasing from interval 2 – 8 and then reaching a plateau from interval 8- 12. Likewise, the interval by strain interaction was significant, $F$ (11, 561) = 12.403, $p < .001$, but the interval by age interaction was not significant, $F$ (22, 561) = 1.464, $p = .080$. The three-way interaction, interval by strain by age, was found to be significant, $F$ (22, 561) = 1.603, $p = .041$. (See Figure 8.) The B6 mice exhibited a low level of immobility during the first two intervals of testing followed by a sharp increase in immobility between intervals two and four. The sharp increase was followed by a plateau in immobility from intervals four through eight. On the other hand, D2 mice displayed higher initial levels of immobility, which shifted more gradually during testing as compared to the B6 mice. At individual 30 s blocks, there were very few differences by age within strains, but there were multiple differences between strains and ages. (See Figure 8.)

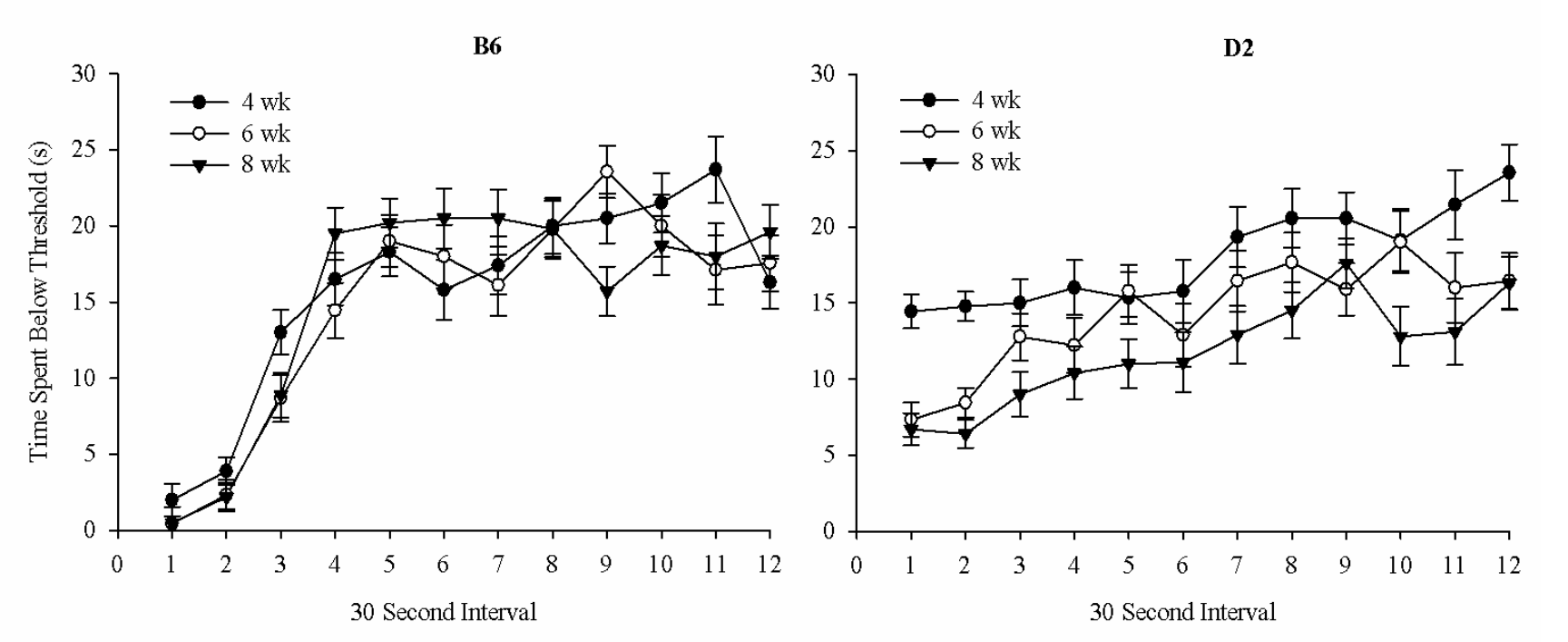


**Figure 8.** Time spent below threshold (immobility) in the tail suspension test by strain, age, and 30 second interval. Data are presented as means ± SEM. The following groups were different at the level, $p < .05$: Interval 1: D2 4wk > all, D2 6wk, 8wk > B6 6wk, 8wk; Interval 2: D2 4wk > all; Interval 4: B6 4wk > D2 6wk, B6 8wk > D2 6wk, 8wk; Interval 5: B6 all > D2 8wk; Interval 6: B6 6wk > D2 8wk, B6 8wk > D2 6wk, 8wk; Interval 7: D2 4wk > D2 8wk, B6 8wk > D2 8wk; Interval 9: B6 6wk > B6 8wk, B6 6wk > D2 6wk; Interval 10: B6 6wk > D2 8wk, D2 4wk, 6wk > D2 8wk; Interval 11: B6 4wk > B6 6wk, B6 4wk > D2 6wk, 8wk, D2 4wk > D2 8wk; Interval 12: D2 4wk > B6 4wk, D2 4wk > D2 6wk, 8wk. 4 wk = four weeks of age, 6 wk = six weeks of age, 8 wk = eight weeks of age, B6 = C57Bl/6J, D2 = DBA/2J.

*Overall time below threshold.* ANOVA did not find an effect of strain on the total time spent immobile during the tail suspension test, $F$ (1, 51) = .647, $p$ = .425. However, there was a significant effect of age, $F$ (2, 51) = 6.845, $p$ = .002. Post hoc testing indicated that 4wk mice spent more time immobile than 6wk or 8wk mice, but the 6wk and 8wk mice did not differ. ANOVA also revealed a significant strain by age interaction, $F$ (2, 51) = 4.947, $p$ = .011. (See Figure 9.) Analysis of simple effects showed that there were no differences among B6 mice based on age. On the other hand 4wk D2 mice spent more time immobile than did their 6wk and 8wk counterparts, who did not differ. Comparing the two strains, B6 and D2 mice did not differ at 4wk or 6wk, but 8wk B6 mice spent more time immobile than did D2 mice of the same age.

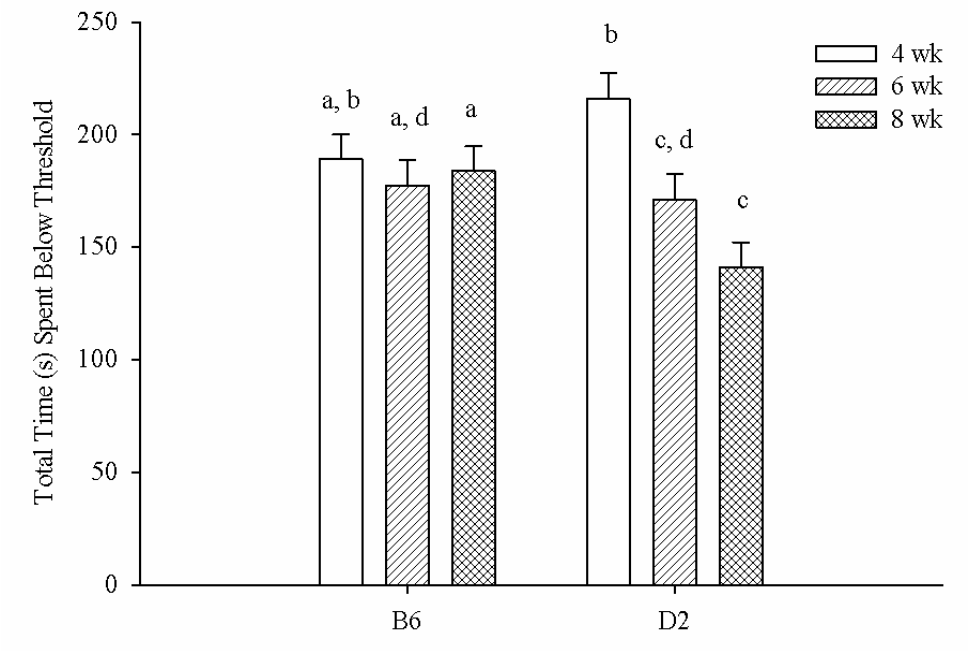


**Figure 9.** Total time spent bellow threshold (immobility) during the tail suspension test by strain and age. Data are presented as means ± SEM. Groups that do not share a common lowercase letter are different at the level, $p < .05$. 4 wk = four weeks of age, 6 wk = six weeks of age, 8 wk = eight weeks of age, B6 = C57Bl/6J, D2 = DBA/2J.

## Discussion and Conclusion

### Discussion of Results of Experiment 1

Examining the effects of acute administration of CDZ on B6 and D2 mice in the elevated zero maze, we found strain differences on all measures, which are similar to those previously reported (Tarantino et al., 2000; Cook et al., 2001). On the other hand, we did not find CDZ to affect activity or latency to enter an open quadrant. Previous reports on the effects of CDZ on activity and latency in the EZM are variable, and a number of authors do not include these measures at all. None the less, it seems that CDZ generally increases or has no effect on activity and either decreases or is without effect on latency (see Table 2). Increased latency is indicative of a heightened anxiety-like state; thus, it is somewhat surprising that CDZ does not produce a consistent decrease in latency. However, this measure has been previously noted as being quite variable and difficult to interpret (Matto et al., 1997).

Considering the principle measure of anxiety-like behavior, time spent in the open quadrants, we did see an effect of CDZ. Oddly, none of the doses tested differed from the saline control, but 7.5 mg/kg of CDZ produced an axiolytic effect in comparison to animals administered 2.5 or 5 mg/kg. While the drug by strain interaction was not significant, visual inspection of the data suggest that the drug treatment effect was largely due to CDZ having an anxiolytic effect in D2 mice but no effect in B6 mice. (See Figure 1.) This notion is supported when separate one-way ANOVAs were performed for each strain. While the number of studies that have examined the effects of CDZ on mice in the EZM is limited, most have found an anxiolytic effect (see Table 2). To our knowledge the only other report on the effects of CDZ on B6 and D2 mice in the EZM is that of

Mathiasen and colleagues (2008). They found CDZ to have no effect in D2 mice but an axiogenic effect in B6 mice at a dose of 20 mg/kg. However, looking at their data overall, it seems this may be an artifact of a sedative effect. Interestingly, when testing these strains on the EPM, CDZ had no effect on B6 mice but an anxiolytic effect in D2 mice (Mathiasen et al., 2008), which is similar to what we have found. Other studies of B6 mice in the EPM also indicate that CDZ is without effect on this strain (Rodgers et al., 2002; Clément et al., 2009; Lalonde and Strazielle, 2010), but exceptions do exist (Belzung et al., 2000; Paterson et al., 2010). Many authors have attributed this lack of response to a floor effect as B6 mice generally exhibit a low level of anxiety-like behavior, but there is evidence that the differential response to CDZ between B6 and D2 mice may be related to differences in GABAergic transmission (Hitzemann and Hitzemann, 1999). Considering this, it would be helpful to examine the differences between strains more thoroughly using receptor specific drugs and additional measures.

Interestingly, Rodgers and colleagues (2002) have additionally reported that 129S2/Sv mice are also unresponsive to the effects of CDZ in the EPM. This, with the present findings and those of others, has major implications for the study of benzodiazepines using transgenic mice as they are frequently produced using B6 blastocysts and 129 embryonic stem cells. While this highlights the importance of considering strain effects in pharmacological research, it also suggests that expanded pharmacological phenotyping of inbred strains could lead to a better understanding of the genetics of GABAergic transmission and the treatment of anxiety disorders.

**Discussion of Results of Experiment 2**

In order to characterize the effects of chronic administration of SNRIX in mice, we administered SNRIX daily from PND 60 – 89. Beginning at PND 90 animals began a four day battery of behavioral testing. Behavior was assayed using the following tests: EZM, open field, hotplate algesia meter, light/ dark box, acoustic startle/ prepulse inhibition, conditioned fear, and the tail suspension test. To determine if these effects would vary according to strain and sex, male and female B6 and D2 mice were used. For clarity of presentation, the results from each of these tests are discussed individually.

Body Weight

The body weight of both sexes and strains generally increased throughout the course of the experiment, and differences were overall, generally small. Although some differences were found to be statistically significant in the present study, the greatest difference between means observed at the same time point (between B6 females and D2 males at PND 60) was only 5.02 g. Strain differences in body weight are often inconsistent, for example some have found B6 mice to weigh more than D2 mice (Liu and Gershenfeld, 2003), while others have found the opposite (Morris et al., 1999). More interestingly, we did not find an effect of SNRIX on body weight. We are unaware of any reports of the effects of SNRIX on body weight in mice. However, ten days treatment with SNRIX in sham operated female bulbectomized rats decreased body weight (Oliveira et al., 2004), but had no effect on body weight in male rats treated for 21 days (Xu et al., 2003). Drugs acting on monoamine systems are known to affect appetite, feeding behavior, and the regulation of body weight, and the effects of antidepressant agents on weight gain appear to be complex (Gobshtis et al., 2007). Further, it is not clear

to what degree the regulation of body weight is similar between clinical and preclinical populations. However, our failure to find an effect of SNRIX on body weight corresponds with reports that SNRIX does not affect weight gain in clinical populations (Deshmukh and Franco, 2003). As the effects of antidepressant drugs on body weight can exert considerable influence on patient compliance and are of particular concern for the treatment of patients with comorbid obesity-related diseases (Deshmukh and Franco, 2003), further investigation would be worthwhile.

Elevated Zero Maze

Extending the pharmacological characterization of the EZM in mice was a primary goal of this experiment. Therefore, mice were tested on this measure the first day following SNRIX administration. Increases in the percentage of time spent in the open quadrants of the elevated zero maze is considered to reflect an anxiolytic effect. We found that B6 mice spent a greater percentage of time in the open quadrants than D2 mice, which concurs with previously reported findings (Cook et al., 2001). However, we did not find a significant effect of SNRIX. Similarly, acute treatment with SNRIX has no effect on behavior in the EZM in female NMRI mice (Troelsen et al., 2005). Interestingly, Troelsen et al. (2005) found duloxetine, another SNRI, to have no effect in mice on the EZM when administered acutely but to have an axiolytic effect after chronic administration. There are numerous possibilities for the difference between the effects of these two drugs, but duloxetine does have a greater affinity for the norepinephrine transporter (NET) at lower doses compared to SNRIX (Troelsen et al., 2005). However, further studies would be needed to determine what effect this might have. The EPM, from which the EZM was derived, similarly has been shown to be generally insensitive to the

effects of antidepressant drugs. Chronic administration of SNRIX in sham operated female bulbectomized rats is without effect on behavior in the EPM (Oliveira et al., 2004). Likewise, imipramine (Cole and Rodgers, 1995), desipramine (Gobshtis et al., 2007), fluvoxamine (Rodgers et al., 1997), paroxetine (Goeldner et al., 2005), and bupropion (Carrasco et al., 2004) have all been found to have no effect on mouse behavior in the EPM. On the other hand, in mice, fluoxetine has been shown to have an anxiogenic effect when administered chronically but an anxiolytic effect if administered acutely (Goeldner et al., 2005), still yet others have found acute administration of fluoxetine to be without effect (Holmes and Rodgers, 2003).

Similar to the percentage of time in the open, we did not find an effect of SNRIX on activity or latency to enter an open quadrant in the EZM. Considering other antidepressants, acute administration of desipramine (Gur et al., 2007), amitriptyline, citatlopram, fluoxetine, paroxetine, and venlafaxine (Toelsen et al., 2005) are without an effect on activity or latency in mice on the EZM. However, with duloxetine, Troelsen and colleagues (2005) found acute, but not chronic, administration increased latency while chronic, but not acute, treatment increased activity in female NMRI mice. Although we did not find an effect of SNRIX on these measures, we did find a main effect of strain for activity and a strain by sex interaction for latency. B6 mice were more active than D2 mice. With regards to latency, male B6 and D2 mice did not differ, while female D2 mice displayed a greater latency than their B6 counterparts. While the relationships between strains on these measures concur with those previously reported, the sex differences seem less consistent (Cook et al., 2001).

Others have suggested that the EZM may be a useful test to detect delayed effects of antidepressants (Troelsen et al., 2005). However, our data suggest that chronic SNRIX is without effects on behavior in the EZM. Generally speaking, both the EZM and the EPM have not demonstrated a robust ability to detect the effects of antidepressant agents, with negative or conflicting results frequently being obtained (vide supra). Further, the elevated plus maze has been shown to be highly sensitive to slight environmental differences (Whalsten et al., 2003). These points and our finding suggest that elevated maze tests of anxiety-like behavior are not a highly efficient method of investigating the effects of antidepressant drugs and a larger number of animals may be needed to achieve the power necessary to detect effects if they exist.

Open Field

We found a significant interaction between sex and strain and no effect of treatment with SNRIX on the total distance traveled in the open field. D2 males and B6 mice of either sex did not differ, but all traveled more than D2 females. This is similar to a previous report that B6 mice travel a greater distance than do D2 mice, however only males were examined (Liu and Gershenfeld, 2003). Reports of the effects of SNRIX in mice on this measure vary greatly with strain. In B6 mice an acute dose of 40 mg/kg SNRIX, but not lower doses tested, increased distance traveled (Kos et al., 2006). On the other hand, acute doses of SNRIX as low as 2.5 mg/kg in NMRI mice (Brocco et al., 2002) and 16 mg/kg in swiss mice (Redrobe et al., 1998) increase distance traveled in the open field. Still yet, using knockout mice maintained on a mixed B6 129SvEv background, it has been reported that in the wild-type controls 20 mg/kg SNRIX administered acutely was without effect but the same dose administered daily for 21 days

produced a decrease in the distance traveled (Mitchell et al., 2006). Our failure to find an effect of SNRIX in B6 mice may due to the highest dosage we tested, 30 mg/kg, being too low to elicit an effect or possibly due to a difference between chronic and acute treatment in this strain. Such variation between strains in the effects of SNRIX highlights the importance of strain selection in pharmacological research, especially when transgenic animal models are used.

We additionally found an interaction between sex and strain on the frequency of rearing. B6 females and D2 males reared the most followed by B6 males and D2 females. This finding is contrary to another that found B6 males to rear more frequently than D2 males, but females were not tested (Liu and Gershenfeld, 2003). We further found an effect of sex on the habituation ratio, with males exhibiting a greater degree of habituation to the open field than females, and an interaction between strain and sex on the percentage of time spent in the corners of the arena. B6 males and females spent the most time in the corners followed by D2 males and then D2 females. We did not find any effects of SNRIX treatment on the frequency of rearing, habituation ratio, or percentage of time spent in the corners of the arena and are unaware of any previous reports of the effects of antidepressant drugs, strain, or sex on these measures.

We found the percentage of time spent in the center of the arena, which is considered a measure of anxiety-like behavior, to vary with strain, sex, and SNRIX treatment. (See Figure 2.) B6 mice spent a greater percentage of time in the center of the arena than did D2 mice irregardless of sex or drug treatment. SNRIX had no effect on D2 mice of either sex, and B6 mice of both sexes that were administered 10 mg/kg SNRIX or saline did not differ. On the other hand, B6 males treated with 30 mg/kg SNRIX tended

to spend a greater percentage of time in the center of the arena, while B6 females given 30 mg/kg SNRIX tended to spend less time in the center as compared to their respective saline controls. This suggests that chronic administration of 30 mg/kg SNRIX produces an anxiolytic effect in B6 males but an anxiogenic effect in B6 females. We are unaware of any previous reports on the effects of SNRIX on this measure. However, others found that chronic fluoxetine treatment reduces center time in B6 males but is without effect in D2 males, although the effect in B6 males was confounded with an effect on activity in the open field (Dulawa et al., 2004). We did not find a significant effect of SNRIX on the percentage of total distance traveled in the center of the arena. This along with the lack of an effect of SNRIX on the total distance traveled suggests that the effect of 30 mg/kg administration on time spent in the center of the arena is not due to differences in activity levels. Still, further investigations would be needed to delineate causal factors behind the strain and sex differences observed here.

Hotplate

We only found a significant effect of strain on hotplate latency, with D2 mice exhibiting a longer latency to display a pain response than B6 mice, which concurs with previous reports (Mogil et al., 1999). We did not find an effect of SNRIX administration. In Swiss mice it has been reported that an acute dose of 16 mg/kg SNRIX is without effect on hotplate latency (Ripoll et al., 2006). However, acute SNRIX administration has been shown to increase latency in ICR mice with an estimated $ED_{50}$ of 46.7 mg/kg (Schrieber et al., 1999). Similarly, fluvoxamime and citalopram, but not escitalopram, have been reported to increase hotplate latency when administered acutely (Schrieber et al., 1999; Schrieber and Pick 2006). It may be the case that the doses tested in the present

study were too low to elicit an effect. However, the lack of an effect of SNRIX in the current study could be due to chronic as opposed to acute drug administration. Unfortunately, acute administration was not examined here, and we are not aware of any previous investigations of the effects of chronic administration of antidepressant drugs on hotplate algesia.

Light-Dark

The percentage of time spent in the light half of the light-dark box is generally recognized as a measure of anxiety-like behavior. We found a significant strain by sex interaction. B6 males and females did not differ and spent a greater amount of time in the light side of the apparatus than did D2 mice. Further, D2 males spent more time in the light than did D2 females. This finding generally concurs with a previous report that male B6 mice display less anxiety-like behavior than D2 males (Liu and Gershenfeld, 2003). Additionally, our results indicated a trend towards an interaction between strain, sex, and SNRIX administration, but this did not reach significance ($F$ (2, 119) = 2.798, $p$ = .065, ES: $f$ = .217; Figure 3). B6 males in the saline group spent a greater percentage of time in the light than did any other treatment group. B6 females and D2 males did not differ, while B6 mice of both sexes and D2 males spent more time in the light than did D2 females. Administration of SNRIX was without effect on D2 mice of both sexes as well as B6 females. On the other hand, both doses of SNRIX tested produced an equivalent anxiogenic response in B6 males as compared to their respective saline control. We are not aware of any previous reports on the effects of SNRIX in the light-dark box in mice. However, in male rats, acute or seven days treatment with 20 mg/kg SNRIX results in an anxiolytic response, but the same dose administered for 14 days is without effect

(Nowakowska et al., 2003). The effects of the acute administration of other antidepressant agents on behavior in the light-dark box in mice have been previously described; however, the reported results have been divergent. Paroxetine (Hascoët et al., 2000) and the tricyclic antidepressant, dothiepin, (Bourin et al., 1996) have an anxiolytic effect, but fluoxetine, imipramine, and maprotiline are without effect (Bourin et al., 1996).

We found a significant interaction between strain and sex on the distance traveled in the light/ dark box. B6 females traveled the greatest distance followed by B6 males and D2 males, while D2 females traveled the least distance of all. Additionally, main effects of strain and sex were found on the percentage of total distance traveled occurring in the light side of the apparatus. B6 were more active than D2 mice, and males were more active than females in the light compartment. We found no effect of SNRIX administration on either the total distance traveled or the percentage of total distance traveled occurring in the light.

Acoustic Startle/ Prepulse Inhibition

We found a significant interaction between strain and sex on the response to the 120 db startle stimulus. B6 males exhibited a greater response than did B6 females followed by D2 males and females, which did not differ. This finding generally concurs with others that have found B6 mice to display a greater startle response than D2 mice (Paylor and Crawley, 1997; Willott et al., 2003), but where examined no effect of sex was observed (Willott et al., 2003). Main effects of strain were found on the percentage of prepulse inhibition of the startle response at 70, 80, and 85 db. In all cases B6 mice exhibited a greater degree of inhibition than did D2 mice. Previous reports on strain

differences in prepulse inhibition have varied. Our findings concur with those of one report (Willott et al., 2003), but an opposite relation between these strains has also been reported (Paylor and Crawley, 1997). It is possible that these differences in results are due to differences in apparatus designs and configurations, as they vary considerably between studies. In the present study, a main effect of sex was found on prepulse inhibition at 80 db, but not 70 or 85 db, with males exhibiting a greater degree of inhibition than females. Additionally, we observed a trend towards an effect of SNRIX treatment on prepulse inhibition at 70 db ($F$ (2, 123) = 2.832, $p$ = .063, ES: $f$ = .215), and a significant effect at 80 db. (See Figure 4.) In both cases, administration of 30 mg/kg, but not 10 mg/kg, SNRIX increased the percentage of prepulse inhibition relative to saline controls. We are not aware of any previous reports of the effects of SNRIX on acoustic startle or prepluse inhibition. However, serotonin agonists are generally without effect in B6 mice tested in a MDMA disrupted prepulse inhibition paradigm (Duwala and Geyer, 2000). Similarly, citalopram and buproprion have no effect on prepulse inhibition and decrease the acoustic startle response, while desipramine increases prepulse inhibition and decreases the startle response in rats treated with amphetamine (Pouzet et al., 2005). Previous reports have indicated that the NET may play a role in acoustic startle and prepulse inhibition in both preclinical (Yamashita et al., 2006) and clinical populations (Quednow et al., 2004). It has previously been reported that chronic administration of 10 mg/kg SNRIX in rats inhibits the activity of the serotonin transporter but not the NET, while 40 mg/kg SNRIX inhibits the activity of both (Béïque et al., 2000). If the same dose dependency exists in mice, this might provide a possible explanation as to why in the present study we found an effect of 30 mg/kg SNRIX but not

10 mg/kg. These reports along with our finding suggests that further investigations into the effects of antidepressant medications, and specifically those acting on noradrenergic systems, on acoustic startle and prepulse inhibition are warranted.

Fear Conditioning

The measure of training suppression of activity is indicative of how successful the training procedure was at decreasing activity relative to baseline activity prior to the shock – tone pairings, with a lower value indicating greater suppression of activity and a value of 1 indicating no change. We found no differences between any treatment groups on the basis of strain, sex, or SNRIX administration for training suppression, and all groups displayed a reduction in activity following training ($M$ = .355, $SEM$ = .032). Context suppression of activity compares activity in the training context relative to the baseline activity recorded the prior day and is indicative of the strength of the association formed between the training context and the unconditioned stimulus (i.e. electric shock). We found a significant effect of strain on context suppression of activity, with B6 mice displaying less activity suggesting an enhanced memory of the context compared to D2 mice, which concurs with previous reports (Logue et al., 1997; Stiedl et al., 1999; Nie and Abel, 2001). Additionally our finding of an interaction between strain and sex replicates a previous report, which found no difference between B6 males and females although D2 females displayed a greater inhibition of activity in the context than did their male counterparts (Bolivar et al., 2001). In the present study we found SNRIX to be without effect on context suppression of activity. We are unaware of any previous reports of the effects of antidepressant agents on fear conditioning. However, citalopram has been reported to reduce contextual conditioning in rats using the conditioned fear stress

paradigm, which does not include cued conditioning (Inoue et al., 1996). As for cued conditioning, we found a significant strain by sex interaction. B6 males and females, as well as B6 males and D2 males, did not differ in their response to the tone. However, D2 females showed a greater reduction in activity in response to the tone than did the other groups. Others have reported a similar interaction where females were tested (Bolivar et al., 2001). However, reports on males alone are more varied with some finding no differences between B6 and D2 mice (Logue et al., 1997; Nie and Abel, 2001), but increased tone suppression of activity in B6 as compared to D2 mice has also been observed (Stiedl et al., 1999). Such variability in findings may, in part, be due to differences in the methods used to score behavior. For instance a number of authors have used visual determination of freezing behavior, where as we have used an automated system to measure general activity. Both methods have advantages; visual scoring is more specific, but the use of an automated system increases throughput and decreases the opportunity for experimenter error. Additionally, we have compared activity during presentation of the tone to baseline activity in the altered context while others have reported only activity during the presentation of the tone. Our results indicate that SNRIX is without effect on cued conditioning; unfortunately, we are not able to compare this with any previous reports.

Tail Suspension

We observed a large number of mice that climbed their tails during the tail suspension test. A central principle of this test is that the stress of the procedure is inescapable (Cryan and Mombereau, 2004), and once the animal climbs its tail, this is no longer the case. Thus, tail climbing is problematic for the model. Little has been has been

reported about tail climbing behavior, other than its occurrence. We therefore decided to analyze the data collected on tail climbing using the between subject factors of the study in a logistic regression analysis. Our analysis did not suggest that strain was predictive of tail climbing, which is contrary to a previous report that B6 mice climb their tail more frequently than D2 mice (Mayorga and Lucki, 2001). We did find that males were more likely to climb their tails than females, but SNRIX treatment was without a significant effect. Tail climbing has generally been treated as a nuisance, and some apparatus claim to avoid the behavior by attaching the animal's tail to a ring rather than a metal plate. However, our results and those of others (Mayorga and Lucki, 2001) indicate that this trait may be influenced by genetic factors and sex, suggesting that it may be worthy of more in depth investigation.

Overall, we did not observe an effect of strain on time spent immobile during tail suspension. Others have reported that B6 mice exhibit more immobility than D2 mice, although differences were small (Liu and Gershenfeld, 2003). We did not find an effect of SNRIX on immobility. Others have reported that in mice acute administration of SNRIX decreases immobility in the tail suspension test (Millan et al. 2001; Liu et al., 2003; Kos et al., 2006) and the forced swim test (Redrobe et al., 1998; David et al., 2001; Millan et al., 2001; Berrocoso et al., 2004). We are unaware of any previous reports of the effects of chronic SNRIX administration in mice. However, in rats, administration of SNRIX for 7 days (Nowakowska et al., 2003; Nowakowska and Kus, 2005) or 10 days (Oliveira et al., 2004) decreases immobility, but administration for 14 days (Nowakowska et al., 2003; Nowakowska and Kus, 2005) or 24 days (Connor et al., 2000) has no effect. It should be noted that the reliability of our results is questionable due to the low number

of subjects tested. In addition to dropping the data for 31.2% of the animals in the study due to tail climbing, the data for 10 additional animals was lost due to a malfunction of the computer used to operate the tail suspension equipment resulting in some treatment cells being comprised of as few as four animals. Thus caution should be used in the interpretation of these results.

Summary and Implications of Findings in Experiment 2

We have demonstrated here that the behavioral effects of chronic administration of SNRIX in mice vary by test, strain, and sex. As has been discussed elsewhere (Dulawa and Hen, 2005), few behavioral tests have been shown to be sensitive to chronic administration of antidepressant drugs. Our results reinforce this notion, as we did not find effects of SNRIX using the EZM, hotplate algesia meter, fear conditioning, or the tail suspension test. However, we have found that treatment with SNRIX was without effect in D2 mice, but produced an anxiolytic effect in B6 males and an anxiogenic effect in B6 females in the open field. On the other hand, our results suggest a non-significant trend towards an anxiogenic effect of SNRIX in B6 males, but not B6 females or D2 mice, in the light/ dark box. Lastly, we found that SNRIX produces an improvement in the inhibition of the acoustic startle response by an 80 db prepulse regardless of strain or sex.

The present findings have several implications for future research. Previously it has been shown that the novelty-induced hypophagia test (Dulawa and Hen, 2005) and the resident intruder paradigm (Mitchell and Redfern, 2005) are sensitive to chronic administration of antidepressant drugs. Our finding that the open field and light/ dark box are sensitive to the chronic administration of SNRIX suggests that these tests may also be

sensitive to the chronic administration of other antidepressants. If so, this would represent a marked improvement in efficiency, as these tests are far less time consuming to perform than either of those previously suggested. Additionally, we have shown that the response of B6 animals to SNRIX is dependent on sex in both the open field and light/ dark box, which suggests that this strain may be useful for future studies on the sex dependent effects of antidepressant agents. Further, our data indicate that B6 and D2 strains differ in their response to SNRIX administration. This highlights the value of testing multiple inbred strains in pharmacological research and the importance of considering background effects when using transgenic models. Further, the difference in strain response suggests that the BXD recombinant inbred lines may be a good choice for mapping quantitative trait loci influencing the effects of antidepressant drugs. Lastly, the present findings underscore the need for further investigations of antidepressant drugs that consider sex, genetic differences, and chronic administration.

**Discussion of Results of Experiment 3**

Noting that there is relatively little information in the literature on age-related behavioral differences in mice during adolescence and seeking to expand upon what is available, we examined the B6 and D2 mice at 4, 6, and 8 weeks of age in six behavioral models. We found almost all measures to vary with strain, and for the most part the relations observed concur with previous reports (Logue et al., 1997; Stiedl et al., 1999; Cook et al., 2001; Nie and Abel, 2001; Liu and Gershenfeld, 2003). Our observations with regard to the effect of age are far more variable. Considering the standard measures of anxiety-related behavior in the present study, we did not find an effect of age on the percentage of time spent in the light side of the light-dark box or in fear conditioning, but

we did find a significant strain by age interaction on the percentage of time spent in the center of the open field and a trend towards an effect of age on the percentage of time spent in the open quadrants of the EZM. However, these two measures depict opposite relations between the age groups. Although not quite reaching significance ($F$ (2, 54) = 2.971, $p$ = .060, ES: $f$ = .331), 8wk mice displayed more anxiety-like activity than 6wk mice in the EZM, but the opposite was seen among B6 mice in the open field. (See Figures 5 and 6.) Looking at the literature, it seems that age-related differences in anxiety-like behavior differ considerably between tasks, and the patterns of variability between tasks differ between mice and rats.

We are not aware of comparable studies of rodents in the EZM, but there are a number of studies on age-related differences in behavior using the EPM. In mice a relatively consistent pattern of results is seen, with most studies finding no age-related differences during adolescence (Hefner and Holmes, 2007; Kota et al., 2007; Peleg-Raibstein and Feldon, 2011) or, similar to our observations in the EZM, adolescent mice display less anxiety-related behavior than adult mice (Adriani et al., 2004; Oh et al., 2009). On the other hand, The reports on rats in the EPM are more variable, finding adolescents less anxious than adults (McCormick et al., 2008; Doremus-Fitzwater et al., 2009a; Kupferschmidt et al., 2010), adults less anxious than adolescents (Elliot et al., 2004; Lynn and Brown, 2009; Lynn and Brown, 2010), or no age-related differences (Doremus-Fitzwater and Spear, 2007; Doremus-Fitzwater et al., 2009b; Eppolito et al., 2010; Villégier et al., 2010).

In the open field, a converse relationship between rats and mice is seen regarding anxiety-like behavior. The reports on mice are variable. Similar to our findings, Hefner

and Holmes (2007) found 8wk B6 mice to display less anxiety-like behavior than 4wk or 6wk mice. However, Oh et al. (2009) found adolescent B6 mice to be less anxious than their adult counter parts while there were no age-related differences in Swiss mice. In contrast, reports on rats predominantly find no age-related differences in anxiety-related behavior in the open field (Lynn and Brown, 2009; Lynn and Brown, 2010; Li et al., 2010), but Cao et al. (2010) has found that adolescent rats are less anxious than periadolescents or adults.

Unfortunately, we are not aware of any previous reports on age-related differences in the light-dark box using mice, but most studies using rats have found no age-related effects (Slawecki, 2005; Slawecki et al., 2006; Kupferschmidt et al., 2010). However, Kupferschmidt et al. (2010) did find adolescents to display a shorter latency to enter the light side of the light-dark box, suggesting reduced anxiety. While we did not include this measure in our analysis, we did find that 4wk mice traveled a greater percentage of distance in the light than did 8wk mice. While the interpretation of this measure is nuanced, it is indicative of increased activity in an aversive environment. It may be that in the light-dark box activity- and anxiety-related measures are more intertwined with regard to age.

If in fact anxiety models differ in their sensitivity to age-related differences in anxiety-like behavior between rats and mice, extrapolation of findings between species would be tenuous, and the selection of the appropriate behavioral model would be imperative to protecting against type II error. However, there are a number of factors that should be considered. For instance, the number of studies examining the behavior of adolescent rodents is limited, and the age-ranges examined between studies vary

considerably. Additionally, much of the available data on behavior during adolescence comes from pharmacological studies, and many of them either do not report baseline differences on the basis of age or analyze age groups separately. Thus, the comparisons presented above are in places based upon estimates of the data presented. Further complicating the issue, it seems that age-related differences may be particularly sensitive to test and pretest conditions. For example, Slawecki (2005) found no differences between adult and adolescent rats in the light-dark box under standard conditions but did find age-related differences when testing was performed under bright lights or when animals had been previously subjected to restraint stress. Unfortunately, in the end, further research and replication will be required to disentangle the relationships between these factors.

We also examined nociception, fear conditioning, and depression-related behavior. We did not see any age-related effects in the hotplate algesia test. Only a few studies have examined nociception during adolescence. Similar to our findings, ICR mice do not display age-related differences in their latency to display a pain response in the tail flick test or the hotplate test (Kota et al., 2007). However, adolescent and adult Sprague-Dawley rats do not differ in the tail flick test (Conway et al., 1998), but periadolescents display a longer latency than do adolescents in the hotplate test (Ingram et al., 2007). While nociception is not directly linked to anxiety-like behavior, it can be indicative of gross abnormalities, and the finding of age-related differences in nociception during adolescence could have implications for pain management in clinical settings.

In fear conditioning, we did not see any age related-differences in training acquisition, conditioning to the training context, or conditioning to the cue. Similarly,

others have found no difference in training acquisition in B6 mice (Pattwell et al., 2011; Peleg-Raibstein and Feldon, 2011) or rats (Land and Spear, 2004; Esmorís-Arranz et al., 2008). On the other hand, Hefner and Holmes (2007) observed 4wk B6 mice to display more freezing at trial 4, but no differences in previous trials. With regards to conditioning to the context, findings are variable. Some have found no age-related differences in B6 mice (Peleg-Raibstein and Feldon, 2011) and rats (Land and Spear, 2004), but others report greater contextual conditioning in adolescent rats as compared to young adults (Esmorís-Arranz et al., 2008). It seems that contextual conditioning may be particularly sensitive to the exact age at which conditioning occurs. Pattwell et al. (2011) found no differences in contextual conditioning at PND 23-27 and PND 49-70, but a deficit at PND 29-33 and a slight deficit at PND 35-39 in B6 mice. Interestingly, when animals were trained at PND 29-33, they showed a deficit when contextual conditioning was tested under standard procedures, but showed normal contextual conditioning when tested 14 days later with no additional training (Pattwell et al., 2011). The findings with regard to conditioning to the cue are quite variable as well. Some have found no age-related differences in B6 mice (Pattwell et al., 2011) and rats (Land and Spear, 2004), but conflicting reports also exist finding adolescent (Hefner and Holmes, 2007) and adult (Peleg-Raibstein and Feldon, 2011) B6 mice to both show superior conditioning to the cue. We are not aware of any previous reports on age-related differences in fear conditioning in mouse strains other than B6. Thus, we are unable to compare our null findings in D2 mice directly to any other reports. Considering the differences between inbred strains among adults, it may be the case that if additional strains were examined strain specific age-related differences would emerge.

In the tail suspension test, we found no age-related differences in B6 mice, but 4wk D2 mice spent more time immobile than 6wk and 8wk D2 mice. Additionally, looking at the progression of immobility across testing, we found that B6 mice displayed a low initial immobility that quickly increased to a plateau. On the other hand, D2 mice, especially in the 4wk group, showed higher initial immobility that increased more gradually. We are not aware of any previous studies looking at age-related differences in D2 mice in depression-related behavior, but a number of reports on B6 mice are available. In contrast to our findings, all previous reports have found age related differences in B6 mice in the tail suspension test (Mason et al., 2009) and the forced swim test (Hefner and Holmes, 2007; Oh et al., 2009; Mason et al., 2009). It is not clear why we did not see an age related effect in B6 mice, but our finding of increased immobility in 4wk D2 mice concurs with others who have found a similar age relationship in B6 and BALB/c mice in the TST (Mason et al., 2009) and in B6, BALB/c, and B6x129s5 F2 mice in the forced swim test (Hefner and Holmes, 2007; Mason et al., 2009). However, the reported age differences among mice are not entirely consistent. Some have reported no differences in the tail suspension test in B6x129s5 F2 mice (Mason et al., 2009) and in the forced swim test using Swiss mice (Oh et al., 2009). Still yet, others have reported B6 adults (Oh et al., 2009) and Swiss adults (Moreira et al., 2005) to spend more time immobile than there adolescent counterparts. None the less, the variability between strains with regards to age-differences suggests that the development of depression-related behavior is likely influenced by genetics, and further experiments in this area may shed light onto the risk factors associated with pediatric/ adolescent depression in humans.

## Conclusion

The experiments presented here are, of course, not without limitations. Experiments 1 and 2 sought to more fully characterize the EZM by examining the acute effects of CDZ and the effects of chronic administration of SNRIX in B6 and D2 mice. In all actuality, this is far from a full pharmacological characterization. Our laboratory is working to extend the findings of Experiment 1 to include two additional classes of anxiolytics, buspirone and fluoxetine, and female animals as well. However, unfortunately, difficulties producing mice prevented full data sets from being available. With regards to Experiment 2, the method of drug administration is less than ideal. Animals were injected daily. Thus, it can be assumed that an effect of handling and injection stress was present, although experienced by all animals. Additionally, drug administration was ceased during behavioral testing. This decision was made largely due to the amount of drug we were provided by the pharmaceutical company and the time constraints presented by the behavioral battery. Although we did find effects of SNRIX administration as late as the third day of testing, it must be acknowledged that the levels of drug on board can not be assumed to be consistent across behavioral tests. Future use of osmotic mini pumps may well be preferable, as it would greatly diminish such concerns. In both Experiments 2 and 3, Animals were tested on a battery of several tests over the course of four days. We are not able to say what effect prior testing had on subsequent testing. However, previous use of this battery in a large mutagenesis screen suggests that phenotypes identified with sequential testing in early pedigrees are reliably reproduced with naïve testing in later pedigrees (Cook et al., 2007). The primary benefit of using such screens is the ability to rapidly test large numbers of animals on a relatively

wide range of behaviors. However, it cannot be discounted that the testing history of the animals should be considered. Lastly, in all three experiments, we have only examined two inbred strains. It would be ideal to extend the current experiments to include additional inbred strains, and examining the 129 strains would be of particular value given their regular use in the generation of transgenic mice.

Looking at the experiments presented here and the literature as a whole, one of the most striking features is the great deal of variability seen between similar tests, animals, and drugs. For example, we found no effect of SNRIX in two measures of anxiety-like behavior (i.e. the EZM and fear conditioning) and seemingly opposite effects, at least in B6 males, in two other measures of anxiety-like behavior (i.e. open field and light/ dark). While such variability is perplexing, it does seem to be the norm in the literature on preclinical anxiety-like behavior. It is possible that different assays are measuring unique aspects of anxiety-related behavior and particular drugs and experimental manipulations only affect some of these. However, using factor analysis to compare multiple measures of anxiety-like behavior often yields results that do not allow for meaningful interpretation or suggest that each measure should load onto an independent factor (Brigman et al., 2009; Philip et al., 2010). Ramos (2008) has noted that the effects seen on any single measure provides only a snapshot of an animal's overall pattern of behavior, and estimates of covariance between tests are highly temporally and contextually dependent. Considering this, it is interesting that in the open field, which is nearly four times the duration of our other measures of anxiety behavior, we see effects of both SNRIX and age-related differences. Considering Experiment 2, looking at only the first five minutes of open field testing, we still find the anxiolytic

effect of SNRIX in B6 males, but the anxiogenic effect in B6 females drops out. It may be the case that extending the test durations used in the other measures of anxiety-like behavior would aid in detecting small to moderate effects.

Overall, the available evidence on anxiety disorders and mood disorders in general, suggests they are the product of complex gene by environment interactions that are further shaped across development. Murine genetic models offer a unique opportunity to explore these relationships and address issues in the treatment of mood disorders. However, the nature of the experimental problem requires that factors with relatively small effect sizes be considered, which can be easily drowned out by experimental noise. Here, attempts have been made to address this by extending the baseline data available on anxiety-like behavior in two common inbred strains of mice, the ultimate goal being to further our understanding of the etiology of these disorders and improve our ability to treat them effectively.

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