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Spatial Learning and Stress Response of Male Rats Prenatally Exposed to Dexamethasone

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SPATIAL LEARNING AND STRESS RESPONSE OF MALE RATS PRENATALLY EXPOSED TO DEXAMETHASONE

by

Joseph Donohoe

Presented to the Faculty of
Bucknell University
In Partial Fulfillment of the Requirements for the Degree of
Master of Science in Biology

Approved: Kathleen Page
Adviser

Marie Pyron
Department Chairperson

4/29/2010

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Dexamethasone is routinely administered to women at risk for a preterm birth in order to enhance fetal lung development and reduce uterine contractions. Research has demonstrated possible behavioral abnormalities in adulthood as a result of dexamethasone treatment. Using nonlinear mixed effects modeling, this study found that prenatal dexamethasone treatment impaired spatial learning and memory of adult male Sprague-Dawley rats. Prenatal dexamethasone treatment also led to more anxiety related behaviors on Elevated Plus Maze testing 1½ hours after a stress challenge. Because the assumptions underlying the independent samples t-test were violated, the randomization test was used to compare groups on the Elevated Plus Maze.

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Prenatal maternal stress has been associated with a variety of psychiatric disorders in adult offspring (Darnaudery & Maccari, 2008; Talge et al., 2007). This has been documented in a number of model organisms, including rats (Morley-Fletcher et al., 2003), mice (Alonso et al., 2000), guinea pigs (Kapoor et al., 2009), and non-human primates (Hauser et al., 2008). Epidemiological evidence from humans exposed to prenatal maternal stress substantiates these findings (Malaspina et al., 2008; Watson et al., 1999), and the disorders implicated are extensive, linking prenatal maternal stress to depression (Watson et al., 1999), anxiety (Nagano et al., 2008), post traumatic stress disorder (Seckl & Meaney, 2005), attention deficit hyperactivity disorder (Van den Bergh et al., 2005), substance abuse (Maccari et al., 2003) and schizophrenia (Walker et al., 2008).

One possible mechanism by which prenatal stress exerts these effects is through excess endogenous glucocorticoids *in utero* (Seckl & Holmes, 2007). Glucocorticoids are steroid hormones secreted by the adrenal cortex as part of the hypothalamic-pituitary-adrenal (HPA) axis. When the HPA axis is activated in response to a stressor, the paraventricular nucleus of the hypothalamus secretes corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) into the hypophyseal portal capillaries. These hormones, in turn, stimulate the release of adrenocorticotropic hormone (ACTH) by the anterior pituitary. The hormone ACTH induces the adrenal cortex to secrete cortisol in humans and corticosterone in the rat, enhancing metabolic activity and increasing blood levels of sugars and other nutrients while also dampening the body’s immune response

(de Kloet et al., 2005). The increase in circulating glucocorticoids then reduces output of CRH and ACTH through negative feedback on the hypothalamus and anterior pituitary, respectively. Activity of the HPA-axis is further modulated by colocalized mineralocorticoid and glucocorticoid receptors in limbic structures in the brain. The hippocampus is particularly important for the negative feedback of the HPA-axis (Van Haarst et al., 1996).

During prenatal development the fetus is largely protected from endogenous circulating maternal glucocorticoids. This protection arises from the glucocorticoid placental barrier, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which catalyzes most active endogenous glucocorticoids into inert 11-keto forms (Kapoor et al., 2008). Minor amounts of maternal glucocorticoids, however, can pass through the placental barrier to the fetus, particularly during periods of elevated maternal stress (Seckl & Holmes, 2007). Conversely, synthetic glucocorticoids like dexamethasone (Dex) and betamethasoneare are able to pass through the fetal placental barrier because they are poor substrates for 11β-HSD2 (Seckl, 2004). Thus, synthetic glucocorticoids are able to mimic increased endogenous maternal glucocorticoids.

It is hypothesized that “prenatal programming” occurs in response to elevated prenatal glucocorticoids, whether they be endogenous maternal glucocorticoids or synthetic glucocorticoids like Dex (Matthews, 2002). This programming has evolutionary advantages for organisms with short life-spans. For instance, if the early postnatal environment is accurately predicted by the fetal milieu then adaptations *in utero* may

increase the organism's chance of survival and reproduction. For longer living species like humans, however, the fetal environment is less likely to forecast realistic conditions during the lifespan (Seckl & Holmes, 2007). Consequently, outcomes such as low birth weight (Welberg & Seckl, 2001) and HPA axis hyperactivity (Shoener et al., 2006) are detrimental rather than advantageous.

Aside from its role as a model for elevated maternal glucocorticoids, Dex has widespread clinical use, being routinely administered to mothers at risk for preterm birth. An NIH panel endorsed the use of maternal corticosteroid therapy in 1995 (NIH Consensus Development Conference, 1995), although in 2002 an NIH update recommended that repeated corticosteroid doses, a practice becoming more common (Brocklenhurst et al., 1999), be restricted only to current clinical trials (Committee on Obstetric Practice, 2002). Despite this, a randomized controlled trial examined the neonatal outcomes of single and multiple course exposure to synthetic glucocorticoids and found the latter to improve neonatal outcomes (Crowther et al., 2006). Dex and other synthetic glucocorticoids are effective during preterm deliveries because they rapidly enhance fetal lung maturation (Ward, 1994), resulting in improved survival rates among preterm infants (Crowley, 1995). Alarmingly though, roughly one-third of women admitted for pre-term labor end up with deliveries that are on term (Steer & Flint, 1999), while at the same time the overall number of synthetic corticosteroids being prescribed is rising (Polyakov et al., 2007). This combination underscores the need for increased understanding of the long term effects of synthetic glucocorticoids like Dex on the

developing offspring, particularly in regard to the impairments in cognition, affect, and behavior that are central to psychiatric disorders.

Prenatal Dex administration during the last third of gestation in rats has been shown to increase male offspring's anxiety behavior, demonstrated by reduced open arm exploration compared to control rats on the Elevated Plus Maze (Hossain et al., 2008). Additionally, research found that prenatal Dex-exposed offspring displayed reduced locomotion and rearing in the Open Field Test, another behavioral measure of anxiety (Wellberg & Seckl, 2001). The same study also showed that prenatal Dex administration during the last third of gestation reduced the offspring's number of open arm entries on the Elevated Plus Maze (Wellberg & Seckl, 2001). Other work has found that prenatal Dex-exposed offspring display a number of anxiety-like behaviors in the Open Field Test, including reduced time spent in the center of the square, reduced rears, increased immobility, and reduced distance traveled (Nagano et al., 2008). The same researchers examined behavioral outcomes on another anxiety related measure: the Light/Dark Choice Test. Prenatal Dex-exposed offspring spent a shorter time in the light chamber relative to controls, demonstrating increased anxiety (Nagano et al., 2008).

Other studies have shown mixed behavioral outcomes. A recent study by Hauser et al. (2009) reported no differences between prenatal Dex-exposed rats and controls across the Forced Swim Test of depression, the Open Field Test, the Elevated Plus Maze, the Morris Water Maze test of learning and memory, and the progressive ratio schedule of reinforcement, which measures the general inability to experience pleasure, or

anhedonia. Nagano et al. (2008) also failed to show any differences between prenatal Dex-exposed animals and controls in the Forced Swim Test. Prepulse inhibition (PPI) and latent inhibition (LI), two markers of schizophrenia, were also unaltered in offspring prenatally exposed to dexamethasone during the last third of gestation (Hauser et al., 2006).

Previous research in Dr. Page's lab has attempted to address these ambiguities by assessing the baseline behavioral differences between prenatal Dex-exposed male rats and control rats. Between 4-6 months, all animals were tested on the Forced Swim Test, the Elevated Plus Maze, the Open Field Test, and the Morris Water Maze. On all but the Morris Water Maze, there were no discernible differences between prenatal Dex-exposed and control animals (all p values > 0.40). Overall spatial learning rates were not significantly different on the Morris Water Maze although, unexpectedly, the prenatal Dex-exposed animals found the submerged platform faster on days 2, 3, and 4. In the current Morris Water Maze literature, when differences are found between control and Dex-exposed animals it is generally the control animals who find the platform quicker (Emgard et al., 2007; Noorlander et al., 2008).

The current research had two goals. First, it addressed baseline learning and memory with the Morris Water Maze. It was unclear whether the original data presented an actual trend toward significance—with the Dex-exposed animals finding the platform quicker on several days than the control animals—or whether there were experimental errors that accounted for the anomalous findings. The second goal was to more closely

analyze anxiety-related behavior in the prenatal Dex-exposed group. As opposed to learned helplessness and depressive behaviors, anxiety-related behavior measured with the Elevated Plus Maze, the Open Field Test, and the Light/Dark Choice Test, among others, have more consistently shown differences between prenatal Dex-exposed animals and control animals. Nonetheless, our initial investigations were unable to detect anxiety-related behavioral differences at baseline levels. It may be that prenatal dexamethasone exposure results in a maladaptive recovery after a stressor. For instance, compared to control rats, prenatal Dex-exposed rats have higher levels of circulating ACTH and corticosterone at both 1 hour (Hauser et al., 2009; Schoener et al., 2006) and 2 hours (Hauser et al., 2009) following restraint stress.

This research attempted to delineate the behavioral correlates of the observed HPA axis hyperactivity in response to stress challenge. After initial Morris Water Maze testing, all rats were subjected to a stress challenge followed by anxiety-related behavior testing. Similar to previous research (Briones-Aranda et al., 2009), the challenge was a 15 minute forced swim stress where rats were placed in a water-filled cylinder deep enough to prevent them from touching the bottom, yet filled to 15 cm from the top in order to elicit feelings of inescapability. At approximately 90 min.s following the swim stress behavioral testing was conducted on these animals using the Elevated Plus Maze. This time point was chosen because of recent work demonstrating increased circulating ACTH and corticosterone levels in prenatal Dex-exposed rats compared to controls one to two hours after a stress challenge (Hauser et al., 2009; Schoener et al., 2006). It was

hypothesized that prenatal Dex-exposure would impair spatial learning and memory on the Morris Water Maze and would lead to an increase in anxiety behavior on the Elevated Plus Maze after a stress challenge.

Materials and Methods

Animals

Sprague-Dawley dams received daily injections (sc) of DEX (125 μg·kg $^{-1}$ · day^{-1}; *n* = 36; Sigma, St. Louis, MO) or vehicle (saline + 0.4% ethanol, *n* = 36) during days 14–19 of gestation, in accordance with previous research (Shoener et al., 2006). On postnatal day 21 male offspring were weaned and housed 2 per group according to litter, for a resulting sample size of 60 rats. Behavioral testing with the Morris Water Maze was initiated at approximately 4 months of age. The stress challenge and Elevated Plus Maze testing were begun 2 weeks after the initial water maze testing. Testing on the Elevated Plus Maze was conducted 1 1/12 hours after the stress challenge in order to correspond with known durations of HPA-axis hyperactivity following a stress challenge in prenatal Dex-exposed male rats (Hauser et al., 2009; Schoener et al., 2006). All behavioral testing was tracked and recorded with ANY-maze software (Stelting, Co., Wood Dale, IL). Controlled lighting (0600 to 1800) and temperature (23°C) were maintained and rats were given food and water ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee at Bucknell University in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals.”

Morris Water Maze

The Morris Water Maze tests spatial learning and memory in rodents (Morris, 1981). The apparatus is a large circular metal tank with an interior diameter of 168 cm and a height of 58 cm. A coordinate system was used that arbitrarily divides the tub into the 4 principle coordinates: north, south, east, and west. The south coordinate is

represented by the area of the tank closest to the door entering the testing room. The tank is painted black and has a painted black 19.5 x 19.5 x 19.5 cm cinderblock placed in the northwest region. On the north, east, and west walls (with the south wall being the door that leads into the testing room), several shapes have been posted as background cues for spatial localization (Vorhees & Williams, 2006). One is three black stripes, one is a black triangle, and the other is a black plus sign. The tank is filled with 23.5 cm of 19-22°C water, putting the waterline 4 cm above the top of the cinderblock platform.

The test is an 8 day experiment: days 1-5 constitute the spatial learning component and day 8 constitutes the memory component. Each day had a randomly chosen starting coordinate that dictates where the rat was originally placed in the water, always facing the tank's wall. After the rat is in the water it has 2 minutes to find the submerged platform. If it does not find the platform in this timeframe the experimenter places the rat on the platform. Every time the rat reaches the platform, whether on its own or by the experimenter, it must remain there for 15 seconds. If it jumps off the platform before the 15 second period has expired the experimenter places it back on the platform. After 15 seconds, the experimenter places the rat in the next randomly chosen coordinate. This sequence occurs 4 times each day (days 1-5), such that each day the rat will have 4 two-minute timeframes to find the platform (which is always in the northwest quadrant). For reference, the coordinate sequence on day 1 was west, north, south, and east, and was different each day of testing. During days 1-5 the main behavioral measurement is the time—averaged across all 4 trials during each day—that the rat took to reach the submerged platform. Thus, each rat will have a single latency measure for each of the

first 5 days of testing. During days 6 and 7 the rat does not undergo any testing. Day 8 is the memory component, in which the submerged platform is removed and only 1 trial is performed. The rat is placed in a predetermined starting coordinate (south for all subjects) and for 30 seconds the amount of time the rat spends in the old submerged platform area (in the northwest quadrant) is recorded.

Forced Swim Stress

Similar to previous research (Briones-Aranda et al., 2009), the stress challenge will consist of day 1 of the Porsolt et al. (1978) Forced Swim Test. Rats are individually placed in a water (25 ±2 °C) filled translucent white, plastic cylinder, 45 cm high and 35 cm in diameter. Water is filled to a height of 30 cm, leaving 15 cm from the water line to the top of the cylinder. This 15 cm avoids the possibility that the rats can escape the tank and helps to induce feelings of helplessness. Rats remain in the water for 15 minutes. Previous research in the Page laboratory confirms the stress inducing effects of this procedure. After 15 minutes of swim stress, rats are immediately removed from the water, patted dry with towels, and allowed to warm under heat lamps for approximately 15 minutes.

Elevated Plus Maze

The elevated plus maze is an assay of anxiety-related behaviors (Walf & Frye, 2007). It is made of plywood and is painted a flat black. The plus shaped maze is 53 cm high and consists of 2 opposite facing open arms and 2 opposite facing closed arms. Each arm is 10 cm wide and extends 50 cm in length from a 10 X 10 cm square in the middle

of the maze. The open arms have a 1 cm wide and ¼ cm high edging along their border in order to prevent rats from falling off the platform. The closed arms are surrounded by 40 cm walls of thin wood. Rats are placed in the middle of the plus maze facing towards an open arm and their behavior is tracked for 5 minutes. More time spent and entries into the open arms are indicative of reduced anxiety while more time spent and entries into the closed arms signify anxious behaviors (Walf & Frye, 2007).

Data Analysis

Morris Water Maze spatial learning data (days 1-5) were analyzed with non-linear mixed effects modeling (NLME) using the statistical computing program R (http://cran.r-project.org). The two groups were based on prenatal condition (control or dexamethasone). The NLME method was chosen prior to data collection based on existing literature documenting its strengths compared to the repeated measures ANOVA (Gueorguieva & Krystal, 2004; Jonsson, Karlsson, & Wade, 2000; Young et al., 2009). For example, Young et al. (2009) used Monte Carlo simulation to compare the repeated measures ANOVA with both linear and nonlinear mixed effects modeling. They created experiments that varied several properties of the data, including the slope effect sizes between the groups, the overall experimental learning rate, and the y-intercept values between the groups. The linear and nonlinear mixed effects models were both more sensitive than the ANOVA approach, meaning that for a given Type I error level the Type II error level was lower, indicating higher power. Also, the nonlinear mixed effects model showed much better goodness of fit than the linear mixed effects model and the ANOVA (Young et al., 2009).

The repeated measure analysis of variance (ANOVA), has many well documented problems including inflated type I error rates, lack of power, and the need for complicated post-hoc tests that are difficult to interpret (Gueorguieva & Krystal, 2004; Young et al., 2009). The repeated measure ANOVA also has the rigid assumption of homogeneity of covariance. This assumption, often referred to as the sphericity assumption, requires the variability to be equal between each measured time point and also requires the correlations between any two time points of a subject to be equal. Using the current Morris Water Maze procedure as an example, the correlation requirement implies that day 1 and day 2 latency times will show the same correlation as day 1 and day 5 latency times (Gueorguieva & Krystal, 2004). But, as Gueorguieva and Krystal (2004) point out, consecutive measurements of a subject are likely to show a greater correlation than measurements that are spaced further apart. When the homogeneity of covariance assumption is not met the F-distribution may not be a valid approximation of the obtained F-statistic. This will lead to increased Type I error rates, or the increased likelihood of declaring a true difference between groups when none exists.

The function used was based on Morris Water Maze data analyzed Young et al., (2009) in which latency time = Ae^{Bt}. In this exponential function, A represents the y-intercept at t (time) = 0 (i.e., the average latency to the platform on day 1) and B represents the rate of exponential change (i.e., the rate of learning across all 5 days; Young et al., 2009). Based on previous water maze data, the starting parameters used were A = 90 seconds and B = -0.2.

The data for each of the other behavioral measures—the Forced Swim Test, the Open Field Test, the Elevated Plus Maze, and day 8 memory data from the Morris Water Maze—were in a two independent groups design. This design would traditionally be analyzed with an independent samples t-test. Briefly, the independent samples t-test follows the familiar hypothesis-testing procedure in that it first produces a test statistic—generally based on the difference between the means of the two groups on the dependent variable of interest—and then compares that test statistic with an established frequency distribution. A p-value is then obtained which reveals the probability of getting the observed test statistic if the null hypothesis were true. For the independent samples t-test the distribution used for comparison is the student's t distribution.

In order for the student's t-distribution to be accurate, several assumptions about the data must be met. The two main assumptions are that the variances are equal for the two groups and that the dependent variable of interest is normally distributed. When these assumptions are violated, however, the student's t distribution is not necessarily an accurate approximation of the population being studied (Erceg-Hurn & Mirosevich, 2008). In this experiment, boxplots and normal probability plots were used to test the assumptions underlying the independent samples t-test.

Rank-based nonparametric tests are often used as an alternative approach when the assumptions of a parametric test are violated. For the independent samples t-test the rank-based alternative is the Mann-Whitney U test. One problem with ranking data is the lack of power, particularly when effect sizes are small (Adams & Anthony, 1996). Another common technique in response to departures from parametric assumptions is to

transform the data. In this scenario, a logarithm or square root transformation is performed and traditional parametric tests are then conducted on the newly transformed data. Erceg-Hurn and Mirosevich (2008) point out that this approach has a number of shortfalls, including difficulty interpreting the transformed results, occasional loss of power, and failure to deal with outliers.

Modern statistical computing makes other options viable though. One technique that does not rely on assumptions about the shape of the distribution or the variability of the samples is the randomization test (Ernst, 2004). This is a re-sampling approach that uses the null hypothesis that group membership has no effect on the dependent variable. In this experiment, for example, the null hypothesis for the randomization test conducted on the Elevated Plus Maze after stress challenge data would state that the assignment of rats into the prenatal Dex-exposed group or the control group had no effect on their anxiety-related behavior. As in the independent samples t-test, the first step is to compute the test statistic based on the difference between the means of the control and Dex-exposed rats. Then the data are reshuffled and randomly assigned to one of the two groups, regardless of each data point's original group. A test statistic is then computed in the same way as was done in the original data set. This procedure—where the data are reshuffled and a test statistic is computed—is repeated many times, often 10,000 or more. A distribution is created based on the 10,000 re-samples and the original test statistic is compared to the newly created re-sampling distribution. Using $\alpha = 0.05$, if the original test statistic is more extreme than 2.5% of the cases at either end of the distribution the null hypothesis is rejected. Thus, the rationale behind hypothesis testing using the

randomization test is the same as with an F- or t-test: the obtained test statistic is compared to a distribution and the corresponding probability value indicates how likely that particular test statistic would be if there were no differences between the groups being studied. The main difference, of course, is the distribution that the original test statistic is compared to.

Several researchers have argued for the use of the randomization test over an F- or t-test in all scenarios, regardless of whether the assumptions of the F- or t-test are violated (Ludbrook & Dudley, 1998; Mewhort, 2005). One study used a Monte Carlo simulation to compare the randomization test with an F-test when the data were normally distributed, when the data were skewed, and when the data had different variances (Mewhort, 2005). Interestingly, the randomization test was never less powerful than the F-test, even when no assumptions of the F-test were violated. The randomization test was more powerful than the F-test when the data were skewed, however (Mewhort, 2005).

Ludbrook & Dudley (1998) argue for the randomization test over F- or t-tests for another reason. They point out that F- and t-tests are based on a population model of inference, where a random sample is drawn from a normally distributed population. Nonetheless, the vast majority of comparative studies in the biomedical sciences define experimental groups not by random sampling from a broader population of interest but by simply randomizing a nonrandom sample into defined experimental groups (Ludbrook & Dudley, 1998). Although random sampling is rare, statistical procedures like the F- and t-tests are used in an overwhelming majority of experiments, despite the fact that random sampling is a fundamental property of the F- and t-distribution's probability models.

Using this logic, Ludbrook and Dudley argue that the randomization model is a more legitimate way to make experimental inferences.

Day 8 memory data were analyzed in a two independent groups design where prenatal condition (control or Dex-exposure) was the independent variable and time spent in the submerged platform region was the dependent variable. Exploratory data analysis were used to reveal whether the assumptions underlying the traditional independent samples t-test were met. If the assumptions were not met the re-sampling based randomization test was used (Mewhort, 2005) and was then analyzed using R. If the t-test was used the procedure was conducted with SPSS version 16.0. The same approach was applied to the data from the Elevated Plus Maze following the stress challenge. Prenatal condition (control or Dex-exposure) was the independent variable and the ratio of open arm time to total time on the apparatus and the ratio of open arm entries to total arm entries were the dependent variables.

Results

Morris Water Maze Data

Nonlinear mixed effects modeling (NLME) was used to analyze the spatial learning data generated from behavioral testing on the Morris Water Maze. The function used by Young et. al. (2009) fit the data adequately, as assessed by the comparatively low akiake information criterion (AIC) and bayesian information criterion (BIC) values. In addition, exploration of the current Morris Water Maze data also revealed violations of assumptions necessary for the ANOVA, specifically the homogeneity of covariance assumption. Mauchly's test of sphericity was used to determine adherence to the homogeneity of covariance assumption. The test demonstrated serious violations of the assumption for each group of animals tested on the Morris Water Maze (all p-values < 0.01).

For reference, preliminary 2009 Morris Water Maze spatial learning data are shown in Figure 1. Using the nonlinear mixed effect model, the difference between control and Dex day 1 latency times was virtually nonexistent ($p = 0.998$). The rate of learning (i.e., the slope difference between the lines) was also not significant ($p = 0.560$). Day 8 memory data, where the submerged platform is removed and the time spent in the platform region is measured, was not collected for these animals due to experimenter error.

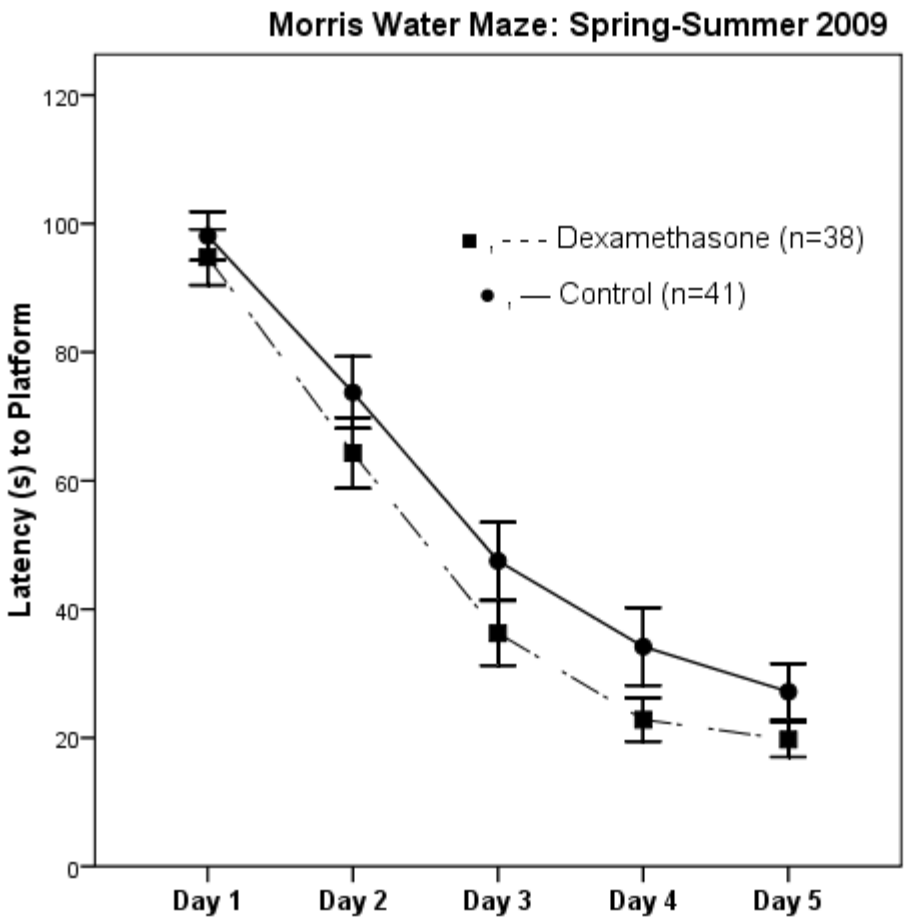

Figure 1. **Preliminary Morris Water Maze spatial learning data**. Neither day 1 latency times nor overall learning rates were significantly different in the two groups. Error bars represent ± S.E.M.

Spatial learning data for the current 2010 experimental animals are displayed in Figure 2. The nonlinear mixed effects model found that the control rat's Day 1 latency time was significantly lower than the Dex-exposed rats latency time ($p = 0.003$). The rate of learning showed a trend toward significance ($p = 0.063$). Two differences are immediately noticeable between this group of rats and the previously tested group. First, in the current group the control rats have lower latency times across each day while in the

previous group the Dex-exposed rats had lower latency times. Secondly, the initial latency values (Day 1) are considerably lower in the current group than in the previously studied animals.

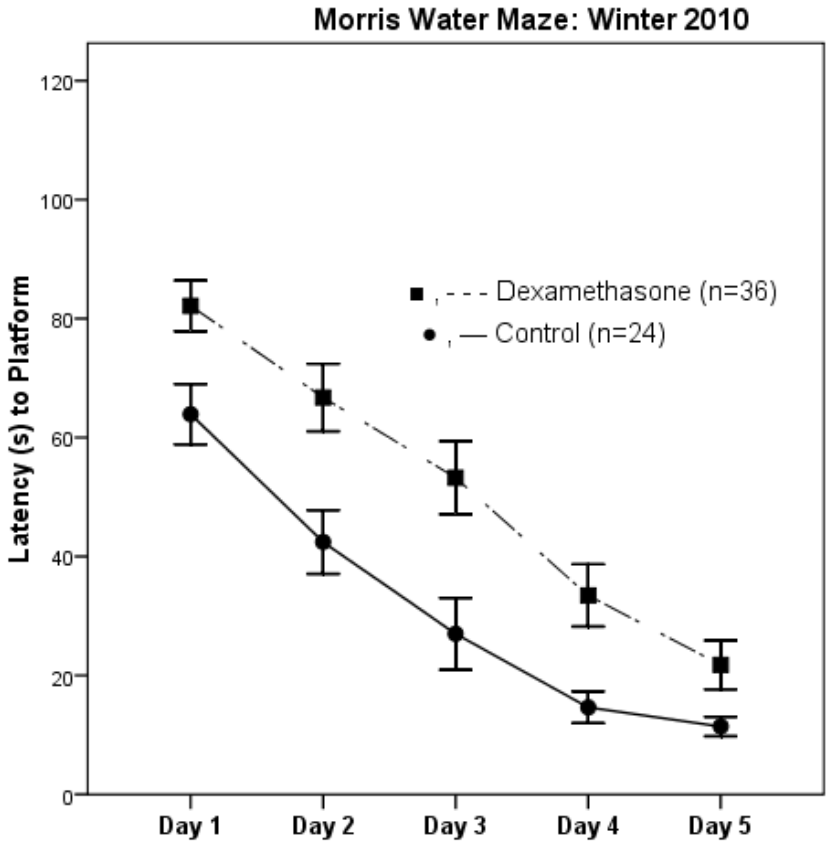

Figure 2. **Morris Water Maze spatial learning data from current 2010 experimental animals**. Day 1 latency times were significantly different and learning rates showed a trend towards significance. Error bars represent ± S.E.M.

The spatial learning data for the current group of 60 rats was combined with the spatial learning data from the previous 79 rats, yielding an overall picture of the effects of prenatal dexamethasone exposure on adult rat's spatial learning (Figure 3). The combined sample had 139 animals. There were no significant differences in day 1 latency time (p = 0.100) or in overall learning rate (p = 0.556) between the control and Dex-exposed rats.

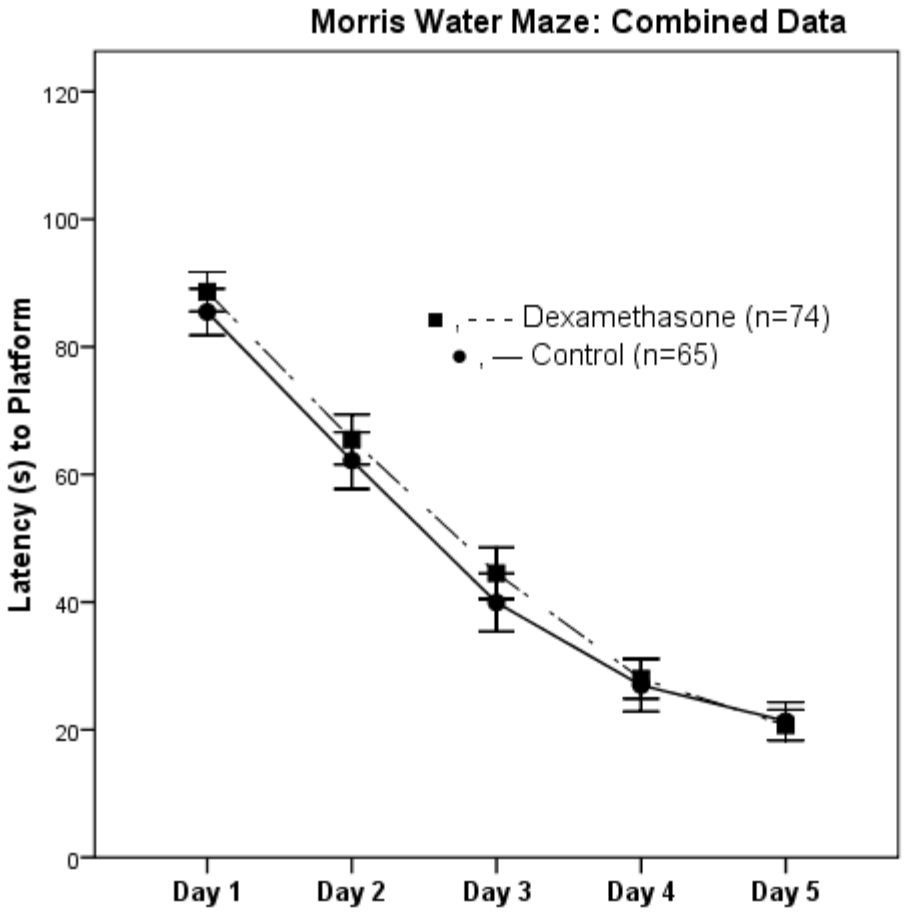

Figure 3. **Morris Water Maze spatial learning data for all animals**. Neither day 1 latency times nor overall learning rates were significantly different between control and Dex-exposed rats. Error bars represent ± S.E.M.

Day 8 Morris Water Maze memory data for the current group of 60 rats is presented in Figure 4. Boxplots of the data reveal that, unlike the 2-independent sample situations examined in the other behavioral measures, the Morris Water Maze day 8 memory data did not have substantial problems with outliers, or the assumptions of being normally distributed and having equal variances (Figure 5). Consequently, the independent samples t-test was employed to test for differences between the groups. The

Dex-exposed animals spent significantly less time in the memory region than the control animals (t (28) = 2.16, p = 0.036).

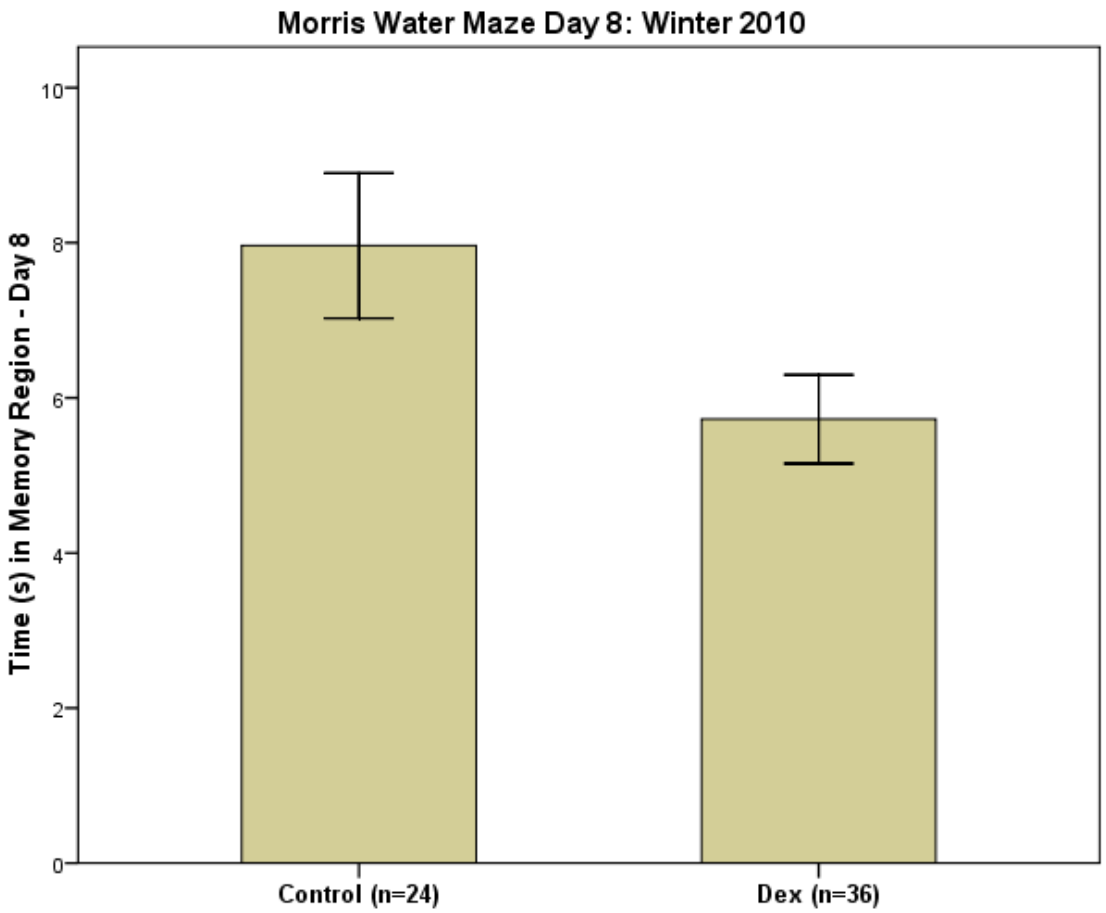

Figure 4. **Morris Water Maze day 8 memory data from the current experimental group**. Dex-exposed rats spent significantly less time in the memory region than the control rats. Error bars represent ± S.E.M.

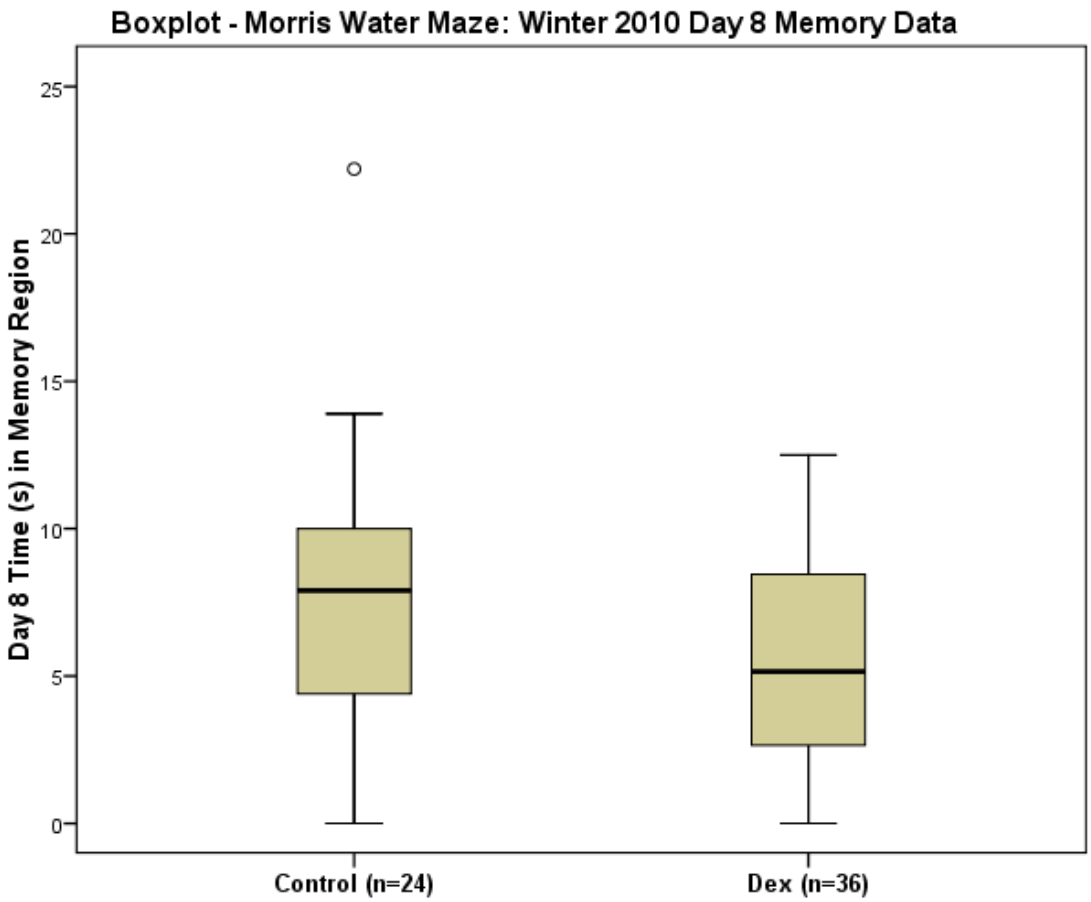

Figure 5. **Boxplots of Morris Water Maze day 8 memory data**. The data were normally distributed and only 1 outlier was detected.

Baseline Elevated Plus Maze, Forced Swim Test, and Open Field Test

As described in the introduction, the baseline behavioral data for the Elevated Plus Maze, the Forced Swim Test, and the Open Field Test displayed no differences between the rats prenatally exposed to dexamethasone and the control rats. Figures 6 through 10 summarize these findings, displaying the main variables of interest for each test. The Open Field Test was only implemented in the summer of 2009, resulting in

smaller sample sizes than the other measures that combined data from the spring and summer.

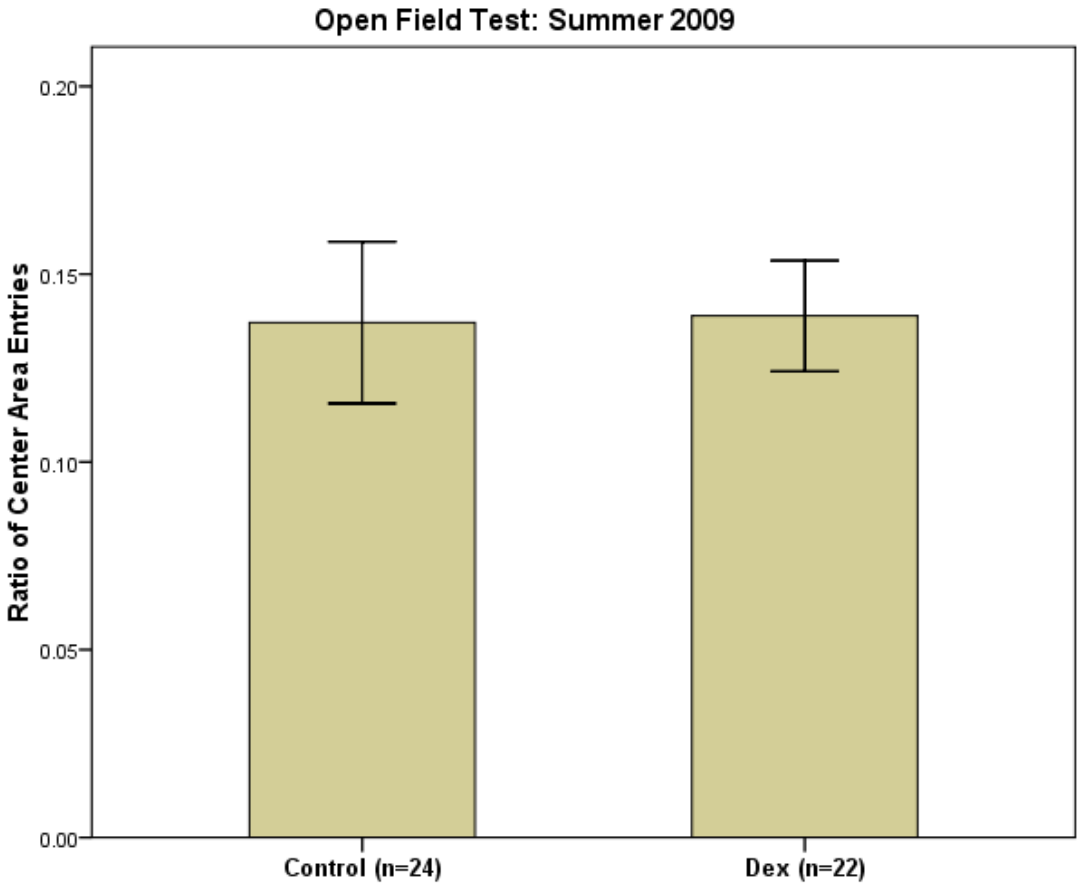

Figure 6. **Open Field Test data from the summer of 2009**. There was no difference between control and dexamethasone rats in the ratio of center area entries to total entries (p = 0.893). Error bars represent ± 1 S.E.M.

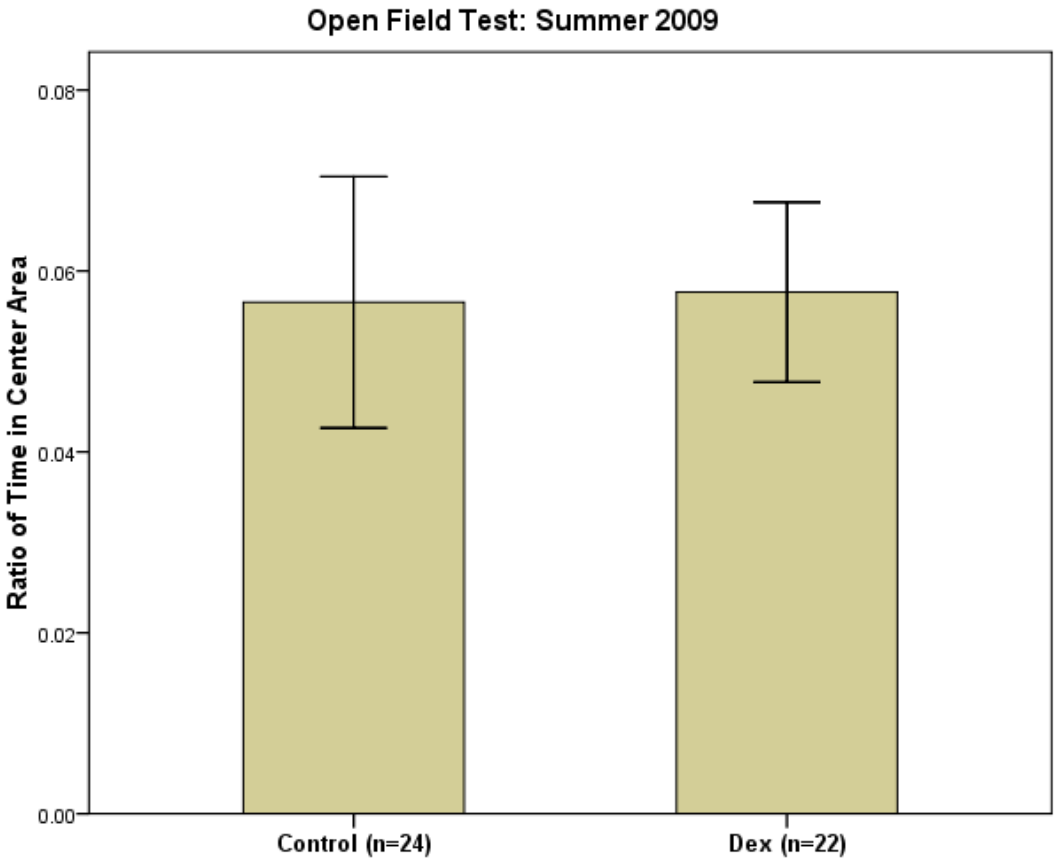

Figure 7. Open Field Test data from the summer of 2009. There was no difference between control and dexamethasone rats in the ratio of center area time to the total time in the apparatus (p = 0.948). Error bars represent ± S.E.M.

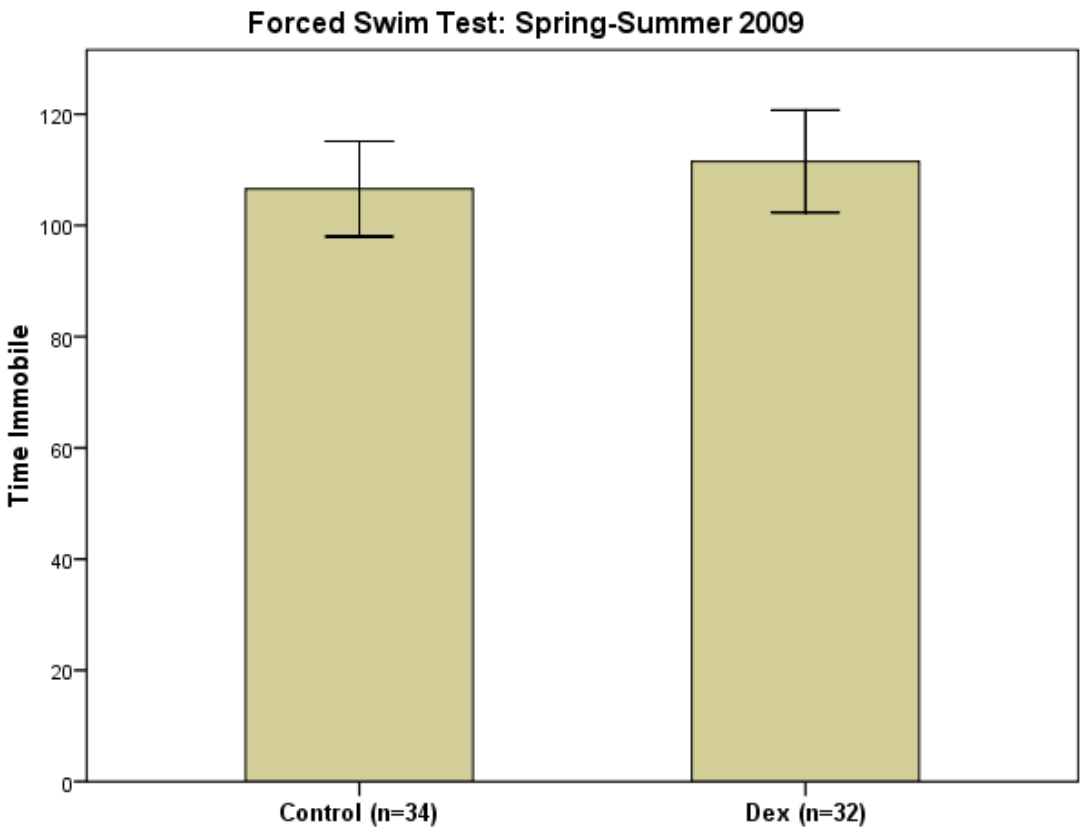

Figure 8. **Forced Swim Test data from the spring and summer of 2009**. There was no difference between control and dexamethasone rats in total time spent immobile (p = 0.706). Error bars represent ± S.E.M.

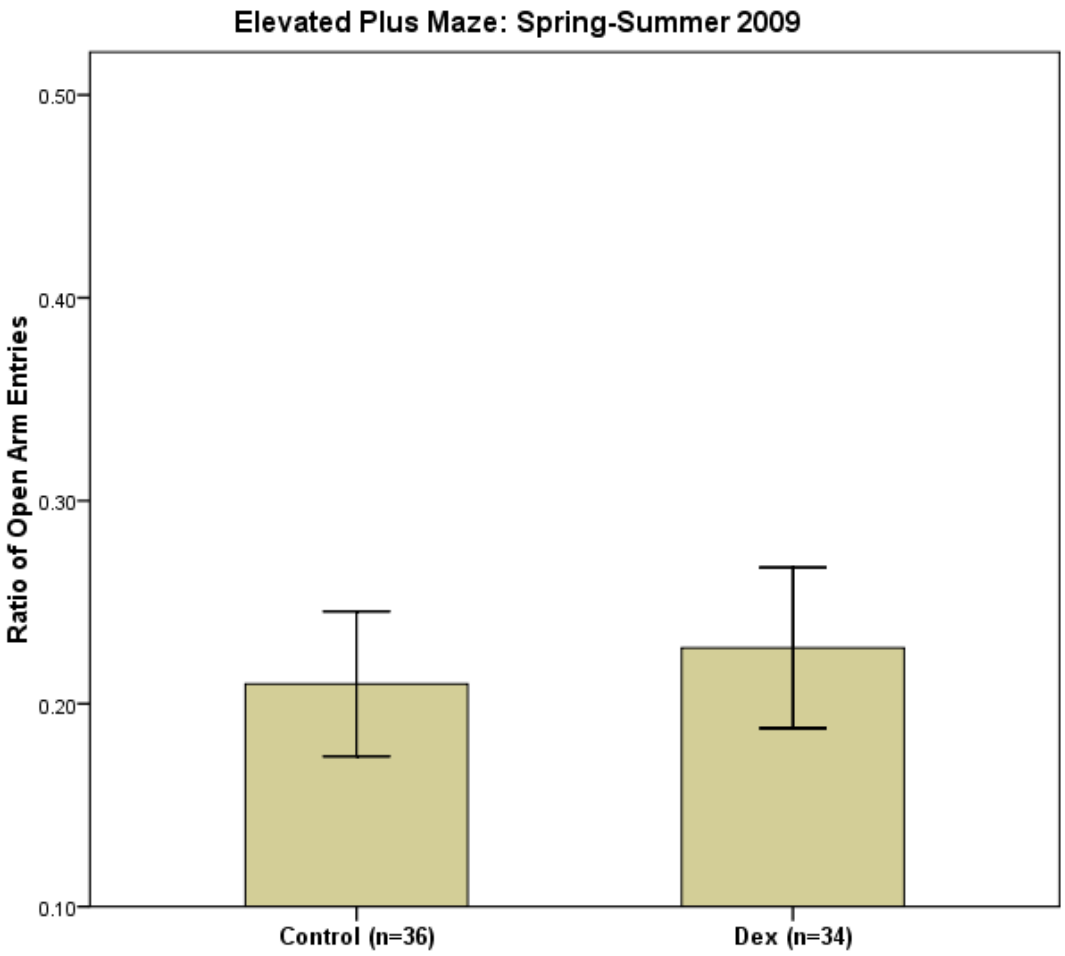

Figure 9. **Elevated Plus Maze data from the spring and summer of 2009**. There was no difference between control and dexamethasone rats in the ratio of open arm entries to total entries ($p = 0.592$). Error bars represent ± S.E.M.

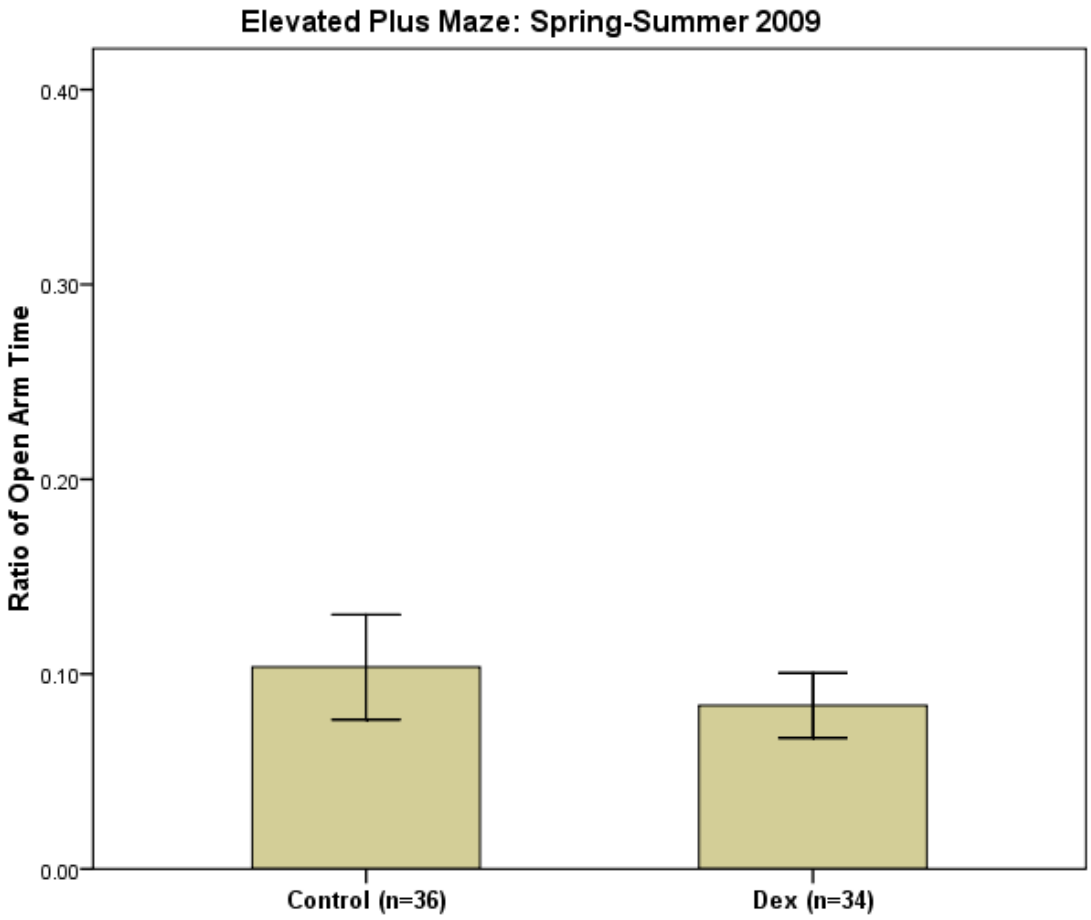

Figure 10. **Elevated Plus Maze data from the spring and summer of 2009**. There was no difference between control and dexamethasone rats in the ratio of time spent in the open arms to the total time in the apparatus ($p = 0.552$). Error bars represent ± S.E.M.

Elevated Plus Maze After Stress Challenge

Rats were exposed to the forced swim stress and then 90 min. later were tested on the Elevated Plus Maze. Data from the two main variables are presented in Figures 11 and 12. Exploratory data analysis revealed that the data violated several of the assumptions necessary for the independent samples t-test. Normal probability plots (figures 13 through 16) indicate that the samples were not normally distributed. This was

true for both the Dex-exposed and control animals across both of the measures of interest. Histograms (figures 17 and 18) and boxplots (figures 19 and 20) also highlight the data's lack of normality. The ratio of time spent on the opens arms for the Dex-exposed group, in particular, was positively skewed. The boxplots also successfully highlighted numerous outliers. Because the assumptions for the independent samples t-test were not met, the randomization test was used to test for differences in the ratio of open arm entries and ratio of time spent in the open arms between control and Dex-exposed rats. Figure 11 displays the differences in the ratio of open arm entries to total entries. Dex-exposed rats had a significantly lower ratio of open arm entries compared to control rats ($p = 0.032$). The differences in the ratio of time spent on the open arms to total time in the apparatus are shown in Figure 12. Dex-exposed rats again showed increased anxiety, with a significantly lower ratio of time spent in the open arms ($p = 0.018$). A useful comparison is between Figures 11 and 12, showing the Elevated Plus Maze data after the stress challenge, and Figures 9 and 10, showing the exact same measures completed by animals not exposed to the stress challenge.

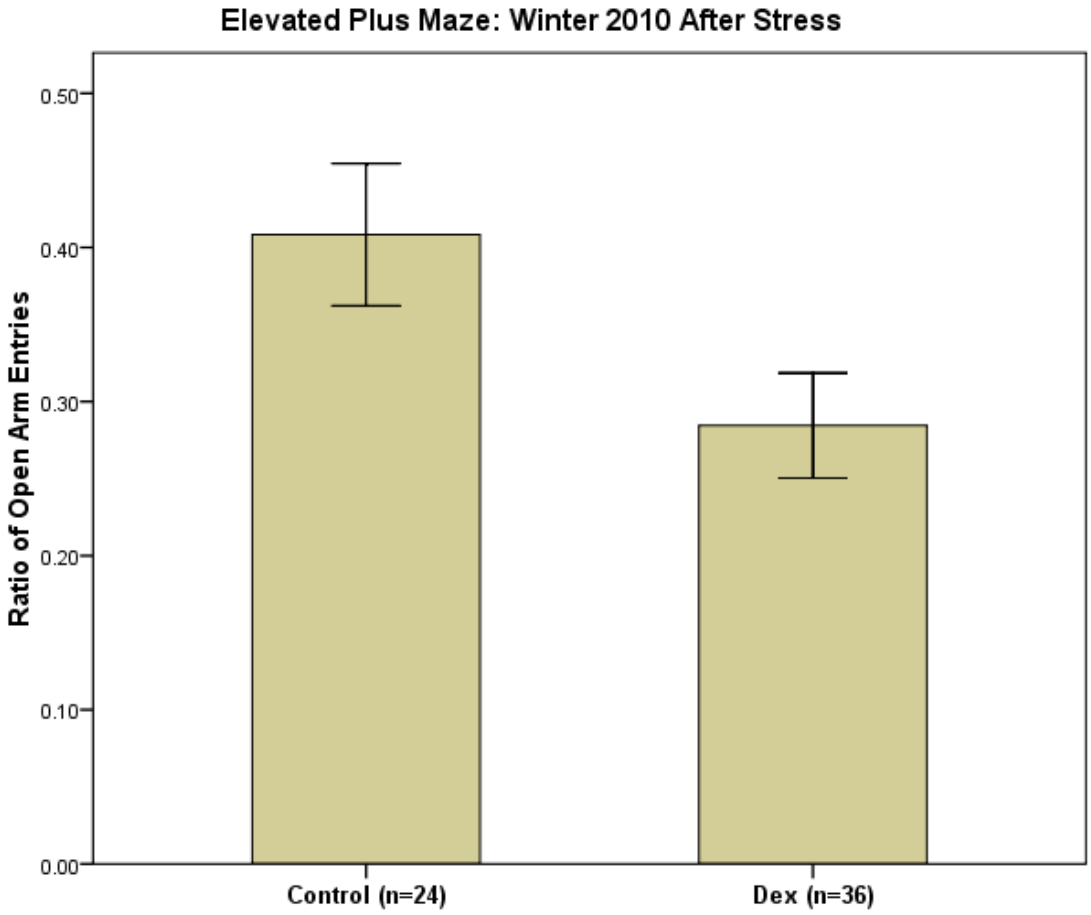

Figure 11. **Elevated Plus Maze data from rats exposed to the forced swim stress**. There was a significant difference between control and dexamethasone rats in the ratio of open arm entries to total entries. Error bars represent ± S.E.M.

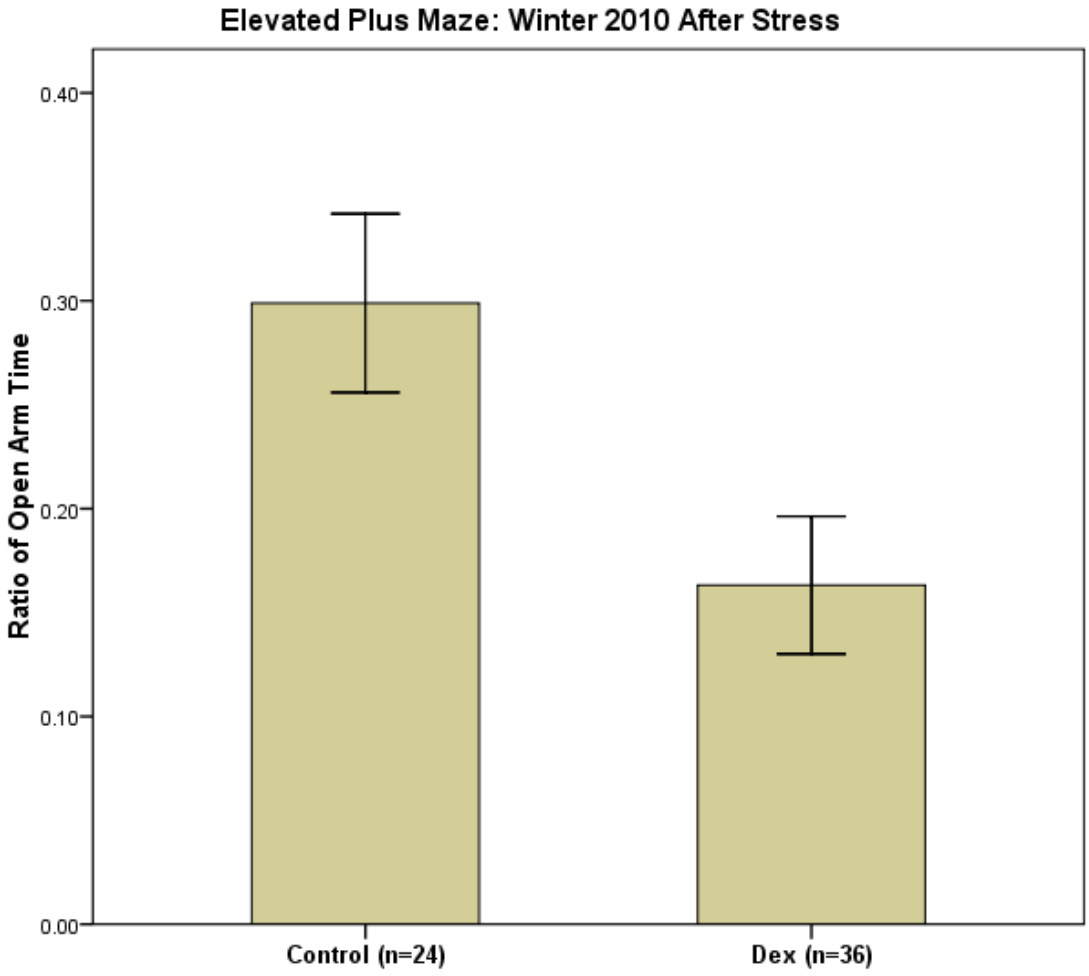

Figure 12. **Elevated Plus Maze data from rats exposed to the forced swim stress**. There was a significant difference between control and dexamethasone rats in the ratio of time spent on the open arms to total time in the apparatus. Error bars represent ± S.E.M.

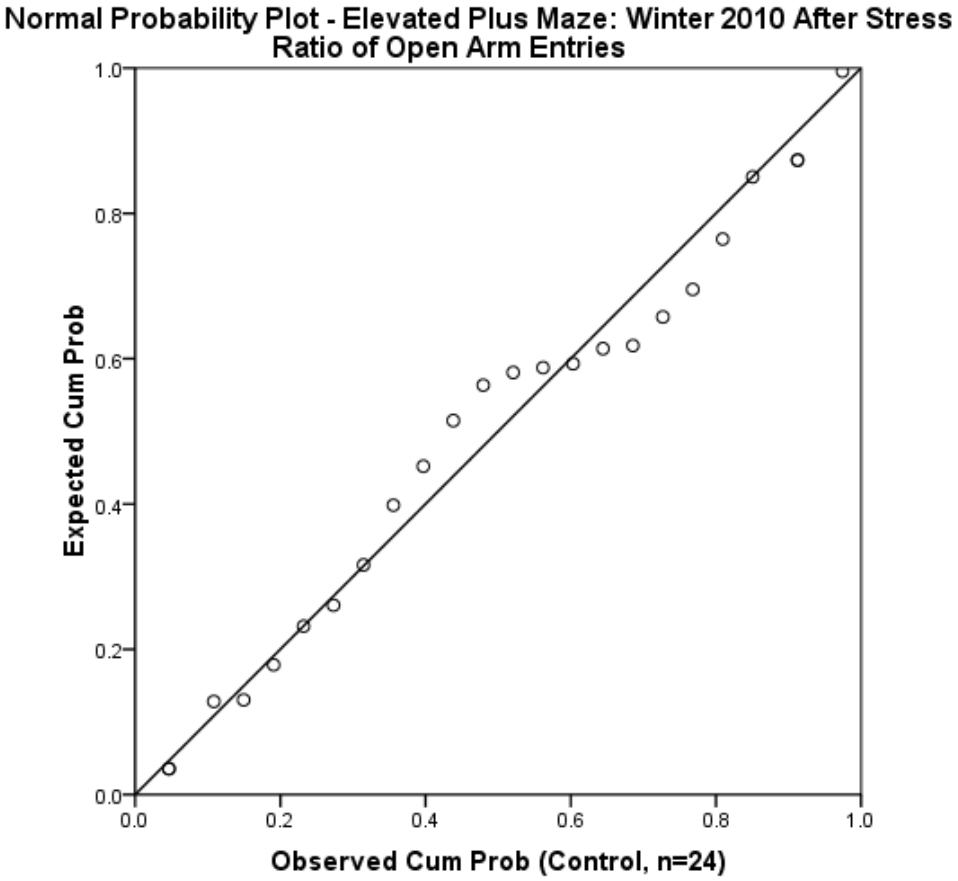

Figure 13. **Normal probability plot:** Control rat's ratio of open arm entries to total entries on the Elevated Plus Maze 90 min. after a forced swim stress challenge.

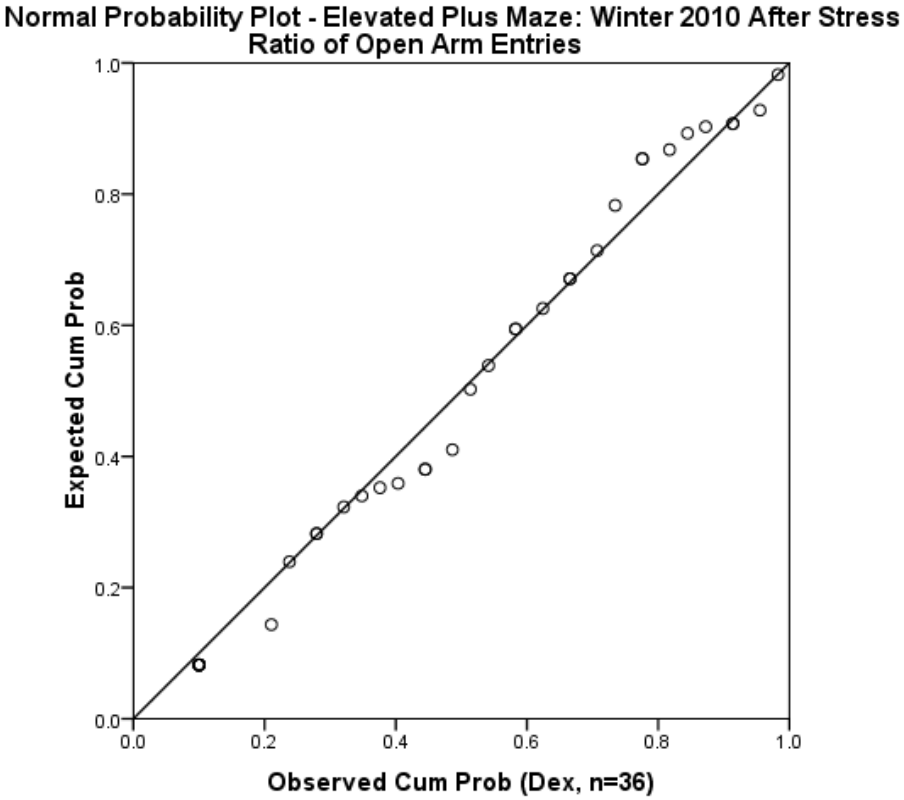

Figure 14. **Normal probability plot**:Dex-exposed rat's ratio of open arm entries to total entries on the Elevated Plus Maze 90 min. after a forced swim stress challenge.

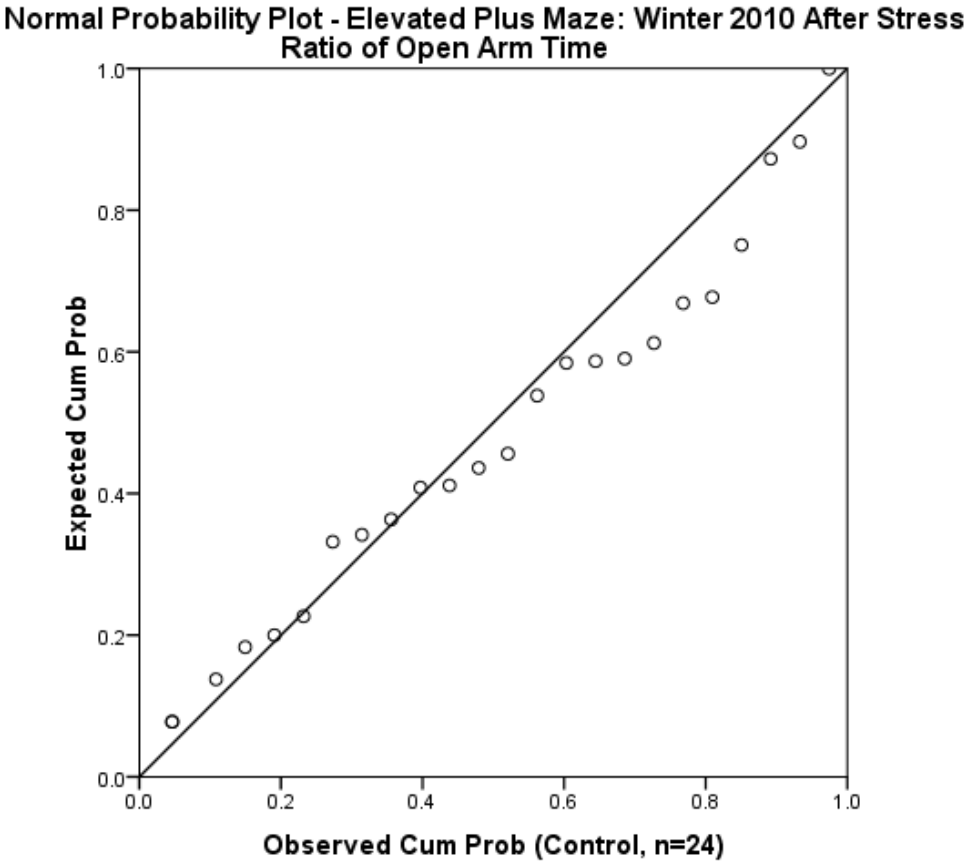

Figure 15. **Normal probability plot**: Control rat's ratio of time spent on the open arms to total time in the apparatus on the Elevated Plus Maze 90 min. hour after a forced swim stress challenge.

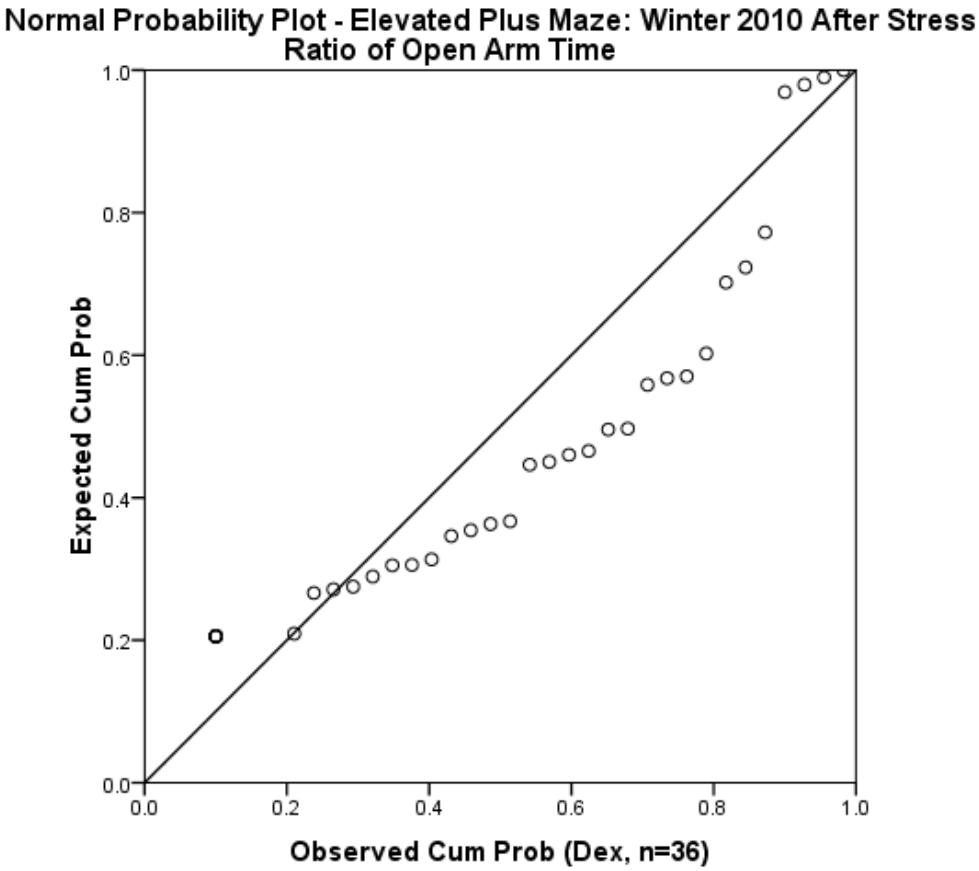

Figure 16. **Normal probability plot**: Dex-exposed rat's ratio of time spent on the open arms to total time in the apparatus on the Elevated Plus Maze 90 min. hour after a forced swim stress challenge.

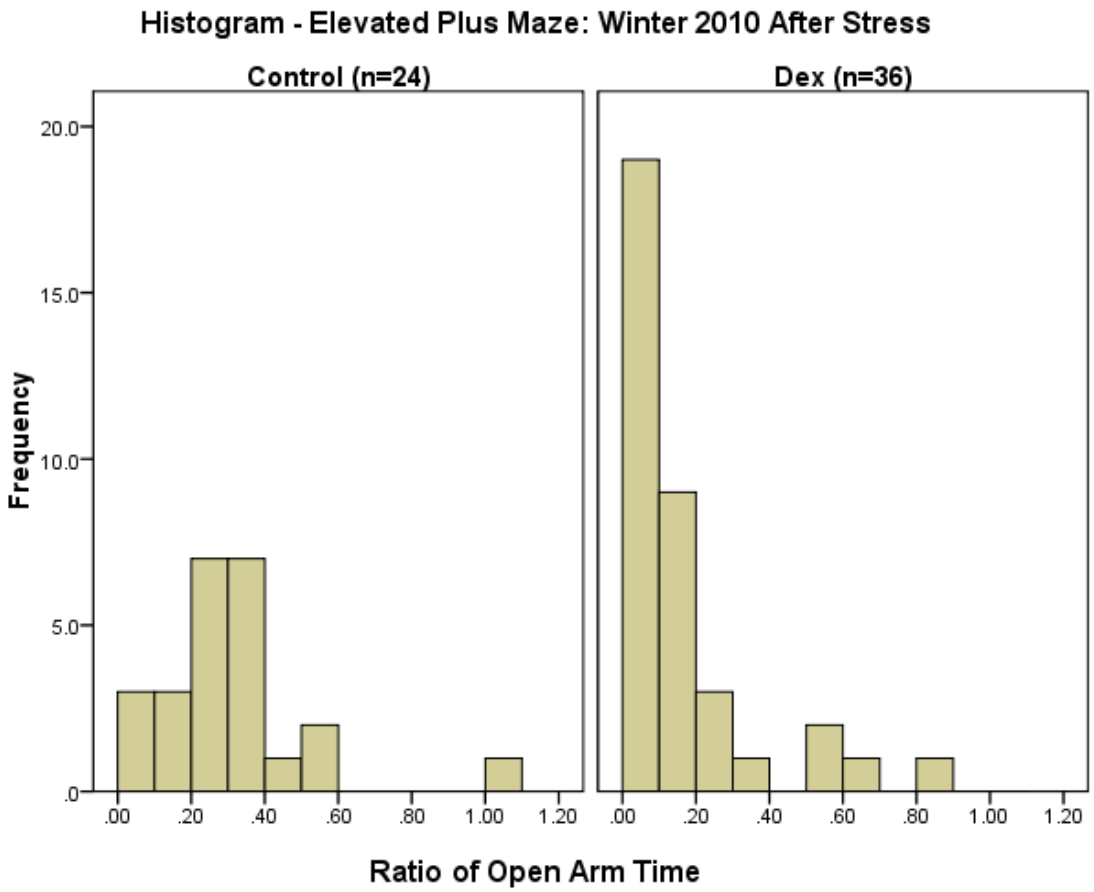

Figure 17. **Histogram:** Ratio of open arm time to total time on the apparatus on the Elevated Plus Maze 90 min. after a forced swim stress challenge.

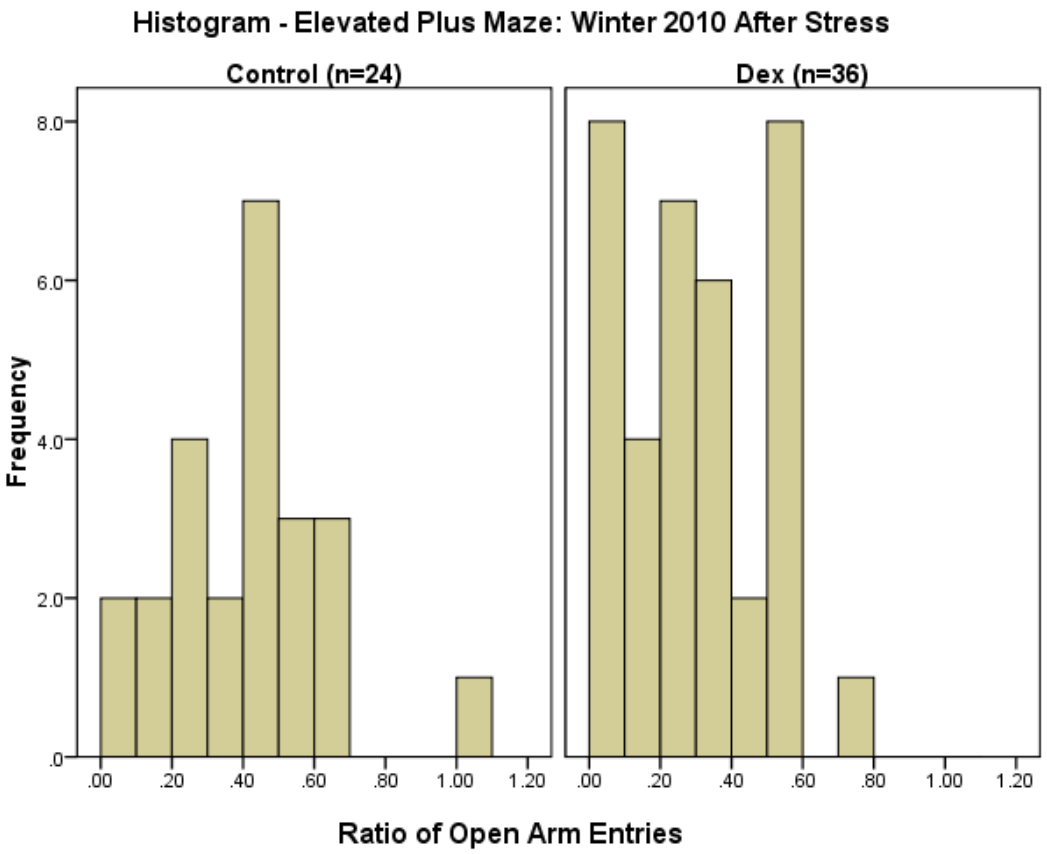

Figure 18. **Histogram**: Ratio of open arm entries to total entries on the Elevated Plus Maze 90 min. hour after a forced swim stress challenge.

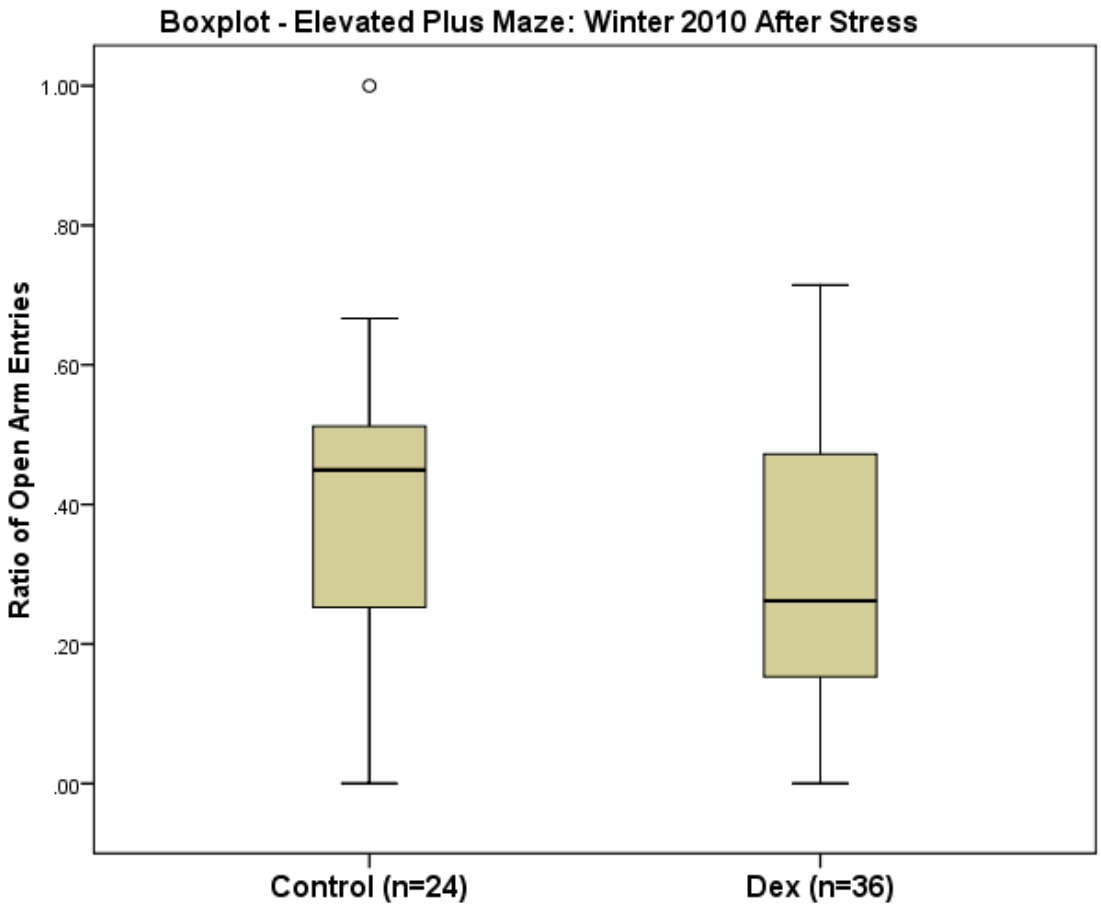

Figure 19. **Boxplots**: Ratio of open arm entries to total entries on the Elevated Plus Maze 90 min. after a forced swim stress challenge.

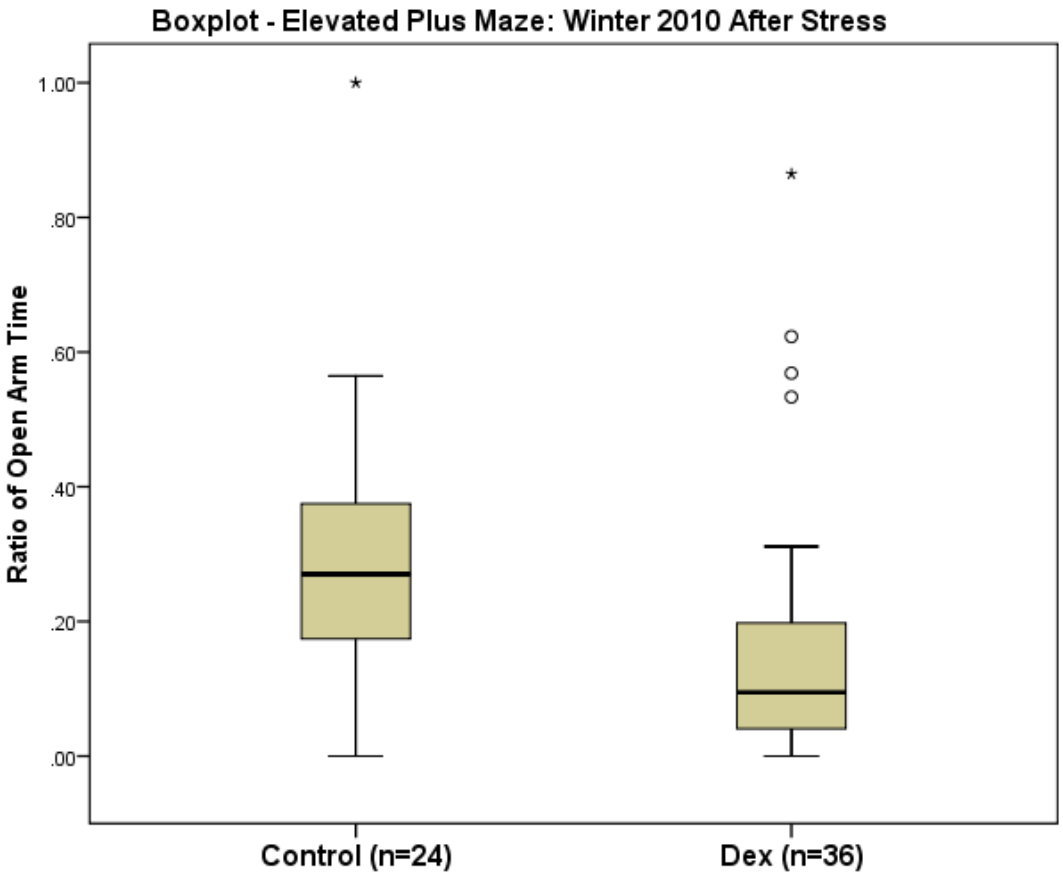

Figure 20. **Boxplots:** Ratio of open arm time to total time on the apparatus on the Elevated Plus Maze 90 min. after a forced swim stress challenge.

Discussion

The behavioral effects of prenatal dexamethasone exposure were studied in 60 adult male Sprague-Dawley rats. Spatial learning and memory were assessed with the Morris Water Maze and showed that the control rats found the submerged platform quicker than the Dex-exposed rats across each day of the test. When combined with the Morris Water Maze data from a previous group of 79 animals, however, no statistically significant differences were found between control and Dex-exposed rats. Explanations for the differences between the current group of 60 rats and the previous group of 79 rats are explored below.

After undergoing a stress challenge, the 60 rats were tested for differences in anxiety-related behaviors on the Elevated Plus Maze. Across both of the main variables measured, the ratio of open arm entries to total arm entries and the ratio of time spent on the open arms to total time in the apparatus, the Dex-exposed rats displayed greater anxiety behaviors than the control animals. This is in contrast to the baseline levels of anxiety in control and Dex-exposed rats, where, in the absence of any stress challenge, there were no differences in anxiety-related behaviors. The lack of any significant findings in the Open Field Test indicate that the behavioral differences on the Elevated Plus Maze after the stress challenge were not a result of different amounts of locomotor activity between the control and Dex-exposed animals.

Current Behavioral Data Compared With Previous Groups

The current research was based in large part on experiments conducted in the Spring and Summer of 2009. This preliminary data examined behavior of control male

rats and male rats prenatally exposed to dexamethasone across the Morris Water Maze, the Elevated Plus Maze, the Forced Swim Test, and the Open Field Test. Several interesting details emerge when comparing the results of these earlier experiments with the results of the current experiments on the Morris Water Maze and the Elevated Plus Maze following a stress challenge. For the Morris Water Maze data there was no real change in experimental procedure when testing the current group of 60 animals: dexamethasone was administered at the same point in gestation, pups were weaned on the same day, water temperature was held constant, and testing took place in the usual 4-6 month old range. Testing even took place during the same 11 a.m. to 3 p.m. time span during the day and only one experimenter conducted the testing. However, the mode of injection was changed from intraperitoneal to subcutaneous and raised from 125ug Dex/Kg to 150 ug Dex/kg.

The most obvious difference in the data for the Morris Water Maze was the reversal of which group had the quicker latency times. In the Spring-Summer 2009 group the Dex-exposed animals reached the platform more quickly than the control animals on each day of the test. This was reversed in the current group of 60 animals, where the control rats had lower latency times compared to the Dex-exposed rats on each day of the test. The outcome of Dex-exposed rats having poorer performance on the Morris Water Maze, as was seen in the current group of 60 rats, would fit more closely with the current literature. Although Hauser et al. (2009) found no effect of prenatal dexamethasone treatment on Morris Water Maze data, other research using both mice and rats has shown that prenatal dexamethasone administration impairs spatial learning and memory on the

Morris Water Maze (Emgard et al., 2007; Noorlander et al., 2008). Since the testing conditions were constant for all groups, experimenter error could be one possible explanation for the discrepancy between the data.

The experience and temperament of the researcher handling the animals during the Morris Water Maze can have an effect on the test's outcomes (Vorhees & Williams, 2006). The animals tested in the Spring-Summer 2009 group were the first animals ever handled and tested by the experimenter. It is plausible that by the time of testing for the current group of 60 animals, the experimenter was more comfortable, and thus less anxious, during testing and handling of the animals. This would result in the animals being less anxious during the Morris Water Maze and, ultimately, in better performance finding the submerged platform. The data confirms this hypothesis. For both the control and Dex-exposed rats, latency times decreased at each day from the Spring-Summer 2009 group to the current group (i.e., day 1 latency time for controls was lower in the current experiment than day 1 latency time for controls in the Spring-Summer 2009 group, and so on for each day of testing). The only exception was a slight increase of 3.8 seconds in latency time for the Dex-exposed animals on day 3 of testing.

Although decreases in latency times between the Spring-Summer 2009 group and the current group were seen across all days of testing, the decreases did vary systematically with day. Presumably, because of the novelty of the testing conditions, the animal’s temperament on day one of testing would be most likely to vary with the temperament of the experimenter. Because day one testing of the Spring-Summer 2009 group was the experimenter’s first experience testing the animals, it seems reasonable to

assume that this day would produce the poorest performance compared to the current group of animals tested. Contrasting the Spring-Summer 2009 data with the current group's data suggests that this is what occurred, as the biggest decrease in latency time from the Spring-Summer 2009 group to the current group came on day one of testing.

In addition to differing by test day, the difference in latency times between the Spring-Summer 2009 group and the current group also differed by assignment to the control or prenatal dexamethasone condition. The difference in latency time for the control group was nearly double the difference in latency time for the prenatal Dex-exposed group for each of the testing days. It appears that the experimenter's increased comfort and relaxed temperament with the current group of animals was more beneficial for the control rats than the rats prenatally exposed to dexamethasone. In other words, the only stress and anxiety that the control animals dealt with during the current experiment was due to the nature of the Morris Water Maze itself, and not from extraneous variables like the experimenter's temperament or a particular treatment. Their latency times dropped substantially as a result. Despite the absence of the experimenter's temperament as an influence on the Dex-exposed animals in the current group, the prenatal dexamethasone treatment itself—with the well documented effect of HPA-axis hyperactivity (Hauser et al., 2009; Schoener et al., 2006)—presented additional stress and anxiety that the control animals did not have. Their latency times were still reduced as a result of the experimenter's increased comfort conducting the test, but their performance did not improve as much as the control animals because of the effect of the prenatal dexamethasone treatment. Morris Water Maze literature demonstrating the adverse

effects of stress on performance substantiates this hypothesis (D'Hooge & De Deyn, 2001).

The Elevated Plus Maze data of the Spring-Summer 2009 group and the current group's Elevated Plus Maze data were also compared. Unlike the Morris Water Maze data discussed above, the experimental procedure on the Elevated Plus Maze did differ between the Spring-Summer 2009 group and the current group. The Spring-Summer 2009 data measured baseline activity, meaning that there were no other treatments or factors introduced. Animals were assigned to a control group or received prenatal dexamethasone treatment and then were tested on the Elevated Plus Maze when they reached adulthood. The same events occurred in the current group of animals, although in this group a stress challenge was also part of the procedure. Ninety minutes before the animals were tested on the Elevated Plus Maze they were subjected to 15 minutes of forced swim stress.

When comparing the Elevated Plus Maze data of the Spring-Summer 2009 group and the current group an overall trend of anxiety behavior regardless of assignment to the control or dexamethasone condition was detected, which was not expected. The two main measures of the Elevated Plus Maze are the ratio of time spent in the open arms to total time in the apparatus and the ratio of open arm entries to total arm entries. For both of these measures, higher ratios mean that the rats are exploring the open arms more and are less anxious (Walf & Frye, 2007). Across both control and dexamethasone conditions the ratios were higher in the current group than in the Spring-Summer 2009 group, despite the current group undergoing the stress challenge before their Elevated Plus Maze testing.

Also intriguing was the finding that the difference between the Spring-Summer 2009 group and the current group was more pronounced in the control condition than the prenatal dexamethasone condition.

One explanation for the increase in the ratio of open arm time and the ratio of open arm entries after the stress challenge has to do with the risk taking nature of the Elevated Plus Maze. The apparatus presents a conflict between the safe and protected areas of the closed arms and the enticing yet dangerous areas of the open arms (Walf & Frye, 2007). Using this description open arm activity can be thought of as a risky behavior, a characterization that prior research has used as well (Estanislau & Morato, 2006). The effects of stress on risk taking behavior have previously been studied and consistently report that stress increases risk taking in males. For example, Preston et al. (2007) stressed human participants by telling them that they would be giving a public speech after their participation in an experiment. This stress group subsequently took more risks in a gambling task than a control group. Similarly, other researchers stressed participants by having them place their hand in ice cold water for 3 minutes (Lighthall et al., 2009). These participants then engaged in greater risk taking than control participants in a computerized game simulating risky behavior. Both of these studies found the influence of stress on risk taking behavior to be sex specific, only affecting males. It is in this context that differences in open arm activity on the Elevated Plus Maze between Spring-Summer 2009 animals and the current animals can be explained. The forced swim stress that the current experimental animals underwent resulted in their increased

propensity for risky behaviors, hence their higher ratio of time spent on the open arms and higher ratio of open arm entries.

Perhaps the most important finding of the study was that the baseline Elevated Plus Maze data collected from the Spring-Summer 2009 group showed no difference in anxiety behavior between the control and Dex-exposed animals while the data collected after the stress challenge did reveal differences. The rats prenatally treated with dexamethasone displayed a significant increase in anxiety behavior compared to the control rats after undergoing a stress challenge. It appears that prenatal dexamethasone exposure alone is not enough to disrupt behavior on the Elevated Plus Maze, but the combination of prenatal dexamethasone exposure and intense stress results in aberrant behavior. This behavioral data fit well with research on the HPA-axis hormones of prenatally treated dexamethasone rats.

Hauser et al. (2009) studied the HPA-axis activity of prenatal Dex-exposure. At basal levels, there was no prenatal effect on either ACTH or CORT. Then the researchers examined the hormonal response after a stress challenge in rats prenatally exposed to Dex. The stress challenge they used was a restraint stress procedure, in which the rat is placed in a restrictive plastic tube for 20 min. The researchers measured ACTH and CORT at 20, 40, 80, and 120 min. after the restraint stress. At each time point following the stress, ACTH and CORT were significantly higher in the Dex-exposed rats compared with control rats (Hauser et al., 2009). Schoener et al. (2006) found similar effects of prenatal Dex-exposure on HPA-axis hormones. Like Hauser et al. (2009), this research studied the response to restraint stress challenge. ACTH and CORT levels were elevated

60 min. after the restraint stress in the Dex-exposed group (Schoener et al., 2006). These researchers also found basal differences in ACTH and CORT between prenatal Dex rats and control rats.

The increased anxiety behavior after stress challenge demonstrated in this research and the previous work on elevated HPA-axis hormones following stress challenge point to impairment in the negative feedback mechanism of the HPA-axis for prenatally Dex-exposed animals. The hippocampus serves as a primary mediator of this negative feedback (Van Haarst et al., 1996). For example, lesion of the hippocampus increases glucocorticoid release following stress (Jacobson & Sapolsky, 1991). Conversely, hippocampal stimulation increases glucocorticoid output (Dunn & Orr, 1984). Following acute stress (e.g., a stress challenge), excess endogenous glucocorticoids act through glucocorticoid receptors (GR) on the hippocampus to inhibit the HPA-axis stress response (Ulrich-Lai & Herman, 2009). In rats prenatally Dex-exposed, GR expression is reduced in the hippocampus (Welberg et al., 2001). The increased anxiety following stress challenge in this study provide a complimentary behavioral profile of the hormonal and molecular evidence of impaired HPA-axis negative feedback in prenatally Dex-exposed rats.

Conclusion and Future Directions

Ambiguity surrounded the literature on the behavioral effects of prenatal dexamethasone exposure in the rat. Several studies report increased anxiety related behaviors as a result of prenatal dexamethasone (Hossain et al., 2008; Wellberg & Seckl, 2001), while other work found no behavioral effects (Hauser et al., 2009; Nagano et al.,

2008). Our research approached the issue of prenatal dexamethasone exposure with a more nuanced analysis of the resulting behavior in adulthood. In our study, anxiety related behavior was found to be increased in the Dex-exposed animals after undergoing a stress challenge, whereas the same behavioral measure found no effect of prenatal dexamethasone on an earlier group tested without the stress challenge These data suggest that the effect on behavior is dependent on subsequent stressors later in life. Spatial learning and memory were also impaired in the prenatal Dex-exposed rats, although this effect was not seen in an earlier group tested on the Morris Water Maze in our lab. It is likely that experimenter error accounts for the discrepancy.

Continued research is needed in several areas. One is in regard to the sex differences. This research used only male rats because females tend to be more resilient to the effects of prenatal dexamethasone exposure than males, although the exact details of this difference are poorly understood (Hossain et al., 2008; Seckl & Holmes, 2007). Another area of future research could focus on treatments that ameliorate the effect of prenatal dexamethasone exposure. Behavioral treatments like the enriched environment (Laviola et al., 2004) and anxiolytic pharmacological treatments (Drago et al., 1999) have been useful in models for investigating the impact of prenatal stress, but research on treatments for the prenatal dexamethasone model are rare. It is possible that these same treatments would be helpful in identifying the precise mechanism of potential clinical treatments and in further elucidating the effects of prenatal dexamethasone.

References

Alonso, S. J., Damas, C., & Navarro, E. (2000). Behavioral despair in mice after prenatal stress. *Journal of Physiology and Biochemistry*, *56*(2), 77-82.

Briones-Aranda, A., Castillo-Salazar, M., & Picazo, O. (2009). Adrenalectomy modifies the hippocampal 5-HT1A receptors and the anxiolytic-like effect of 8-OH-DPAT in rats. *Pharmacology, Biochemistry and Behavior, 92*,182–189.

Brocklehurst, P., Gates, S., McKenzie-McHarg, K., Alfirevic, Z., & Chamberlain, G. (1999). Are we prescribing multiple courses of antenatal corticosteroids? A survey of practice in the UK. *British Journal of Obstetrics and Gynaecology, 106*, 977–979.

Committee on Obstetric Practice. (2002). ACOG committee opinion: antenatal corticosteroid therapy for fetal maturation. *Obstet. Gynecol. 99*, 871–873.

Crowley, P. (1995) Antenatal corticosteroid therapy: a meta-analysis of the randomized trials, 1972–1994. *Am J Obstet Gynecol, 173*, 322–355.

Crowther, C. A., Haslam, R. R., Hiller, J. E., Doyle, L. W., Robinson, J. S., Australasian Collaborative Trial of Repeat Doses of Steroids (ACTORDS) Study Group. (2006). Neonatal respiratory distress syndrome after repeat exposure to antenatal corticosteroids: a randomised controlled trial. *Lancet, 367*, 1913–1919.

Darnaudery, M., & Maccari, S. (2008). Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. *Brain Research Reviews*, *57*(2), 571-585.

de Kloet, E. R., Joels, M., & Holsboer, F. (2005) Stress and the brain: From adaptation to disease. *Nature Reviews Neuroscience*, *6*(6), 463-475.

Drago, F., Di Leo, F., Giardina, L. (1999). Prenatal stress induces body weight deficit and behavioural alterations in rats: the effects of diazepam. *Eur. Neuropsychopharmacol. 9,* 239–245.

Dunn, J. D. & Orr, S. E. (1984). Differential plasma corticosterone responses to hippocampal stimulation. *Exp. Brain Res. 54*, 1–6.

Estanislau, C. & Morato, S. (2006). Behavior ontogeny in the elevated plus-maze: prenatal stress effects. *Int. J. Dev. Neurosci. 24*, 255–262.

Gueorguieva, R., & Krystal, J. H. (2004). Move over ANOVA: Progress in analyzing repeated-measures data and its reflection in papers published in the Archives of General Psychiatry. *Archives of General Psychiatry, 61*, 310–317.

Hauser, J., Feldon, J. & Pryce, C. R. (2006). Prenatal dexamethasone exposure, postnatal development, and adulthood prepulse inhibition and latent inhibition in Wistar rats. *Beahvioural Brain Research, 175*(1), 51-61.

Hauser, J., Knapman, A., Zurcher, N. R., Pilloud, S., Maier, C., Diaz-Heijtz, R., et al. (2008). Effects of prenatal dexamethasone treatment on physical growth, pituitary-adrenal hormones, and performance of motor, motivational, and cognitive tasks in juvenile and adolescent common marmoset monkeys. *Endocrinology, 149*(12), 6343-6355.

Hauser, J., Feldon, J., & Pryce, C. R. (2009). Direct and dam-mediated effects of prenatal dexamethasone on emotionality, cognition and HPA axis in adult Wistar rats. *Hormones and Behavior, 56*(4), 364-375.

Hinojosa, F. R., Spricigo, L., Izidio, G. S., Bruske, G. R., Lopes, D. M., & Ramos, A. (2006). Evaluation of two genetic animal models in behavioral tests of anxiety and depression. *Behavioural Brain Research, 168*(1), 127-136.

Hossain, A., Hajman, K., Charitidi, K., Erhardt, S., Zimmermann, U., Knipper, M., & Canlon, B. (2008). Prenatal Dexamethasone Impairs Behavior and the Activation of the BDNF Exon IV Promoter in the Paraventricular Nucleus in Adult Offspring. *Endocrinology, 149*, 6356-6365.

Jacobson, L. & Sapolsky, R. M. (1991). The role of the hippocampus in feedback regulation of the hypothalamo-pituitary-adrenocortical axis. *Endocr. Rev. 12,* 118–134.

Kapoor, A., Kostaki, A., Janus, C., & Matthews, S. G. (2009). The effects of prenatal stress on learning in adult offspring is dependent on the timing of the stressor. *Behavioural Brain Research, 197*(1), 144-149.

Kapoor, A., Petropoulos, S., & Matthews, S. G. (2008). Fetal programming of hypothalamic-pituitary-adrenal (HPA) axis function and behavior by synthetic glucocorticoids. *Brain Research Reviews, 57*(2), 586-595.

Laviola, G., Rea, M., Morley-Fletcher, S., Di Carlo, S., Bacosi, A., De Simone, R., Bertini, M., & Pacifici, R. (2004). Beneficial effects of enriched environment on adolescent rats from stressed pregnancies. *Eur. J. Neurosci. 20,* 1655–1664.

Ludbrook, J., & Dudley, H. (1998). Why permutation tests are superior to t and F tests in biomedical research. *American Statistician, 52,* 127-132.

Maccari, S., Darnaudery, M., Morley-Fletcher, S., Zuena, A. R., Cinque, C., & Van Reeth, O. (2003). Prenatal stress and long-term consequences: Implications of glucocorticoid hormones. *Neuroscience and Biobehavioral Reviews, 27*(1-2), 119-127.

Malaspina, D., Corcoran, C., Kleinhaus, K. R., Perrin, M. C., Fennig, S., Nahon, D., et al. (2008). Acute maternal stress in pregnancy and schizophrenia in offspring: A cohort prospective study. *Bmc Psychiatry, 8.*

Manly, B. F. J. (1991). Randomization and Monte Carlo Methods in Biology. New York: Chapman & Hall.

Matthews, S. G. (2002). Early programming of the hypothalamo-pituitary-adrenal axis. *Trends Endocrinol Metab, 13*, 373–381.

Mewhort, D. J. K. (2005). A comparison of the randomization test with the F test when error is skewed. *Behavior Research Methods, 37,* 426-435.

Morris, RGM. (1981) Spatial localization does not require the presence of local cues. *Learning and Motivation 12*, 239-260.

Morley-Fletcher, S., Rea, M., Maccari, S., & Laviola, G. (2003). Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. *European Journal of Neuroscience, 18*(12), 3367-3374.

Nagano, M., Ozawa, H., & Suzuki, H. (2008). Prenatal dexamethasone exposure affects anxiety-like behaviour and neuroendocrine systems in an age-dependent manner. *Neuroscience Research, 60*(4), 364-371.

NIH Consensus Development Conference. (1995). Effects of corticosteroids for fetal maturation and perinatal outcomes. *Am J Obstet Gynecol, 173,* 253–344.

Preston, S. D., Tansfield, R. B. S., Buchanan, T. W., & Bechara, A. (2007). Effects of anticipatory stress on decision making in a gambling task. *Behavioral Neuroscience 121,* 257–263.

Polyakov, A., Cohen, S., Baum, M., Trickey, D., Jolley, D., & Wallace, E. M. (2007). Patterns of antenatal corticosteroid prescribing 1998–2004. *Aust N Z J Obstet Gynaecol, 47*(1), 42–45.

Porsolt, R. D., Anton, G., Blavet, N., & Jalfre, M. (1978). Behavioral despair in rats - new model sensitive to antidepressant treatments. *European Journal of Pharmacology, 47*(4), 379-391.

Seckl, J. R. (2001). Glucocorticoid programming of the fetus: Adult phenotypes and molecular mechanisms. *Mol Cell Endocrinol, 185,* 61–71.

Seckl, J. (2004). Prenatal glucocorticoids and longterm programming. *Eur J Endocrinol, 151*, U49–U62.

Seckl, J. R., & Holmes, M. C. (2007). Mechanisms of disease: Glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. *Nature Clinical Practice Endocrinology & Metabolism, 3*(6), 479-488.

Seckl, J. R., & Meaney, M. J. (2006). Glucocorticoid "programming" and PTSD risk. *Psychobiology of Posttraumatic Stress Disorder: A Decade of Progress, 1071*, 351-378.

Shoener, J. A., Baig, R., & Page, K. C. (2006) Prenatal exposure to dexamethasone alters hippocampal drive on hypothalamic-pituitary-adrenal axis activity in adult male rats. *Am J Physiol Regul Integr Comp Physiol, 290*, R1366–R1373.

Steer, P. & Flint, C. (1999). ABC of labour care: preterm labour and premature rupture of membranes. *Br Med J, 318*, 1059–1062.

Talge, N. M., Neal, C., Glover, V., & Early Stress Transnational Res Pre. (2007). Antenatal maternal stress and long-term effects on child neurodevelopment: How and why? *Journal of Child Psychology and Psychiatry*, *48*(3-4), 245-261.

Ulrich-Lai, Y. M. & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nat. Rev. Neurosci., 10*, 397-409.

Van den Bergh, B. R. H., Mennes, M., Oosterlaan, J., Stevens, V., Stiers, P., Marcoen, A., et al. (2005). High antenatal maternal anxiety is related to impulsivity during performance on cognitive tasks in 14-and 15-year-olds. *Neuroscience and Biobehavioral Reviews*, *29*(2), 259-269.

Van Haarst, A. D., Oitzl, M. S., & De Kloet, E.R. (1997). Facilitation of feedback inhibition through blockade of glucocorticoid receptors in the hippocampus. *Neurochem Res 22*, 1323–1328.

Vorhees, C. V. & Williams, M. T. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature Protocols*, *1*(2), 848-58.

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols*, *2*(2), 322-328.

Walker, E., Mittal, V., & Tessner, K. (2008). Stress and the hypothalamic pituitary adrenal axis in the developmental course of schizophrenia. *Annual Review of Clinical Psychology*, *4*, 189-216.

Watson, J. B., Mednick, S. A., Huttunen, M., & Wang, X. Y. (1999). Prenatal teratogens and the development of adult mental illness. *Development and Psychopathology*, *11*(3), 457-466.

Ward, R. M. (1994). Pharmacologic enhancement of fetal lung maturation. *Clinics in Perinatology*, *21*, 523–542.

Welberg, L. A. M. & Seckl, J. R. (2001). Prenatal stress, glucocorticoids and the programming of the brain. *Journal of Neuroendocrinology*, *13*, 113–128.

Young, M. E., Clark, M. H., Goffus, A., & Hoane, M. R. (2009). Mixed effects modeling of Morris water maze data: Advantages and cautionary notes. *Learning and Motivation*, *40*(2), 160-177.