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Behavioral Sex Difference in Healthy Sprauge-Dawley Rats

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Abstract:

Recently, the NIH has pushed for both sexes of a species to be incorporated into behavioral experiments. This push came as a result of an unchecked exclusion of females in experimentation. In rat studies, it is a common argument that females are more variable than males due to the presence of their estrous cycle. This study set out to evaluate this claim, and provide sex differences data for a variety of motor, anxiety, and cognitive behavioral tests. No sex differences in motor or anxiety behavior were found between the sexes. Males performed significantly better on the spontaneous alternation test of spatial memory, but had no differences on other cognitive tasks including novel object recognition and the T-maze learning test. While the estrous cycle of females was not monitored in the current study, no significant sex differences in variability of behavioral responding were found, disputing the claim that females are more variable than males. The results of this study will help to dispel the bias in rat model research, and thus encourage the production of more accurate behavioral data.

Introduction

Modern preclinical scientific research relies heavily on the use of animal models. However, there is an overwhelming sex bias in favor of using male animals in neuroscience research (Capello & Butcher, 2014). A literature review of 2,000 animal studies showed that male bias in nonhuman studies is evident in 8 of 10 major biological disciplines, including neuroscience, pharmacology, general biology, and behavioral sciences (Beery & Zucker, 2011). Neuroscience research in particular is highly skewed towards the use of male subjects with a ratio of 5.5:1 male-only versus female-only studies. As such, the National Institute of Health has recently pushed for equal sex incorporation in preclinical studies (Clayton & Collins, 2014) in order to help fill in current gaps in knowledge.

The Sprague-Dawley rat is the most widely-used outbred rat strain in animal research and is frequently used in motor, anxiety, and cognitive analyses. Use of male rats predominates in neuroscience research. However, sex differences have been observed in a number of behaviors that are frequently assessed only in male rats including: wheel running, aggression, food intake (Goy & McEwen, 1980), and especially learning and memory (Dalla & Shores, 2009). While past studies show no major sex differences in basic motor performance in healthy rats (Roislien et al.,

2009; Walker et al., 2001), literature on sex differences in anxiety and cognitive behaviors is more variable. Some have reported that males are more anxious (Donner & Lowry, 2013; Goy & McEwen, 1980; Johnston & File, 1991; Zimmerburg & Farley, 1993), less anxious (Donner & Lowry, 2013; Palanza, 2001), have better memory performance (Astur, Tropp, Sava, Constable, & Markus, E. J2004; Roof & Stein, 1999), or have no difference in memory performance (Harris et al., 2008; Einon, 1980) when compared to females.

One common explanation for these differences and for the use of male over female rats is that the presence of females' estrous cycle is thought to enhance behavioral variability, making them a less viable model (Beery & Zucker, 2011). There is some credence to this belief. Past studies have shown evidence that the estrous cycle affects spatial memory and learning (Korol et al., 2004; Warren & Juraska, 2000) and anxiety (Marcondes et al., 2001). However, while female sex hormones might influence behavior, there is little evidence that they create more variable data. Additionally, male sex hormones also influence behavior. For instance, testosterone in males naturally causes less activity and more defecation in open field tests (Blizard, Lippman, & Chen, 1975).

Despite potential differences, it is important that the bias against female animals in research is reduced since it is believed that the rise of irreproducibility in biomedical research may be due, in part, to this failure (Clayton & Collins, 2014). Furthermore, sex bias may contribute to problems translating animal studies to humans. Therefore, this study set out to evaluate basal sex differences for a variety of motor, anxiety, and cognitive behavioral tests. This study excluded observation of the estrous cycle in females to test their variability when not accounting for effects of the cycle. We hypothesized that females would be more variable than males on anxiety and cognitive tests and would show no differences in variability on motor tests.

Methods

1. Animals

Motor, anxiety, and cognitive tests were conducted over a 9 week period using adult male (n=6) and female (n=6) Sprague-Dawley rats bred at Binghamton University. Male rats weighed 375-450 g and female rats weighed 240-275 g. Rats were housed in same-sex pairs in plastic cages (46 cm x 22 cm x 20 cm), with ad libitum access to standard lab chow and water. Rats were food restricted to 85-90% of free feeding weight for tasks requiring food restriction. The colony room was kept at 20-22°C and had 12/12 dark and light cycles (on at 0700 and off at 1900). Animals were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee at Binghamton University (Protocol # 736-14) and the "Guide for the Care and Use of Laboratory Animals" (Institute for Laboratory Animal Research, National Academies Press 2011).

2. Behavioral Testing

2A. Motor Tests

2A1. Gait Analysis

Sex differences in gait parameters were analyzed using a computer-assisted CatWalk (Noldus, TM) according to a protocol adapted from Westin, Janssen, Sager, and Temel (2012). Light from a green fluorescent tube reflected off of the glass walkway floor (127.0 cm x 20.3 cm) mounted over a digital camera. When the rats' paws made contact with the glass, the contacted area was illuminated. A digital camera captured and digitized the images for analysis of several parameters including: stride length (the distance between the front and hind paws when stepping), traversal speed (in cm/s), and base of support (BOS, the distance between paws in cm) for front

and back paws. Rats were tested up to 3 days, until they completed 3 successful trials. A trial was deemed successful when the rat traversed the catwalk without pausing.

2A2. Rotarods

Rotarods (San Diego Instruments) were used to assess locomotor ability according to the protocol of Monville, Torres, and Dunnett (2006). The apparatus consisted of an electric motor controlled by an integrated computing panel to rotate rollers (i.e., the rods) in 4 individual lanes separated by stainless steel walls. The larger, middle lanes (lane width: 10.8 cm, rod diameter: 8.0 cm) were used to test male rats. Female rats were tested in the outer lanes (lane width: 8.1 cm, rod diameter: 6.0 cm) due to their smaller size. Photobeam detectors below the rod detected latency to fall. A foam landing pad was positioned 60 cm under the rods. The landing area was enclosed to prevent escape.

Rats were placed perpendicularly to the axis of rotation, such that they had to walk forward to remain on the rotating rod. During habituation, rats were placed on the rod while it rotated at a constant speed of 5 rotations per minute (rpm). Rats were habituated to the rod until they stayed on the rod for 180 s period for 2 consecutive trials. On test days, the rod speed accelerated from 0-40 rpm over 240 s. Three trials/test days were recorded for a total of 6 trials. Data were expressed as the average latency to fall off the rod (s).

2A3. Locomotor Activity Chambers (LMC)

LMC were used to assess spontaneous motor activity and anxiety-like behavior. Using a modified protocol from Ostock et al.(2015), rats were placed individually into a plexiglass box (40 cm x 40 cm x 30 cm) surrounded by a 15 x 15 photocell array connected to a computer running Versamax and Versadat software to record and analyze locomotor activity. The center of the chamber was illuminated while the testing room itself was dark. Motor activity data were pooled

into 12 5-min time bins for 1 hour. Data were expressed as average movement number and average time spent in the center of the chamber (a measure of anxiety).

2B. Anxiety Tests

2B1. Marble Burying

The marble burying test was used to assess anxiety-like behaviors, where more marbles buried is indicative of higher neophobia and anxiety (Gulinello, 2007). Rats were placed in a plastic cage (51.0 x 41.0 x 21.0 cm) with bedding 6 cm high. Red, green, blue, and yellow marbles were placed 6 cm apart on top of the bedding. Rats were habituated to the cage for 20 minutes without marbles present, then were placed back in home cage for 6 minutes. During the test phase, rats were placed into the cage with marbles for 30 minutes without researcher presence. After 30 min, rats were returned to their home cage and the number of marbles buried were counted. Data were expressed as the average number of marbles buried >75%.

2B2. Elevated Plus Maze (EPM)

The EPM was used to assess anxiety-like behavior. The apparatus consisted of a plus maze raised 91.0 cm off the ground with two open arms (across from each other) and two arms enclosed with walls 20.0 cm high. Each arm measured 50.0 cm long and 10.0 cm wide, and the center of the maze was 10 x 10 cm. Rats were placed in the center of the maze facing an open arm, and allowed to explore the maze for 5 minutes. The test was videotaped for later analysis and researchers left the room. Data were expressed as time (s) spent in the open arms, closed arms, and number of head dips. More time on the open arms and more head dips indicate less anxiety (Sullivan et al., 2014).

2C. Cognitive Tests

2C1. Spontaneous Alternation

Spontaneous alternation assesses spatial memory and exploratory behavior in rats (Stefani, Nicholson, & Gold, 1999). Rats were food deprived to encourage exploratory/foraging behavior. The testing room was rich in visual spatial cues and in the center of the room was a 4-arm plus maze (arm dimensions: 46.4 cm, length x 14.0 cm, width) constructed of clear Plexiglas sidewalls (17.0 cm high) with a black plexiglas floor. Using a protocol adapted from Sakakibara et al. (2014), rats were placed in the center of the maze, and were allowed to explore the maze for 20 minutes.

Experimenters recorded the number and sequence of arm entries made by the rats. An arm entry was defined as when all four paws of the rat were fully in one arm of the maze. A rat made an arm re-entry by entering one arm, then another arm, and then proceeding back to the previous arm (for example, an arm re-entry on the data sheet would look like 3, 2, 3). A rat made a spontaneous alternations by entering all four arms without revisiting an arm (for example, 1, 2, 3, 4 and not 1, 2, 3, 1). More spontaneous alternations corresponds with better spatial memory, and the higher the number of arms that are entered, the higher the exploratory behavior/hyperactivity. Data were expressed as percent spontaneous alternations (# of successful alternations / # of possible alternations) and percent arm re-entries (# of arm re-entries / # of potential arm re-entries).

2C2. T-Maze Discrimination Task

The T-maze discrimination test measures learning strategies (Vetreno, Anzalone, & Savage, 2008). A standard plus maze was modified for this task by blocking 1 arm. Thus, the maze consisted of a start box (21.1 x 13.9 cm) located in the long arm of the maze (66.1 cm), and 2 short arms (46.4 cm). Maze walls were 17 cm high.

Prior to testing, rats were food deprived to encourage maze exploration. During habituation, the entire maze was baited with honey-nut cheerio pieces. Rats were placed into the start box for 15 seconds, then the door was removed and rats were allowed to explore the maze

freely. On test days, one arm was baited. After the rat was placed in the start box for 15 seconds, the wall was removed and rats were given 45 seconds to choose an arm to enter. For each rat either the left or right arm was always baited (with baited-arm counterbalanced between and within the sexes). If a rat chose the baited arm, it was placed back in the start box after eating its reward. If it entered the empty arm, it was blocked in the end of the arm for 15 seconds as "punishment" before being returned to the start box for the next trial. Rats that did not make a choice within 45 seconds were returned to the start box for the next trial.

The test day consisted of 3 phases. Phase 1 consisted of the rats reaching criterion (i.e., 7 consecutive entries into the baited arm, following a minimum of 15 trials). Following 7 consecutive entries, a probe trial was completed, during which time the maze orientation was rotated 180° such that the start box was opposite from its original location, and both arms were baited. Two more probe trials occurred after a set of 15 normal trials each. This test evaluated learning type used to acquire food rewards during the probe trials. If a rat turned in the same direction (i.e., always made a left turn) during probe and normal trials, the behavior was termed response learning. If the rat turned towards the correct spatial location in the room, the behavior was termed place learning (Vetreno et al., 2008). Data were expressed as the percentage that either sex on average demonstrated place learning on each probe trial, as well as percent choice accuracy (rat's ability to choose the correct arm). One male rat failed to learn the task and was excluded from final analyses.

2C3. Novel Object Recognition (NOR)

The NOR task tests recognition memory in rats (Antunes & Biala, 2011). The NOR test had three stages: habituation, familiarization, and test phase. Rats were habituated first to the testing room in their home cages for 20 minutes, and then to the empty plexiglass testing chamber

(40 cm x 40 cm x 30 cm) for 10 minutes. Lego blocks and super balls were used as the investigation objects. The familiarization phase consisted of placing the rats into the chambers with two identical objects inside. Rats were placed in the apparatus against the center of the wall opposite the objects, with backs facing the objects, in order to prevent coercion to explore the objects. The rats were allowed to explore for 5 minutes. The rats were then removed, placed in a holding cage for 1 hour, and then returned to the chambers to begin the test phase. During the test phase, there were still two objects in the chambers, but one was identical to the one in the familiarization phase, while the other was novel. Rats were once again allowed to explore for 5 minutes. In the familiarization and testing phases, the objects were placed at opposite ends of the chambers. Researchers left the room while all testing commenced and rats were filmed so that data could be analyzed later. Parameters assessed were the amount of time a rat spent investigating an object as well as the number of approaches a rat made towards an object. A rat was deemed investigating an object *only* if its snout was pointed directly towards the object (within 2 cm) or if the rat was moving the object purposefully.

3. Data Analysis

Behavioral data were analyzed using independent samples t-tests and mixed-design ANOVAs with the alpha level set to 0.05. Levene's tests for equality of variance were also run for each behavior to evaluate the claim that female rats show more variability in responding than male rats.


Results

1. Motor

1A. Gait Analysis

Independent-samples t-tests were used to analyze gait parameters. BOS was significantly wider for males versus females for both the forepaws [$t(10)$= 2.86, $p = 0.017$] and hindpaws, [$t(10)$= 3.56, = 0.005] (Figure 1A). No significant differences existed between males and females in regards to stride length [$t(10)$= 0.12, $p = 0.906$] or run speed [$t(10)$= 1.40, $p = 0.191$] (Figure 1B, 1C).

1B. Rotarods

Habituation data where RPM was held constant (day 1) were analyzed using a 2 (sex) $\times$ 3 (trial) mixed design ANOVA (Figure 1D). A significant main effect of sex [$F(1,10)$= 6.34, $p = 0.030$] and trial [$F(2,20)$= 10.50, $p = 0.001$] existed where females performed better than males, and performance increased over time. An interaction between trial and sex also existed [$F(2,20)$= 8.526, $p = 0.002$] where females stayed on the rod significantly longer than males during the first 2 trials. Male performance significantly improved over subsequent trials [Trial 1 vs. 2 $p = 0.650$; Trial 1 vs. 3 $p < 0.0001$; Trial 2 vs. 3 $p < 0.0001$], while female performance did not significantly change with each trial [Trial 1 vs Trial 2 $p = 0.787$; Trial 1 vs. Trial 3 $p = 0.875$; Trial 2 vs. Trial 3 $p = 0.669$]. Rotarod data from test days where RPM increased from 0-40 RPM (days 2-3) were analyzed using a 2(sex) $\times$ 3(trial)$\times$ 2(day) mixed design ANOVA (Figure 1D). Main effects of sex [$F(1,10)$= 3.74, $p = 0.080$], trial [$F(2,20)$= 3.26, $p = 0.060$], and day [$F(1, 10) = 0.29$, $p = 0.60$] failed to reach significance. There was no interaction between sex, trial, and day [$F(2, 20) = 1.24$, $p = 0.310$].

1C. LMC

A 2 (sex) × 12 (time) mixed design ANOVA was used to analyze movement number in the LMC (Figure 1E). A significant main effect of time was found for movement number [$F(11, 110) = 33.04$, $p < 0.0001$] where rats moved less over time regardless of gender. However, there was no main effect of sex [$F(1,10) = 0.786$, $p = 0.396$] and no interactions between sex and time on movement number in the LMC [$F(11,110) = 1.22$, $p = 0.283$].

Figure 1: Motor Measures
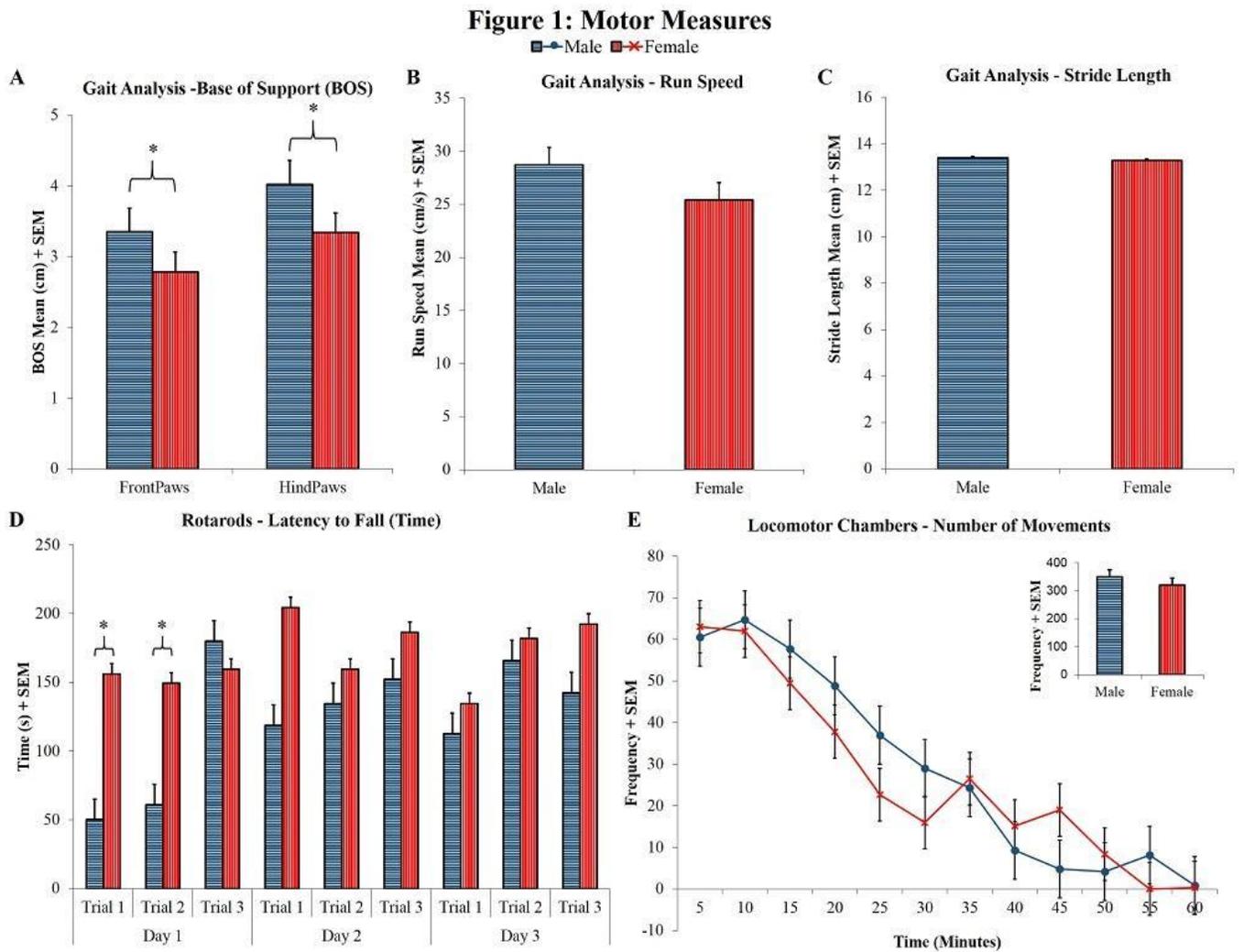
On the first day (habituation), RPM were held constant at 5 RPM. On days 2 and 3, RPM accelerated 0-40 RPM over 4 min and latency to fall was recorded. The time course of average number of movements (E) and total number of movements (inset) made while exploring the LMC is shown. *$p < 0.05$ males vs. female

2. Anxiety and Compulsive Behavior

2A. LMC

A 2 (sex) $\times$ 12 (time) mixed design ANOVA analyzed the time spent in the center of the LMC (Figure 2A). A significant main effect of time bin on center time was found [$F(11,110) = 10.34$, $p < 0.0001$] where rats moved less over time regardless of gender. However, there was no main effect of sex [$F(1, 10) = 3.48$, $p = 0.092$] and no interaction between sex and time for time spent in the center of the LMC [$F(11,110) = 1.74$, $p = 0.074$].

2B. Marble Burying

An independent samples t-test showed there was no difference in the total number of marbles buried between male and female rats [$t(10)= 1.17$, $p = 0.272$] (Figure 2B).

2C. EPM

Independent samples t-tests revealed no difference between the sexes for time spent in the open [$t(10)= 0.28$, $p = 0.785$] or closed maze arms [$t(10)= 0.66$, $p = 0.522$] (Figure 2C). In addition, there was no difference in head dips [$t(10)= 1.27$, $p = 0.23$] (Figure 2D).

Figure 2: Anxiety Measures
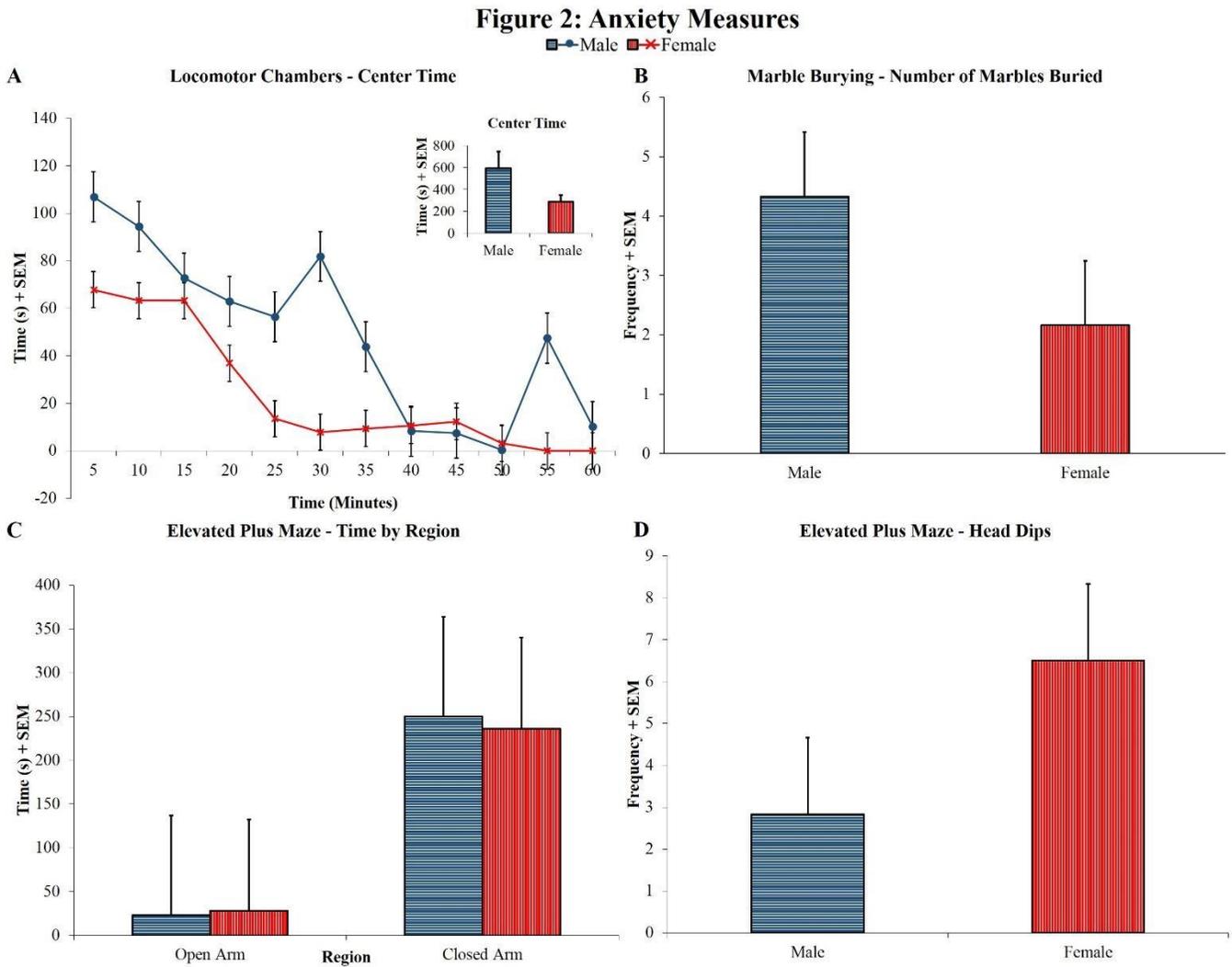
Figure 2: Anxiety Measures in male (n = 6) and female (n = 6) rats: Average time (A) by male and female rats spent in the center of the LMC was graphed over 5 minute intervals, and total time spent in the center is shown in the inset. The average number of marbles buried by both sexes in the marble burying test is shown (B). The average time rats spent exploring each region of the EPM is shown (C), along with the average number of head dips (D). Head dips are the number of times that a rat peered over the side of an open arm.

3. Memory and Learning

3A. Spontaneous Alternation

An independent-samples t-test demonstrated that male rats had a higher percentage of spontaneous alternations than female rats [t(10) = 2.31, p = 0.0433] (Figure 3A). In addition, a

non-significant trend was shown for percent arm re-entries where females re-entered previously visited arms more than males [t(10) = 2.00, p = 0.072] (Figure 3A).

3B. T-Maze Discrimination

An independent-samples t-tests analyzed the percent choice accuracy (choosing the correct arm) on the T-Maze test (Figure 3B). Choice accuracy [Male: M = 81.27, SD = 6.28; Female: M = 81.18, SD = 6.81] did not differ between the sexes. Spearman's rho analyzed learning type to determine if it correlated with sex (data not shown). However, no correlation existed, [ρ(11)= -0.331, p = 0.35].

3C. NOR

A 2 (sex) x 2 (object: novel vs. familiar) mixed design ANOVA was used to evaluate the amount of time spent with the novel and familiar objects (Figure 3C). There was no main effect of sex on time spent interacting with the objects [F(1,10) = 0.713, p = 0.418], however, there was a main effect of object [F(1, 10) = 6.27, p = 0.031] where all rats spent more time with the novel than familiar object. The interaction between object and sex was not significant [F(1,10) = 0.150, p = 0.707].

Figure 3: Cognitive Measures
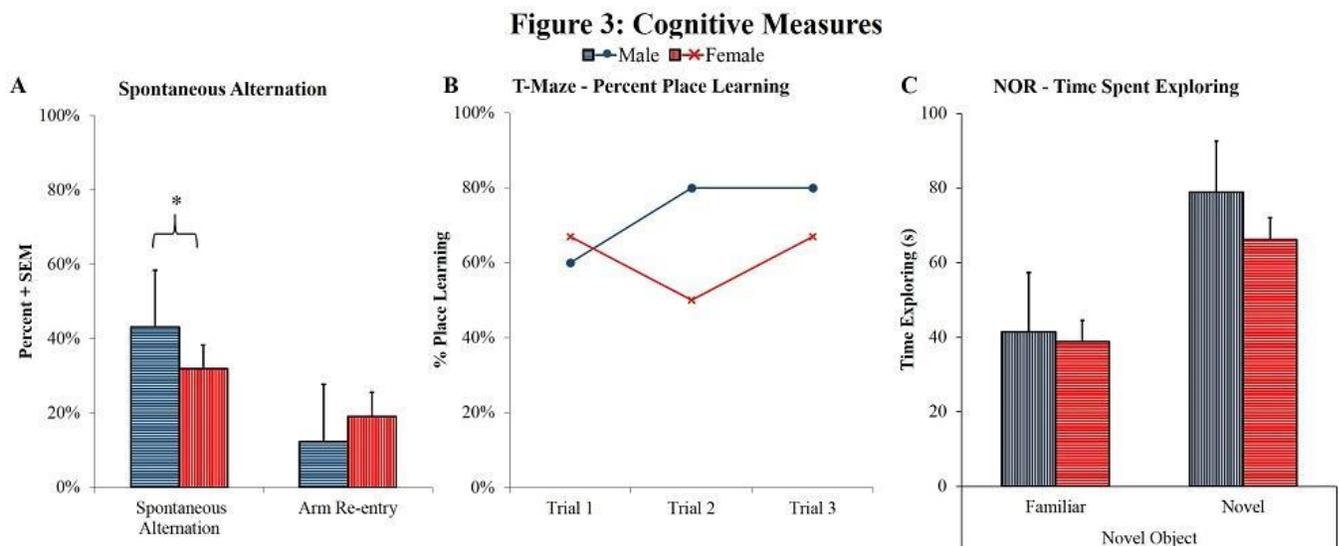
4. Variability

Levene's tests of homogeneity of variance were used to evaluate the sex differences in behavioral variability. There were significant differences in variability on the forelimb BOS measure of Gait Analysis and in time spent investigating familiar and novel objects (Table 1). There were no differences in variability on all other behavioral tests (Table 1).

Table 1. Comparison of sex differences in behavioral variability

Behavioral Measure	Standard Deviations		Levene's test of homogeneity of variance	
	Male	**Female**	**F value**	**p value**
Motor				
Rotarods- day 1	42.94	36.46	0.01	0.95
Rotarods- day 2	31.93	45.70	1.71	0.22
Rotarods- day 3	29.68	40.07	0.06	0.81
Total Movement time in LMC	1.17	0.82	0.48	0.51
Gait analysis- Stride Length	1.45	1.60	0.17	0.69
Gait analysis- Run Speed	2.54	4.61	2.56	0.14
Gait analysis- BOS forelimbs	0.19	0.40	4.54	**0.06**
Gait analysis- BOS hindlimbs	0.23	0.36	1.02	0.37
Anxiety				
Marbles burried	3.93	2.32	0.71	0.42
EPM- open arms	29.16	35.05	0.06	0.81
EPM- closed arms	27.45	44.96	0.69	0.42
EPM- head dips	2.32	6.66	2.06	0.18
Total Center time in LMC	63.53	75.50	0.01	0.95
Cognition				
% alternation	11.47	8.83	0.87	0.37
% arm re-entry	6.65	5.25	0.38	0.55
NOR- Time at familiar object	38.68	13.93	4.49	**0.06**
NOR- Time at novel object	33.56	14.42	4.56	**0.06**

Discussion

The purpose of this study was to collect sex differences data for Sprague-Dawley rats on several common behavioral tests in three main areas: motor ability, anxiety, and cognition. Overall,

we confirmed previous work showing limited differences in motor ability and found a male-advantage for spatial memory. Importantly, our data also brought evidence against the popular assumption that females have more variance in performance than males, caused by their estrous cycle (Beery & Zucker, 2011).

Motor

Several parameters of motor ability were assessed, including frequency of spontaneous movements, gait, and coordination. Female and male rats performed similarly across most motor tasks. No sex differences were found in the average number of spontaneous movements in the LMC, coinciding with previous studies (Walker et al., 2001; Elliott et al., 2005). Gait and coordination were measured using gait analysis and rotarods. Consistent with past work, male and female rats performed similarly in regard to stride length and run speed for gait analysis (Parker & Clarke, 1990) and on latency to stay on the rotarods during trials where the rod increased speed from 0-40 RPM (Parsania et al. 2014). The lack of differences in variability between the sexes refutes the claim that the female's estrous cycle increases their variability in terms of motor tests.

Males did have a significantly wider BOS and females trended towards being more variable on this parameter. This is likely explained by the size difference between the sexes since males in this study were approximately 100 grams heavier than females. Interestingly, during rotarod habituation females outperformed males, suggesting males learned the task slower than females. However, a study that set out to calibrate the rotarods' rotational acceleration mentioned that body size could be an extraneous variable (Bohlen et al., 2009), which might suggest the size difference between our rats had an effect. Females were tested using the smaller rods due to their size, and therefore also traveled less distance than males. Thus, males may have become fatigued quicker, resulting in females performing better. Overall, these parameters suggest male and female rats

move the same way, though the physiology of male and female rats should be considered for motor tests where size might be an issue.

Anxiety

Sex differences in anxiety-like behavior remains controversial, with some studies showing females are more (Belviranli, Atalik, Okudan, & Gokbel 2012; Palanza 2001) or less (Brotto, Bair, & Gorzalka, 2000; Goy & McEwen 1980; Johnston & File 1991; Zimmerburg & Farley 1993) anxious than males. We used 3 separate tests to evaluate anxiety-like behaviors: an adapted open-field-like test, marble-burying, and the EPM. Across all tests, we found no difference in anxiety-like behaviors between the sexes. Time spent in the center of the illuminated LMC was used as a proxy of anxiety-like behavior in the current study, in which the more time spent in the center of the chamber indicates less anxiety. Our data failed to reach significance, but showed a nonsignificant trend towards males showing less anxiety like behavior. This is in line with previous studies showing males spend more time than females in the center of an open field, suggesting they are less anxious than females (Belviranli et al., 2012; Palanza, 2001), but opposes others demonstrating the opposite (Brotto, Bair, & Gorzalka, 2000).

The estrous cycle in females is known to impact anxiety-like behaviors (Frye et al., 2000; Marcondes et al., 2001), and may account for some of the discrepancies observed in our anxiety behavior results. Our findings revealed no sex differences in time spent in the open arms nor in head dips on the EPM, while past studies have shown females to spend more time in the open arms than males, suggesting less anxiety in females (Goy & McEwen, 1980; Johnston & File, 1991; Marcondes et al., 2001; Zimmerburg & Farley, 1993). These findings may be mediated by sex-hormones since, when comparing across the estrous cycle, female rats in proestrus spend more time in the open arms of the maze and less in diestrus (Marcondes et al., 2001).For example, it is

possible no females were in proestrus during the EPM, and thus, behaved similarly to males. This may be the case for our marble burying results as well, where one past study linked female hormones like progesterone and estrogen to compulsive burying (Schneider & Popik, 2007); unfortunately, there are no previous studies on sex differences in marble burying with which to compare our results.

Further research concerning sex differences in anxiety is greatly needed, and modern anxiety tests may require re-evaluation based on conflicting data from different anxiety tests. These tests may assess different aspects of anxiety, or even test behaviors besides anxiety, like hyperactivity or exploratory behavior. Marble burying, for example, is linked to anxiety, compulsive behavior, and impulsivity (Thomas et al., 2009; Reimer et al., 2015), affecting the accuracy of results interpretation.

Cognition

Spatial navigation memory and learning were tested using the spontaneous alternation and T-maze discrimination tests. Males completed more successful alternations (visiting each of the 4 arms in sequence without repeating an arm) and trended towards having less arm re-entries than female rats, indicating that males have better spatial memory. This finding is consistent with a large body of evidence showing males perform better on spatial memory tests than females (Astur Ortiz, & Sutherland, 1998; Astur et al., 2004; Einon, 1980; Roof & Stein, 1999).

T-maze discrimination was conducted to determine whether male and female rats use different strategies for spatial navigation and spatial memory in the current work. Results showed no sex differences in learning speed or strategy. Both males and females learned at the same rate and practiced place learning more often than response learning. A past study done using the Olten maze, a task similar to T-maze discrimination, found no sex differences as well (Einon, 1980).

However, it has been found that females practice place learning during proestrus and response learning during estrus (Korol et al., 2004). Another study found that spatial learning in females has been shown to be best during estrus when estrogen and progesterone are low, and worst during proestrus when estrogen and progesterone are high (Warren & Juraska, 2000). Despite these findings, we found no sex differences in variability on T-maze nor in spontaneous alternation.

Recognition memory, or the ability to recognize a previously encountered stimulus, was measured using the NOR test. Male and female rats both spent more time interacting with the novel object than the familiar object during the test phase of the experiment, as shown elsewhere (Cyrenne & Brown, 2011). Previous studies have shown that female rats are better at discriminating between novel and familiar objects than male rats in the NOR test (Saucier et al., 2008; Sutcliffe et al., 2007). In contrast, no sex differences were observed for recognition memory in the current work. Surprisingly, however, a trend towards higher variability within the males was seen for time spent with the novel object.

Variability and the Estrous Cycle

This study also set out to verify the claim of females being more variable than males. Overall, there were no differences in variability between the sexes; however, nonsignificant trends were seen for females to be more variable than males in forelimb BOS and less variable than males on the NOR test. Since we were not monitoring the estrous cycle, we cannot discount the potential role of stage in the hormonal cycle for our behavioral results. The mean estrous cycle length is approximately 4 days in rats (Marcondes, 2001), and since testing was conducted over 2 months, each female went through multiple cycles. It is possible that all females were in a particular stage of the estrous cycle at the same time due to chance, which may have affected some of our results that conflicted with past studies, such as the EPM and NOR.

Some have speculated that the Whitten effect, wherein females grouped together synchronize estrous cycles as a result of male pheromones (Carlson, 2011; Dalal et al., 2001), could affect results in studies including females. However, while there is extensive evidence for synchrony in mice, evidence indicates that cycles do not synchronize in rats (Lohmiller & Swing, 2006; Schank, 2001).Overall, lack of variability caused by potential estrous cycle synchrony is unlikely, though further experimentation needs to be done in order to fully verify these claims.

Conclusion

These findings support that there are few sex differences in basic motor ability between male and female rats but also highlight the need for further evaluation of sex differences in anxiety and cognitive tests. Importantly, we reject our hypothesis that females are more variable than males on cognitive and anxiety tests, and accept the hypothesis that there would be no sex differences in variability on motor tests. However, further research on female behavioral variability while monitoring the estrous cycle is important. Regardless, the need for equal inclusion of females in behavioral studies is unquestionable. Males are not always equal to females, and researchers should use caution when generalizing results from one sex for both sexes, as this may cause issues with translatability of animal data to humans. We hope our results can be used as a baseline for future studies incorporating females and/or studying sex differences, and help influence more efficient experimentation with regards to such studies, resulting in more accurate data output.

References

Antunes, M., & Biala, G. (2011). The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cognitive Processing, 13*(2), 93-110.

Astur, R. S., Ortiz, M. L., & Sutherland, R. J. (1998). A characterization of performance by men and women in a virtual Morris water task: A large and reliable sex difference. *Behavioural Brain Research, 93*(1), 185-190.

Astur, R. S., Tropp, J., Sava, S., Constable, R. T., & Markus, E. J. (2004). Sex differences and correlations in a virtual Morris water task, a virtual radial arm maze, and mental rotation. *Behavioural Brain Research, 151*(1), 103-115.

Beery, A. & Zucker, I. (2011). Sex bias in neuroscience and biomedical research. *Neuroscience & Biobehavioral Reviews, 35*(3), 565-572.

Belviranli, M. Atalik, K.E.N., Okudan, N., & Gokbel, H. (2012). Age, sex, and anxiety affect locomotor activity in rats. *Proceedings of Measuring Behavior*, 500-503.

Blizard, D. A., Lippman, H. R., & Chen, J. J. (1975). Sex differences in open-field behavior in the rat: the inductive and activational role of gonadal hormones [Abstract]. *Physiology & Behavior, 14*(5), 601-608.

Bohlen, M., Cameron, A., Metten, P., Crabbe, J.C., & Wahlsten, D. (2009). Calibration of rotational acceleration for the rotarod test of rodent motor coordination. *Journal of Neuroscience Methods, 178*(1), 10-14.

Brotto, L., Barr, A., & Gorzalka, B. (2000). Sex differences in forced-swim and open-field test behaviours after chronic administration of melatonin. *European Journal of Pharmacology, 402*(1–2), 87-93.

Capello E., & Butcher, G.Q. (2014). Assessment of the male sex bias in Parkinson's research on nonhuman subjects: A meta-analysis. *IMPULSE: The Premier Undergraduate Neuroscience Journal*, 1-6.

Carlson, N. R., & Carlson, N. R. (2011). *Foundations of behavioral neuroscience*. Boston: Allyn & Bacon.

Clayton, J. A., & Collins, F. S. (2014). NIH to balance sex in cell and animal studies. *Nature*, *509*(7500), 282-283.

Cyrenne, D. M., & Brown, G. R. (2011). Ontogeny of sex differences in response to novel objects from adolescence to adulthood in lister-hooded rats. *Developmental Psychobiology, 53*(7), 670-676.

Dalal S. J., Estep J. S., Valentin-Bon I. E. and Jerse A. E. (2001). Standardization of the Whitten Effect to induce susceptibility to *Neisseria gonorrhoeae* in female mice. *Contemporary Topics in Laboratory Animal Science, 40*, 13-17.

Dalla, C., & Shores, T. J. (2009). Sex differences in learning processes of classical and operant conditioning. *Physiology & Behavior, 97*(2), 229–238.

Donner, N. C., & Lowry, C. A. (2013). Sex differences in anxiety and emotional behavior. *Pflügers Archiv - European Journal of Physiology, 465*(5), 601-626.

Einon, D. (1980). Spatial memory and response strategies in rats: age, sex and rearing differences in performance. *The Quarterly Journal of Experimental Psychology, 32*(3), 473-489.

Elliott, B. M., Faraday, M. M., Phillips, J. M., & Grunberg, N. E. (2004). Effects of nicotine on elevated plus maze and locomotor activity in male and female adolescent and adult rats. *Pharmacology Biochemistry and Behavior, 77*(1), 21-28.

Frye C.A., Petralia, S.M., Rhodes, M.E. (2000). Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3alpha,5alpha-THP. *Pharmacology Biochemistry and Behavior, 67*(3), 587-96.

Goy, R.W., & McEwen, B.S. (1980). *Sexual differentiation of the brain: Based on a work session of the Neurosciences Research Program*. Cambridge: MIT Press.

Gulinello, M. (2007). *Behavioural Protocols and Training: Marble Burying*. Albert Einstein College of Medicine.

Harris, A. P., D'eath, R. B., & Healy, S. D. (2008). Sex differences, or not, in spatial cognition in albino rats: Acute stress is the key. *Animal Behaviour, 76*(5), 1579-1589.

Johnston, A. & File, S. (1991). Sex differences in animal tests of anxiety. *Physiology & Behavior, 49*(2), 245-250.

Korol, D. L., Malin, E. L., Borden, K. A., Busby, R. A., & Couper-Leo, J. (2004). Shifts in preferred learning strategy across the estrous cycle in female rats. *Hormones and Behavior, 45*(5), 330-338.

Llaneza, D. C., & Frye, C. A. (2009). Progestogens and estrogen influence impulsive burying and avoidant freezing behavior of naturally cycling and ovariectomized rats. *Pharmacology Biochemistry and Behavior, 93*(3), 337-342.

Lohmiller, J.J. & Swing, S.P. (2006). Reproduction and Breeding. In M.A. Suckow, S.H. Weisbroth, C.L. Franklin (Eds.), *The Laboratory Rat* (2nd ed., 147-164) Elsevier Academic Press.

Marcondes, F.K., Miguel, K.J., Melo, L.L., & Spadari-Bratfisch, R.C. (2001). Estrous cycle influences the response of female rats in the elevated plus-maze test. *Physiology & Behavior, 74*(4-5), 435-440.

Monville, Christelle et al. (2006). Comparison of incremental and accelerating protocols of the rotarod test for the assessment of motor deficits in the 6-OHDA model. *Journal of Neuroscience Methods, 158*, 219–223

Ostock, C. Y., Hallmark, J., Palumbo, N., Bhide, N., Conti, M., George, J. A., & Bishop, C. (2015). Modulation of l-DOPA's antiparkinsonian and dyskinetic effects by α2-noradrenergic receptors within the locus coeruleus. *Neuropharmacology, 95*, 215-225.

Palanza, P. (2001). Animal models of anxiety and depression: How are females different? *Neuroscience & Biobehavioral Reviews, 25*(3), 219-233.

Parker, A. J., & Clarke, K. A. (1990). Gait topography in rat locomotion. *Physiology & Behavior, 48*(1), 41-47.

Parsania, S., Shabani M., Moazzami K., Razavinasab M., Larizadeh M., Nazeri M., Asadi-Shekaari, M., & Kermani, M. (2014). Gender difference in motor impairments induced by chronic administration of Vinblastine. *Iranian Journal of Basic Medical Sciences 17*(6), 433-440.

Reimer, A. E., de Oliveira, A. R., Diniz, J. B., Hoexter, M. Q., Chiavegatto, S., & Brandão, M. L. (2015). Rats with differential self-grooming expression in the elevated plus-maze do not differ in anxiety-related behaviors. *Behavioural Brain Research, 292*, 370-380.

Røislien, J., Skare, Ø., Gustavsen, M., Broch, N. L., Rennie, L., & Opheim, A. (2009). Simultaneous estimation of effects of gender, age and walking speed on kinematic gait data. *Gait & Posture, 30*(4), 441-445.

Roof, R. L., & Stein, D. G. (1999). Gender differences in Morris water maze performance depend on task parameters. *Physiology & Behavior, 68*(1), 81-86.

Sakakibara, Y., Kasahara, Y., Hall, F. S., Lesch, K., Murphy, D. L., Uhl, G. R., & Sora, I. (2014). Developmental alterations in anxiety and cognitive behavior in serotonin transporter mutant mice. *Psychopharmacology, 231*(21), 4119-4133.

Schank, J. C. (2001). Do Norway rats (Rattus norvegicus) synchronize their estrous cycles? *Physiology & Behavior, 72*(1-2), 129-139.

Schneider, T., & Popik, P. (2007). Attenuation of estrous cycle-dependent marble burying in female rats by acute treatment with progesterone and antidepressants. *Psychoneuroendocrinology*, *32*(6), 651-659.

Stefani, M. R., Nicholson, G. M., & Gold, P. E. (1999). ATP-sensitive potassium channel blockade enhances spontaneous alternation performance in the rat: a potential mechanism for glucose-mediated memory enhancement. *Neuroscience, 93*(2), 557-563.

Sullivan, R.M., Dufresne, M.M., Siontas, D., Chehab, S., Townsend, J., & Laplante, F. (2014). Mesocortical dopamine depletion and anxiety-related behavior in the rat: Sex and hemisphere differences. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 54*, 59-66.

Sutcliffe, J., Marshall, K., & Neill, J. (2007). Influence of gender on working and spatial memory in the novel object recognition task in the rat. *Behavioural Brain Research, 177*(1), 117-125.

Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L. A., & Paylor, R. (2009). Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology, 204*(2), 361-373.

Vetreno, R. P., Anzalone, S. J., & Savage, L. M. (2008). Impaired, spared, and enhanced ACh efflux across the hippocampus and striatum in diencephalic amnesia is dependent on task demands. *Neurobiology of Learning and Memory, 90*(1), 237-244.

Walker, Q. D., Cabassa, J., Kaplan, K. A., Li, S. T., Haroon, J., Spohr, H. A., & Kuhn, C. M. (2001). Sex differences in cocaine-stimulated motor behavior: Disparate effects of gonadectomy. *Neuropsychopharmacology, 25*(1), 118-130.

Warren, S. G., & Juraska, J. M. (2000). Sex differences and estropausal phase effects on water maze performance in aged rats. *Neurobiology of Learning and Memory, 74*(3), 229-240.

Westin, J.E, Janssen, M.L., Sager, T.N., & Temel, Y. (2012). Automated gait analysis in bilateral Parkinsonian rats and the role of l-DOPA therapy. *Behavioural Brain Research 226*, 519–528.

Zimmerberg, B., & Farley, M. J. (1993). Sex differences in anxiety behavior in rats: Role of gonadal hormones. *Physiology & Behavior, 54*(6), 1119-1124.