Rat Shipping and Stress Acclimation

by

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Abstract
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Transporting animals between facilities can cause "shipping stress". Tracking shipping stress and acclimation for rats in research is important for animal welfare and science. Fifty-six female Sprague Dawley rats were monitored to determine if frequent handling accelerates or retards acclimation. Acclimation was determined by glucose and corticosterone parameters, body weight and exploratory behavior up to 7 days after shipping. The rats lost 8.5% of their body weight during shipment. No significant differences were noted between the not handled and handled groups on body weight and glucose; however differences were significant by study day and corticosterone. Corticosterone levels reduced significantly four days after arrival to the new facility. There was a handling effect observed for behavioral end points including more time spent in the center of the arena, more contacts with the novel object and more entries to the open arm of the elevated Y maze. For experiments that require the animals to be stable physiologically, the results of this study support a 48-hour acclimation period for body weight to return to that of pre-shipment levels and 72 hours for experiments involving behavioral measures. This study supports previous research that different personality (behavioral syndromes) types react differently to the same situation. Reactive animals may not adapt as quickly or easily to transportation, housing conditions or experimental procedures as their proactive counterparts.

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Introduction: animals in biomedical research

The use of animals in biomedical research is necessary to advance knowledge of cures and treatments of diseases affecting humans and animals. Researchers can study human diseases in a controlled environment using animals before introducing potential treatments to humans. Vertebrate animal models are used in the early phases of biomedical research due to similarities of their anatomy and physiology with humans. Animals models can replicate different aspects of illnesses and diseases of humans, and are capable of responding to potential treatments and cures to these illnesses and diseases. Common research animals such as rodents have a short life cycle, which allows for the benefit of studying areas of interest not only throughout an animal's entire life cycle but also across generations. The Animal Welfare Act (2002) mandates that any research with animals be documented in a study rationale protocol to describe in detail the procedure to be done. The proposal is reviewed by an institutional animal care and use committee (IACUC). The IACUC ensures the proposed study is justified, (i.e. that is has the potential to advance scientific knowledge or benefit patients) and that it is done in a humane manner.

The Animal Welfare Act regulates the source and sale of animals in research as well as assures humane treatment of animals during transportation, by having standard regulations to control temperature, lighting, shelter, space, food and water during transit (Animal Welfare Act, 2002). Although mice and rats are not a covered species under the Act, most institutions follow these guidelines for them. Even with standards in place, transportation for animals is stressful and transporting animals between facilities can

cause "shipping stress". Prior to transportation, animals are removed from their home cage and placed in a shipping crate. Animals may be in a shipping crate with members of the same cohort or different cohort. Within the crate they may be separated from other animals with the use of a divider, or they may all be together. Either situation can potentially cause stress for an individual. Animals can be transported to the receiving institution by air or truck. Typically animals are packed in transportation crates the afternoon prior to shipping. During this time and in cases where travel time is more than 24 hours and there is an overnight lag, the shipping crates are placed in designated holding facilities. During transportation social groups are disrupted and environmental changes including temperature, light, sounds, smells and motion are encountered.

Upon arrival to the new facility rodents may be housed in unfamiliar style cages, on different bedding or fed a different diet than at the breeding facility. These variations make stress unavoidable and may result in disruptions in the cardiovascular, endocrine, immune, central nervous and reproductive systems (Obernier & Baldwin, 2006). These disruptions in psychophysiological measures may impact research results if experiments are conducted prior to the animal recovering from the effects of transportation (van Ruiven et al., 1996). It is important that animals are allowed to acclimate and recover from shipping stress for animal welfare as well as validity of experimental data.

Habituation to the new facility is important for animal welfare so that animals are not distressed at the start of procedures. Habituation is needed to obtain better results, so that the effects measured are not lost in the noise of a fight or flight response.

Stress and Approaches to Studying It

Stress has been defined in many different ways. For this study, stress will be defined as an "effect produced by acute events referred to as stressors that induce an alteration in the subject's biologic equilibrium" (Reinhardt & Reinhardt, 2006). Stress can be slight or severe, temporary or long lasting and different stressors can evoke different behavioral and physiological responses. Stress can originate when an organism loses predictability and control over its environment (Wiepkema & Koolhaas, 1993). Environmental stimuli that result in an imbalance of homeostasis (internal physiological equilibrium) (Chrousos & Gold, 1992) are referred to as "stressors" and animals exhibit a "stress response" as a result (Mostle & Palme, 2002). Organisms counteract stressors with any number of adaptive responses which consist of a series of physiological, hormonal and behavioral changes in order to reestablish homeostasis. Acute short term stressors are associated with behavioral responses of orientation, alarm and acute vigilance, and physiological components include tachycardia, increased respiration and increased glucose metabolism and glucocorticoid levels.

Adrenal Axis

Within seconds of encountering a stressor, catecholamines are secreted from the sympathetic nervous system which triggers responses such as arousal, alertness, focused attention and aggression. During stressful situations, the corticotrophin-releasing hormone (CRH) in the paraventricular nucleus of the hypothalamus coordinates a series of physiological and behavioral responses. The CRH enhances arousal, and produces effects such as freezing and other fear related behaviors (Erickson et al., 2003) and can lead to increases in heart and respiratory rate as well as in blood pressure and glucose and

plasma corticosteroids levels (Chrousos & Gold, 1992). Activation of the stress response system leads to behavioral and peripheral changes that improve the ability of the organism to adjust and increase its chances for survival. The stress response system is of limited duration and is meant to be temporarily beneficial to the organism to get it through the immediate situation. The stress response takes place in the hypothalamic-pituitary-adrenal axis (HPA). When an animal confronts a stressor, it is detected in the cortex of the brain. The cortex releases a neuronal signal to the hypothalamus which in turn releases a hormone that signals the pituitary. The pituitary sends the hormonal signal adrenocorticotropin (ACTH) to the adrenal gland. As a result the adrenal gland releases glucocorticoids (in rats, corticosterone) (Romero, 2005). The release of glucocorticoids is the last step in the cascade and happens approximately 3-5 minutes after the introduction of the stressor. This release of glucocorticoids allows the animal to cope with the event by initiating changes in metabolism. This increase in glucocorticoids improves fitness by redirecting energy resources and behavior, thus defending the organism against the stressful condition (Mostle & Palme, 2002).

Rats have been shown to have a 24-hour corticosterone rhythm, reaching peak value 2 hours before the beginning of the dark cycle (Atkinson & Waddell, 1997). The relationship between the stressor and amount of glucocorticoids released is complementary; corticosterone levels increase with the stimulus, the more aggressively perceived the stressor is the more glucocortoids are released. Acclimating animals to stressors can result in a lower glucocorticoid response. When an animal is acclimated, they no longer respond to familiar stressors. Glucocorticoids are excreted in blood, urine

and feces. Common approaches to studying stress include evaluating corticosterone levels, glucose levels, body weight and behavior.

Behavioral Tests

Several behavioral tests have been developed to measure emotionality and anxiety related behaviors in rodents. Two common tests to determine the anxiety levels of rodents are the open field arena and the elevated Y maze. Rodents have a natural aversion to open spaces, height and novelty and prefer enclosed areas that offer shelter. Typically rodents will freeze when exposed to novel stimuli. Thigmotaxis (wall hugging) and freezing are thought to be evolutionary anti-predator avoidance behaviors because it is difficult for predators to spot non-moving animals (Harries et al., 2009).

An open field arena tests the conflict between exploration and aversion to open areas (Schmitt & Hiemke, 1998). The test can be used as a one time measure of anxiety or it can be repeated to test memory and habituation. Time spent along the periphery walls is a measure of timidity, and freezing indicates high stress (Walsh & Cummin, 1976).

The open field arena should be several times larger than the home cage and marked off into areas of equal size to measure ambulation across sections. Often times, a novel object will be placed in the center of the arena to encourage exploration as rats prefer novel or moved objects over familiarity (Sutcliffe et al., 2006). Rats are placed in the arena for a predetermined length of time and their behavior is monitored. Parameters that can be measured are ambulation, freezing, time spent along the periphery compared to time spent in the center, contacts with novel object (if applicable), urination, defecation, rearing and grooming. Rats prefer walls to open field, so animals who spend most of the time along the periphery (Figure 1A) are categorized as more anxious than those that

spend the greatest time in the center of the field (Figure 1B). As habituation occurs, exploration declines.

The elevated Y maze combines openness with elevation. A typical elevated Y maze test has two enclosed arms and one open arm. Rodents have a fear of open spaces and heights, therefore rats are reluctant to enter the open arm of the maze and typically avoid them. For this maze, rodents are placed in the closed arm with their nose facing the open arm and their behavior is recorded for 5 minutes (Walf & Frye, 2007). Anxious animals will spend all of their time in the closed arms of the maze (Figure 2A) and less anxious animals will enter out on to the open arm (Figure 2B). Thigmotaxis is positively correlated with corticosterone levels and when confined to open arms rats exhibit fear behaviors such as freezing, defecation and an increase in plasma corticosteroids (Pellow et al., 1985). Studies have shown that the open space of the elevated maze is the main cause of fear since the rat is unable to perform thigmotaxis (Treit et al., 1993). Pellow, et al. (1985) reported that in the elevated maze, rats confined to open arms have significantly higher corticosterone levels (51.6 ± 6.51 µg/ 100 ml) compared to those confined to closed arms (24.4 ± 3.15 µg/ 100 ml) suggesting that rats prefer the closed arm of the maze and that the open arm is more stressful.

Rodents have a preference for protected areas as well as a strong motivation to explore novel environments and objects. The assumption for both of these tests is that emotionality and exploration are inversely related. Therefore low fear animals would show high exploration in an open arena and would tend to enter out to the open arm of the Y maze. Both of these tests have been well documented and validated with the use of anxiolytic compounds. When administered, aversion toward open spaces decreases and

exploration increases. Rodents with increased open arm entries on mazes also have been shown to have increased center entries in an open field arena (Walf & Frye, 2007)

There is strong evidence that genetic factors significantly influence reactivity to stress. One study concluded that BALB/c mice were the least anxious of the strains tested (Trullas & Skolnick, 1993) while another listed them as intermediately anxious (Griebel et al., 2000). PVG/OlaHsd (PVG) rats displayed lower levels of activity in test arenas as well as fewer entries into open arms of plus mazes than Hsd: Sprague DawleySD (SPRD) rats (Schmitt & Hiemke, 1998).

Sex Differences in the Stress Response and Behavioral Tests

The HPA axis in rodents is influenced by sex steroids with females having higher glucocorticoid levels than males (Viau, 2002). Corticosterone levels in male rats have not been reported to significantly differ from estrous or post estrous females, but were significantly lower (two fold) than females in proestrus. Among female rats, higher corticosterone levels have been reported during proestrus (Atkinson & Waddell, 1997). Proestrus is the stage where the estrogen levels are the highest. This finding coincides with the claim that estrogen has been suggested to enhance adrenocortical responses to stress (Figueiredo et al., 2006).

In addition to higher baseline corticosterone levels (Kirschbaum et al., 1999), female rats also have a larger corticosterone secretion after ACTH injections and enhanced responses to stressors than males (Kirschbaum et al., 1992). Plasma corticosterone has also shown to rise more quickly in females rats (Beiko et al., 2004).

Figueiredo et al. (2002) supports this finding, reporting that stress response is dependent on estrous cycle variations. Interestingly, male rats are reported to exhibit

more fear related behaviors in an open field arena and reduced entries into the open arm of mazes than females (Fernandes et al., 1999) suggesting that caution should be used in interpreting results from female rats in tests validated on males. Pare and Redei (1993) also reported superior performance among female rats on open field tests. This performance suggests that female rats are less vulnerable to stressors compared to males. The majority of the published literature regarding females and behavior tests support this finding. There was no difference in the performance of male and female rats in the plus-maze test (Marcondes et al., 2001). Females in proestrus and estrus, however, have been reported to have higher entries in open arms than females in metestrus and diestrus (Diaz-Veliz et al., 1997), while no differences have been observed between proestrus and diestrus animals (Nomikos & Spyraki, 1988). Male Lewis rats exhibited lower corticosterone levels than female Lewis rats (Griffin & Whitacre, 1991). This difference was also documented in Sprague-Dawley rats (Griffin & Whitacre, 1991).

Age has been reported to affect behavioral parameters. Younger rats are more fearful than adult rats. A critical period of 60 to 120 days of age has been identified as a range for behavioral differences in Sex. During this age range, male rats have been shown to exhibit more fear-related behaviors such as a decrease in ambulation and an increase in defecation in the open field test. Males have also been reported to have fewer entries in the open arm of plus mazes than female rats of the same age (Imhof et al., 1993). There were no differences between the sexes after 120 days. A different study reported younger male rats to be more active than older males and that this effect was not seen in female rats (Todorovic et al., 2003). In the open field arena, studies have shown either no differences as well as significant difference in Sex.

In several mouse strains including Balf and B6 males are more active than females in the open field arena tests (Leppanen et al., 2006; Palanza et al., 2001) and yet other strains have shown no difference between Sexs (Bolivar et al., 2000). These studies varied in their procedure of arena shape and testing time of day, which most likely contributed to the different results. There does not appear to be a difference in the baseline anxiety levels between male and females in behavior tests such as the plus maze (Andrews, 1996). Female rats perform better than male rats at standard novel object recognition tests (substituting a familiar object with a novel object and scoring exploratory behavior) (Sutcliffe et al., 2006). Male adolescent Sprague-Dawley rats are more active in a novel environment and approach novel object faster than adult males of the same strain (Stansfield & Kirstein, 2005).

Most studies involving the HPA function have been performed with males because they are believed to represent a stable hormonal environment. However, testosterone release changes as a function of social and reproductive status in response to stress (Viau & Meaney, 1996). Circulating testosterone levels most likely is a contributing factor to the differences reported in young males and adult males.

Acclimation/transportation

A seven to 10-day acclimatization period is advised after transport before animals are used in research; however in practice acclimatization varies from two days to two weeks (Stemkens-Sevens et al., 2009). *The Guidance on the Transport of Laboratory Animals* states that acclimatization is necessary for recovery before procedures begin (Swallow et al., 2005). Body weight in transported rodents has been shown to decrease during transportation upon arrival to the new research facility. Monitoring the effects of

shipping stress and acclimation for rats in research is important for animal welfare and science. To obtain accurate research results and minimize shipping stress, it is important that rats acclimate to their surroundings before participating in experiments.

Previous Studies on Shipping Stress

Studies on shipping stress have included species ranging from African elephants to agricultural animals and laboratory mice, and only a few studies have incorporated research rodents. The few articles found on transportation stress in laboratory rodents differ greatly in methodology or present broad ranges in the results. Studies have reported that it can take between 2-12 days for parameters used in measuring stress to return to pre-shipment levels. One study looking at transportation stress in laboratory rodents suggests corticosterone returns to normal within two to four days (van Ruiven et al., 1996). A telemetry study conducted with guinea pigs reported a period of 10-12 days to return to pre-shipping heart rate, body temperature, and activity levels (Stemkens-Sevens et al., 2009).

Implanted radiotelemetry transmitters in rats were used to measure heart rate, body temperature and activity levels before and after transport. Rats were socially paired prior to transport and were not mixed throughout transport or upon arrival to the novel facility (Capdevila et al., 2007). Social pairs are not typical prior to shipment, nor are keeping animals separate during shipment. Although this study yielded evidence supportive of a 3-day acclimation period, these circumstances are not the norm.

Van Ruiven et al. (1998) explored transportation stress adaptation for rat nutritional studies. Results showed a decrease in blood corticosterone levels one day after transport before returning to normal levels 3 days after transport. Blood was not taken upon arrival

so it is not known if there was a peak in corticosterone between transport and the one day after arrival. Tuli et al. (1995) reported corticosterone levels returning to baseline in mice after 24 hours from transportation within the facility, however from behavioral observations they reported that after 4 days their behaviors had not stabilized.

Handling

Handling animals prior to enrolling them in studies is common. Handling can desensitize them to being picked up and can be tailored to mimic any procedures that might be stressful by performing mock sessions. These training sessions habituate the animals to routine handling and specific procedures, which should avoid unnecessary stress during the study. Rat studies involving handling have reported conflicting results. Some have shown that handling has increased locomotor activity in rats (Reboucas & Schmidek, 1997; Schmitt & Hiemke, 1998) while others have shown no handling effect (Meerlo et al., 1999; Vallee et al., 1997). One study showed Wistar rats that were handled daily had lower corticosterone levels on day 15 of handling than on day 1 (Dobrakovava & Jurcovicova, 1984). In another study rats that were handled three times a day for 14 days had lower corticosterone levels than single handled rats (Dobrakovova et al., 1993) and exploratory activity has been shown to increase with handling (Schmitt & Hiemke, 1998). In an effort to determine acclimation following transportation, Obernier and Baldwin (2006) highlighted physiological changes in rodents after transportation. Transportation times ranged between eighteen and eighty-eight hours. Rats showed an increase in plasma corticosterone levels for less than 48 hours, but a body weight recovery range of one to seven days.

Damon et al. (1986) showed a significant difference in nephrotoxic responses to uranium ore between handled acclimated and non-acclimated rats. Rats that were acclimated for 21 days the toxic dose was 220-650 mg/kg, while rats that were not acclimated, the toxic dose was 3-8 mg/kg. Guinea pigs require 14-17 days for heart rate, body temperature and activity to return to pre-transportation levels (Stemkens-Sevens et al., 2009). After 5 hours of transport, rats recovered body weight, heart rate, activity levels and body temperature after a 3-day acclimation period (Capdevila et al., 2007). Handling in hamsters has been shown to be negatively correlated with corticosterone (Gebhardt-Henrich et al., 2007). Based on these results it has been recommended that hamsters be acclimated to handling prior to blood collection. This handling stress has also been reported in mice where bleeding them after catching by hand presumably caused corticosterone to rise (Shim et al., 2008). Handled mice did not have different locomotor activity levels than mice that were not handled prior to open field testing (Gariepy et al., 2002).

In all of these studies handling procedures (type, length and duration) varied, which may be the root cause of the varying results or it may be attributed to behavioral syndromes.

Behavioral Syndromes

Two elements in animal behavioral research are cross-species and within-species comparisons (Gosling, 2001). Different species exhibit different behavioral strategies in standardized conditions, and individuals within the same species have shown variation in their coping strategies. In human studies, these variations are referred to as traits or personality types. Historically, animals shown to have displayed these variations have

been categorized as outliers, or as exhibiting non-adaptive behavior (Carere & Eens, 2005). Personality research on animals can be quantified objectively by coding behaviors or rating traits across different situations and stages of an animal's life (Gosling, 2001). Each species or individual within a species expresses different traits, which can be objectively measured and categorized. Individual's responses to standardized challenges should be measured for consistency (Bell, 2007) and when behavior is the same regardless of the situation, there is a correlation, which allows us to label animals as having a specific behavioral syndrome. Behavioral syndromes are "a suite of correlated behaviors reflecting between-individual consistency in behavior across multiple (two or more) situations" (Sih et al., 2004a). Gosling (2001) lists four behavioral syndromes that have been studied repeatedly in both humans and animals; aggression, activity level, sociability and fearfulness.

Behavioral syndromes provide an explanation for those individuals displaying non-optimal behavior because their behavioral type is the basis for reactions to situations. An individual will reap the benefits of their type in favorable situations but may suffer the costs in unfavorable situations (Sih et al., 2003). For example, an active or bold individual will exhibit this type of behavior regardless of predation. In low predator areas this is optimal resulting in higher feeding rates, but in high predation areas this behavior is sub-optimal resulting in higher mortality for prey species (Sih et al., 2004b). Aggression is optimal for juvenile female fishing spiders (*Dolomedes fimbriatus*) for growth; however as adults this aggression can result in sexual cannibalism, and is therefore sub-optimal (Arnqvist & Henriksson, 1997).

Studies have shown that behavioral syndromes can be adaptive within populations as a result of the environment. Juvenile poeciliid fish (*Brachyraphis episcope)* differ in boldness depending on location. Predation risks differ between downstream and upstream, and as a result fish living in the higher predation environment are less bold than those found living in the lower predation environment (Brown et al., 2007).

On the contrary, Sih et al.(2003) showed that behavioral correlations across situations result in salamander larvae (*Ambystoma barbouri*) spending much of their time out of refuge and exposed in fish pools where they are vulnerable to predation. Larvae are exposed in pools in the daytime when they feed, and at nighttime they drift out of the pools. The daytime need to feed has overridden the risk of predation (Sih et al., 2003).

Reale et al. (2000), tested temperament in individual bighorn sheep (*Ovis Canadensis*) and found that temperament was domain specific. Bold ewes reproduced earlier and had higher weaning success than shyer ewes. Temperament however was not related to reproductive status, age or body mass. Similarly, individual differences were found to be context-dependent in the pumpkinseed sunfish (*Lepomis gibbosus)*. Fish that were bold to approach a threatening object were not bold to approach a novel food source (Coleman & Wilson, 1998). Measures of shyness and boldness in rainbow trout (*Oncorhynchus mykiss*) also proved to be context specific. Trout that were bold for foraging and feeding were not bold in an exploration task (Wilson & Stevens, 2005).

Studies have also shown that social learning can affect behavior. Bold rainbow trout, observing other bold individuals of the same species remained bold in their responses to approaching novel stimuli. Bold fish who observed shy fish in the same context became more cautious and shy individuals showed not change in their response to novel stimuli

when observing bold fish; however they became bolder (approached novel stimuli faster) when observing other shy fish (Frost et al., 2007). This study contradicts most literature regarding behavioral syndromes, by suggesting that behavior may not be fixed and one dimensional. Animals may exhibit boldness or shyness; however within each of these categories they may be able to adapt their behavior to be "less bold" or "less shy" depending on the situation. The proactive-reactive axis personality type takes into account exploratory behaviors, aggression, fear and response to the environment. Proactive animals can be bold, aggressive, active and exploratory and tend to thrive in a stable environment. Reactive animals can be sensitive to stimuli, cautious and slow to adjust to environmental changes (Sih et al., 2004b).

In sum, the literature shows that cohorts of animals may need different acclimation times depending on their age, sex, strain, transport conditions, and conditions at the study site. Furthermore, individual animals within a cohort may have different acclimation times due to behavioral syndromes.

The aim of this project was to determine an acclimation period for a cohort of female Sprague Dawley rats after 48 hours in transit, and to investigate if frequent handling accelerates or retards acclimation. Parameters assessed include glucose and corticosterone parameters, body weight, and exploratory behavior using an open arena object exploration task and an elevated Y maze. Reaction to novel stimuli and exploration data was collected to determine if an individual could be categorized as proactive or reactive. A secondary objective was to determine which parameters could be used for future studies to determine acclimation time for different strains, sex, ages or laboratories.

Methods

Animals

Fifty-six Female Sprague Dawley rats 225-250g (approximately 57-70 days old) were acquired in two different shipments of 28 animals each from Charles River Laboratories (Portage, MI).

Shipping

The vendor labeled each rat with an individual identification number (1-28) on its tail with a black marker. Each animal was weighed and packed in a cardboard shipping crate. Each shipping crate contained 10 rats, a gel pack for food and water and a wood shaving substrate. Transit time from Portage, MI to Boston, MA, from time of packing for transit to time of arrival and housing, is approximately 48 hours. Rats were randomly assigned upon arrival to one of two habituation groups; a group that would not be handled and a group that would be handled. Upon arrival, rats were weighed and bled for a glucose reading.

Housing

Rats were housed two per cage in individually ventilated Tecniplast rat cages (24.7 cm X 40.64 cm X 18.42 cm) made of polysulfone with a stainless steel wire bar lid and polysulfone cage lid. The cage substrate was Maple Sani Chip Bedding (Harlan Teklad) and rats were fed irradiated 18% protein Rodent Diet (Harlan Teklad 2018). Each cage had a polycarbonate red tube for enrichment/shelter. Food was free choice.

Room conditions were at a set temperature of $70^{\circ} \pm 1^{\circ}$ Fahrenheit with 50% humidity. There was a 12:12 light cycle with lights turning on at 7:00 am and off at 7:00 pm. The air flow in the room was set at 12 air changes per hour.

Assays

Blood was collected at the same time each study day under brief manual restraint via saphenous stick method (Beeton et al., 2007). Glucose levels were read by a One Touch glucometer at the time of blood collection. The One Touch glucometer was validated by comparing readings from the meter to those sent to the laboratory at Merck Research Laboratories. For corticosterone levels, blood was collected in heparinized microhematocrit tubes and spun on a micro-hematocrit centrifuge for 5 minutes; plasma was stored at -20º C for analysis. Corticosterone levels were determined using the IDS Corticosterone EIA kit from Immunodiagnostic Systems Limited.

Open Field Arena with Novel Object

The open field arena used was from Plastic Craft and is made of black acrylic (94 cm x 94 cm with 30.5 cm high walls) with a Lego block secured in the center grid to serve as the novel object. The arena was divided into nine equal sections (30.5 cm x 30.5 cm). Animals were placed in the lower right corner of the arena and were left in the arena for 15 minutes. A camera was mounted atop the arena to record the rats' activity. The arena was cleaned with 70% ethanol alcohol after each trial to eliminate olfactory cues from previous trials.

Elevated Y Maze

The elevated Y Maze was made of black acrylic from Plastic Craft and consisted of one enclosed arm (35.6 cm x 15.2 cm with 30.5 cm high walls) and one open arm (35.6 cm x 15.2 cm). The maze was supported on a table (78.75 cm above the floor). Rats were placed in the closed arm of the maze with their nose facing the open arm. Entering the open arm was achieved when the rat had all four feet out onto the open arm. The rat

was removed from the maze once they entered the open arm or after 5 minutes. The elevated Y maze was cleaned with 70% ethanol alcohol after each trial to eliminate olfactory cues from previous trials.

Analysis

Data obtained from the open field arena data were recorded and analyzed using the software *Cleversys Topscan* (Cleversys, Inc). Parameters analyzed were distance traveled in the arena; time spent in the center of the arena and the number of times the rat made contact with the novel object.

Statistical Analysis

Statistics were analyzed using PASW statistics (SPSS) version 18.0. Parametric statistics were calculated using a two-way ANOVA and non-parametric statistics were calculated using a Kruskall-Wallis and Mann-Whitney tests. Significant results were examined with the Tukey's post-hoc test. Parametric correlations were computed using the Pearson correlation coefficient, and non-parametric correlations were computed using Spearman correlation coefficient. Differences were considered significant at the 5% level ($p<0.05$). The study design entailed merging the results of two different cohorts of rats. Preshipment body weight, arrival body weight, glucose and corticosterone were compared between the cohorts to validate this merging (Table 1). The only significant difference was in arrival body weight, the first cohort averaged 212.93 g (SD = 14.02) and the second cohort averaged 204.34 g (SD = 12.33). Body weight was not shown to correlate with any other parameter, and both groups gained weight throughout the study in a similar trend (Figure 4). Therefore the groups were combined as planned.

Study Design

Study days chosen were selected to bracket the acclimation times reported in previous literature. The study tested 56 rats that were divided into two cohorts of 28 rats each. Each cohort was categorized into two habituation subgroups; not handled and handled. Fourteen rats comprised the not handled group, and fourteen rats comprised the handled group. The group of 4 rats from the not handled group on day 0 served as a baseline for both habituation groups. Rats in the not handled group were left undisturbed and handled only on the scheduled test day. Rats in the handled group were handled daily for 3 minutes up to their scheduled test day. Handling consisted of picking the rat up by the tail and cradling the animal to emulate the saphenous stick technique for blood collection position for 2 minutes (cradling the rat with the animal's nose tucked in the bend of the caretakers wrist) (Figure 4). On the assigned test day, the rat cage was placed under a heat lamp for 3 minutes to help plump saphenous vein for easier blood withdrawal. After 3 minutes, the rat was handled for blood collection, weighed and placed in the open arena for the object habituation task. Rats were handled by two experimenters so that blood collection and open field arena testing was done simultaneously with the rat's cage mate. Following the open arena task rats were placed in the Y Maze until they entered the open arm. If rats did not enter the open arm within 5 minutes they were removed from the maze. On day 7, all rats were weighed, and had blood collected for a final glucose reading (Table 2, 3).

All procedures were approved by the Merck Research Laboratories and Hunter College Institutional Animal care and Use Committees.

Results

Physiological Parameters

Physiological parameters are presented in Table 4.

Body Weight

Rats lost an average of 8.5% of their body weight during shipment; the average pre-shipment body weight for the rats was 233.39g (SD = 6.33), which decreased to 221.51 g (SD = 15.70) upon arrival to our facility. The weight loss from the time they were packed and transported to arrival at the new facility was significant, [t (54) = 4.117, p <0.001].

There was a significant main effect of study day on body weight for the not handled rats, [F (3, 28) = 7.31, p <0.001]. Body weight increased with study day, and post-hoc comparisons indicated that the body weight of rats on arrival day 0 (M= 209.311g, SD = 14.12) were significantly lower than day 7 (M= 243.89 g, SD=23.88), as were the rats on day 2 (M=235.34 g, SD= 12.3) compared to rats on day 7. There was a significant main effect of study day on body weight for the handled rats, [F (3, 28) = 16.9, p <0.001]. Rats on arrival day 0 weighed significantly less than rats on day 2 (M = 228.58g, SD = 6.74), day 4 (M = 237.44g, SD = 12.76), day 7 (M = 247.52g, SD = 9.53) and days 2 and 7. There was no main effect for habituation on body weight, although both groups of rats gained weight throughout the study (Figure 5).

Glucose

There was no significant difference in glucose levels by study day and habituation groups (Figure 6).

Corticosterone

There was a significant effect of study day on corticosterone for the not handled rats, [F (3, 28) = 4.08, p = 0.02]. Corticosterone decreased with study day, and post-hoc comparisons indicated that mean corticosterone levels on arrival day 0 (M= 709.22 ng/ml, SD=314.35) was significantly higher than the mean corticosterone levels for rats on day 7 (M=353.06 ng/ml, SD=200.17) as well as on days 2 (M=706.51 ng/ml, SD= 227.6) and 7. There was no main effect for habituation on corticosterone. However, corticosterone decreased by study day for both groups (Figure 7).

Behavior

Behavioral parameters are presented in Table 5.

Open Field Arena

There was a significant main effect on study day and distance traveled in the open field arena for not handled rats, [F (3, 28) = 6.48, p = 0.02]. Distance traveled within the arena increased significantly with study day. Post-hoc comparisons indicated that the mean distance traveled for rats on arrival day 0 (M=18.2 m, SD = 13) was significantly shorter on days 4 (M= 43.17 m, SD=14.23) and 7 (M= 39.82 m, SD=6.1). This effect was also seen with handled rats, [F (3, 28) = 10.04, p = < 0.000]. Distance traveled within the arena increased significantly with study day. Post-hoc comparisons indicated that the mean distance traveled for rats on arrival day 0 (M=18.2 m, SD = 13) was significantly shorter on days 4 (M= 57.38 m, SD=13.27) and 7 (M= 52.26 m, SD=15.55).

There was a significant main effect on habituation and distance traveled in the open field arena [F (1, 56) = 5.64, p = 0.02]. Rats on day 7 in the handled group traveled

further than rats on day 7 in the not handled group, [t (14) =-2.109, $p = 0.022$]. Both habituation groups showed a similar trend, where distance increased over time (Figure 8).

Time spent in the center of the open field arena increased significantly with study day for handled rats, [χ^2 (3, N=32) =7.5, $p = 0.05$]. Rats on arrival day 0 spent less time in the center of the arena than rats on day 7 [t (30) =-2.68, $p = 0.01$] (Figure 9).

There was a main effect of study day on the number of contacts with the novel object for handled rats, [χ2 (3, N=32) =9.86, p = 0.02]. Rats upon arrival day 0 made fewer contacts with the novel object than rats on day 7 [t (30) =-3.72, $p < .001$] (Figure 10).

Elevated Y Maze

Eighty-three percent of the handled group entered the open arm of the elevated Y maze compared to thirty-one percent of entries from the not handled group. Entries to the open arm of the maze increased significantly with study day for the handled rats [χ^2 (3, N=32) =16.7, $p < 0.001$]. Rats on day 0 (M= 0.13 entries, SD= 0.35) entered the open arm less frequently than rats on day 4 ((M= 0.63 entries, SD= 0.52) and day 7 (M= 0.88 entries, SD= 0.35). There was a habituation effect for entries to the open arm; rats in the handled group had significantly more entries on day 4, [t (14) =2.73, $p = 0.006$] and day 7, [t (14) = 2.19, $p = 0.046$] compared to rats from the not handled group on the same days (Figure 11).

Habituation Effects

There was a handling effect, [t (54) = -3.673, $p < 0.001$] with distance traveled in the open field arena. A Mann-Whitney test showed a significant difference in the time handled rats spent in the center of the open field arena, (U=248, p=0.02) and the number

of contacts with the novel object (U=266.5, p=0.05) as compared to not handled rats.

Correlations Among Parameters

Physiological parameters did not correlate on any study day (Table 6, Figures 12-14). Behavioral parameters however were strongly correlated (Figures 15-17). Upon arrival, day 0, there was a positive correlation between time spent in the center of the arena and the number of contacts with the novel object [r (8) = 0.95, $p < 0.00$] (Table 7A).

On day 2, for the not handled rats, time spent in the center of the open field arena positively correlated with the number of contacts with the novel object, [r (8) =0.93, $p < 0.001$] and distance, [r (8) = -0.89, $p = 0.003$]. The number of contacts with the novel object positively correlated with distance, [r (8) = 0.82, $p = < 0.01$], and a negative correlation with corticosterone, [r (8) = -0.72, $p = 0.04$]. The rats within the handled group showed a positive correlation between time spent in the center of the open field arena and the number of contacts with the novel object, [r (8) = 0.95, $p = < 0.00$] (Table 7B).

On study day 4, for the not handled rats time spent in the center of the open field arena positively correlated with the number of contacts with the novel object, [r (8) =0.93, $p = < 0.001$]. The number of entries out to the open arm of the elevated Y maze negatively correlated with corticosterone, [r (8) = -0.74, $p = 0.04$]. The handled rats had a positive correlation with time spent in the center of the open field arena and the number of contacts with the novel object, [r (8) =0.94, $p = < 0.00$]. There was a negative correlation for time spent in the center of the open field arena and corticosterone [r (8) = -0.76, $p = 0.03$] (Table 7C).

On study day 7, for the not handled rats time spent in the center of the open field arena positively correlated with the number of contacts with the novel object, [r (8) =0.95, p = < 0.000] as well as distance traveled in the center of the open field arena, [r (8) = 0.88, p = 0.04]. Distance traveled in the center of the open field arena was also positively correlated with the number of contacts with the novel object, [r (8) =0.90, p = < 0.000]. The handled rats showed a positive correlation with time spent in the center of the open field arena and the number of contacts with the novel object, [r (8) =0.98, p = < 0.000], as well as the number of entries to out to the open arm of the elevated Y maze, , [r (8) =0.72, p = 0.05]. Entries out to the open arm of the elevated Y maze also had a positive correlation with the number of contacts with the novel object, [r (8) =0.81, p = < 0.02] (Table 7D).

Behavioral Syndrome Parameters

Correlations

Correlations were computed to determine the relationship, if any, among all parameters. Glucose and body weight did not correlate with any parameter. There were correlations between behavioral parameters and corticosterone. There was a correlation between distance traveled in the open field arena and time spent in the center of the open field arena, [r (56) =0.66, p = < 0.000], the number of contacts with the novel object, [r (56) =0.68, p = < 0.000], and entries out to the open arm of the elevated Y maze [r (56) = -0.41, p = < 0.01]. There was a correlation between time spent in the center of the open field arena and the number of contacts with the novel object, [r (56) =0.91, p = < 0.000], as well as with corticosterone, [r (56) = -0.27, p = 0.04]. Corticosterone correlated with time spent in the center of the open field arena, [r (56) = -0.27, p = 0.04]. There was a

correlation between the number of contacts with the novel object and the number of entries out to the open arm of the elevated Y maze [r (56) = -0.26, p = < 0.05] (Table 8).

Comparisons were made to determine if there were any differences in these parameters between the rats who did not enter the open arm of the elevated Y maze and those that did (Table 9). Rats who entered the open arm of the elevated Y maze had significantly lower corticosterone levels than those who did not enter [t (54) = -2.43, p = 0.02]. They also traveled significantly longer distances in the open field arena, [t (54) = -3.57, p = < 0.001], spent more time in the center of the open field arena, [t (54) = - 2.32, p = 0.02], and had more contacts with the novel object, [t (54) = -2.39, p = 0.02] than those rats who did not enter the open arm of the elevated Y maze. There were no significant differences in any of these parameters within the group of rats that entered the open arm of the elevated Y maze or within the group that did not enter the open arm of the Y maze (Table 10).

Discussion

The rats in our study demonstrated further evidence of shipping stress and its effects on physiology and behavior. One immediate indicator was the average 8.5% weight loss rats incurred from pre-shipment to arrival. Rats regained their pre-shipment body weights by study day 2.

Glucose measures did not provide a good indicator for stress or acclimation, as glucose remained constant and within the normal range throughout the study. Corticosterone levels however decreased with time, i.e., study day, showing the longer the animals were housed at our facility the lower their corticosterone levels. Rats appear to acclimate four days after arrival using corticosterone as a parameter. Previous studies reported a decline in corticosterone levels one day after arrival to a new facility and a return to "normal" corticosterone levels 2-4 days after transport (Tulie et al., 1995; Van Ruiven et al., 1996), however there no reference for normal levels of corticosteroids was given. In the current study, there was a decline in corticosterone 2 days after arrival and a significant decline in corticosterone levels by day 4.

Distance traveled in the open field arena increased over time, with distance increasing each day. There was no significant difference in travel time observed between day 4 and day 7. This result suggests a 4-day acclimation period for distance traveled in the open field arena.

Time spent in the center of the arena increased significantly by day 4; however, animals in the handled group spent more time in the center of the open field arena than the not handled rats on day 7. The number of contacts with the novel object compliments this data; the object was secured in the center of the arena therefore time spent in the

center of the open field arena and the number of contacts with the novel object follow the same trend. Animals that spend the longest time in the center of the arena are regarded as less anxious or proactive, and the results here support this conclusion.

Previous findings have shown that corticosterone is involved in fear behaviors in the elevated Y maze (Korte & Beoer, 2003). Entries out to the open arm of the elevated plus maze did increase with study day as corticosterone decreased. The results from this study support findings that handling increases exploration, which is travel distance and time spent in the center in an open field arena, as well as the number of entries to the open arm of the elevated Y maze. These parameters also suggest a 4-day acclimation period for both handled and not handled rats for distance traveled in the open field arena, as well as a 4-day acclimation period for handled rats using entries out to the open arm as a measure.

There were significant differences in the behavioral parameters and corticosterone levels between the rats that entered open arm of the elevated Y maze and those that did not. On the other hand, there were no significant differences in behavioral parameters and corticosterone within the group rats that entered the open arm of the elevated Y maze, and the same was found (no significant differences in parameters) within the group of rats that did not enter the open arm of the elevated Y maze. Therefore latency to enter the open arm of the elevated Y maze was not a factor, whether they entered or not was. There were no significant differences in any parameters among rats that entered the open arm in 4 seconds (shortest latency observed) and 140 seconds, (longest latency observed). In this study, proactive explorers entered the open arm of the elevated Y maze, traveled longer distances in the open field arena, spent more time in the center of the arena, and

had more contacts with the object. The findings of this study parallel the exploratory behavior of the juvenile male great tits (*Parus major)* where fast explorers have an active style of coping with stress and slow explorers show a passive coping style (Verbeek et al., 1996) This has also been found in pigs where active coping animals contact a novel object sooner than passive coping pigs. Passive coping pigs also show consistent differences in behavioral, physiological and endocrine responses in stressful situations (Hessing et al., 1994). In rats, proactive coping styles have been associated with low corticosterone levels and reactive with high corticosterone levels (Koolhaas et al., 2001).

Previous studies have suggested that early life experiences can assist in developing an individual's personality to prevailing environmental conditions (Groothuis & Carere, 2005; Frost et al., 2007). In the pumpkinseed sunfish there was an intermediate class of fish that were neither shy nor bold; they ignored a novel stimulus until the threat got too close at which time they fled or approached (Coleman & Wilson, 1998). In the present study, there was a group of rats whose behavior classified them as proactive explorers upon arrival or within the 7 day study (entry to the maze, exploration in the arena, and contact with novel object). A second group of rats remained reactive throughout the duration of the study (no entry to maze, no or minimal exploration in the arena and contacts with the novel object). Upon arrival, there was one (out of eight) individual that exhibited proactive behavior, and on day 7 there were five (out of 16) individuals that exhibited reactive behavior. Like the pumpkinseed sunfish, there was an intermediate group of rats which became proactive with acclimation or handling (six individuals on day 2, 12 on day 4 and 11 on day 7), while others remained reactive (10 individuals on day 2, four on day 4 and five on day 7). Previous work has shown that selection for a

particular behavior at a young age may affect that behavior (Bell & Stamps, 2004). In the current study, rats were juveniles, and it is possible that acclimation and handling shaped their behavior.

Behavioral syndromes are stable when two or more behaviors correlate with each other, resulting in a disposition or temperament which is a tendency for an individual to behave a certain way in a number of circumstances. Coping styles are important to how animals deal with stress and challenging situations (Groothuis & Carere, 2005). Identifying the behavioral syndrome for the species or individuals in the laboratory can be an important factor in research. Limited plasticity, shown by the consistent behavior across situations may affect the strategy an individual uses to cope with a given environment or situation (Drent et al., 2002). The degree to which a species or individual is able to cope may have animal welfare implications (Carere & Eens, 2005).

The ability to identify behavioral syndromes in a population of animals may help advance research. Researchers have been able to identify high responders and low responders to novelty in the Nijmegen rat by their performance on 3 behavioral paradigms. Genetics studies have linked this behavior with a predisposition for drug addiction in humans. The coping strategies used by these rats are similar to that in humans. The response to novelty and sensitivity to drugs of abuse was shown to be correlated, and as a result it has become common to use high responders and low responders to novelty to search for mechanisms underlying individual differences in drug abuse (Cools & Gingras, 1997).

This study supports previous research that different personality types react differently to the same situation. Reactive animals may not adapt as quickly or easily to

transportation, housing conditions or experimental procedures as their proactive counterparts. This variation in adaptation can be a potential confound in experimental data. It is important to identify an acclimation period because if animals are placed on a study too soon results may be compromised. Skewed data as a result of a stressed animal may produce misleading results. On the other hand, holding animals for a longer period than necessary to acclimate can become costly in terms of equipment, husbandry, veterinary care, space and resources elsewhere needed. For experiments that require the animals to be stable physiologically, the results of this study support a 48-hour acclimation period for body weight to return to that of pre-shipment levels and 72 hours for a significant decline in corticosterone. For experiments that involve exploratory behavior, the animals should be allowed to acclimate for a minimum of four days.

Age, sex and strain of animals in certain types of research may play an important role. Female rats have shown to be less vulnerable to stressors compared to males, and females respond differently behaviorally and physiology depending on their stage of estrous. Age can also confound behavioral parameters. Younger rats are more fearful and more active than adult rats.

Another important variable to be considered in future research is animals' circadian activity and their related day/night time habits. This study was conducted during the diurnal phase, although rats are a nocturnal species. Almost all manipulations in laboratory animal models are performed during this phase and in lighted rooms. Some laboratories house certain research models in a reverse light cycle in attempt to counteract this confound, however there is no guarantee that all physiological functions change along with the circadian cycle to accommodate this reverse light cycle (Bertoglio

& Carobrez, 2002). One study measuring anxiety reported no change in circadian behavior in rats kept in a reverse light cycle (Jones & King, 2001). Rats have been shown to explore more in lower illumination conditions than higher (Bertoglio & Carobrez, 2002). Rats that were tested under both diurnal and nocturnal conditions in the forced-swim test were significantly different in their behavioral and physiological responses. Rats tested during the nocturnal phase were more active, showed lower physiological stress responses and were less agitated by handling than the rats that were tested in the diurnal phase (Kelliher et al., 2000). Eighty percent of control rats in an avoidance training task were successful in learning avoidance behavior when trained in the dark compared to only sixty percent of rats that were successful in learning avoidance behavior when trained during the daytime (Catala et al., 1983). Studies with *Aplysia fasciata,* a nocturnal species showed no long term memory on tasks that were trained during the day, suggesting the circadian clock correlates with memory and activity periods (Lyons et al., 2005). All of these studies suggest that ideal circumstances for testing nocturnal animals would be during their active phase, however there are limitations to this option and reverse light cycles do not necessarily change physiological components.

Certain traits may allow animals to acclimate quicker and easier to specific procedures. Choosing traits specific to experimental procedures may benefit both animals and research. Animals should be allowed sufficient time to overcome shipping stress and its effects on physiology and behavior. Acclimation time may vary depending on species, strain, age, sex, transport conditions, conditions at the study site as well as the criteria for acclimation. Physiological parameters such as body weight and corticosterone

as well as behavioral parameters such as distance traveled in an open field arena and entries out to the open arm of the elevated Y maze can be used to help determine an acclimation period appropriate for the research animal and experimental model. Having an appropriate acclimation period enhances animal welfare and scientific validity.

Table 1. *Comparing Arrival Parameters for each Cohort before Merging Data.*

	Parameter			
	Pre-shipment Body Weight (g)	Arrival Body Weight (g)	Arrival Glucose (mg/dL)	Arrival Corticosterone (ng/ml)
Cohort 1 (n = 28)	233.75 (6.51)	221.51* (14.03)	114.00 (16.65)	736.82 (253.52)
Cohort 2 (n = 28)	233.04 (6.24)	204.34* (12.33)	117.11 (14.44)	681.62 (425.13)

$p < .05^{*}$

Note. Table presents means and standard deviations (in parentheses).

Table 2. *Schedule for Animals in the "Not handled" Group.*

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Animals	1-4							
Weight	x							x
Blood Collection	x							x
Open Field Arena	x							
Elevated Y Maze	x							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Animals	5-8							
Weight	x		x					x
Blood Collection	x		x					x
Open Field Arena			x					
Elevated Y Maze			x					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Animals	13-16							
Weight	x				x			x
Blood Collection	x				x			x
Open Field Arena					x			
Elevated Y Maze					x			
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Animals	21-24							
Weight	x							x
Blood Collection	x							x
Open Field Arena								x
Elevated Y Maze								x

Note. Table presents study design for animals in the not handled group.

Table 3. *Schedule for Animals in the "Handled" Group.*

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Animals	9-12							
Weight	x		x					x
Handle	x	x	x					
Blood Collection	x		x					x
Open Field Arena			x					
Elevated Y Maze			x					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Animals	17-20							
Weight	x				x			x
Handle	x	x	x	x	x			
Blood Collection	x				x			x
Open Field Arena					x			
Elevated Y Maze					x			
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Animals	25-28							
Weight	x							x
Handle	x	x	x	x	x	x	x	x
Blood Collection	x							x
Open Field Arena								x
Elevated Y Maze								x

Note. Table presents study design for animals in the handled group.

Table 4. *Mean Physiological Parameters by Study Day and Habituation.*

	Not handled			Handled		
	Body Weight (g)	Glucose (mg/dL)	*Corticosterone (ng/ml)*	Body Weight (g)	Glucose (mg/dL)	*Corticosterone (ng/ml)*
Study Day 0 (n = 8)	209.31 (14.12)	125.50 (20.20)	709.22 (325.38)			
Study Day 2 (n = 16)	235.33 (12.33)	131.38 (16.35)	706.51 (227.59)	228.58 (6.74)	132 (15.98)	576.30 (189.17)
Study Day 4 (n = 16)	242.68 (14.65)	134.38 (16.25)	581.69 (147.55)	237.44 (12.76)	131.75 (12.48)	450.66 (200.60)
Study Day 7 (n = 16)	243.89 (23.88)	127.50 (10.92)	353.05 (200.17)	247.52 (9.53)	128.75 (8.53)	408.02 (174.61)

Note. Table presents means and standard deviations (in parentheses). Each study day totals 16 animals, 8 represent the not handled habituation group and 8 represent the handled habituation group.

Table 5. *Mean Behavioral Data by Study Day and Habituation.*

	Not handled			Handled		
	Distance Traveled in Arena (m)	Time Spent in Center of Arena (s)	Number of Contacts with Novel Object	Distance Traveled in Arena (m)	Time Spent in Center of Arena (s)	Number of Contacts with Novel Object
Study Day 0 (n = 16)	18.21 (13.37)	8.56 (9.98)	2.25 (2.61)			
Study Day 2 (n = 16)	31.77 (13.81)	15.22 (16.74)	5.88 (7.02)	38.54 (19.49)	18.36 (18.14)	6.63 (7.29)
Study Day 4 (n = 16)	43.17 (14.23)	20.40 (14.37)	10.75 (7.76)	57.38 (13.27)	23.35 (9.21)	10.88 (6.78)
Study Day 7 (n = 16)	39.82 (6.05)	13.28 (12.13)	6.38 (6.07)	52.26 (15.54)	28.81 (14.98)	12.41 (7.41)

Note. Table presents means and standard deviations (in parentheses). Each study day totals 16 animals, 8 represent the not handled habituation group and 8 represent the handled habituation group.

Table 6A-D. *Correlation Coefficients Among Physiological Parameters by Study Day and Habituation.*

A. Arrival Day 0

	Body Weight	Glucose
Body Weight		-0.08
Corticosterone	0.35	0.15

B. *Study Day 2*

Not handled

	Body Weight	Glucose
Body Weight		0.18
Corticosterone	0.15	0.50

Handled

	Body Weight	Glucose
Body Weight		-0.26
Corticosterone	0.6	0.12

C. Study Day 4

Not handled

	Body Weight	Glucose
Body Weight		0.38
Corticosterone	-0.42	0.42

Handled

	Body Weight	Glucose
Body Weight		0.19
Corticosterone	0.14	0.47

D. Study Day 7

Not handled

	Body Weight	Glucose
Body Weight		0.5
Corticosterone	0.37	-0.11

Handled

	Body Weight	Glucose
Body Weight		0.32
Corticosterone	-0.33	-0.07

Table 7A-D. *Correlation Coefficients Among Behavioral Parameters and Corticosterone by Study Day and Habituation.*

A. Arrival Day 0

	#Contacts with object	Time in Center	Distance Traveled	Y Maze	Corticosterone
#Contacts with object		0.95*	0.62	0.35	-0.37
Time in center	0.95*		0.42	0.35	-0.38
Distance traveled	0.62	0.42		-0.21	0.47
Y Maze	0.35	0.35	-0.21		-0.23

$p < .05^{*}$

B. *Study Day 2*

Not handled

	#Contacts with object	Time in Center	Distance Traveled	Y Maze	Corticosterone
#Contacts with object		0.93*	0.82*	0.34	0.72*
Time in center	0.93*		0.89*	0.37	0.68
Distance traveled	0.82*	0.89*		0.45	0.58
Y Maze	0.34	0.37	0.45		0.56

$p < .05^{*}$

Handled

	#Contacts with object	Time in Center	Distance Traveled	Y Maze	Corticosterone
#Contacts with object		0.98*	0.62	0.09	-0.37
Time in center	0.98*		0.42	0.05	-0.38
Distance traveled	0.62	0.42		-0.21	-0.47
Y Maze	0.09	0.5	-0.21		-0.23

$p < .05^{*}$

C. *Study Day 4*

Not handled

	#Contacts with object	Time in Center	Distance Traveled	Y Maze	Corticosterone
#Contacts with object		0.93*	0.58	0.02	-0.66
Time in center	0.98*		0.6	0.08	-0.56
Distance traveled	0.58	0.6		-0.55	-0.13
Y Maze	0.02	0.08	-0.55		-0.74*

$p < .05^{*}$

Handled

	#Contacts with object	Time in Center	Distance Traveled	Y Maze	Corticosterone
#Contacts with object		0.94*	0.63	0.19	-0.55
Time in center	0.94*		0.58	0.24	-0.76*
Distance traveled	0.63	0.58		0.04	-0.11
Y Maze	0.04	0.24	0.09		0.31

$p < .05^{*}$

D. *Study Day 7*

Not handled

	#Contacts with object	Time in Center	Distance Traveled	Y Maze	Corticosterone
#Contacts with object		0.95*	0.90*	0.24	0.21
Time in center	0.93*		0.88*	0.09	0.04
Distance traveled	0.90*	0.88*		0.06	0.04
Y Maze	0.24	0.09	0.06		-0.04

$p < .05^{*}$

Handled

	#Contacts with object	Time in Center	Distance Traveled	Y Maze	Corticosterone
#Contacts with object		0.98*	0.24	0.81*	0.12
Time in center	0.98*		0.34	0.72*	0.26
Distance traveled	0.24	0.34		-0.12	0.37
Y Maze	0.81*	0.68	-0.12		0.16

$p < .05^{*}$

Table 8. *Correlation Coefficients Among Behavioral Parameters and Corticosterone to Assess Relationships for Behavioral Syndromes.*

	Parameter				
	Contacts with Novel Object	Time in Center of Arena	Distance Traveled in Arena	Y Maze Open Arm Entries	Corticosterone
Contacts with Novel Object		0.91*	0.68*	-0.26*	-0.28*
Time in Center of Arena				-0.23	-0.27*
Distance Traveled in Arena		0.65*		-0.41*	-0.25
Y Maze Open Arm Entries					0.28*

$p < .05^{*}$

Note. Table presents correlation coefficient value.

Table 9. *Comparing Behavioral Parameters and Corticosterone with Entries out to the Open Arm of the Elevated Y Maze.*

	No Entry	Entered
Distance Traveled in Arena (m)	31.77 (14.61)	47.44 (14.61)
Time Spent in Center of Arena (s)	13.55 (12.38)	22.38 (15.65)
Number of Contacts with Novel Object	5.54 (5.99)	9.90 (7.45)
Corticosterone (ngl/ml)	621.95 (275.13)	470.43 (188.28)

Note. Table presents means and standard deviations (in parentheses).

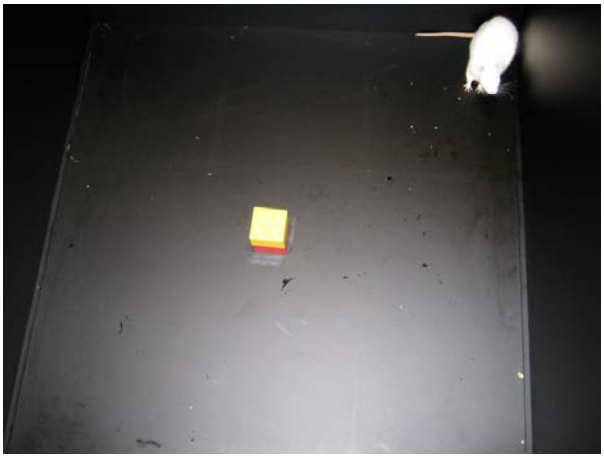
Table 10. *Comparing Entries out into the Open Arm of the Elevated Y Maze to assess behavioral syndromes. Proactive Animals enter the open arm and reactive animals do not.*

	Not handled		Handled	
	No Entry	Entered	No Entry	Entered
Study Day 0 (n = 8)	7	1		
Study Day 2 (n = 16)	7	1	3	5
Study Day 4 (n = 16)	4	4	0	8
Study Day 7 (n = 16)	4	4	1	7

Note. Each study day totals 16 animals, 8 represent the not handled habituation group and 8 represent the handled habituation group.

Figure 1. Open Field Arena Test. Thigmotaxis (A) is a measure of anxiety, while exploration (B) and contacts with novel object are characteristics of an acclimated rat.

A



B

Figure 2. Elevated Y Maze. Freezing behavior in the closed arm of the elevated y maze is an example of an anxious behavior (A) while entering out on to the open arm (B) is a demonstration of an acclimated rat behavior.

A

B

Figure 3. Body weight trends for Pre-shipment, arrival and final study day by cohort.

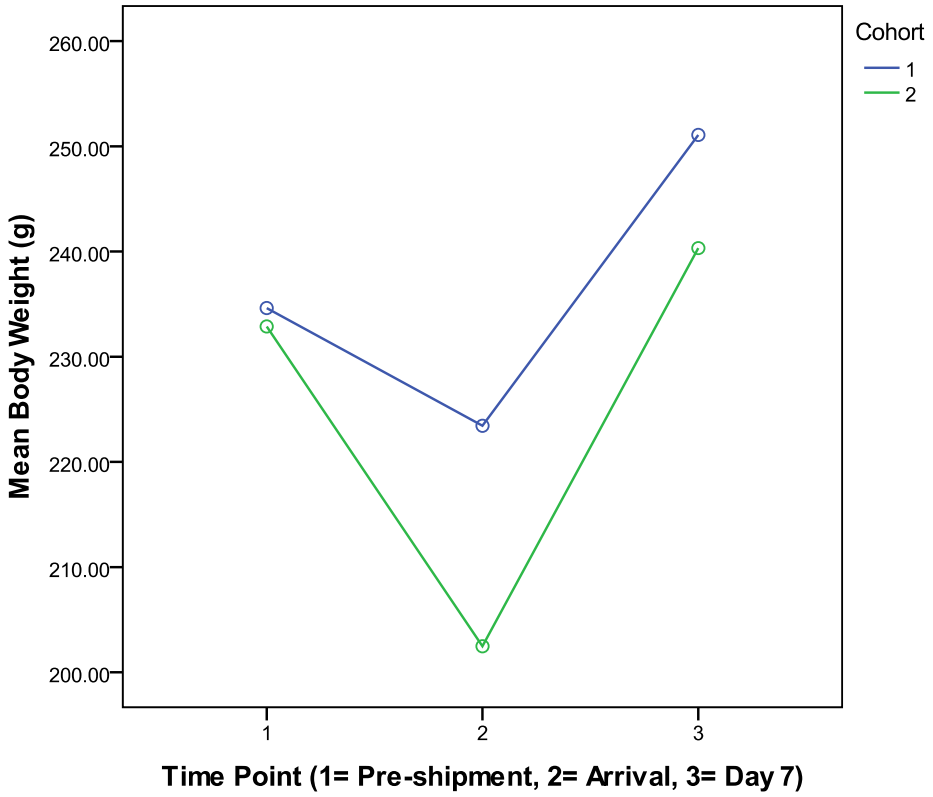


Figure 4. Handling technique. Rats in the "handled" acclimation group were handled daily for 2 minutes by experimenters. The handling technique is the cradle position for saphenous stick blood collection.

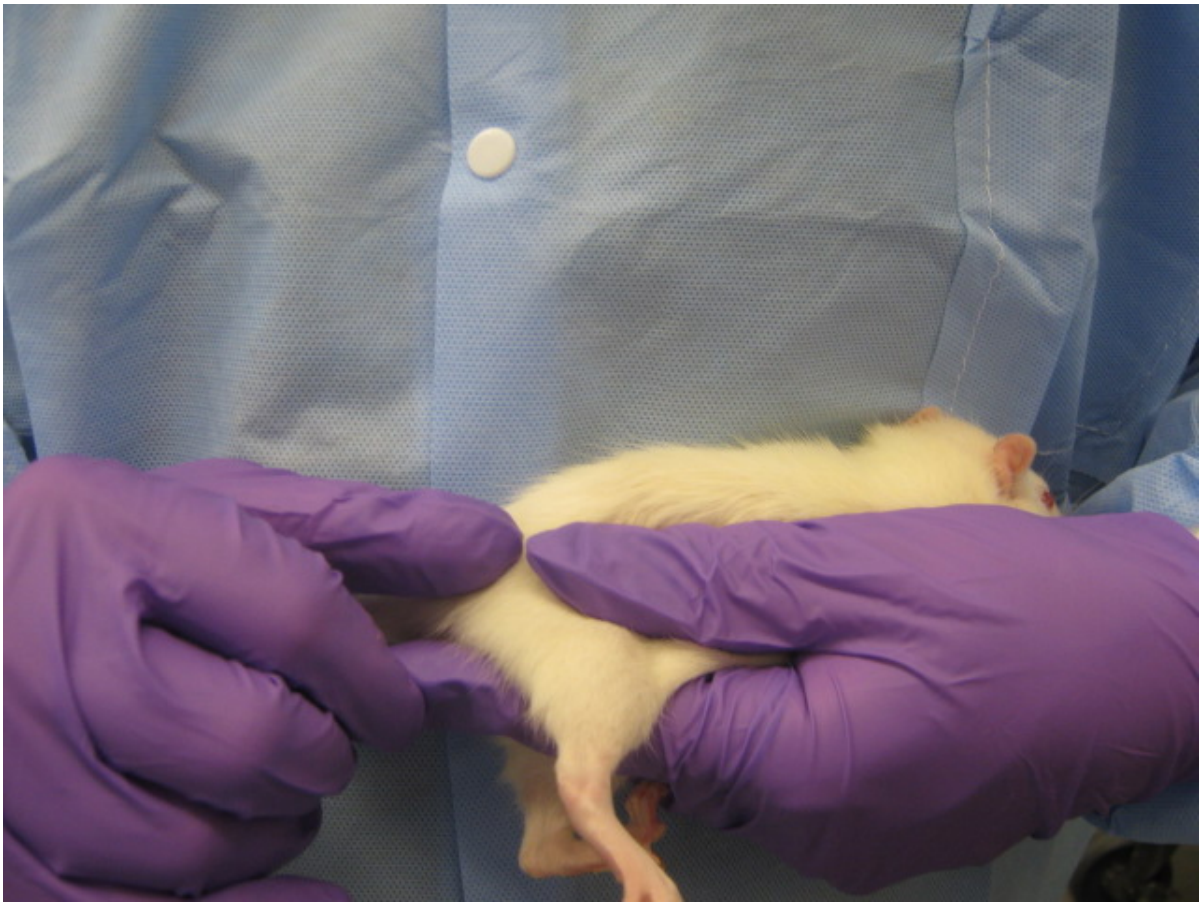


Figure 5. Body weight trends for both cohorts by study day and habituation. Both the not handled and handled rats gained weight throughout the study. The red line indicates the pr-shipment weights of rats.

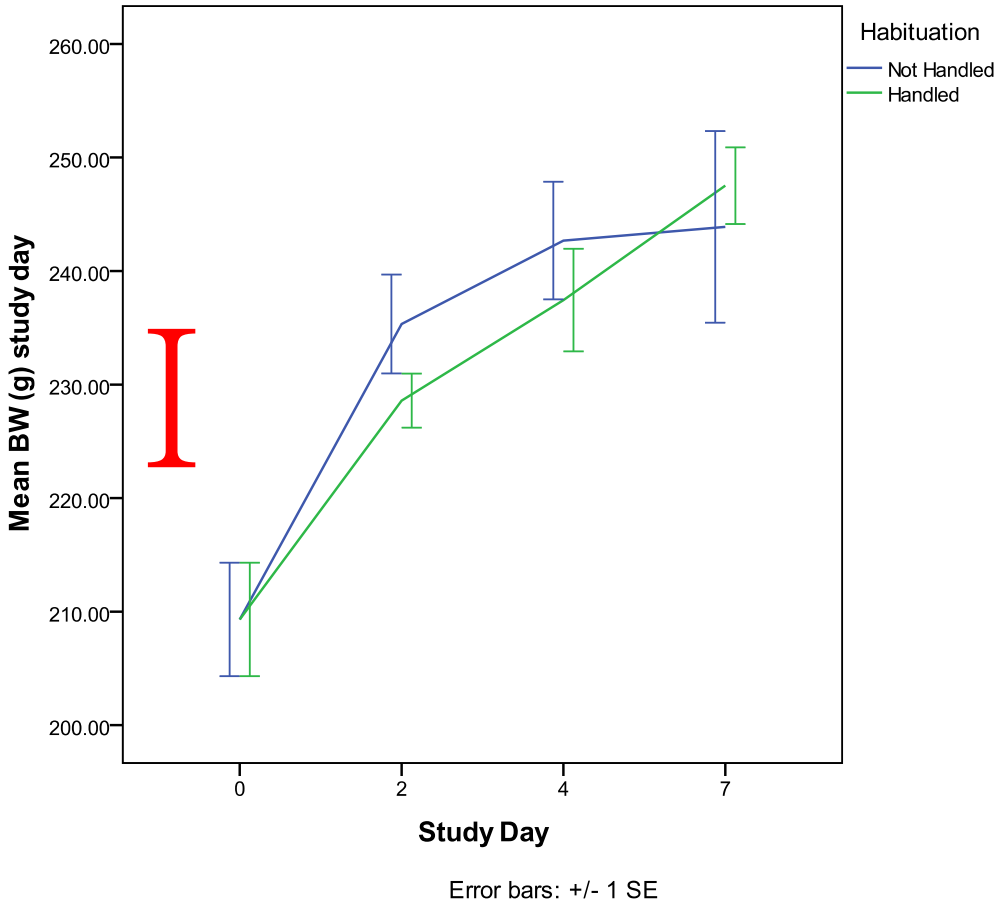


Figure 6. Glucose by study day and habituation. There was no significant difference found in glucose levels by study day or in either habituation group during the study.

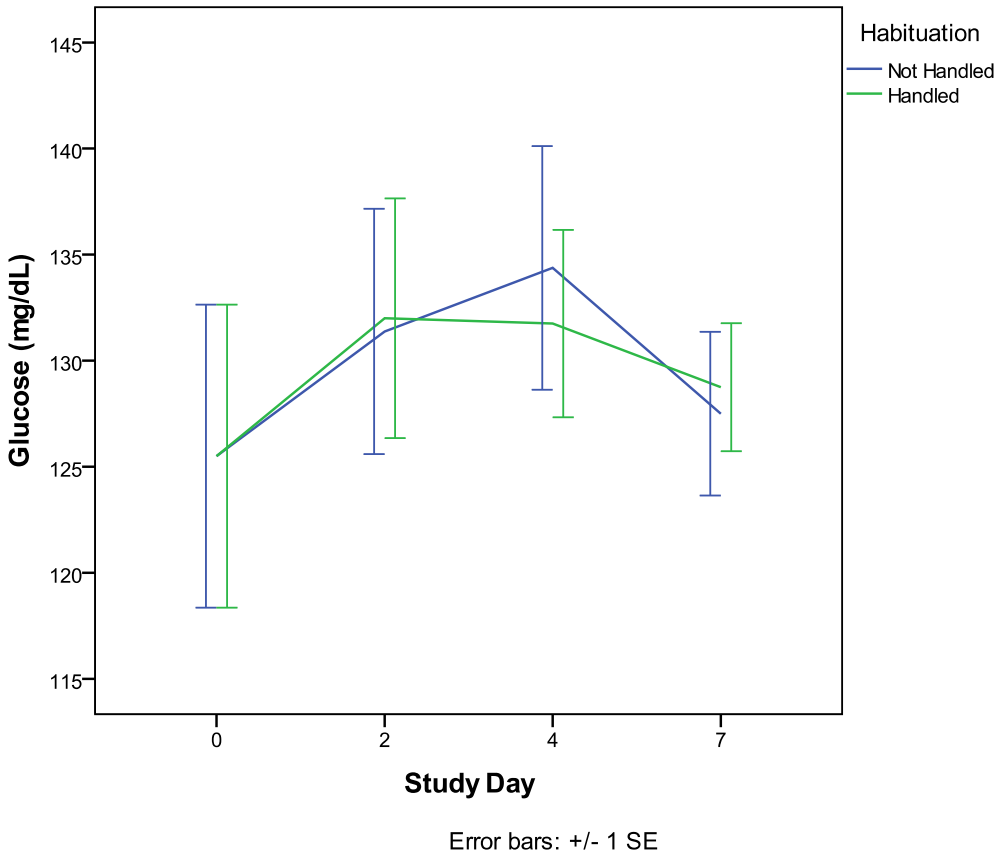


Figure 7. Corticosterone by study day and habituation. There was a significant effect of study day on corticosterone for the not handled rats. Corticosterone decreased with study day, and mean corticosterone levels upon arrival day 0 were significantly higher than the mean corticosterone levels for rats on days 2 and 7.

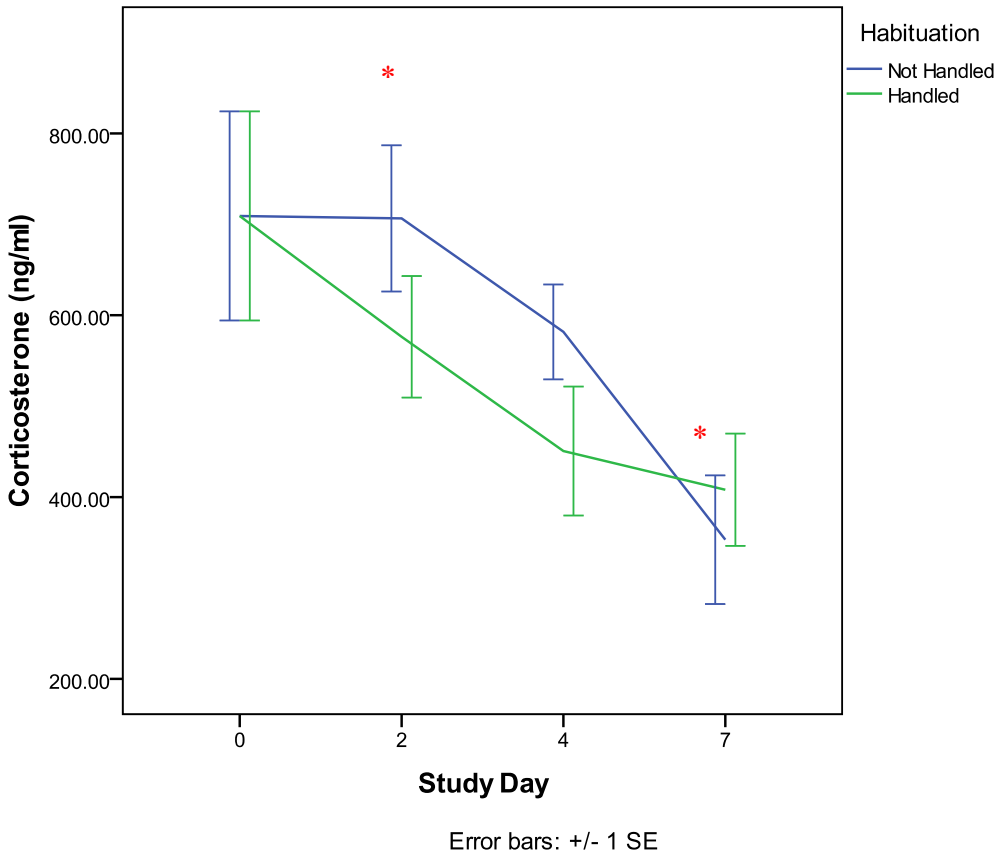


Figure 8. Distance traveled in the Open field arena by study day and habituation. Both habituation groups increased distance traveled in the open field arena. Rats on day 7 in the handled group traveled further than rats on day 7 in the not handled group.

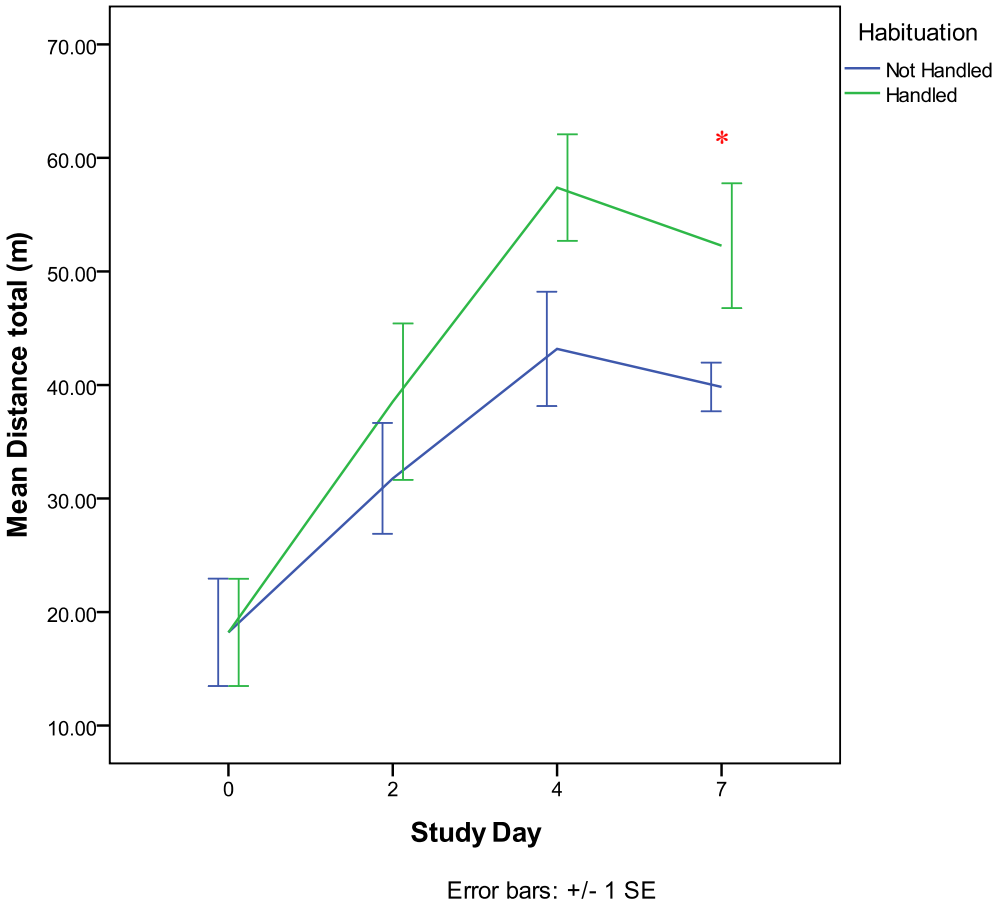


Figure 9. Time spent in the center of the open field arena by study day and habituation. Time spent in the center of the open field arena increased significantly with study day for handled rats. Rats on arrival day 0 spent less time in the center of the arena than rats on day 7.

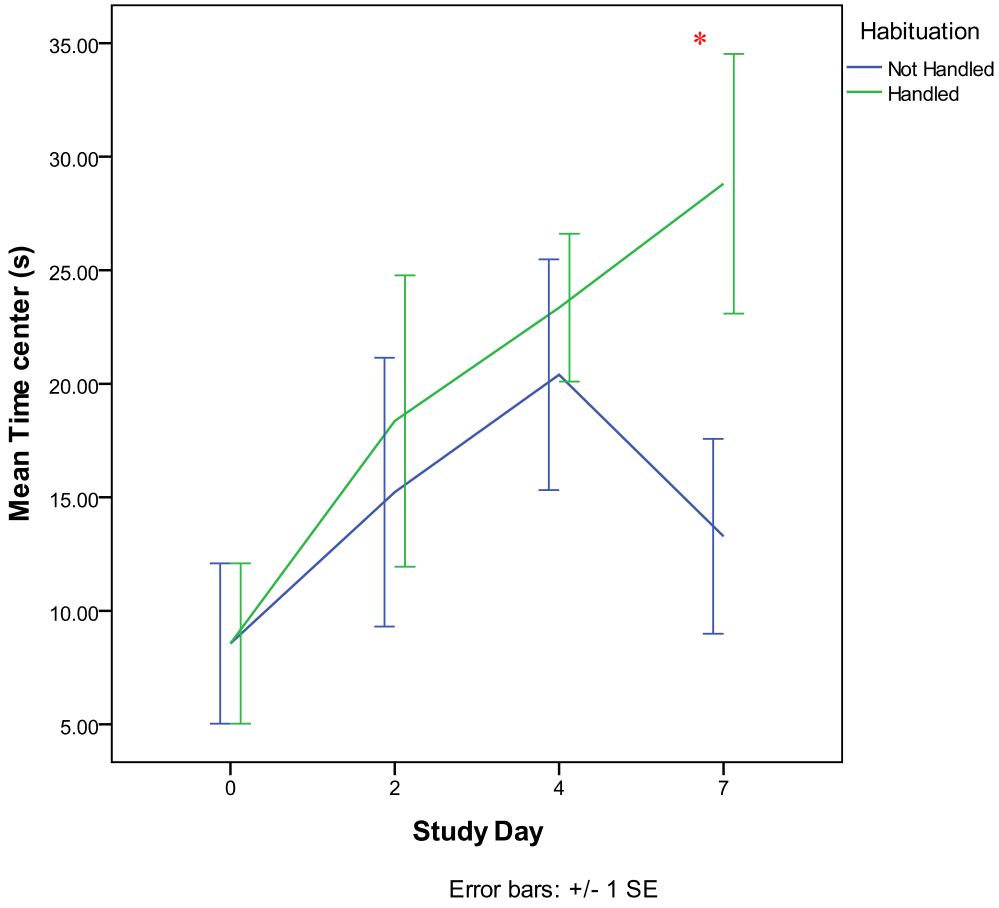


Figure 10. Number of contacts with the novel object in the open field arena by study day and habituation. There was a main effect of study day on the number of contacts with the novel object for handled rats. Rats upon arrival day 0 made fewer contacts with the novel object than rats on day 7.

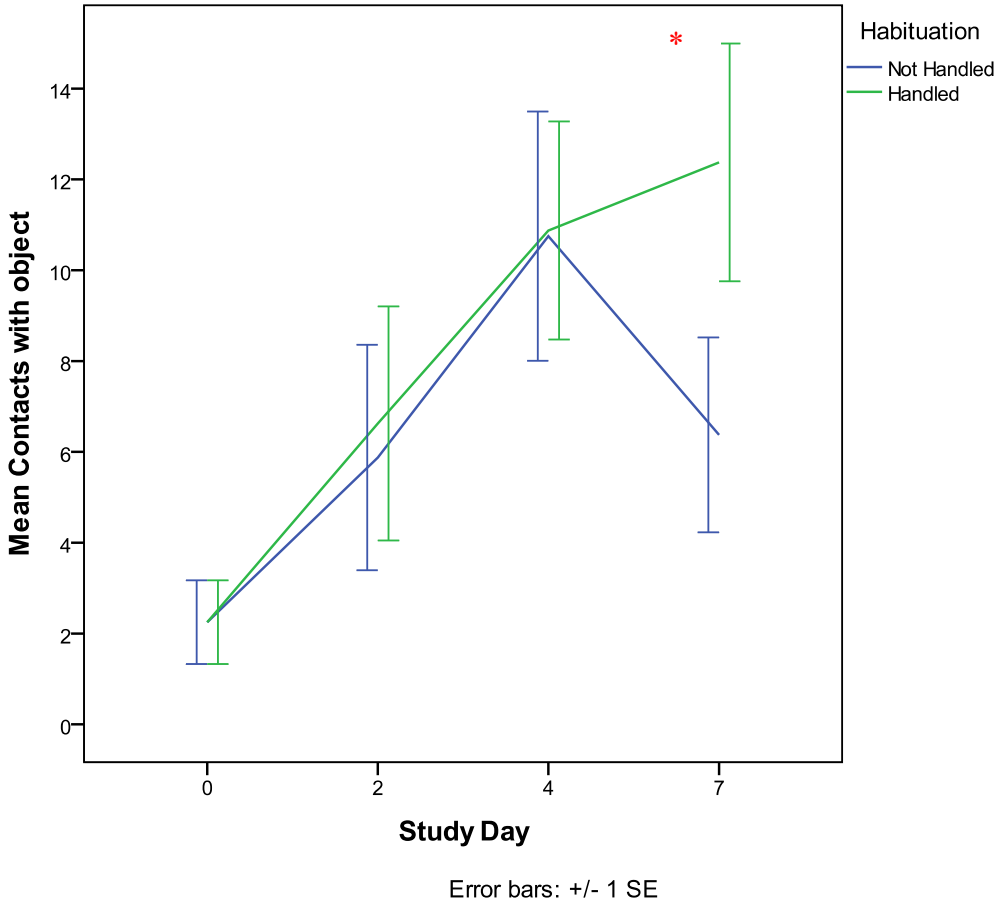


Figure 11. Entries out to the open arm of the elevated Y maze by study day and habituation. Entries to the open arm of the maze increased significantly with study day for the handled rats. Rats on day 0 had significantly fewer entries to the open arm than rats on days 4 and 7. There was a habituation effect for entries to the open arm; rats in the handled group had significantly more entries on days 4 and 7 compared to rats from the not handled group on the same days.

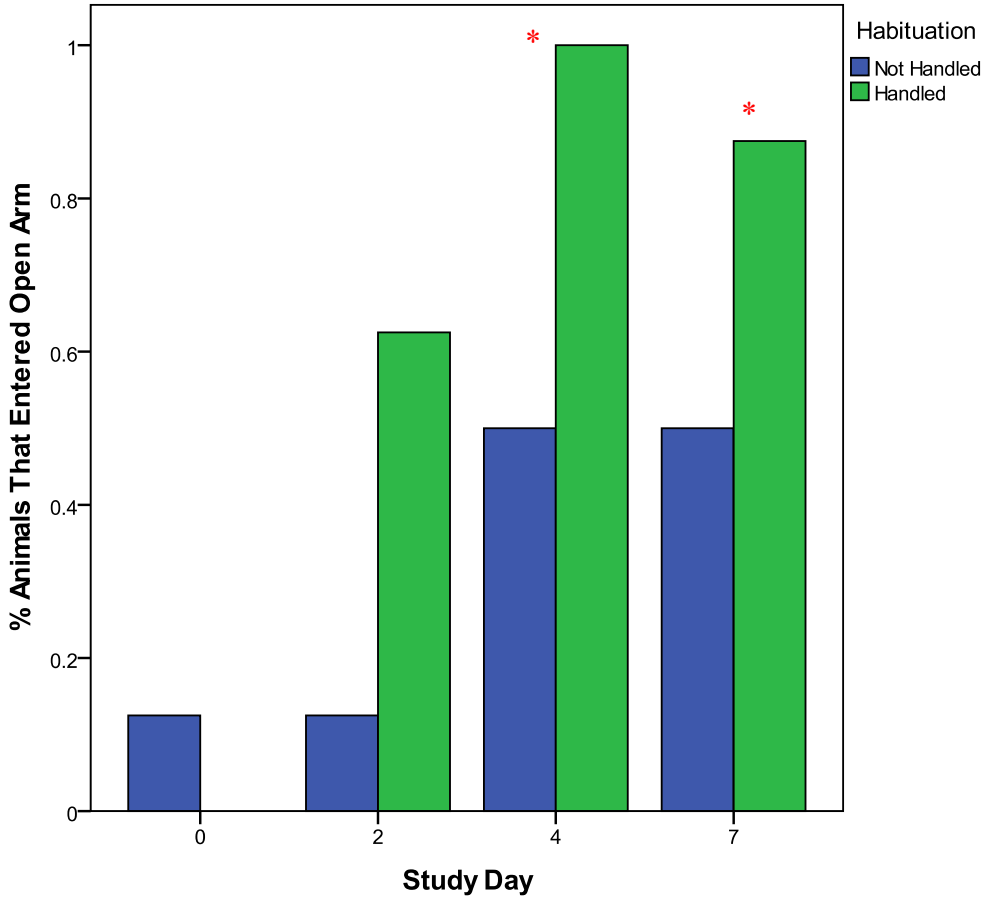
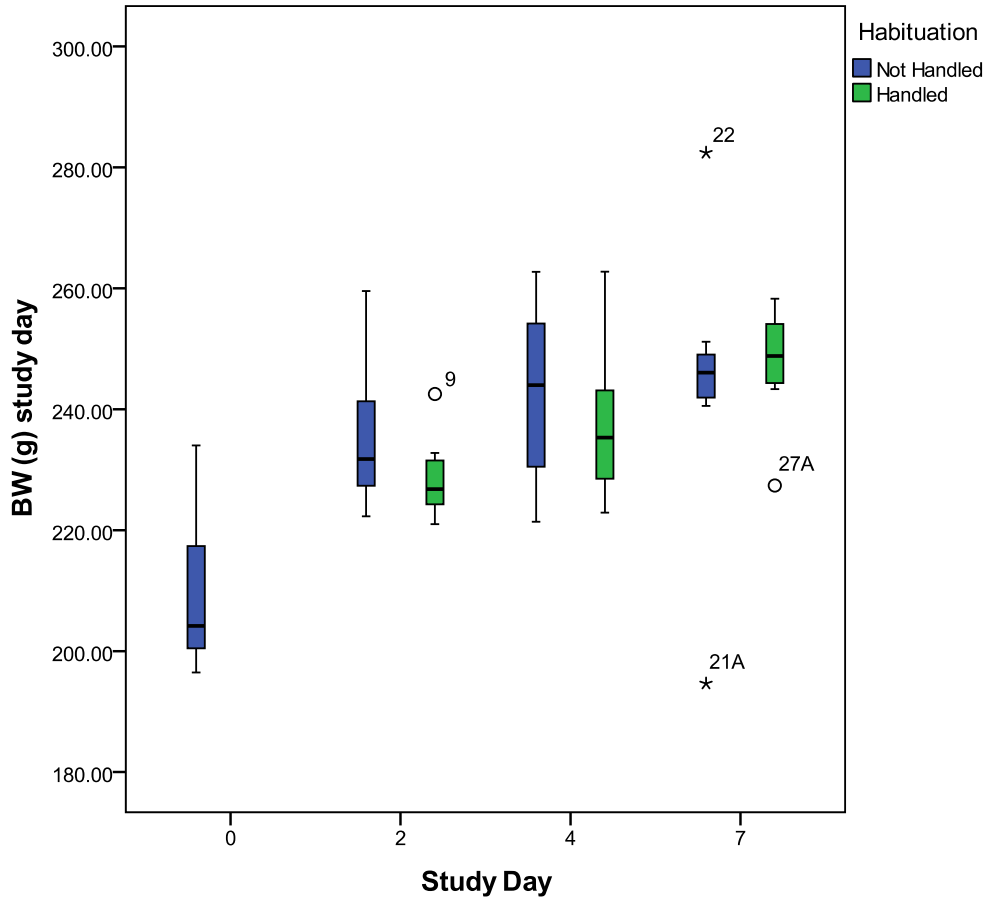
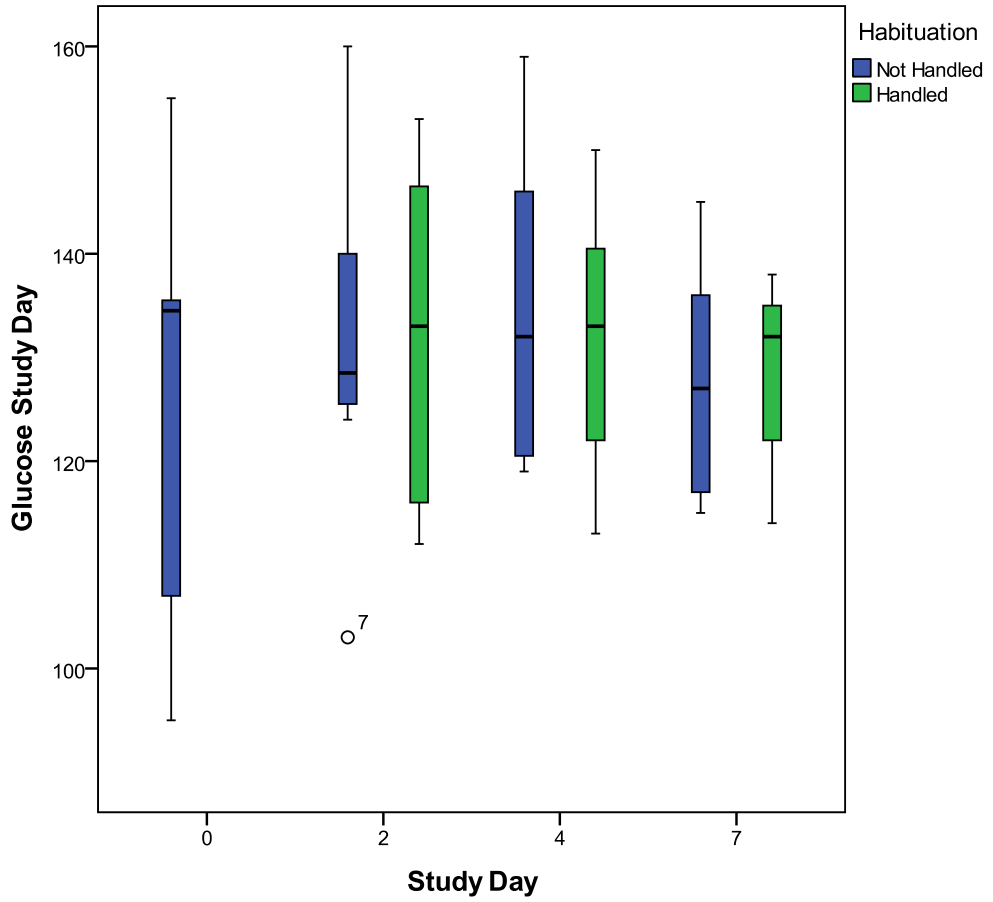


Figure 12. Box plot for body weight data by study day and habituation.



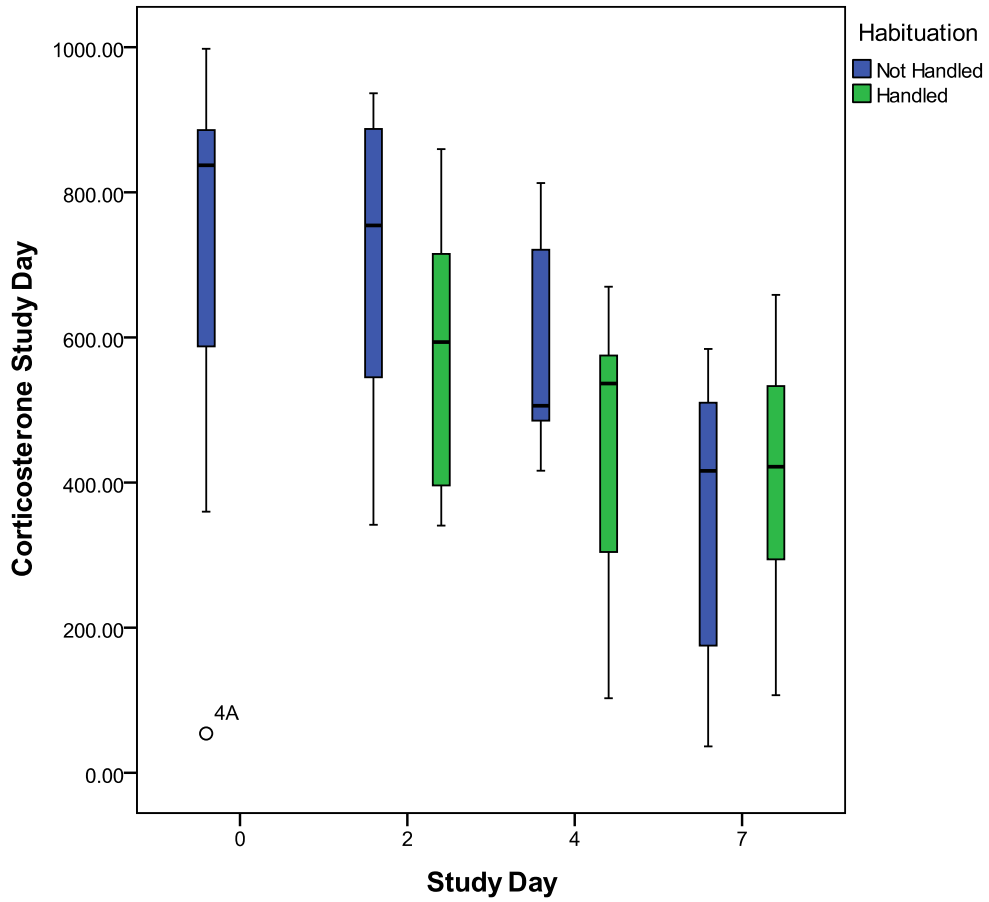
Note. 0 denotes individual outliers by rat identification number for Not handled cohort
* denotes individual outliers by rat identification number for Handled cohort

Figure 13. Box plot for glucose data by study day and habituation.



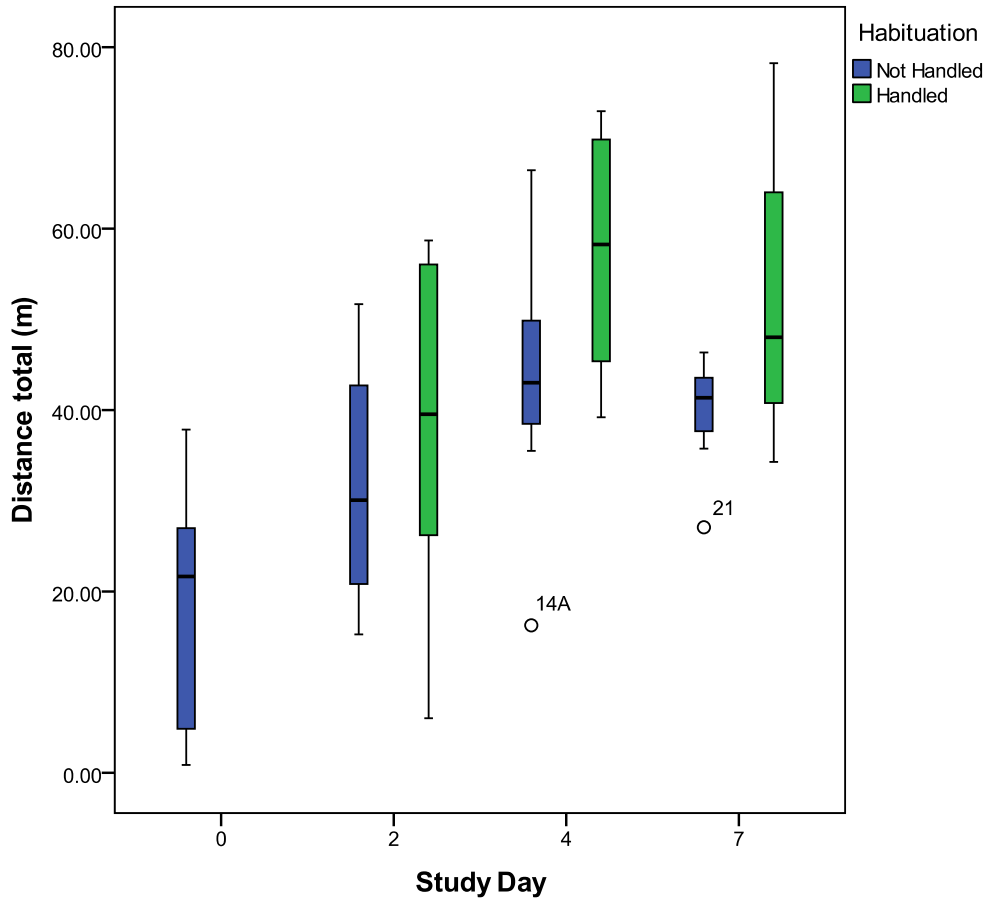
Note. 0 denotes individual outliers by rat identification number for Not handled cohort
* denotes individual outliers by rat identification number for Handled cohort

Figure 14. Box Plot for Corticosterone Data by Study Day and Habituation.



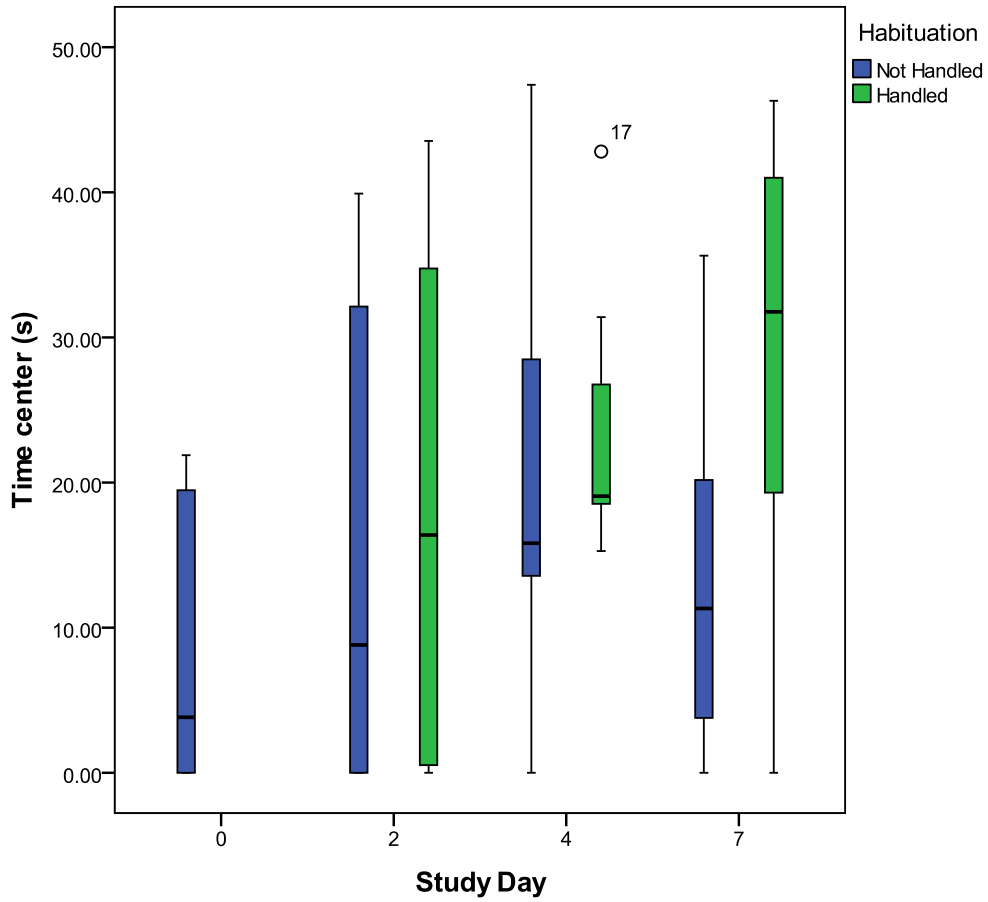
Note. 0 denotes individual outliers by rat identification number for Not handled cohort
* denotes individual outliers by rat identification number for Handled cohort

Figure 15. Box Plot for Distance Traveled in Open Field Arena by Study Day and Habituation



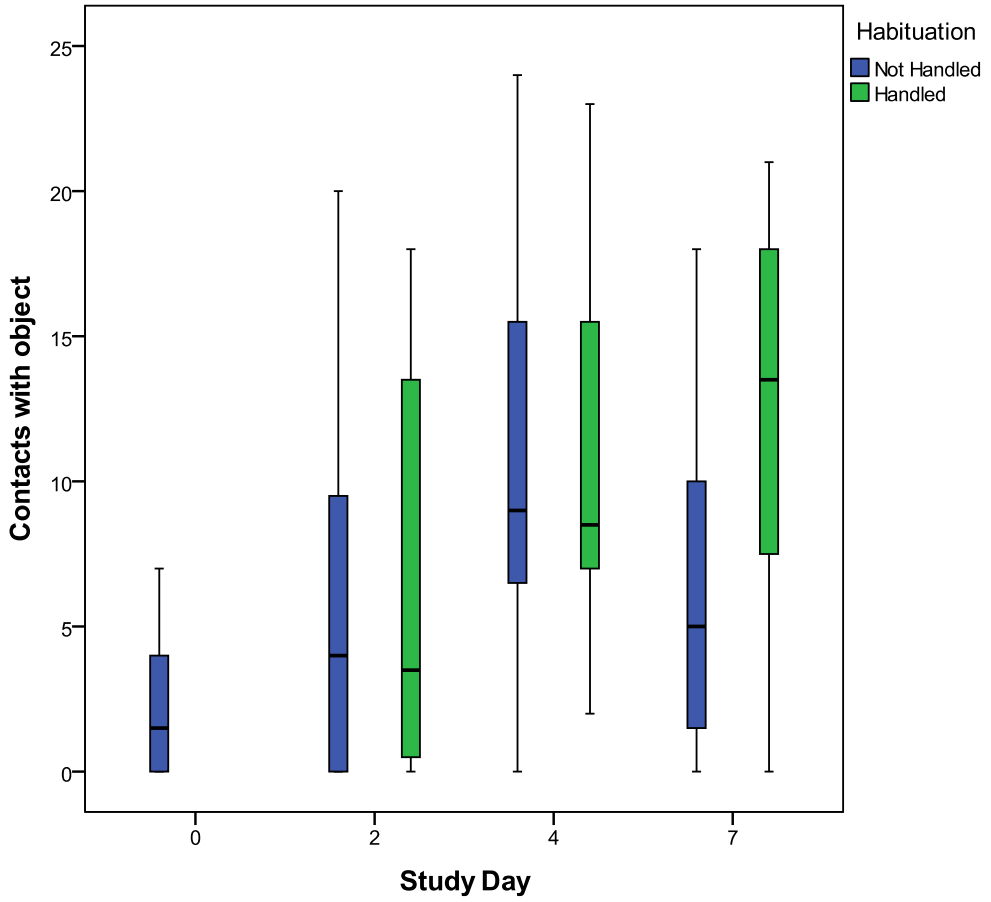
Note. 0 denotes individual outliers by rat identification number for Not handled cohort
* denotes individual outliers by rat identification number for Handled cohort

Figure 16. Box Plot for Time Spent in the Center of the Open Field Arena by Study Day and Habituation



Note. 0 denotes individual outliers by rat identification number for Not handled cohort
* denotes individual outliers by rat identification number for Handled cohort

Figure 17. Box Plot for the Number of Contacts with the Novel Object by Study Day and Habituation



Note. 0 denotes individual outliers by rat identification number for Not handled cohort
* denotes individual outliers by rat identification number for Handled cohort

References

Andrews JS (1996). Possible confounding influence of strain, age and Sex on cognitive performance in rats. *Cognitive Brain Research* **3,** 251-267.

Arnqvist G & Henriksson S. 1997. Sexual cannibalism in the fishing spider and a model for the evolution of sexual cannibalism based on genetic constraints. *Evolutionary Ecology* **11,** 255-273.

Atkinson H & Waddell B. (1997). Circadian variation in basal plasma corticosterone and Adrenocorticotropin in the rat: sexual dimorphism and changes across the estrous cycle. *Endocrinology* **138,** 3842-3848.

Beeton C, Garcia, A, & Chandy, G. (2007). Drawing blood from rats through the Saphenous vein and by cardiac puncture. *Journal of Visualized Experiments* **(7),** 266.

Beiko J, Lander R, Hampson E, Boon F, & Cain D. (2004). Contribution of sex differences in the acute stress response to sex differences in water maze performance in the rat. *Behavioural Brain Research* **151,** 239-253.

Bell A. (2007). Future directions in behavioural syndromes research. *Proceedings of the Royal Society of Biological Sciences* **274,** 755-61.

Bell A, & Stamps A. (2004). Development of behavioural differences between individuals and populations of sticklebacks, *gasterosteus aculeatus. Animal Behavior* **68,** 1339-1348.

Bertoglio L, & Carobrez A. (2002). Behavioral profile of rats submitted to session 1 session 2 in the elevated plus-maze during diurnal/nocturnal phases and under different illumination conditions. *Behavioural Brain Research* **132,** 135-143.

Bolivar V, Caldarone B, Reilly A, & Flaherty L. (2000). Habituation of activity in an

open field: A survey of inbred strains and F1 hybrids. *Behavior Genetics* **30(4),** 285-293.

Brown C, Burgess F, & Brathwaite V. (2007). Heritable and experiential effects on boldness in a tropical poeciliid. *Nature* **62,** 237-243.

Burchfield, S. (1979) The stress response: A new perspective. *Psychosomatic Medicine* **41, No. 8.**

Capdevila S, Giral M, Ruiz de la Torre JL, Russell RJ, & Kramer K. (2007) Acclimatization of rats after ground transportation to a new animal facility. *Laboratory Animals* **41**, 255-261.

Carere & Eens. (2005). Unraveling animal personalities: how and why individuals consistently differ. *Behaviour* **142,** 1149-1157.

Catala M, Pallardo F, Roman A, Villanueva P, & Vina Giner J. (1983). Effect of pinealectomy and circadian rhythm on avoidance behavior in the male rat. *Physiology & Behavior* **34,** 327-333.

Chrousos G & Gold P. (1992). The concepts of stress and stress system disorders. *Journal of the American Medical Association* **267 (9),** 1244-1247.

Coleman K & Wilson D. (1998). Shyness and boldness in pumpkinseed sunfish: Individual differences are context-specific. *Animal Behaviour* **56,** 927-936.

Cools A & Gingras M. (1997). Nijmegen high and low responders to novelty: a new tool in the search after the neurobiology of drug abuse liability. *Pharmacology Biochemistry and Behavior* **60 (1)** 151-159.

Damon Eg, Eidson AF, Hobbs CH & Hahn F. (1986). Effect of acclimation on nephrotoxic response of rats to uranium. *Laboratory Animal Science* **36,** 24-7.

Diaz-Veliz G, Alarcon T, Espinoza C, Dussaubat N, & Mora S. (1997). Ketanserin and anxiety levels: influence of Sex, estrous cycle, ovariectomy and ovarian hormones in female rats. *Pharmacology Biochemistry and Behavior* **58,** 637-642.

Dobrakovova M & Jurcovicova J. (1984). Corticosterone and prolactin responses to repeated handling and transfer of male rats. *Experimental Clinical Endocrinology* **5,** 21-27.

Dobrakovova M, Kvetnansky R, Oprsalova Z, & Jeova D. (1993). Specificity of the effect of repeated handling on sympathetic-adrenomedullary and pituitary-adrenocortical activity in rats. *Psychoneuroendocrinology* **18(3),** 163-174.

Drent P, van Oers K, van Noordwijk A. (2002). Realized heritability of personalities in the great tit (*Parus major*) *Proceedings: Biological Sciences* **270,** 45-51.

Erickson K, Drevets W, & Schulkin J. (2003). Glucocorticoid regulation of diverse cognitive functions in normal and pathological emotional states. *Neuroscience and Biobehavioral Reviews* **27,** 233-246.

Fernandes C, Gonzalez M, Wilson C & File S. (1999). Factor analysis shows that female rat behaviour is characterized primarily by activity, male rats are driven by sex and anxiety. *Pharmacology Biochemistry and Behavior* **64(4),** 731-738.

Figueiredo H, Dolgas C, & Herman J. (2002). Stress activation of cortex and Hippocampus is modulated by sex and stage of estrus. *Endocrinology* **143,** 2534-2540.

Figueiredo H, Ulrich-Lai Y, Choi D, & Herman J. (2006). Estrogen potentiates adrenocortical responses to stress in female rats. *American Journal of Physiology and Endocrinology and Metabolism* **292,** 1173-1182.

Frost A, Winrow-Giffen A, Ashley P, & Sneddon L. (2007). Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proceedings of The Royal Society of Biological Sciences* **274,** 333-339.

Gariepy J, Rodriguiz R, & Joens B. (2002). Handling genetic and housing effects on the mouse stress system, dopamine function and behavior. (2002). Journal *of Pharmacology, Biochemistry and Behavior* **73(1),** 7-17.

Gebhardt-Henrich, SG, Fischer, K, Hauzenberger, A, Keller, P, & Steiger, A. (2007) The duration of capture and restraint during anesthesia and euthanasia influences glucocorticoid levels in male golden hamsters. *LabAnimal **36,** **(4).***

Gosling S. (2001). From mice to men: what can we learn about personality from animal research? *Psychological Bulletin* **1,** 45-86.

Griebel G, Belzung C, Perrault G, & Sanger DJ. (2000). Differences in anxiety-related behavior and sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology* **148,** 164-170.

Griffin A & Whitacre C. (1991). Sex and Strain differences in the circadian rhythm fluctuation of endocrine and immune function in the rat: implications for rodent models of autoimmune disease. *Journal of Neuroimmunology* **35,** 53-64.

Groothuis T & Carere G. (2005). Avian personalities: characterization and epigenesis. *Neuroscience & Biobehavioral Reviews* **29,** 137-150.

Hall J & Gold G. (1986). The effects of training, epinephrine, and glucose injections on plasma glucose levels in rats. *Behavioral and Neural Biology* **46,** 156-167.

Harries, A, D'Eath R, & Healy S. (2009). Environmental enrichment enhances spatial cognition in rats by reducing thigmotaxis (wall hugging) during testing. *Animal*

Behaviour **77,** 1459-1464.

Hessing M, Hagelso A, Schouten W, Wiepkema P, & van Beek J. (1994). Individual behavioral and physiological strategies in pigs. *Physiology and Behavior* **55,** 39-46.

Imhof J, Coelho Z, Schmitt M, Morate G, & Carobrez P. (1993). Influence of Sex and age on performance of rats in the elevated plus maze apparatus. *Behavioural Brain Research* **56,** 177-180.

Jones N, & King S. Influence of circadian phase and test illumination on pre-clinical models of anxiety. (2001). Physiology *and Behavior* **72,** 99-106.

Kelliher P, Connor T, Harkin A, Sanchez C, Kelly J, & Leonard B. (2000). Varying responses to the rat forced-swim test under diurnal and nocturnal conditions. *Physiology & Behavior* **69,** 531-539.

Kirschbaum C, Wust S, & Hellhammer D. (1992). Consistent sex differences in cortisol responses to psychological stress. *Psychosomatic Medicine* **54,** 648-657.

Kirschbaum C, Kudielka B, Gaab J, Schommer N, & Hellhammer D. (1999). Impact of Sex, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosomatic Medicine* **61,** 154-162.

Koolhaas J, de Boer S, Buwalda B, van der Vegt B, Carere C, & Groothuis A. (2001). How and why coping systems vary among individuals. *Welfare in Animals including Humans* 197-209.

Korte M & Boer S. (2003). A robust animal model of state anxiety: fear potentiated behaviour in the elevated plus maze. *European Journal of Pharmacology* **463,** 163-175.

Leppanen P, Ravaja N, & Ewalds-Kvist S. (2006). Twenty-three generations of mice

bidirectionally selected for open-field thigmotaxis: selection response and repeated exposure to the open field. (2006) *Behavioral processes* **72(1),** 23-31.

Lyons L, Rawashdeh O, Katzoff A, Susswein A, & Eskin A. (2005). Circadian modulation of complex learning in diurnal and nocturnal *Aplysia*. *PNAS* **35,** 12589-12594.

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

Meerlo P, Sgoifo A, De Boer SF, & Koolhas JM. (1999) Long lasting consequences of social conflict in rats: behavior during the interaction predicts subsequent changes in daily rhythms of heart rate, temperature, and activity. *Behavioral Neuroscience* **113(6)** 1283-1290.

Mostle E & Palme R. 2002). Hormones as indicators of stress. *Domestic Animal Endocrinology* **23,** 67-74.

Nomikos G & Spyraki C. (1988). Influence of oestrogen on spontaneous and diazepam-induced exploration of rats in an elevated plus maze *Neuropharmacology* **27,** 691-696.

Obernier J & Baldwin R. (2006) Establishing an Appropriate Period of Acclimatization following Transportation of Laboratory Animals. *ILAR Journal* **47 (4)** 364-369**.**

Palanza P, Gioiosa L, & Parmigiani S. (2001). Social stress in mice: Sex differences and effects of estrous cycle and social dominance. *Physiology and Behavior* **73(3)** 411-420.

Pare W & Redei E. (1993). Sex Differences and Stress Response of WKY Rats. *Physiolog and Behavior* **54,** 1179-1183.

Pellow S, Philippe C, File S, & Briley M. (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods* **14,** 149-167.

Reale D, Gallant B, Leblanc M & Festa-Bianchet M. (2000). Consistency of temperament in bighorn ewes and correlates with behaviour and life history. *Animal Behaviour* **60,** 589-597.

Reboucas R & Schmidek W. (1997). Handling and isolation in three strains of rats affect open field, exploration, hoarding and predation. *Physiology and Behavior* **62(5),** 1159-64.

Reinhardt V & Reinhardt A. (2006). Variables, refinement and environmental enrichment for rodents and rabbits kept in research institutions: making life easier for animals in laboratories. Animal Welfare Institute.

Romero MH. (2005). Physiological stress in ecology: lessons from biomedical research. *TRENDS in Ecology and Evolution* **19(5),** 249-255.

Sachs BD. (1981). Is stress due to shipment of animals a confounding variable in developmental research? *Developmental Psychobiology* **14**(**2),** 169-171.

Schmitt U & Hiemke C. (1998). Strain Differences in open-field and elevated plus maze behavior or rats without and with pretest handling. *Pharmacology Biochemistry and Behavior* **59(4)** *807-811.*

Shim SB, Lee SH, Kim CK, Kim BG, Kim YK, Jee WS, Lee SH, Sin JS, Bae CJ, Lee BC, Jang MK, Cho JS, Chae KR, & Hwang, DY. (2008). The effects of long-duration, low temperature ground transportation on physiological and biochemical indicators of stress in mice. *LabAnimal* **37 (3).**

Sih A, Kats L, Maurer E. (2003). Behavioural correlations across situations and the evolution of antipredator behaviour in a sunfish-salamander system. *Animal Behavior* **65,** 29-44.

Sih A, Bell A & Johnson C. (2004a). Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution* **19 (7), 372**-378.

Sih A, Bell A, Johnson C, Ziemba R. (2004b). Behavioral syndromes: an integrative overview. *The Quarterly Review of Biology* **79 (3),** 241-277.

Stansfield K & Kirstein C. (2005). Effects of novelty on behavior in adolescent and adult rat. *Developmental Psychobiology* **48,** 10-15.

Stemkens-Sevens S, van Berkel K, Greeuw I, Snoeijer B, & Kramer K. (2009). The Use of radiotelemetry to assess the time needed to acclimatize guinea pigs following several hours of ground transport. *Laboratory Animals* **43,** 78-84.

Sutcliffe J, Marshall K, & Neill J. (2006). Influence of Sex on working and spatial memory in the novel object recognition task in the rat. *Behavioural Brain Research* **177,** 117-125.

Swallow J, Anderson D, Buckwell A, Harris T, Hawkins P, Kirkwood J, Lomas M, Meacham S, Peters A, Prescott M, Owen S, Quest R, Sutcliffe R, & Thomson K. (2005). *Guidance on the transport of laboratory animals*. Report of the transport working group established by the laboratory animal science association (LASA). *Laboratory Animals* **39,** 1-39.

Todorovic C, Dimitriejevic M, & Stanojevic S. (2003). Correlation between age-related changes in open field behavior and plaque forming cell response in DA female rats. *International Journal of Neuroscience* **113(9),** 1259-1273.

Treit D, Menard J, & Royan C. (1993). Anxiogenic stimuli in the elevated plus maze. *Pharmacology, Biochemistry and Behavior* **44(2),** 463-469.

Trullas R & Skolnick P. (1993). Differences in fear motivated behaviours among inbred mouse strains. *Psychopharmacology* **111**, 323-331.

Tuli JS, Smith JA, & Morton DB. (1995). Stress measurements in mice after transportation. *Laboratory Animals* **29**, 132-138.

United States Code. (2002). Animal Welfare Act and Animal Welfare Regulations. Official US. Code, 7 U.S.C. Sections 2131-2159.

Vallee M, Mayo W, Dellu F, Moal M, Simon H, & Maccari S. (1997). Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring correlation with stress-induced corticosterone secretion. *Society for Neuroscience* **17 (7),** 2626-2636.

Verbeek M, Boon A, and Drent P. (1996). Aggressive behaviour and dominance in pair-wise confrontations of juvenile male great tits. *Behaviour* **133,** 945-963,

van Ruiven R, Meijer, GW, van Zutphen LFM, & Ritskes-Hoitinga, J. (1996). Adaptation period of laboratory animals after transport: a review. *Scandinavian Journal of laboratory Animal Science 23***,** 185-190.

van Ruiven R, Meijer GW, Wiersma A, Baumans V, van Zutphen LFM, & Ritskes-Hoitinga J. (1998). The influence of transportation stress on selected nutritional parameters to establish the necessary minimum period for adaptation in rat feeding studies. *Laboratory Animals* **32**, 446-456.

Viau V. (2002). Functional cross-talk between the hypothalamic-pituitary-gonadal and adrenal axes. *Journal of Endocrinology* **14,** 506-513.

Viau V & Meaney M. (1996). The inhibitory effect of testosterone on hypothalamic-pituitary-adrenal responses to stress is mediated by the medial preoptic area. *The Journal of Neuroscience* **16(5),** 1866-1876.

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

Walsh R & Cummin R. (1976). The open-field test: A critical review. *Psychological Bulletin* **83(3),** 482-504.

Wiepkema P & Koolhaas J. (1993). Stress and animal welfare. *Animal Welfare* **2,** 195-218.

Wilson A & Stevens E. (2005). Consistency in context-specific measures of shyness and boldness in the rainbow trout, *Oncorhynchus mykiss. Ethology* **111,** 849-862.