Meta-Analysis of Rodent Behavior in Various Brain Injury and Disease Models

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A Meta-Analysis of Rodent Behavior in Various Brain Injury and Disease Models

by

Earl C. Thorndyke III

A Thesis submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy in Clinical Psychology

September 2013
Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree Doctor of Philosophy.

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I would like to express sincere gratitude to my committee members, whose invaluable guidance permitted this project to come to fruition. Thank you for your unflagging patience and all of the knowledge and skill you have given me. I also owe a debt of thanks to all of the researchers who carefully gathered the data used here over the course of more than a decade. I would finally like to thank my family and friends, who have given me their unwavering support and kind words through long years of graduate education.
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ABBREVIATIONS

BrdU  Bromodeoxyuridine
CCI  Controlled Cortical Impact
TBI  Traumatic Brain Injury
ABSTRACT OF THE THESIS

A Meta-Analysis of Rodent Behavior in Various Brain Injury and Disease Models

by

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Master of Arts, Graduate Program in Psychology
Loma Linda University, September 2013
Dr. Richard E. Hartman, Ph.D., Chairperson

The objective of this study was to identify patterns and profiles present in rodent behavioral assay data to inform methodology of future animal research. Domains of spatial learning, motor function, activity, and emotionality were examined. This study first identified how rodent performance on commonly used behavioral and neurocognitive assays varies by species, sex, and age. Significant differences were observed in spatial learning curves, patterns of motor function, emotionality and activity in rats and mice. Overall, males and females had similar performances on spatial learning and motor function, but sex and species interactions were also observed, indicating varied sex differences in rats and mice. This study also identified neurocognitive profiles specific to rodent models of cortical and subcortical brain injury that grossly resemble deficits observed in human brain injury. This study also used exploratory factor analysis to examine the latent factor structure present in behavioral assay data. The factor structure indicated the prominent contribution of strategic learning and activity level to performance across behavioral domains, explaining more than 50 percent of total variance. The potential for use of this information to design an abbreviated neurocognitive battery was discussed.
CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Introduction

Despite the fact that approximately 95% of the estimated 25 million lab animals used annually in the U.S. are rats or mice (Humane Society, 2009; USDA, 2010), very few studies have focused specifically on evaluating behavioral testing procedures for these animals, although some studies have focused on specific performance differences between species (Wishaw & Tomie, 1996), sex (Jonasson, 2005), or age. Such studies have so far been relatively limited in focus and sample size, generally evaluating a single domain of performance on a single task between two demographic variables. Considering the high rate of rodent use in critical biomedical research (Foundation for Biomedical Research, 2010), a study using a relatively large sample of rodent behavioral data could provide insight into performance differences across rodent species, sex, and age, as well as provide information about intercorrelated behavioral test performance. If animal test performance is attributable to one or two performance factors, a more streamlined and cost effective protocol for neurocognitive assessment of rodents may be possible.

Differences Between Rat and Mouse Performance

Many procedures for behavioral testing in rodents are utilized without modification for both rats and mice alike, yet it is not entirely clear that both species are equally suited to these tasks. Significant differences in task performance and trends in performance could indicate that one species provides a better model for translation to humans.
A small body of research has indicated that rats may provide a significantly better model of human behavior than mice, despite their increased cost for experimentation. A review by Wishaw, Metz, Kolb, and Pellis (2001) concluded that mice have a simpler behavioral repertoire when compared to rats. Mice were found to have more reflexive behaviors, possibly because faster brain maturation renders them less dependent on developing complex social behaviors.

Rats have been found to recruit more new dentate gyrus cells than mice in a fear-conditioning task, evidencing significantly larger numbers of new hippocampal cells one week after conditioning, which were identified by BrdU, a synthetic analogue for thymadine that is incorporated into the DNA of cells created after BrdU administration. All new cells, including neurons, contain BrdU after administration and can be marked and visualized with immunohistochemistry. These rats were also found to have more dense hippocampi than mice and demonstrated learning behaviors significantly faster than mice (Snyder et al, 2009). These findings indicate that rats acquire knowledge at a higher rate than mice, meaning that they may more closely resemble how humans learn.

Conversely, however, mice have more synapses per neuron in all layers of the cortex than rats, although the number is still less than the number of synapses evident in humans (DeFelipe, 2011).

Rats also demonstrate greater flexibility in spatial learning across testing paradigms, including the Morris Water Maze and the Radial Arm Maze. The Morris Water Maze is the gold-standard test of spatial learning and memory in rats and mice (Hartman et al, 2001). This test consists of a platform placed at various locations within a
large round tank of opacified water that contrasts with the color of the animal’s fur (see Figure 1).

*Figure 1.* Morris Water Maze apparatus with mouse completing cued trial (on platform).

The animal is placed in the water near the periphery of the tank and is intrinsically motivated to find a way to escape the water because immersion in water is aversive. Initially the platform is visible above the water, allowing the animal to visually locate the platform. In later trials, the platform is slightly submerged, requiring the animal to rely upon spatial cues to locate the platform. The animal’s swim is tracked by overhead
camera, and the Ethovision computer program records the swim path making swim time, distance, and strategy data available for analysis. This test typically includes one day of cued trials (platform visible) and three days of spatial trials (submerged platform), which provides data regarding learning and working memory (Hartman et al, 2001).

During the cued trials, five blocks of two trials each are conducted, with varying release points around the periphery of the tank. Each block consists of one release point near the platform and one far from the platform. Each trial lasts a maximum of 60 seconds or until the animal locates the platform, whichever comes first. If the animal fails to locate the platform within 60 seconds, it is placed on the platform to encourage spatial learning. The animal is removed from the tank and relevant spatial cues between trials. During the spatial trials, five blocks of two trials each are conducted on three consecutive days. The procedure resembles the cued task on each of those days, except that the platform is slightly submerged and is not visible to the animal, and the platform changes position on each of the days of the spatial trials.

The Radial Arm Maze is another protocol used to measure spatial learning and memory in rodents under dry conditions. It consists of eight arms of uniform length radiating from a central platform (see Figure 2). Food is located at the end of each arm, but is recessed so as not be visible from the central platform. An animal, once placed on the central platform, is free to move about the apparatus and retrieve food from each arm. After entering each arm, the animal must return to the central area before choosing another arm. The ratio of novel arm entries to repeated arm entries is measured and is an indicator of spatial learning. Unlike the water maze, which motivates escape by
application of an aversive stimulus, interaction with the radial arm maze relies upon appetitive motivation on the part of the animal.

Figure 2. A typical radial arm maze with eight arms. Photo, by Mcole13, 2008, used under GNU Free Documentation License: http://creativecommons.org/licenses/by-sa/3.0/deed.en.

In a comparison of spatial learning on a dry land task, the Radial Arm Maze, mice and rats demonstrated learning at a similar rate. When the Morris Water Maze was used to assess spatial learning, rats significantly outperformed mice, whose performance was impaired (Wishaw & Tomie, 1996). Moreover, the rats also demonstrated improved problem solving strategies over time relative to mice, indicating that they were learning to learn, while mice showed no such problem solving strategy improvement. In comparing rat and mouse performance on the water maze task, swim speed was use as a covariate due to the fact that rats’ larger size and superior swim speed may permit them to locate the platform more quickly, even without spatial memory for the platform.
location. Even though strain differences have been observed in both rats (e.g., Linder & Schallert, 1988) and mice (e.g. Royle, Collins, Rupniak, Barnes, & Anderson, 1999) due to varying swim speed and visual acuity, their ability to conduct an organized search with high accuracy indicates rats are better problem solvers than mice in the water. Rats, like humans, can learn complex behavior flexibly across varying situational demands (Wishaw, 2001).

The rotarod test of rodent motor function has been repeatedly demonstrated to be a sensitive measure of change in motor performance in both rats and mice; analyses of fall latency (time to fall off the rotating cylinder) have demonstrated performance deficits at both mild and moderate levels of brain injury, as well as significantly different performances between injury levels (Hamm, Pike, O’Dell, Lyeth, & Jenkins, 1994; Cendelin, Korelusova, & Voseh, 2008). The rotarod test consists of a rotating cylinder placed approximately one foot above a tray. The animal is required to walk forward to stay on this cylinder as it rotates in order to avoid falling (see Figure 3).

This test measures coordination and balance across three conditions of two trials each: steady rotation (5 rpm), accelerating (5 rpm initial speed accelerating by 5 rpm every five seconds) and faster accelerating rotation (5 rpm initial speed, accelerating by 5 rpm every 3 seconds). Fall latency is recorded, with trial duration of one minute maximum for the first condition and 120 seconds for conditions two and three.

A direct comparison of rat and mouse performance on a rotarod protocol is not yet available: however, some data suggest that learned strategic motor behavior plays a role in rotarod performance for rats while physical conditioning does not. In a study by Buitrago, Schulz, Dichgans, and Luft (2004), rats that trained on a running wheel in
addition to repeated exposure to the rotarod task did not demonstrate improved performance compared to animals that were exposed to the rotarod alone. The rats made intra-session improvements that had increased carryover to following session across multiple task exposures, indicating that the rats were learning a strategy for improved task performance rather than gaining increased physical fitness.

*Figure 3.* Rotarod apparatus with four lanes and laser fall sensors.

Additionally, rats have demonstrated a greater ability to learn complex and coordinated asymmetrical motor behavior than mice in a test of reaching behavior and environmental manipulation (Wishaw, 1986). As a result of rats' aforementioned complex behavioral repertoire and ability to rapidly learn motoric strategies, rats may
also evidence higher rotarod performance when directly compared to mice, and could, therefore, create a more sensitive model for human motor learning in which both strategic motor behavior and gross motor ability affect task performance, as would be the case in humans.

The elevated zero-maze test is a popular tool for measuring anxiety-like behavior in rodents, where time spent in the sheltered dark zones of the apparatus is considered an indicator anxiety-like behavior. The apparatus for this test is a raised circular ring about three inches wide. One half of this ring is enclosed, providing a “safe” environment. The other half is open and under bright lights, representing a more “threatening” environment. The proportion of time spent in the enclosed portion of the apparatus is recorded (see Figure 4).

Figure 4. Typical zero-maze apparatus with illumination.
For rodents, which are inclined to seek shelter in small, enclosed hiding places, the dark, enclosed zones of the apparatus represent a safe, hidden environment for the animal while the brightly lit open sections represent exposure and are anxiogenic. Part of the popularity of the zero-maze is due to the fact that it seems to be resistant to test order effects in a behavioral test battery (Blokland et al, 2012). Unlike other dark-preference tests of emotionality, such as the radial arm maze, the zero-maze has no enclosed dead ends and requires the animal to cross a lit part of the field to enter another enclosed area (Shepherd, 1994). It has repeatedly been shown to be a sensitive measure of anxiety-like behavior in both mice (Bailey & Crawley, 2009) and rats (Shepherd, 1994), where performance can be significantly manipulated with anxiolytic agents of anxiety inducing environmental factors such as bright light and loud noises (Cryan & Sweeney, 2011). Despite its frequent use without modification in mice and rats both, no direct comparison of rat and mouse performance on the zero-maze test is currently available.

Analysis of repeated exposure to the zero-maze task in mice indicates that mice have an increasing tendency to prefer the dark zones across three trials with increasing latency to enter lit zones (Cook, 2002). Because dark preference and proportion of time spent there is considered an anxiety analogue, these results would seem to indicate that repeated exposures produce increased negative emotionality in mice, with more anxiety-like behavior as the task becomes more familiar. In humans, however, repeated exposure to a novel ambiguous stimulus leads to reduced perceptions of anxiety (Lester, Field, & Meris, 2011), a process that may be more accurately modeled by rat behavior.
The open-field activity test examines spontaneous movement over 30 minutes in an open box with high sides (see Figure 5). This test is also recorded via overhead video and provides data about distance travelled, time spent moving, velocity, and time spent in the center of the field and at the periphery of the field (Hartman et al, 2001).

Figure 5. Overhead image of rats performing open field activity, boxes arranged for four animals. The box in the upper right shows the animal’s 30-minute path.

In a study of rat behavior in an open field, where rats were provided access to a light/dark field from their home cage after an initial involuntary exposure to the field, their voluntary exploration of the field significantly increased across subsequent days (Aulich, 1976). These results concerning changes in behavior across novel environment exposure suggest that rodent species differentially retain and apply information, gathered from the initial exposure onward, to determine behavior on subsequent task exposures. This means that even behavior on a test of supposed emotionality, when repeated, is at
least in part a learned behavior. No direct comparison of rat and mouse data is available; future study is needed to identify species deifferences.

**Performance Differences between Male and Female Animals**

The existence of rodent sex performance differences on behavioral tests and their magnitude should they exist at all, remains unclear as the issue has been somewhat contentious in the literature. Although comparisons of performance between male and female animals are often conducted directly, they are also often conducted by manipulation of animal sex characteristics such as castration of male animals (Slob, Huizer & Van der Werff Ten Bosch, 1986) or administration of testosterone to females (Roof, 1993). Such experimental paradigms have proven successful in manipulating differential performance experimentally, yet nevertheless results remain mixed overall. In humans, males have often demonstrated relatively consistent advantages in spatial tasks (Voyer, Voyer, & Bryden, 1995). This pattern has also been noted in animals, although somewhat less consistently. Animal behavior data that reflect such a trend will better model human spatial abilities.

In an examination of male and female mouse performance on the Morris Water Maze, Ramsey and Pittenger (2010) observed no significant sex differences; however other studies have found sex differences on both the water maze and Radial Arm Maze (Roof, 1993). A meta-analysis of rodent sex differences by Jonasson (2005) concluded that overall there was evidence for spatial sex differences despite the fact that the majority of reviewed studies retained the null hypothesis of no difference. A reported caveat was that sex differences varied widely between rat and mouse strains and across
animal ages. Nevertheless, the nature and magnitude of observed sex differences remains inconsistent and unclear.

**Latent Factor Structure and Battery Composition**

Exploratory and confirmatory factor analytic studies have been performed on different aspects of animal behavior using single behavioral tests. Such analyses of animal emotionality have identified multiple factors present in data from open field and elevated plus maze, including decision making, territory marking, and fear responses, and autonomic balance (Royce, 1977; Wall & Messier, 2001). Factor analytic studies of other domains of behavior have not yet been conducted. Furthermore, no single study has yet explored the existence of latent factors that might underlie performance across behavioral domains. Some data do suggest that subjection to a comprehensive behavioral battery may affect performance on individual assays, and that individual tests performed on naïve animals possess greater sensitivity and therefore greater ethological validity.

Animals that are chronic test subjects seem to change their behavior, assuming a decreased inclination to escape immersion in water by locating the water maze platform (McIlwain, Merriweather, Yuva-Paylor, & Paylor, 2001). This behavioral shift indicates that rodents repeatedly exposed to testing as part of a behavioral battery are by virtue of their participation less suitable and sensitive models of changes in spatial learning ability. Similar findings have been reported for participation in tests of rodent emotionality, where repeatedly tested animals demonstrated reduced indicators of fear-related behaviors and also reduced exploratory behaviors (Voikar, Vasar, & Rauvala, 2004).

Such findings indicate that, in addition to consuming significant time and
personnel resources, batteries of behavioral assays may reduce external validity and sensitivity. It is therefore ideal to generate a comprehensive yet parsimonious means of behavioral testing that will minimally alter rodent behavior by virtue of participation in the battery. To achieve this goal, an exploratory factor analytic study of rodent behavioral data should be conducted that redundant measures and procedures might be deleted on the basis of their high correlation or communality with other behavioral measures.

**Age-Related Differences in Behavioral Assay Performance**

Age-related changes in behavioral assay performance have been observed in rats (Kadar, Silbermann, Brandeis, & Levy, 1990) and mice (Lambert & Gower, 1992). Overall such age-related changes have been reported only as incidental findings in the execution of other experimental aims, or have been limited to only one or two behavior domains. Few studies have examined changes across domains of behavior in either a cross-sectional or longitudinal design, with two exceptions by Lambert and Gower.

In mice, age-related decline has been reported across behavioral domains. An early study by Lamberty and Gower (1990) found that mice demonstrated decreased ability to learn on the Morris water maze on a cross-sectional comparison of three, six, nine, and 12 month-old mice. Although escape latencies were not noted to increase significantly between groups, path length was significantly greater for the 12 month-old group relative to the others. Significantly decreased open field activity was observed in 12-month old mice relative to younger age groups, leading to the conclusion that significant age-related changes could be observed between young adult mice and old
mice, and that these changes initially occurred sometime between nine and 12 months of age.

A follow-up study observed the nature of behavioral changes in 12, 17, and 22 month-old animals (Lamberty & Gower, 1992). This study found further reduced levels of function in each successively older group. Specifically motor abilities significantly decreased and spatial learning became relatively impaired from middle to old age, although significant changes in swim speed were absent.

Hartman, Lekic, Rojas, Tang, and Zhang (2009) observed similar age-related trends in mice with decreased time spent moving in the open field test between young adult controls and older adult controls, although the level of change was only approaching significance. Similarly Hartman et al (2005) found trends toward increased path length across water maze blocks in mice with preservation of swim speed.

Studies of rat brains have provided insight into some of the possible etiological factors underlying changes. In comparisons 35 day old and 18 month old rats, far fewer new BrdU marked cells were observed in the dentate gyri of the older animals compared to the younger group, though new cells were evident in both groups (Seki & Arai, 1995). Decreased numbers of BdrU marked cells were observed in the hippocampi of rats, in the context of exposure to the radial arm maze, where older rats demonstrated impaired learning curves relative to their youthful counterparts and had smaller hippocampi (Kadar, Silbermann, Brandeis, & Levy, 1990). Another study of rat motor neurons found that older animal’s motor neurons, beyond being reduced in overall number, had smaller soma, fewer dendritic connections, and less myelination, possibly indicating decreased sensorimotor function (Hashizume, Kanda, & Burke, 1988). As of the present time, no
study has thoroughly investigated a wide range of neurobehavioral differences in old mice; further investigation is needed to identify to what extent older and adult mice differ on behavioral assays.

**Behavioral Assay Profiles in Brain Injury Models**

Rodents are commonly subjects in a variety of experimental models of brain injury. Some of these models include procedures to replicate traumatic brain injury, commonly through direct impact to the cortex (e.g. Ajao et al, 2012; Bertolizio, 2011) and more subcortical injuries, such as hemorrhagic and ischemic stroke (Lekic et al, 2012). These various rodent models, which are typically designed to replicate single, or at least simplified, pathobiological processes in brain injury, often produce behavioral profiles with similarly impaired learning, motor function, and decreased activity and exploration (Cernak, 2005). Because these models typically generate broadly similar impairments in rodents and somewhat belie the complexity of brain injury processes, the precise extent to which cortical and subcortical injury models produce reliably distinct neurobehavioral profiles is unclear.

Ajao et al (2012) investigated the behavioral sequelae of a single, controlled impact of a rounded metal rod onto the parietal cortex of juvenile rats over two months post injury. Injured animals were found to have significant motor deficits, left turn bias on the water maze, increased anxiety-like behaviors, and reduced exploration of a novel environment. These impairments were observed to continue to adulthood. There were no significant differences in spatial learning and memory between injury and sham injury animals overall.
Another controlled cortical impact (CCI) study in rats by Abdel Baki, Kao, and Keleman (2009) had divergent findings from Ajao et al (2012). These animals were also subjected to CCI directly onto the parietal lobe to induce mild and moderate injuries. No difference was found between injury group and sham animals on open field activity, tests of motor function, and a passive avoidance task. A test of active learning on an avoidance task found that animals with mild injuries and sham injuries learned the task equivalently well, but that learning was relatively impaired for animals with moderate injuries. Although TBI generated by CCI typically induces wide ranging impairments in behavior across domains for mild, moderate, and severe injuries (Bertolizio et al, 2011), not all studies have found clear profiles even when similar procedures were used to induce injuries.

Animal behavior in subcortical brain injury models is typically characterized by significant learning deficits in the absence of severe long-term motor dysfunction, when compared to cortical injury models (Hua et al., 2002). An experiment by Hartman, Lekic, Rojas, Tang, and Zhang (2009) investigated outcomes following intracerebral hemorrhage in rats. They found behavioral outcomes similar to those often reported in cortical TBI but without apparent motoric dysfunction. The authors noted decreased swim speed, reduced activity in the open field, and impaired spatial learning and memory at two weeks post injury. In a neonatal model of germinal matrix hemorrhage in rats, Lekic et al. (2012) found behavioral deficits across all measured domains that persisted to adulthood.

Similar results have been found in other subcortical injury models; Chen et al. (2011) induced hypoxic-ischemic injury by unilateral carotid ligation followed by
prolonged exposure to a reduced oxygen environment. Although spatial learning and memory were the only behavioral domains formally evaluated in this experiment, no obvious motor or swim deficits were noted; however, untreated injury group animals did demonstrate significant impairments of spatial learning and memory on the water maze relative to sham injury animals.

It is presently unclear if these rodent models of brain injury have behavioral deficits that correspond to human injury or if behavioral tests are able to capture behavioral deficits that would be expected given the nature of the rodent brain injury.

**Scientific Objectives and Hypotheses**

Past investigations of rodent behavioral performance have identified differential levels of performance according to sex and species (rat and mouse) in direct comparisons, yet these experimental protocols have typically used limited sample sizes to investigate behavioral performance in a single domain of function. Observed sex differences in rodents reflect trends observed in human experiments, but results to date have been mixed. Furthermore, no comparative study of rodent behavior following cortical and subcortical injury models has been conducted, and the extent to which gold-standard behavioral tests reflect deficits associated with brain damage.

Previous findings regarding rodents’ spatial learning and motor function have indicated differences between rats and mice, where rats perform better than mice overall on measures of ability, possibly due to a superior ability to flexibly implement strategic behaviors within tasks with improved problem solving strategies over time, indicating that they were learning to learn. If such an ability to learn and generalize strategic
problem solving behaviors underlies behavioral test performance, a single construct could underlie performance on behavioral tests in multiple domains. At this time, no factor analytic study exists that has explored the possibility of the existence of such a construct.

The Behavioral Neuroscience Laboratory at Loma Linda University, in conjunction with its affiliates, has a large accumulation of animal behavioral data from many research projects involving mice and rats using a variety of experimental models of brain injury and disease. These data were compiled into a single database to facilitate statistical analysis of animal behavior across experiments. Effectively, this created a large sample of rat and mouse data that can be analyzed to examine species (rats versus mice), sex, and age differences in behavioral test performance. Details of performance patterns will also be available in terms of relative differences in learning curves and across levels of difficulty between rats and mice and male as well as male and female animals. It will then be possible to identify any of sex differences vary between rats and mice in an interaction effect. It is also possible to use this database to identify a factor structure, should one exist, in animal behavioral performance. The goal of this research project is to increase the cost-efficiency behavioral research in rodent models by making them shorter and less labor intensive.

**Hypotheses**

**Hypothesis One**

Regarding species differences, rats will have significantly better spatial learning on spatial water maze trials than mice. Swim speed will be used as a covariate in this analysis because rats’ larger size and increased speed in the water may allow them to
locate the platform more rapidly than mice, making a direct comparison between rats and mice inappropriate. Rats are also hypothesized to have greater motor aptitude on the rotarod test. Performances on other behavioral assays, including anxiety-like behavior and activity, will not be significantly different between species. There will be no interaction effect observed between species and sex.

**Hypothesis Two**

Regarding sex differences in behavior, male animals will evidence significantly better spatial learning scores than female animals, but performance on other behavioral assays will not evidence a significant difference between sexes. There will be no interaction effect observed between sex and species.

**Hypothesis Three**

With regard to behavioral differences between younger and older animals, young animals will evidence significantly better spatial learning scores than older animals, who will also demonstrate reduced motor ability when compared to younger animals. There will be no significant difference in motor aptitude or anxiety-like behavior between age groups.

**Hypothesis Four**

Regarding brain injury models, animals with untreated cortical injuries (injured controls) will exhibit significantly poorer motor function, significantly better spatial learning and memory, and equivalent levels of activity and less anxiety-like behavior
when compared to animals with untreated subcortical injuries. Compared to control (sham) animals, both injury groups will exhibit poorer motor function and spatial learning. Control animals will not differ from injury animals on level of anxiety. Controls and cortically injured animals will have less anxiety like behavior than subcortically injured animals.

**Hypothesis Five**

Factor analysis of behavioral assay data from tests of multiple domains will identify an underlying factor structure. An exploratory factor analysis of behavioral data will us to identify a reduced number of factors, indicating that a latent variable (or variables) will underlie performance on multiple behavioral assays.

**Variables and Operational Definitions**

*Independent Variables*

1. Species:
   a. Rat
   b. Mouse
2. Sex:
   a. Male
   b. Female
3. Age:
   a. Young: Animals less than or equal to three months of age.
   b. Old: Animals older than three months of age.
4. Injury:

   a. Cortical injury: Animals that received cortical injuries via controlled cortical impact

   b. Subcortical injury: Animals that received injuries via induced hemorrhage, ischemia, or hypoxia that produce subcortical lesions.

**Dependent Variables**

1. Spatial learning index: Average water maze cumulative distance (cm) from target across blocks. Lower distances indicate greater proximity to target platform overall and therefore better spatial learning.

2. Water maze cumulative distance from platform: Per block average cumulative distances (cm) from target. The two trials per spatial water maze block will be averaged for all five blocks.

3. Water maze entries to target zone: The average raw number of times during probe trials the animal entered the quadrant that previously contained the escape platform.

4. Swim speed: Average swim speed in cm/s during water maze trials.

5. Motor aptitude index: Average rotarod fall latency (seconds) across all three conditions. Longer latencies indicate better performance.

6. Rotarod fall latency: Average fall latency (seconds) for each of the three Rotarod conditions, steady rotation, slow acceleration, and fast acceleration.

7. Anxiety Index: Average proportion of total time spent in dark on elevated zero maze.

8. Activity Index: Total average duration (seconds) of movement in openfield test.
9. Openfield time in center: Proportion that the animal spent in the center area of the apparatus.
CHAPTER TWO

METHODS

Behavioral Test Protocols and Data Used

The primary aim of the current project was to analyze rat and mouse performance using a battery of behavioral tests including Morris Water Maze, elevated zero maze, open-field activity, and rotarod tests, as previously described that was collected over approximately 12 years in the course of 26 previous experiments.

Experimental subjects consisted of a total of 699 animals, 209 of which were rats and 490 of which were mice. Overall, 519 of the animals were male and 180 were female. There were 155 younger animals and 544 older animals. There were 54 animals with subcortical brain injuries, and 12 with cortical brain injuries. Varied breeds of rats and mice, including wild type animals, comprised the sample.

Because the data to be used in the proposed study were gathered over a period of several years and in multiple laboratory environments with diverse research goals, exact implementations of behavioral tests have varied, as have the apparatuses used. Although quite similar overall with consistent design, speeds, test intervals, and trial times, some experiments deviated from standard administration and used extended versions of behavioral tests to examine change over time (i.e. additional spatial blocks in water maze; repeated exposure to open-field activity, etc). For those cases, only the portions of data that correspond to standard administration were used. In other cases, experimenters omitted data not applicable to the research aims at hand (i.e. did not record time in center of open-field). In those cases, missing values were excluded from relevant analyses.
**Statistical Analyses**

The proposed research project involved a variety of *post hoc* analyses to address each of the specific aims. Only control animals were used to minimize the influence of necessarily impaired learning in transgenic mice. For violations of the assumption of homogeneity of error variance, as evaluated by Levene’s test, a root transformation was applied and the assumption was met. For violations of sphericity, assessed by Mauchly’s test of sphericity, a Huynh-Feldt correction was applied when epsilon was > .75 and the Greenhouse-Geisser correction was applied when epsilon was < .75. When main effects were significant for repeated measures analyses, planned contrasts were analyzed and the Scheffe correction was applied. A Bonferroni correction was applied to control for the possibility of family-wise error, where all a priori analyses conducted for each hypothesis were considered to constitute a family, and the accepted alpha level was correspondingly adjusted.

In order to test the first two hypotheses, a series of three 2 (sex) x 2 (species) between-subjects ANOVAs and a 2 (sex) x 2 (species) ANCOVA were conducted to evaluate potential species and sex differences as well as any possible interaction between sex and species. For the 2 x 2 ANCOVA, species and sex were independent variables and spatial learning index score was the dependent variable with swim speed as a covariate. The first two-way ANOVA also included sex and species as independent variables with motor aptitude index as the dependent variable. The second two-way ANOVA included sex and species as independent variables and anxiety index scores as the dependent variable. The final two-way ANOVA again included sex and species as the independent variables with the activity index as the dependent variable.
Further testing for the first hypothesis using the same sample utilized a mixed-model ANOVA to compare the learning curves of mice and rats across spatial learning blocks. Species and block were the independent variable while spatial water maze cumulative distance from the platform was the dependent variable for each of the five spatial water maze blocks. Additional analysis of species differences in motor function involved a 2 (species) x 3 (rotarod block) mixed ANOVA to compare rotarod performance across all three rotarod conditions to evaluate species differences in performance across different levels of task difficulty. Species and rotarod block were the independent variables and fall latency was the dependent variable. The Bonferroni adjusted p-value for hypothesis one was $p < .008$.

Additional testing for the second hypothesis involved two more mixed-model ANOVAs. The first was a 2 (sex) x 5 (water maze block) mixed ANOVA used to compare the learning curves of male and female animals across all spatial learning blocks. Sex and block were the independent variables while spatial water maze cumulative distance from the platform was the dependent variable. Testing for sex differences in motor function involved a 2 (sex) x 3 (rotarod block) mixed ANOVA to compare rotarod performance across all three rotarod conditions. Sex and block were the independent variables and fall latency for each level of rotarod difficulty was the dependent variable. The Bonferroni adjusted p-value for hypothesis two was $p < .008$.

Evaluating the third hypothesis required four analyses. The first was a 2 (age) x 5 (water maze block) mixed ANOVA to compare the learning curves of old and young animals across all water maze spatial learning blocks. Another 2 (age) x 3 (rotarod block) mixed ANOVA was used to compare rotarod performance across all three rotarod
conditions to evaluate age differences in performance across different levels of task difficulty. Age and block were the independent variables and fall latency for each level of rotarod difficulty was the dependent variable. An ANOVA included levels of age as independent variables and anxiety index scores as the dependent variable. Another ANOVA again included levels of age as the independent variables with the activity index as the dependent variable. The Bonferroni adjusted $p$-value for hypothesis three was $p < .0125$.

Similarly, testing the fourth hypothesis first was tested using a 3 (injury status) x 5 (water maze block) mixed ANOVA to compare the learning curves of cortical and subcortical injury groups with control animals across all spatial learning blocks. Injury status and block were the independent variables and cumulative distance from the platform was the dependent variable. A 3 (injury status) x 3 (rotarod block) mixed ANOVA was used to compare rotarod performance across all three rotarod conditions to evaluate injury model differences in performance across different levels of task difficulty. Brain injury status (cortical, subcortical, and control) and block were the independent variables and fall latency for each level of rotarod difficulty was the dependent variable. An ANOVA used injury status as the independent variable and anxiety index scores as the dependent variable. Another ANOVA again used injury status as the independent variables with the activity index as the dependent variable. The Bonferroni adjusted $p$-value for hypothesis three was $p < .0125$.

In order to address the fifth hypothesis, exploratory factor analysis was conducted. Parallel analysis was used to determine the number of factors to extract. In this procedure, the eigenvalues from 11 factors (equal to the number of variables in the
analysis) in observed animal data were generated in SPSS using principle axis extraction, then compared to eigenvalues from analyses of multiple random data sets with the same number of cases and variables as the animal behavioral dataset as proposed by Horn (1965). The 95th percentile of the random dataset eigenvalue distributions was used as the cutoff criterion to identify animal data factor eigenvalues that exceeded random chance (Crawford et al., 2010). Factors with eigenvalues exceeding those found in random data were identified as significant factors representing latent variables and were analyzed based on the variables loading highly onto each factor. Based on the mean correlation between factors, a higher order factor was extracted.
CHAPTER THREE

RESULTS

Hypothesis One

The hypothesis that rats would evidence significantly better learning than mice was not confirmed (Figure 6). Because the sphericity assumption was violated, $\chi^2(9) = 27.64, p < .001$, where $\varepsilon > .75$, the Huynh-Feldt correction was used. The main effect for species was not significant at Bonferroni-corrected alpha level, where rats outperformed mice at a level approaching significance, $F(1,272) = 5.98, p > .008, \eta^2 = .022$. An analysis of spatial learning index scores indicated that there was not a significant interaction between sex and species, indicating that there is not differential learning between sexes when comparing rats and mice, $F(1,272) = 1.029, p > .008, \eta^2 = .004$ (Figure 7).

There was a significant main effect for performance across spatial water maze blocks, $F(3.86,1050.93) = 75.86, p < .001, \eta^2 = .22$ (Figure 8). The interaction between species and water maze block was significant with a small effect size, $F(3.86, 1050.93) = 8.916, p < .001, \eta^2 = .03$. Because there was a significant effect for water maze block, planned within-subjects contrasts were analyzed. There was a significant change between blocks one and two as well as two and three, but contrasts were not significant for contrasts between block three and four or four and five, indicating flattened learning for the last three blocks (Table 1). Contrasts for the interaction between species and water maze block were also analyzed, revealing that rats and mice had similar learning and performance on the first three blocks, but the learning curve for mice flattened relative to
rats between blocks three and four, indicating that rats continued to learn more over repeated exposures than mice (Table 1).

Figure 6. Main effect for species on spatial water maze performance
Figure 7. Spatial learning index scores for male and female animals between species.
Figure 8. Learning curves for rats and mice across spatial water maze blocks.

Table 1

Tests of within-subjects contrasts for water maze block and block/species interaction.

<table>
<thead>
<tr>
<th>Block comparison</th>
<th>F</th>
<th>Effect size ($\eta^2$)</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM block</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 v. 2</td>
<td>99.56</td>
<td>.27</td>
<td>Yes</td>
</tr>
<tr>
<td>2 v. 3</td>
<td>9.99</td>
<td>.04</td>
<td>Yes</td>
</tr>
<tr>
<td>3 v. 4</td>
<td>1.77</td>
<td>.10</td>
<td>No</td>
</tr>
<tr>
<td>4 v. 5</td>
<td>3.10</td>
<td>.01</td>
<td>No</td>
</tr>
<tr>
<td>Block x Species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 v. 2</td>
<td>1.78</td>
<td>.18</td>
<td>No</td>
</tr>
<tr>
<td>2 v. 3</td>
<td>0.68</td>
<td>.41</td>
<td>No</td>
</tr>
<tr>
<td>3 v. 4</td>
<td>6.34</td>
<td>.01</td>
<td>Yes</td>
</tr>
<tr>
<td>4 v. 5</td>
<td>.52</td>
<td>.47</td>
<td>No</td>
</tr>
</tbody>
</table>

The hypothesis that rats would have significantly better motor performance than mice was confirmed. Because the sphericity assumption was violated, $\chi^2(2) = 71.56$, $p <$
.001, where $\varepsilon > .75$, the Huynh-Feldt correction was used. The main effect for species was significant, where rats had better motor performance overall than mice, with a large effect size, $F(1,201) = 5184.02, p < .001, \eta^2 = .48$ (Figure 9). An analysis of motor aptitude index scores indicated that there was a significant interaction effect between sex and species, $F(1,248) = 22.31, p < .001, \eta^2 = .083$, suggesting that there is differential motor performance between sexes when comparing rats and mice (Figure 10). There was a larger performance discrepancy between male and female mice than there was for male and female rats. There was also a significant main effect for performance across levels of rotarod difficulty with a large effect size, $F(1.56,312.51) = 143.30, p < .001, \eta^2 = .42$ (Figure 11). The interaction between species and rotarod block was significant with a medium effect size, $F(1.56,312.51) = 31.32, p < .001, \eta^2 = .14$. Mouse performance differed significantly between the steady and slow acceleration conditions, while rat performance did not change as much between these conditions. Because there was a significant effect for rotarod block, planned within-subjects contrasts were analyzed. There was a significant change between all blocks, indicating that level of rotarod difficulty discriminated level of motor ability. An interaction effect between block and species was observed for all levels where rats and mice did not have different levels of performance on the steady condition, but did for blocks with an acceleration component (Table 2). Rats and mice did not have significantly different performance on the steady speed rotarod block $F(1,201) = 3.48, p > .05, \eta^2 = .01$. A significant difference was found for subsequent slow acceleration block performance, where rats had significantly longer fall latencies than mice $F(1,201) = 185.69, p < .001, \eta^2 = .43$. Rats also had significantly
longer fall latencies on the fast acceleration block $F(1, 201) = 164.43, p < .001, \eta^2 = .37$ (Figure 11).

![Bar chart showing fall latencies for rats and mice.](image)

*Figure 9.* Main effect of species on rotarod performance

![Bar chart showing motor aptitude index scores for male and female rats and mice.](image)

*Figure 10.* Motor Aptitude Index scores for male and female animals between species.
Figure 11. Fall latency for rats and mice across rotarod blocks of increasing difficulty.

<table>
<thead>
<tr>
<th>Rotarod Block</th>
<th>Fall Latency (seconds) +/ SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steady</td>
<td>85 ± 0.37</td>
</tr>
<tr>
<td>Slow Accel</td>
<td>78 ± 0.38</td>
</tr>
<tr>
<td>Fast Accel</td>
<td>72 ± 0.40</td>
</tr>
</tbody>
</table>

Tests of within-subjects contrasts for rotarod block and block/species interaction.

<table>
<thead>
<tr>
<th>Block comparison</th>
<th>F</th>
<th>Effect size ($\eta^2$)</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR block steady v. slow accel</td>
<td>72.72</td>
<td>.27</td>
<td>Yes</td>
</tr>
<tr>
<td>slow accel v. fast accel</td>
<td>106.37</td>
<td>.37</td>
<td>Yes</td>
</tr>
<tr>
<td>Block x Species steady v. slow accel</td>
<td>44.81</td>
<td>.18</td>
<td>Yes</td>
</tr>
<tr>
<td>slow accel v. fast accel</td>
<td>13.44</td>
<td>.06</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The hypothesis that levels of anxiety-like behavior would not differ between rats and mice was not supported. The assumption of homogeneity of variances was met after a square root transformation, $F(3,396) = 3.48, p > .05$. A significant main effect was
observed where rats exhibited significantly more anxiety-like behavior than mice; the effect size was large, $F(1,396) = 206.16, p < .001, \eta^2 = .342$. The interaction between sex and species was not significant, $F(1,396) = 4.85, p > .008, \eta^2 = .001$, such that males exhibited more anxiety-like behavior than females in rats, but the trend was reversed in mice (Figure 12).

![Graph showing Anxiety Index scores for male and female animals between species.](image)

*Figure 12.* Anxiety Index scores for male and female animals between species.

The hypothesis that there would not be significant differences in activity levels between rats and mice was not supported. The assumption of homogeneity of variances was met, $F(3,334) = .835, p > .05$. Mice were found to have been significantly more active than rats, $F(1,334) = 78.54, p < .001, \eta^2 = .190$. The interaction between species and sex was not significant (Figure 13).
Hypothesis 2

The hypothesis that males would evidence significantly better spatial learning than females was not confirmed, $F(1,272) = 2.517, p > .008, \eta^2 = .09$ (Figure 14).

Because the sphericity assumption was violated, $\chi^2(9) = 40.66, p < .001$, where $\varepsilon > .75$, the Huynh-Feldt correction was used. There was a significant main effect for performance across spatial water maze blocks with a medium effect size, $F(3.77,1024.70) = 49.25, p < .001, \eta^2 = .15$ (Figure 15). An analysis of spatial learning index scores indicated that there was not a significant interaction effect between sex and species, suggesting that there is not differential learning between species when comparing males and females (Figure 7). The interaction between species and water maze block was not significant at the Bonferroni-corrected alpha level, $F(3.77, 1024.70) = 3.08, p > .008, \eta^2 = .01$. 

Figure 13. Activity Index scores for male and female animals between species.
Because there was a significant effect for water maze block, planned within-subjects contrasts were analyzed. There was a significant change between blocks one and two as well as two and three, but contrasts were not significant between block three and four, indicating a variation in the expected learning curve (Table 3). Comparison between blocks four and five indicated significant change.

*Figure 14. Main effect of sex on spatial water maze performance*
Figure 15. Learning curves for males and females across spatial water maze blocks.

Table 3

Tests of within-subjects contrasts for water maze block and block/sex interaction.

<table>
<thead>
<tr>
<th>Block comparison</th>
<th>F</th>
<th>Effect size (η²)</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM block</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 v. 2</td>
<td>71.11</td>
<td>.20</td>
<td>Yes</td>
</tr>
<tr>
<td>2 v. 3</td>
<td>9.14</td>
<td>.03</td>
<td>Yes</td>
</tr>
<tr>
<td>3 v. 4</td>
<td>1.48</td>
<td>.01</td>
<td>No</td>
</tr>
<tr>
<td>4 v. 5</td>
<td>10.85</td>
<td>.04</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The hypothesis that males and females would not have significantly different motor performance was supported, $F(1,201) = 4.106, p > .008, \eta^2 = .02$ (Figure 16). Because the sphericity assumption was violated, $\chi^2(2) = 88.27, p < .001$, where $\epsilon < .75$, the Greenhouse-Geisser correction was used. An analysis of motor aptitude index scores indicated that there was a significant interaction effect between sex and species, $F(1,248)$
= 22.31, \( p < .001 \), \( \eta^2 = .083 \), suggesting that there is differential motor performance between species when comparing males and females (Figure 10). Male and female rats did not have significantly different overall motor performance \( F(1,88) = 1.76, p > .05, \eta^2 = .02 \), but female mice significantly outperformed male mice \( F(1,167) = 29.73, p < .001, \eta^2 = .15 \).

There was also a significant main effect for performance across levels of rotarod difficulty with a large effect size, \( F(1.47,296.28) = 111.46, p < .001, \eta^2 = .36 \) (Figure 17). The interaction between sex and rotarod block was not significant, \( F(1.47,296.28) = 1.18, p > .008, \eta^2 = .01 \). Because there was a significant effect for rotarod block, planned within-subjects contrasts were analyzed. There was a significant change between all blocks, indicating that level of rotarod difficulty discriminated level of motor ability for both males and females (Table 4).

![Figure 16. Main effect of sex on overall rotarod performance.](image-url)
Figure 17. Fall latency for males and females across rotarod blocks of increasing difficulty.

Table 4

Tests of within-subjects contrasts for rotarod blocks for males and females.

<table>
<thead>
<tr>
<th>Block comparison</th>
<th>F</th>
<th>Effect size (η²)</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR block steady v. slow accel</td>
<td>72.72</td>
<td>.27</td>
<td>Yes</td>
</tr>
<tr>
<td>slow accel v. fast accel</td>
<td>106.37</td>
<td>.37</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The hypothesis that levels of anxiety-like behavior would not differ between males and females was confirmed, $F(1,396) = .25, p > .008, \eta^2 < .01$ (Figure 18). The assumption of homogeneity of variances was met after a square root transformation, $F(3,396) = 3.48, p = .062$. The main effect for sex was not significant. The interaction
between sex and species was not significant, $F(1,396) = 4.85, p > .008, \eta^2 = .001$ (Figure 12).

![Chart showing Anxiety Index scores for male and female.](chart.png)

*Figure 18. Main effect of sex on Anxiety Index scores.*

The hypothesis that there would not be significant differences in activity levels between males and females was not supported. The assumption of homogeneity of variances was met, $F(3,334) = .835, p = .475$. Females were found to have been significantly more active than males, $F(1,334) = 15.68, p < .001, \eta^2 = .05$ (Figure 19). The interaction between sex and species was not significant (Figure 13).
Figure 19. Main effect of sex Activity Index score.

Hypothesis Three

The hypothesis that younger animals would evidence significantly better spatial learning across all spatial water maze trials was not confirmed. Because the sphericity assumption was violated, $\chi^2(9) = 31.78, p < .001$, where $\varepsilon > .75$, the Huynh-Feldt correction was used. In fact, the opposite effect from hypothesized performance was observed. The main effect for age was non-significant, but older animals outperformed younger animals at a level approaching significance, $F(1,272) = 3.346, p > .0125, \eta^2 = .01$ (Figure 20). The main effect for water maze block was significant, with a small effect size, $F(3.83, 1042.54) = 17.22, p < .001, \eta^2 = .06$. The interaction between age and water maze block was significant with a small effect size, $F(3.83, 1042.54) = 6.66, p < .001, \eta^2 = .02$ (Figure 21).
Because there was a significant effect for water maze block and the block x age interaction, planned within-subjects contrasts were analyzed. Significant changes were observed between blocks one and two, but remaining contrasts were not significant, most likely due to the relatively flattened learning curve produced by younger animals. Contrasts for the interaction between species and water maze block were also analyzed, revealing that younger animals had flatter overall learning curve than older animals and had sporadic block-to-block performance (Table 5).

Figure 20. Main effect of age on spatial water maze performance.
Figure 21. Learning curves for older and younger animals across spatial water maze blocks.

Table 5

Tests of within-subjects contrasts for water maze block and block/age interaction.

<table>
<thead>
<tr>
<th></th>
<th>Block comparison</th>
<th>F</th>
<th>Effect size ($\eta^2$)</th>
<th>Significant?</th>
</tr>
</thead>
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<tr>
<td>WM block</td>
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<td>36.79</td>
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</tr>
<tr>
<td></td>
<td>2 v. 3</td>
<td>.229</td>
<td>&lt;.01</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>3 v. 4</td>
<td>1.37</td>
<td>&lt;.01</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>4 v. 5</td>
<td>1.45</td>
<td>&lt;.01</td>
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<td>Block x Age</td>
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<td>&lt;.01</td>
<td>No</td>
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</tr>
<tr>
<td></td>
<td>3 v. 4</td>
<td>.84</td>
<td>&lt;.01</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>4 v. 5</td>
<td>9.58</td>
<td>.03</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The hypothesis that younger animals would have significantly better motor performance than older animals was not confirmed. The reverse trend was found where
older animals had longer fall latencies across blocks. The main effect for age was significant, where older animals had better motor performance overall than younger animals, with a small effect size, $F(1,201) = 13.38, p = .001, \eta^2 = .06$ (Figure 22). Because the sphericity assumption was violated, $\chi^2(2) = 89.94, p < .001$, where $\varepsilon < .75$, the Greenhouse-Geisser correction was used. There was a significant main effect for performance across levels of rotarod difficulty with a large effect size $F(1.47,295.11) = 88.671, p < .001, \eta^2 = .31$. The interaction between age and rotarod block was not significant, $F(1.47,295.11) = 3.26, p > .0125, \eta^2 = .02$ (Figure 23). Because there was a significant effect for rotarod block, planned within-subjects contrasts were analyzed. There was a significant change between all blocks, indicating that level of rotarod difficulty discriminated level of motor ability (Table 6).

*Figure 22. Main effect of age on rotarod performance*
Figure 23. Fall latency for older and younger animals across rotarod blocks of increasing difficulty.

Table 6

Tests of within-subjects contrasts for rotarod blocks across animals of all ages.

<table>
<thead>
<tr>
<th>Block comparison</th>
<th>F</th>
<th>Effect size ($\eta^2$)</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR block</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>steady v. slow accel</td>
<td>61.19</td>
<td>.23</td>
<td>Yes</td>
</tr>
<tr>
<td>slow accel v. fast accel</td>
<td>31.66</td>
<td>.14</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The hypothesis that levels of anxiety-like behavior would not differ between older and younger animals was not supported. The assumption of homogeneity of variances was met, $F(1.398) = .880, p > .05$. A significant difference was observed where older animals exhibited significantly more anxiety-like behavior than their younger counterparts; the effect size was small, $F(1.398) = 36.23, p < .001, \eta^2 = .08$ (Figure 24).
Finally, the hypothesis that there would not be significant differences in activity levels between older and younger animals was not supported. The assumption of homogeneity of variances was met, $F(1,336) = 3.697, p > .05$. Older animals were found to have been significantly less active overall than younger animals, with a small effect size, $F(1,336) = 24.02, p < .001, \eta^2 = .07$ (Figure 25).

*Figure 24. Main effect of age on Anxiety Index score.*
Hypothesis Four

The hypothesis that animals with untreated cortical brain injuries would demonstrate significantly better spatial learning and memory than those animals with untreated subcortical injuries, and that control animals would perform better than both groups, across all spatial water maze trials was confirmed (Figure 26). Because the sphericity assumption was violated, $\chi^2(9) = 26.34, p < .05$, where $\varepsilon > .75$, the Huynh-Feldt correction was used. The main effect for injury type was significant with a medium effect size, $F(1,82) = 83.74, p < .001, \eta^2 = .19$. Comparisons of the three groups indicated that sham animals ($M = 1726.26, 95\% \text{ CI} [-484.55, 3937.07]$) performed better than the animals with cortical injuries ($M = 5120.09, p < .05, 95\% \text{ CI} [3363.12, 6877.07]$), and both of those groups performed significantly better than animals with subcortical injuries.
(M = 7000.93, p < .05, 95% CI [5958.61, 8042.98]). The main effect for water maze block was significant, indicating significant change between water maze blocks overall $F(3.69, 303.33)=18.01, p < .0125, \eta^2=.07$. The interaction between injury type and water maze block was not significant, $F(3.69, 303.34) = 1.07, p > .125, \eta^2 = .02$ (Figure 27) indicating that slop of the learning curves did not differ significantly between the three groups. Because the main effect of water maze block was significant, planned within-subjects contrasts were analyzed (Table 7).

*Figure 26. Main effect of injury type on spatial water maze performance.*
Figure 27. Learning curves for animals with cortical and subcortical injuries across spatial water maze blocks.

Table 7

Tests of within-subjects contrasts for water maze block across injury types.

<table>
<thead>
<tr>
<th>Block comparison</th>
<th>F</th>
<th>Effect size ($\eta^2$)</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM block</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 v. 2</td>
<td>20.69</td>
<td>.20</td>
<td>Yes</td>
</tr>
<tr>
<td>2 v. 3</td>
<td>3.52</td>
<td>.04</td>
<td>No</td>
</tr>
<tr>
<td>3 v. 4</td>
<td>.65</td>
<td>&lt; .01</td>
<td>No</td>
</tr>
<tr>
<td>4 v. 5</td>
<td>.27</td>
<td>&lt; .01</td>
<td>No</td>
</tr>
</tbody>
</table>

The hypothesis that animals with subcortical injuries would have significantly better rotarod performance than cortically injured animals was confirmed. Because the sphericity assumption was violated, $\chi^2(2) = 54.21$, $p < .001$, where $\varepsilon < .75$, the Greenhouse-Geisser correction was used. The main effect for injury type was significant.
with a large effect size, $F(1,70) = 30.15, p < .001, \eta^2 = .46$ (Figure 28). Comparisons indicated that animals with cortical injuries, $M = 16.08, 95\% \text{ CI } [9.53, 22.64]$) had significantly worse performance on the rotarod than sham animals ($M = 43.46, p < .05, 95\% \text{ CI } [38.45, 48.46]$) and animals with subcortical injuries ($M = 42.80, p < .05, 95\% \text{ CI } [40.44, 45.16]$), whose performances were not significantly different. There was a significant main effect for performance across levels of rotarod difficulty with a large effect size $F(1.30, 90.73) = 38.21, p < .001, \eta^2 = .35$. The interaction between injury type and rotarod block was not significant, $F(2.59, 90.73) = 1.47, p > .0125, \eta^2 = .02$ (Figure 29). Because there was a significant effect for rotarod block, planned within-subjects contrasts were analyzed. There was a significant change between all blocks, indicating that level of rotarod difficulty discriminated level of motor ability across injury types (Table 8). The absence of an interaction between rotarod block and injury type indicates that animals with both injury types had relatively consistent block to block changes on the rotarod.

*Figure 28. Main effect of brain injury type on rotarod performance.*
Figure 29. Fall latency for older and younger animals across rotarod blocks of increasing difficulty.

Table 8

Tests of within-subjects contrasts for rotarod blocks across injury types.

<table>
<thead>
<tr>
<th>RR block</th>
<th>Block comparison</th>
<th>F</th>
<th>Effect size (ɳ²)</th>
<th>Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>steady v. slow accel</td>
<td>20.07</td>
<td>.22</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>slow accel v. fast accel</td>
<td>38.14</td>
<td>.35</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

The hypothesis that animals with subcortical injuries would exhibit more anxiety-like behavior than sham animals and cortical injuries was supported. The assumption of homogeneity of variances was met, F(2,100) = 1.02, p=.364. The main effect of injury type was significant with a large effect size, F(2,100) = 42.43, p < .001, ɳ² = .48 (Figure
On comparisons, a difference was observed where subcortically injured animals ($M = .83, 95\% \text{ CI} [.787, .880]$) exhibited significantly more anxiety-like behavior than their counterparts with cortical injuries ($M = .54, p < .001, 95\% \text{ CI} [.492, .584]$) and sham injuries ($M = .581, p < .001, 95\% \text{ CI} [.491, .672]$). Cortical injury and sham groups did not differ significantly, $p > .05$.

![Figure 30. Main effect of injury type on Anxiety Index score.](image)

The hypothesis that there would not be significant differences in activity levels injury types was not supported. The assumption of homogeneity of variances was met, $F(2,117) = .266, p = .471$. There was a significant main effect of injury type on activity index scores, $F(2,117) = 33.01, p < .001, \eta^2 = .36$ (Figure 31). Planned comparisons indicated that animals with subcortical injuries ($M = 798.04, 95\% \text{ CI} [743.73, 852.39]$) had lower levels of activity on the open field compared to animals with cortical ($M = 1119.61, p < .001, 95\% \text{ CI} [1060.72, 1178.49]$) and sham injuries $M = 1029.44, p < .001,$
95% CI [940.15, 118.73]). The cortical injury group did not differ from the sham injury group, $p > .05$.

**Figure 31.** Main effect of injury type on Activity Index score.

**Hypothesis Five**

In order to conduct exploratory factor analysis to address hypothesis five, first an R-matrix was generated using data from 311 cases to examine intercorrelations between the variables and to detect possible multicollinearity (Table 9). A review of this correlation matrix indicated that all variables had more than one significant correlation to another variable. No two variables were correlated at a level greater than .9, meaning there were no observed occurrences of multicollinearity.

These findings suggested that there might be latent variables in the behavioral assay data. The 311 cases used exceeded the minimum number of 300 cases and 10 cases per variable recommended by Tabachnick and Fidell (2013). The Kaiser-Meyer-Olkin
measure of sampling adequacy was .83, indicating an adequate sample for factor analysis. The assumption that the correlation matrix was not an identity matrix was met, $\chi^2(310) = 1834.492$, $p<.001$.

Parallel analysis revealed that the eigenvalues for the first three factors extracted from observed data exceeded the 95th percentile of eigenvalues from the randomly generated data (Table 10), suggesting that there were three significant factors and supporting the fifth hypothesis (Figure 33). Therefore three factors were extracted by principle axis extraction with Promax rotation (Table 11). An analysis of the factor structure matrix yielded pertinent information about latent variables in the behavioral assay data. All rotarod variables and block five water maze variables had salient loadings on the first factor. All water maze variables had salient loadings on the second factor; water maze block five data cross-loaded onto this factor. Open field time in center and zero maze data loaded saliently onto the third factor.

The average of the absolute values of the correlations between the three extracted factors was .374, indicating that a higher order factor was present. A single factor was then extracted by principle axis extraction (Table 12). All variables had salient loadings onto this factor, with the exception of anxiety index scores and activity index scores.
### Intercorrelations of behavioral assay data across domains (R-matrix).

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Anxiety Index</td>
<td></td>
<td>-0.40**</td>
<td>0.32**</td>
<td>0.59**</td>
<td>0.63**</td>
<td>-0.10</td>
<td>-0.08</td>
<td>-0.17*</td>
<td>-0.31**</td>
<td>-0.34**</td>
<td>-0.12</td>
</tr>
<tr>
<td>2. Activity Index</td>
<td></td>
<td></td>
<td>-0.002</td>
<td>-0.31**</td>
<td>-0.31**</td>
<td>-0.02</td>
<td>0.02</td>
<td>0.05</td>
<td>-0.21**</td>
<td>0.15*</td>
<td>-0.12</td>
</tr>
<tr>
<td>3. Rotarod (steady)</td>
<td></td>
<td></td>
<td></td>
<td>0.44**</td>
<td>0.53**</td>
<td>-0.28**</td>
<td>-0.31**</td>
<td>-0.27**</td>
<td>-0.39**</td>
<td>-0.36**</td>
<td>0.30*</td>
</tr>
<tr>
<td>4. Rotarod (slow accel)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.86**</td>
<td>-0.15*</td>
<td>-0.23**</td>
<td>-0.26**</td>
<td>-0.32**</td>
<td>-0.42**</td>
<td>0.27*</td>
</tr>
<tr>
<td>5. Rotarod (fast accel)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.10</td>
<td>-0.26**</td>
<td>-0.25**</td>
<td>-0.34**</td>
<td>-0.40**</td>
<td>-0.20</td>
</tr>
<tr>
<td>6. Spatial WM Block 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.66*</td>
<td>0.62**</td>
<td>-0.60**</td>
<td>0.53**</td>
<td>-0.09</td>
</tr>
<tr>
<td>7. Spatial WM Block 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.69**</td>
<td>0.68**</td>
<td>0.60**</td>
<td>-0.08</td>
</tr>
<tr>
<td>8. Spatial WM Block 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.74**</td>
<td>0.67**</td>
<td>-0.32*</td>
</tr>
<tr>
<td>9. Spatial WM Block 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.72**</td>
<td>-0.29*</td>
</tr>
<tr>
<td>10. Spatial WM Block 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.216</td>
</tr>
<tr>
<td>11. OF Time in Center</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Correlation significant at the 0.01 level.  
* Correlation significant at the 0.05 level.
Table 10

*Initial eigenvalues from observed data with the 95\textsuperscript{th} percentile of eigenvalues generated from randomly observed data.*

<table>
<thead>
<tr>
<th>Factor</th>
<th>Observed Data</th>
<th>Random Data (95\textsuperscript{th} %ile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.700</td>
<td>1.391</td>
</tr>
<tr>
<td>2</td>
<td>1.417</td>
<td>1.285</td>
</tr>
<tr>
<td>3</td>
<td>1.244</td>
<td>1.199</td>
</tr>
<tr>
<td>4</td>
<td>1.103</td>
<td>1.137</td>
</tr>
<tr>
<td>5</td>
<td>0.882</td>
<td>1.079</td>
</tr>
<tr>
<td>6</td>
<td>0.671</td>
<td>1.028</td>
</tr>
<tr>
<td>7</td>
<td>0.524</td>
<td>0.974</td>
</tr>
<tr>
<td>8</td>
<td>0.454</td>
<td>0.920</td>
</tr>
<tr>
<td>9</td>
<td>0.411</td>
<td>0.882</td>
</tr>
<tr>
<td>10</td>
<td>0.339</td>
<td>0.834</td>
</tr>
<tr>
<td>11</td>
<td>0.255</td>
<td>0.775</td>
</tr>
</tbody>
</table>
Figure 32. Observed-data eigenvalues for all thirteen extracted factors to the 95\textsuperscript{th} percentile plotted with eigenvalues from randomly generated data.
Table 11

*Rotated item loading matrix for significant extracted factors with amount of variance explained per factor.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotarod Slow Acceleration</td>
<td><strong>.819</strong></td>
<td>-.403</td>
<td>.265</td>
</tr>
<tr>
<td>Rotarod Fast Acceleration</td>
<td><strong>.786</strong></td>
<td>-.301</td>
<td></td>
</tr>
<tr>
<td>Rotarod Steady</td>
<td><strong>.779</strong></td>
<td>-.543</td>
<td>.329</td>
</tr>
<tr>
<td>Water maze Block 5</td>
<td>-.535</td>
<td>.448</td>
<td>-.215</td>
</tr>
<tr>
<td>Water maze Block 3</td>
<td>-.333</td>
<td><strong>.750</strong></td>
<td>-.406</td>
</tr>
<tr>
<td>Water maze Block 4</td>
<td>-.419</td>
<td><strong>.637</strong></td>
<td>-.227</td>
</tr>
<tr>
<td>Water maze Block 1</td>
<td>-.241</td>
<td><strong>.565</strong></td>
<td>-.205</td>
</tr>
<tr>
<td>Water maze Block 2</td>
<td>-.333</td>
<td><strong>.427</strong></td>
<td></td>
</tr>
<tr>
<td>Open Field Time in Center</td>
<td>.297</td>
<td>-.336</td>
<td><strong>.544</strong></td>
</tr>
<tr>
<td>Activity Index</td>
<td></td>
<td></td>
<td><strong>.514</strong></td>
</tr>
<tr>
<td>Anxiety Index</td>
<td>.172</td>
<td>.163</td>
<td><strong>-.399</strong></td>
</tr>
<tr>
<td>Variance Explained (%)</td>
<td>33.64</td>
<td>12.88</td>
<td>11.31</td>
</tr>
<tr>
<td>Cumulative Variance Expl. (%)</td>
<td>33.64</td>
<td>46.52</td>
<td>57.83</td>
</tr>
</tbody>
</table>

Note: Salient variable loadings in bold. Any loadings < .100 are suppressed.
Table 12

*Variable loadings for the higher order factor.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor Loading</th>
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<tbody>
<tr>
<td>Rotarod Steady</td>
<td>.798</td>
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<td>Rotarod Slow Acceleration</td>
<td>.725</td>
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<tr>
<td>Rotarod Fast Acceleration</td>
<td>.625</td>
</tr>
<tr>
<td>Water maze Block 5</td>
<td>-.585</td>
</tr>
<tr>
<td>Water maze Block 4</td>
<td>-.578</td>
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<tr>
<td>Water maze Block 3</td>
<td>-.565</td>
</tr>
<tr>
<td>Water maze Block 1</td>
<td>-.418</td>
</tr>
<tr>
<td>Open Field Time in Center</td>
<td>-.396</td>
</tr>
<tr>
<td>Water maze Block 2</td>
<td>-.393</td>
</tr>
<tr>
<td>Anxiety Index</td>
<td>.229</td>
</tr>
<tr>
<td>Activity Index</td>
<td></td>
</tr>
</tbody>
</table>

Note: Salient variable loadings in bold. Any loadings < .100 are suppressed.
CHAPTER FOUR

DISCUSSION

Overall, the results of this study show how rodent performance on commonly used experimental behavioral assays varies according to species, sex, age, and type of brain injury, informing future experimental methodology. This study also supported distinct behavioral profiles for animals in cortical and subcortical brain injury models, which were found to resemble injury profiles in humans. Finally, this study indicates a factor structure in behavioral assay data, where motor and spatial memory tests each loaded on their own factor, and data from three behavioral tests loaded onto a single higher order factor. This finding increases the previous level of understanding of how behavioral test performance is related across domains of learning, motor function, activity, and emotionality and has implications for the composition of future rodent behavioral test batteries.

In general, these findings extend the existing body of literature by making thorough comparisons between species across behavioral domains that were absent from previous literature. This study elucidated more clearly the nature and extent of sex differences, in addition to explicating sex differences across species. Small samples and a limited range of comparisons and behaviors have plagued previous studies of these species and sex differences.

Overall, the first hypothesis was partially supported. Rats did not exhibit significantly better spatial and learning on repeated task exposures relative to mice, when accounting for swim speed. A comparison of the respective learning curves for rats and mice, however, yielded more interesting information. Rats had a steeper learning curve
that was sustained across learning trials. Rats and mice both exhibited significant learning between their first and second water maze attempt, but mice made negligible improvements thereafter while rats continued to make gains in spatial knowledge, possibly representing the improvements in strategy that have been previously documented in rats (Wishaw, 2001). Rats’ learning curve more closely resembles the way that humans learn across repeated trials.

The fact that rats make steady gains from block to block may make them better experimental subjects in future experiments where spatial learning and memory are of particular interest, or where the ability to detect subtle performance changes in this domain is desirable, as flattening of the learning curve may be more readily apparent for rats than mice.

As was hypothesized, rats also demonstrated significantly better motor abilities overall than mice. The magnitude of this difference was large. Further, the magnitude of performance differences between sexes was statistically insignificant for rats, but was quite large for mice, where female animals had fall latencies more than 50 percent longer than their male counterparts. This performance pattern has implications for testing motor function when mice are used as experimental subjects; due to highly discrepant motor ability between male and female mice, both sexes should be used when motor function is a dependent variable so that the impact of experimental protocols on both males and females might be evaluated independently. Both rats and mice exhibited differential performance on each successive rotarod condition, confirming previous findings that the rotarod is useful for discriminating level of motor function in both rats and mice.
Additionally, the average fall latencies for mice were near the floor of the rotarod’s measurement range for the most difficult condition: fast acceleration. This means that an experimental condition affecting motor function could cause mice to easily fall below the test’s ability to measure change in motor function on the rotarod (they would fall almost immediately). This could cause an underestimate of the magnitude of changes in motor function when mice are used as experimental subjects.

Activity levels were significantly different in rats and mice, contrary to the first hypothesis, where mice were significantly more active than rats on the open field test. This direct comparison had not been made in any earlier study, and thus there was no reason to suspect that activity levels would differ. This finding extends earlier data by increasing the understanding about how rats and mice differ on behavioral assay performance. Mice may have been more active as they continued to explore and attempt to escape the apparatus, while rats may have recognized the futility of escape, quickly became bored, and moved about the field less. It is important for future researchers to have an understanding of baseline behaviors in rodents to evaluate what change has occurred as well as inform possible reasons for that change.

The portion of the first hypothesis that stated the level of anxiety-like behavior would not differ between rats and mice was also not supported; rats had higher levels of anxiety-like behavior. The precise etiology of this difference is not known at the present time, but may be related to the above findings regarding differential activity levels in rats and mice. Rats may have spent less time moving around the zero maze apparatus than mice, causing them spend a larger proportion of time in the dark and thus appear relatively more anxious. Rats may have moved and explored less than mice because they
were less inclined to expose themselves to the potential danger of an unsheltered area once they found that there was no reward for doing so.

Overall, rats may be better subjects in experimental models than mice due to the fact that they produce somewhat more consistent data, have smaller sex differences, and have performance patterns on the rotarod and water maze that better permit the detection of changes due to experimental conditions.

The second hypothesis was also supported in part. First, the hypothesis that males would evidence better spatial learning and memory than females was not supported. Although males performed nominally better on spatial blocks overall, the size of the difference was not significant. An analysis of the respective learning curves for males and females yielded an interesting aberration in the learning curve of female animals wherein performance was reduced (greater total distance from target platform) on block four, but then continued to improve again on block five. The reason for this fluctuation in performance is unclear at this time, but may warrant further investigation into the swim strategy of female animals. It is possible that females animals might have become fatigued by this point in the task, increased floating in order to rest, then recovered sufficiently to swim for the platform again in block 5.

Males did not have significantly better motor performance overall, as was hypothesized; their overall levels of performance were similar. Given the previously discussed interaction effect between sex and species, where no sex differences were observed for rats, a homogenous experimental sample of either male or female rats would likely generalize to rats on the whole. For mice, where significant sex differences in motor function were observed, a sample with both male and female animals is necessary.
to quantify the effects of an experimental condition on motor function in both male and female mice. Previous findings were confirmed that the rotarod discriminates adequately between levels of task difficulty for both male and female animals.

The portion of the second hypothesis that stated that levels of anxiety-like behavior would not differ between male and female animals was supported. Male and female animals exhibited very similar levels of anxiety-like behavior overall. This finding, which extends previous literature, indicates that a homogenous sample of either males or females is likely to be informative about changes in anxiety-like behavior in future experiments, and that findings can be generalized to both males and females. Because the size and nature of sex differences did not differ significantly for either rats or mice, the aforementioned conclusion may apply to both species.

The final portion of the second hypothesis, that activity levels would not differ between males and females, was not supported. Females were significantly more active overall than males. Although the reason for this distinction remains unclear, differential levels of activity may have resulted from hormonal differences in male and female animals. This finding extends earlier findings by providing additional data about sex differences in rodents. The size of the effect in this case, however, is quite small, so the practical implications of this result are likely to be minimal.

Overall, the third hypothesis was not supported. The results of analyses addressing this hypothesis indicated that there either were not significant differences between old and young animals, or that the observed differences ran in a direction contradictory to the hypothesized relationship. These findings are not, however, likely to accurately represent the nature of performance differences between age groups due to
study limitations. The nature of the available data regarding the age of animals at the time of experimentation, in addition to how age groups were defined in this study, effectively resulted in the comparison of juvenile animals to adult/old animals, rather than a true younger adult versus older adult comparison. Consequentially, this study compared neurologically immature animals with mature adult animals. Although spatial water maze block one performances were very similar, the older (adult) animals demonstrated better spatial learning overall with a learning curve that suggests improvement between consecutive blocks. The younger (juvenile) animals demonstrated sporadic performance from block to block. This result suggests that juvenile animals are not likely to produce usefully consistent data on the Morris water maze compared to adult animals.

Similarly, the hypothesis that younger animals would have better motor performance than older animals was not supported. Older animals had significantly better motor performance at all levels, although the effect size was small. Older animals may have had better motor performance due to greater physical maturity, strength, and superior coordination, as well as a better ability to have a strategic approach to the task. The rotarod again effectively discriminated motor function at all levels for animals of all ages, confirming earlier findings. Younger animals’ performance at the most difficult level of the task was near the floor of the range of possible fall latencies, which may result in a compressed range of scores and a reduced capacity to detect the true magnitude of change in motor function when juvenile animals are used in experiments.

Older animals exhibited significantly more anxiety-like behavior than younger animals, spending a greater proportion of their time in the darkened zones of the zero maze apparatus. Younger animals may have been more inclined to engage in risky
behavior unnecessarily than older animals by moving about an exposed environment similar to the manner in which juvenile humans engage in more risky behaviors than their more experienced and mature adult counterparts.

Lastly, the hypothesis that activity levels would not vary significantly between age groups was not supported, where older animals were less active overall on the open field test. The trends observed in the two previous findings may actually be explained by activity levels. The less active older animals may not have explored the zero maze apparatus as extensively as the younger animals, causing them to appear to be relatively more anxious on the test as opposed to having some internal state of fearfulness or hiding behavior, as has been the traditional interpretation of a large proportion of time spent in the dark portion of the zero-maze.

The fourth hypothesis, that there would be distinct neurobehavioral profiles for animals with cortical versus subcortical brain injuries, was partially supported. The results of behavioral tests produced results that reflected changes in function of the specific structures that were damaged in the injury model, meaning that the rodent behavioral assays were able to capture behavioral change in focal brain injury models. As hypothesized, animals with cortical brain injuries exhibited significantly better performance on a spatial learning task than those animals that had sustained subcortical injuries, most likely due to the fact that these animals had less hippocampal involvement in their injuries. Animals with sham injuries outperformed both injury groups on all water maze blocks, indicating that both cortical and subcortical injury models produced some decrement to spatial learning and memory relative to controls. Both injury groups evidenced learning curves with significant block-to-block improvements. The animals
with subcortical injuries, however, had greater overall distance from the platform from the outset at block one, suggesting that they were also less able to initially locate the platform, and never quite achieved the same level of performance as animals with cortical injuries.

This pattern of findings is similar to that observed in humans. In fact, previous studies have shown that controlled cortical impact typically leads to spatial learning deficits in rodents (Ajao et al., 2012; Hamm, Dixon, Gbadebo, Singha, Jenkins, & Lyeth et al., 2009). When humans have traumatic brain injuries, learning difficulties are ordinarily prominent in global rather than focal injuries (Bullinger, 2002). Human brain injuries with significant subcortical involvement, especially of the hippocampus and cingulate, typically result in severe impairments in learning and memory (Fontaine, Azouvi, Remy, Bussel, & Samson, 1999). This means that the pattern of memory deficits here observed in rodents successfully models the types of cognitive impairments observed in humans with similar types of injury.

The hypothesis that animals with subcortical brain injuries would have significantly better motor function than animals with more cortical injuries was supported. Sham animals had overall rotarod performance that was similar to that of animals with subcortical injury, contrary to the hypothesis. Rotarod performance differences between injury groups were of relatively large magnitude. Animals with subcortical injuries and sham injuries had broadly consistent performance across rotarod blocks one and two with an overall decline in performance for the fast acceleration condition. Animals with subcortical injuries did not demonstrate a significant decrement to motor function in general. Animals with cortical injuries evidenced a significant
change in performance between the steady and slow acceleration condition, but fall latency did not change much between the two acceleration conditions.

The observed pattern of performance indicates that these rodent brain injury models are representative of the broad pattern of neurocognitive deficits observed in human brain injury. Although human brain injury has tremendously heterogeneous cognitive sequelae, motor deficits are observed in approximately one-third of focal traumatic brain injuries involving the cortex and a much larger proportion of more diffuse and severe injuries (Walker & Pickett, 2007). The findings of this study indicate that the nature of motor deficits observed in rodents successfully models the behavioral deficits observed in humans after traumatic brain injury.

The part of hypothesis four that stated animals with subcortical injuries would have higher levels of anxiety than both animals with cortical injuries and sham injuries was supported. Animals with subcortical injuries demonstrated significantly more anxiety-like behavior than their counterparts with cortical injuries and sham injury animals alike. Animals with cortical injuries and sham injuries did not differ significantly in anxiety level. The etiology of this difference is likely to be damage sustained by the limbic structures of animals with subcortical injuries, while these structures remained intact in animals without injury and those with cortical injuries.

Finally, the hypothesis that activity levels would not vary between injury types was not supported. Animals with cortical injuries were found to be significantly more active. Animals with subcortical injuries may have been less likely to initiate movement than animals with cortical and sham injuries due to damage of subcortical structures.
The fifth hypothesis, that exploratory factor analysis would represent a reduced number of factors, was confirmed; three significant factors were extracted, as was a single higher order factor. An interesting and useful factor structure was identified in the behavioral assay data as a result of the factor analysis.

The first extracted factor had salient variable loadings for variables from the rotarod, specifically all three of the rotarod blocks, as well as the fifth block of spatial water maze trials. It explained approximately 34 percent of the total variance in behavioral assay data. Higher variable loadings suggested a stronger relationship to the factor; in this case, rotarod variables were most closely related to this factor and the water maze variable was somewhat less related. This water maze variable was negatively related to the other variables loaded on the same factor, as higher rotarod scores represent better motor function while a lower cumulative distance from the platform represents better learning. Therefore better rotarod performance was also associated with better water maze block five performance.

As was previously reported, the mice that comprised the majority of this study’s sample had a learning curve that was flattened in the last two blocks of the water maze. Therefore, their performance on the fifth block of the water maze may have been more influenced by their swimming skills (which would be suggestive of good motor function) than by their learning at that late point in the test. This conclusion explains why block five of the water maze would load saliently on the first factor with motor data as well as cross-load onto the second factor with the remaining water maze data. Given the very high loadings of the more difficult blocks rotarod data, a test of motor function with a
component of strategic learning, this first factor was determined to represent Strategic Motor Function.

The second extracted factor had salient variable loadings for all five blocks of spatial water maze data. This factor accounted for a further 13 percent of observed variance in behavioral assay data. For factor two, blocks three and four from the water maze had the strongest relationship to the factor. For the mice that composed the majority of the sample for the factor analysis, the greatest amount of learning occurred between blocks two and four, with less learning between blocks four and five. This means that the cognitive load was highest for these blocks of the water maze that loaded highest on this factor and somewhat lower for blocks one, two, and five, which were less strongly associated with factor two. Like the rotarod, the water maze requires strategic learning from block to block. Animals must not only learn the location of the platform in space, but the animals that perform best are also more efficient at searching for the platform. Due to the apparently strong relationship of the water blocks engendering the highest cognitive load to this factor, this second factor may represent Strategic Learning.

The third factor had salient loadings for time spent in the center of the open field, the activity index, and the anxiety index, where the anxiety index was inversely related to the other two variables. The composition of this factor supports the previous supposition of this study that animals that are more active on the open field test also tend to appear to be less anxious on the zero maze. Thus the zero-maze might be considered to be more a test of activity level or exploration than of true test anxiety-like behavior. Animals may choose to avoid exploring both the open field and the zero-maze apparatus, which are potentially dangerous, in the absence of any motivation to the contrary. This behavior
might occur without any appreciable fearfulness on the part of the rodent. This factor therefore may represent Activity Level.

The mean of the absolute values of the correlation between the three extracted factors exceeded .30 and was thus suggestive of a higher order factor, so a single factor was extracted. This factor had salient loadings for nine of the 11 variables included in the factor analysis. All three rotarod blocks had the highest loadings onto this higher order factor. Water maze variables collectively had the second highest set of (inverse) variable loadings where the blocks loaded in reverse order. This suggests that water maze blocks that had had higher levels of cognitive load on the rodents were more strongly associated with this factor in general. The time spent in the center of the open field also loaded inversely onto the higher order factor, suggesting that animals that had better performance on the rotarod and water maze also tended to spend less of their time in more exposed center of the open field.

Both the rotarod and water maze require coordinated motor function for task completion, though pure motor ability alone is not likely to account for the strong relationship here observed between rotarod and water maze performance. Past research has demonstrated that repeated rotarod exposure and training improves performance beyond what can be explained by physical fitness and motor training on a similar task (Buitrago et al., 2004; Cendelin et al., 2008). Similarly, animals exposed to the water maze protocol for a second time subsequent to a significant interval from their first exposure retain some memory of the task that benefits performance. Their learning curves are steeper overall, indicating they learn the location of the platform more rapidly the second time around (Cendelin et al., 2008; Blokland et al, 2012). These findings
indicate that some strategic learning, or learning to learn, affects performance on both the water maze and rotarod. The structure of the higher order factor also indicated that animals that had greater strategic learning ability also spent less time in the center of the open field; possibly these animals were carefully avoiding exposing themselves to a more dangerous portion of their environment, an avoidance of risk that could be associated with intelligence and a tendency toward self preservation. Overall, the variables that had salient loading onto the higher order factor appear be related to an ability to learn from the environment in an organized and strategic fashion so as to avoid unpleasant immersion in water, falls from the rotarod apparatus, and unnecessary exposure to a risky environment. The higher order factor may therefore be Strategic Learning Ability.

The items loading onto the higher order factor has implications for future behavioral testing and battery composition. Rotarod and water maze performance are highly related, so data from the water maze test may be able to provide information about motor function either in addition to or instead of data from the rotarod. It is possible that swim speed may serve as an acceptable indicator of motor function. Unfortunately, it was not possible to include swim speed in the exploratory factor analysis conducted for this study, as doing so would have limited the size of the sample to an unacceptable degree. Swim speed data was available for too few animals that also had data available for all other assays. Future studies should therefore evaluate the nature of the relationship between swim speed and motor function.

This study found that there were three salient factors in behavioral assay data, Strategic Motor Function, Strategic Learning, and Activity Level, although together these factors only account for approximately 58 percent of the total variance in test
performance. On the basis of these findings, it is not possible to identify a reduced battery of behavioral assays that would reliably capture the same amount of information as the standard battery used for the factor analysis in this study: water maze, rotarod, zero maze, and open field activity.

However, not all researchers consider identification of rodent behavioral outcomes a primary research objective. Often the extensive time and budget resources required for administration of this comprehensive battery are not available. In these cases, a strategically designed battery could identify initial behavioral outcomes and indicate the need for future behavioral testing without consuming too many resources. A streamlined battery consisting of the Morris Water Maze and open field activity test could provide preliminary information about rodent behavior across domains, but would not provide the full amount of information generated by the complete four test battery described above. The water maze could potentially provide information about learning and motor function and the open field test could provide information about activity and an indication of anxiety-like behavior.

This study had several limitations. Data collection occurred over the span of several years in multiple experiments and laboratory environments. Although test protocols were theoretically the same, there may have been subtle variations in test administration. The sample for this study comprised several breeds of rats and mice; breed differences possibly introduced additional variability in performance. A number of the observed effect sizes were quite small. In those cases, significant findings are of questionable practical relevance.
Future research is necessary to confirm and extend some of this study’s findings. The data regarding animal age limited the capacity of this study to elucidate neurocognitive changes in old age; the comparison was essentially made between juveniles and adults of varying age. Future research is required to identify performance profiles unique to adult and older adult animals. Confirmatory factor analysis should be conducted on behavioral assay data to improve the understanding of the factor structure of behavioral test performance. It would be beneficial to include swim speed in such a confirmatory model to better identify the relationship of coordinated motor function to strategic learning and determine whether or not swim speed could be used as a proxy for tests of motor function.

In summary, this study provided evidence to show how rodents differ according to species, sex, age, and type of injury, informing how rats and mice might be more effectively used in future experiments. Rats and mice have similar abilities on a measure of spatial learning and motor function, although rats exhibited the ability to continue to learn with greater exposure while mice did not. Notably, sex differences were present and were not equal for both rats and mice. Mice demonstrated more pronounced sex differences in motor ability than rats, while the reverse was true for spatial learning. This means that a homogenous male sample is preferable where motor function is of primary interest. Although rats had greater sex differences on the water maze, they also produced a more consistent learning curve, meaning that rats are preferable subjects when spatial learning is of primary interest. Overall rats produced more consistent data that was not near the floor of tests protocols, making them better experimental subjects overall.
Rodent models of brain injury were found to have distinct cognitive and behavioral profiles depending on the brain structures involved that broadly resemble the cognitive deficits and behavioral changes observed in humans. This finding confirms that rodent models of brain injury are, to some extent, appropriate behavioral models of human brain injury.

Finally, results of an exploratory factor analysis suggested that rodents’ activity levels and ability to learn strategic behaviors play a significant role in how these animals perform on behavioral tests across domains of learning, motor function, activity and emotionality. The three significant extracted factors only explain about 58 percent of animal performance together; therefore paring a comprehensive behavioral test battery down to fewer tests is not desirable. However, on occasions where behavioral outcomes are of secondary interest, or when time and budget resources limit testing (i.e. pilot study) the water maze and open field activity test may be suitable preliminary indicators of neurocognitive function across domains.
REFERENCES


