

5-14-2010

Individuals Differences in Exploratory Behavior of Prairie Voles, *Microtus ochrogaster*

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Individuals Differences in Exploratory Behavior of Prairie Voles, *Microtus ochrogaster*

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A Dissertation Submitted to The Graduate School at the University of Missouri – St.
Louis in partial fulfillment of the requirements for the degree
Doctorate of Philosophy in Biology

March 2010

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ACKNOWLEDGEMENTS

First and foremost, it has been a joy to have studied with the scientists in this department. They have been amazing role models and leaders in academia. Of special note are Dr. Patricia Parker, Dr. Elizabeth Kellogg, Dr. Charles Granger, Dr. Patrick Osborne, Dr. Robert Marquis, and Dr. Godfrey Bourne. Each one has made an indelible impression on me as I paved my own path in science, science education, and science outreach. Plus, I appreciate your support (financial and moral) along the way. My peers are among the best scientists in the world, too. Many thanks to all of my fellow biology graduate students I have come to know. Finally, a special thank you to my lab mates (The Tang Gang) have been amazingly helpful and supportive every step of the way: Jaynie Doerr, Debbie Boege-Tobin, Elizabeth Congdon, Susan Gustafson, Courtney, Endermule, Jorge Hurtado, Derrick Hildreth, Marcella Fernandez, Laura Riley, Gena Sbeglia, Javeri Hernandez, and Tim Lescher.

I am most appreciative of the many research assistants I had. I owe a very big thank you to Alecia Hoyt – who was my first and best assistant. She set the bar way too high for most people to reach. Thank goodness I met more great undergraduates who offered assistance, without whom I would not have been able to collect as much data as I did: Meital Laks, Adrian Jeffries, Dianna Voorhies, Anna Lynn Harris, Kimberly Immer, Evynn Johnson-Craven, and Dominique Crave. I am also indebted to my siblings who were my very first research assistants – my siblings: Jasmine, Allison, and Robert Clayton. My first year of graduate school they helped me locate carry bound scientific journals, photocopy papers, set up experiments, and clean up the lab. They are the best scientific helpers in the world. I love you guys, my little bundles of inclusive fitness.

I would like to acknowledge the voles. Without them, this research would not have been possible. Related to that, thank you to Nancy Solomon and her lab, including Loren Hayes, Kristen Lucia and Todd Levine for providing breeding stock. Additional thanks to Mr. Steve Buck, Dr. Lowell Getz, and the Ferguson Family of the University of Urbana-Champaign, Illinois, for their help when I was in the field trapping and collecting voles for the study.

I am also grateful for great guidance. Thank you to Dr. Karen Norberg for her many selfless hours of assistances with statistics. Plus, thank you to my dissertation committee: Dr. George Taylor, Dr. Stan Braude, Dr. Lon Wilkens, and especially have been a great team of committee members. Finally, thank you Dr. Zuleyma Tang-Martínez, my advisor. When we met in 1999, she told me I would be her last Ph.D. student. Neither of us realized how true that statement would be. It's been a fun learning experience. Thanks!

And last, but certainly not least, I am thankful for the all of the prayers and words of support and encouragement I have received from friends, family, and blog readers. You all have really kept me on my toes and I have enjoyed every moment of sharing my personal science journey with you.

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INDIVIDUALS DIFFERENCES IN EXPLORATORY BEHAVIOR OF PRAIRIE VOLES, *MICROTUS OCHROGASTER*

Individual differences in behavior are significant because they serve as the substrate for natural selection. Within the Behavioral Syndromes framework, researchers study individual differences in behavior of animals. Behavioral Syndromes are defined as correlations between behaviors in different environmental contexts or testing situations. In this study, I examined the effects of litter size and sex ratio, familial relationships, as well as age and sex on exploratory behavior of prairie voles, *Microtus ochrogaster*. Exploratory behavior, defined as spontaneous behavioral responses to unfamiliar stimuli, was examined in three novel situations: an open-field with novel objects, a two-way novel choice apparatus, and a complex maze. Each test was found to measure a different exploratory behavior axis: the open-field test with novel objects measured interactive behavior, the exploratory maze measured general activity behavior, and the two-way novel choice test measured proactive/reactive behavior in response to novel environments. No correlation of behavioral responses across the three tests was found, thus providing no evidence of an overall exploratory behavioral syndrome in this species. On the other hand, there was considerable individual variation in behavior within each test and some of this variation could be explained by the independent variables examined. Litter size and to a smaller degree, age explained exploratory behavior in the open-field. Subjects from large, socially complex litters and young subjects were less interactive in the open-field with novel objects than subjects from smaller litters and older subjects. In the maze, subjects who were the only ones of their sex in a litter entered the maze sooner than subjects from all other litter compositions; there also was a tendency for females to travel longer distances within the maze than males. However, I found no relationship between behavior in the two-way novel choice apparatus and the independent variables of interest. Across all three tests, most subjects across families demonstrated similar behavioral tendencies, as a result I concluded that the general character of this population of prairie voles includes being highly interactive, more active, and proactive. Overall, the results of this study raise questions about the interpretation of behavioral responses and the identification of behavioral syndromes.

CHAPTER ONE

EXAMINING INDIVIDUAL DIFFERENCES AND EXPLORATORY BEHAVIORAL RESPONSES IN PRAIRIE VOLES: IN SEARCH OF A BEHAVIORAL SYNDROME.

ABSTRACT

Behavioral syndromes, also called behavioral phenotypes or profiles, are defined as correlations between behaviors in different environmental contexts or testing situations. But how does one accurately measure and determine what behavioral syndrome a subject demonstrates? The popularity of behavioral syndrome research has yielded many different methods of examining and interpreting behavioral phenotypes. For example, many research teams have identified behavioral syndromes by correlating behaviors from different tests representing different contexts such as exploration, foraging or social interaction; however many of these same researchers failed to test whether there is any correlation of behaviors within a single context. As a result, some other researchers question whether correlated behaviors across contexts are truly related or if those behavioral correlations are artifacts. My objective was to determine *if* prairie voles, *Microtus ochrogaster*, demonstrate correlated behaviors in different situations within a single context – exploration. Exploratory behavior responses were examined in three novel situations: an open-field with novel objects, a two-way novel choice apparatus, and a complex maze. For each situation, behavioral responses were identified by key dependent variables determined by Principal Components Analysis. Three different exploratory responses emerged: the open-field test with novel objects measured interactive behavior, the complex maze measured general activity behavior, and the two-way novel choice test measured proactive/reactive behavior in response to novel

environments. For each test, subjects were ranked from low to high exploratory tendency, thus creating three exploratory behavioral responses. The exploratory behavioral responses were compared and there was no correlation across tests, thus providing no evidence of an overall exploratory behavioral syndrome in this species. In light of these findings, each exploratory test appears to measure different and uncorrelated aspects of exploratory behavior. Recently, an increasing number of studies of behavioral syndromes similarly have failed to find a correlation of behaviors across tests *or* contexts. These results raise questions about the ability to identify personality types in animals and the validity of behavioral syndromes as a general attribute of animal behavior.

Key words: individual differences, behavioral phenotypes, behavioral syndromes, exploratory behavior, open-field test, prairie vole

INTRODUCTION

A fundamental assumption of behavioral ecology is that individuals vary in their behavior (Loughry & Lazari 1994). These individual differences in behavior are significant because they may signal an important or unique response to stimuli and serve as the substrate for natural selection. Nonetheless, behavioral ecologists traditionally approached the study of animal behavior assuming most animals behaved optimally, with the expectation that individual variation in behavior would be slight. In other words, we expected most individuals to exhibit behaviors that fall approximately within the mean value for the population with relatively limited variation around the mean (Wilson *et al.* 1993). Subjects responsible for skewed or outlying behavioral measures were often dismissed as aberrant (Drummond & Gordon 1979). Such examples of unusual or extreme individual variation were generally regarded as the result of a mistake on the part of the researcher and/or an inconvenience because too much variation frustrates our ability to demonstrate clear patterns in behavior (Groothuis & Carere 2005). However, seminal studies by Clark and Ehlinger (1987) and Wilson *et al.* (1993) redirected the attention of ethologists to the importance and adaptive significance of individual variation of behavior.

Following these publications, several ethology and behavioral ecology research groups began studying individual differences in animal behavior. Borrowing terminology from psychology was common and in early published works, researchers used terms such as ‘animal personality’, ‘animal temperament’, and ‘personality types’ to describe individual variation in behavior (Lyons *et al.* 1988; Budaev 1997). For example, developmental psychologists have proposed that the shy-bold continuum may be a

fundamental axis in human behavioral variation (Kagan *et al.* 1988) and Wilson *et al.* (1994) applied the concept of a ‘shyness - boldness continuum’ to animal subjects. As more behavioral ecologists became involved in studying individual differences in behavior, many followed in their footsteps, also examining shy-bold tendencies of other species. Later, the terminology was revised to reflect ecological perspectives, with Budaev (1997) adopting the operationally-defined term ‘behavioral phenotype’ as the statistical tendency for each individual to behave consistently across situations and over time. Later, Sih proposed the concept of ‘behavioral syndromes’ (Sih *et al.* 2004a, b) and Groothuis & Carere (2005) offered the term ‘behavioral profile’ to describe consistent behavioral tendencies or ‘dispositions’ that transcend behavioral contexts and focused on correlated behaviors (Bell & Stamps 2004; Bell 2007).

However, with animal personality research being rooted in psychology, the study of behavioral types was examined in one of two ways, via 1) the emotional component or 2) the response activation component analysis (Fairbanks 2001). An emotional component study is concerned with the emotional state of the animal and examines shyness, anxiety, and behavioral inhibition (Fairbanks 2001). For example, studies with fish that measure individual behaviors along a shy-bold or aggressive-passive continuum (e.g., pumpkinseed fish, *Leopomis gibbosus*, Wilson *et al.* 1993) examine the emotional component of behavior. Likewise, experiments with laboratory mice that allow subjects to move freely from a familiar environment, such as a home cage, to an unfamiliar open-field, called a ‘free exploration test’, are used to evaluate neophobic and exploratory behaviors (Kopp *et al.* 1999). In contrast, a response activation component study examines the physical responses of subjects to an experimental situation (Fairbanks

2001). For example, studies that measure latency and response times in novel settings or with novel objects examine the response activation component of behavior (Fairbanks 2001). This type of study is not concerned with inferences about internal states of behavior; rather, in this case operational definitions of individual behavior would measure the tendency of subjects to behave ‘quickly vs. slowly’ or ‘impulsively vs. considered’.

Clearly, individual variation in behavior is a requisite for studying behavioral syndromes, with individual distinctiveness being the tool to determine the behavioral phenotype of individuals. Presently, much of the research in behavioral syndromes assigns a behavioral profile to subjects along a continuum. Many of these behavioral continua are given labels referring to the presumed motivational, emotional or psychological state of the animal (e.g. anxiety-fear, risk-prone vs. risk-averse, aggressive-social vs. non-aggressive-non-asocial, agonistic-active vs. non-agonistic-passive, bold-aggressive vs. shy-non-aggressive) as opposed to the response activation components of behavior (Wilson *et al.* 1993). Such emotionally biased terms and the methods used to examine individual behavior may or may not reveal the true behavioral tendencies of subjects (Boissy 1995; Bell 2007). Moreover, behavioral syndrome labels that focus on the emotional component of personality types e.g., shy, bold, anxious or fearful, can be ambiguous and difficult to measure (Groothuis & Carere 2005).

Behavioral syndromes cannot be fully evaluated until context and stability are also taken into account. Context refers to a functional behavioral category such as feeding, exploration, anti-predator, courtship and mating, or parental care. Broad behavioral syndromes involve correlations of behaviors from tests from two or more of

these categories. Domain-specific behavioral syndromes are measured as correlations of behaviors from two or more tests representing a single context (Sih *et al.* 2004a, b).

In addition to context, behavioral responses are classified according to their stability. Behavioral responses that only occur in specific situations are called state responses (Kopp *et al.* 1999). A state response differs dramatically from a trait response which is defined as a behavioral response that carries over from one situation to another and is considered to be a stable characteristic of the behavior of an animal (Kopp *et al.* 1999). These components of the definition of behavioral syndromes are closely related though not always fully explained in individual studies. For example, studies that have investigated individual variation in behavior across multiple ecological contexts (e.g., a social dyad test and a locomotor test, or an anxiety test and a predator-cue response test) are examples of a broad behavioral syndrome and a trait response, i.e. correlations of behaviors across more than one contextual category (e.g., Benus *et al.* 1991; Helsing *et al.* 1993; Koolhaas *et al.* 2001; Malmkvist & Hansen 2002). Likewise, studies that have investigated individual variation in behavior within a single ecological context (e.g., novel situation exploration, Verbeek *et al.* 1994; threat response, Coleman & Wilson 1998; human handling, Reale *et al.* 2000) are examples of a domain-specific behavioral syndrome and a trait response, i.e. correlations of behaviors across tests within the same context.

Studying individual differences in behavior and investigating behavioral syndromes can inform our understanding of the maintenance of individual variation in behavioral types (Sih *et al.* 2004a). Many of the earliest research studies focused on identifying correlated suites of behaviors from multiple contexts (Sih *et al.* 2004b); but it

was not always very clear why certain contexts and experimental tests were chosen or the intrinsic connection between the tests. As a result behavioral syndrome research is complicated because researchers must address two important issues: 1) determining the criteria for comparing behavioral responses in two or more situations, and 2) determining which behaviors or contexts are best or most appropriate to study. Bell (2007) examined these fundamental issues and presented two approaches that specifically addressed the concerns of behavioral ecologists and ethologists. The first, **candidate approach**, involves examining relationships between behaviors already commonly known to be associated from previous studies or in other species. The second or **ecological approach** involves studying behaviors selected because they are plausibly relevant to the fitness consequences of the ecology of the species of interest. Additionally, in the ecological approach behavior can be scored along an axis.

Taking these recommendations of Bell (2007) into account, my study investigates the presence or absence of correlated behaviors during exploration of different kinds of novel environments. Exploratory behavior, defined as the tendency to investigate novel environments or stimuli (Renner 1987; Hughes 1997; Draai *et al.* 2001; Dingemanse *et al.* 2004), has often been proposed as a behavioral profile or behavioral phenotype (Benus *et al.* 1991; Hessing *et al.* 1994; Verbeek *et al.* 1994, 1996, 1999; Wilson *et al.* 1994; Reale *et al.* 2000; Dingemanse *et al.* 2004; Groothuis & Carere 2005; Bolhuis *et al.* 2005; Fox *et al.* 2009). Behavioral reactions to unfamiliar situations are a distinctive source of individual variation in humans and other animals (Kagan *et al.* 1988). Moreover, exploration of novel environments and novel objects are also a relatively well-studied behavioral context with ecological implications for many species, (e.g., sunfish, *Lepomis*

gibbosus Wilson *et al.* 1993; great tits, *Parus major*, Verbeek *et al.* 1996, Dingemanse & de Goede 2004, Carere *et al.* 2005, Groothuis & Carere 2005; wood mice, *Adopemus sylvaticus*, Stopka & Macdonald 2003; laboratory rats, *Rattus norvegicus*, Whishaw *et al.* 2006). Intrinsic or spontaneous exploration is defined as exploratory behavior absent of obvious motivations such as hunger, predation risk, or reproduction (Renner 1987; Hughes 1997). It includes behavioral responses that allow individuals to gather information about their local environment that could potentially introduce them to mates, food, shelter sites, and predators. Measures of exploratory behavior include reactivity to the environment and activity or locomotor behavior (Russell 1973) and both can be scored along an axis. Differentiating activity and exploratory behavior in a testing situation is not always obvious (Groothuis & Carere 2005), though both are important behaviors. Although they are related, ‘activity’ measures the movement of an animal in an environment, whereas ‘exploration’ measures approach to or investigation of novel objects or aspects of the environment. Moreover, exploratory behavior may have important consequences for the life of the individual and can be key to survival and reproductive success (Verbeek *et al.* 1994).

In the present study, I observed a cross-section of behaviors of individual prairie voles in a single context but under different conditions (Sih *et al.* 2004b). Thus, my study can be considered a domain-specific examination of a trait response. Exploration of novel settings is the singular context and I was looking for correlations of behavioral responses to the novel testing apparatuses. The objectives of this study were to determine if a) individual behavioral responses in different test situations are correlated and b) if these differences contribute to an overall exploratory behavioral syndrome. To

accomplish this objective, I scored exploratory behavioral responses, based on key dependent variables from each test. Then, these scores were compared to one another. If an overall exploratory behavioral syndrome exists, then I expect to find a relationship among the individual exploratory scores.

GENERAL METHODS

1. Animals

Male and female first through third generation, lab-reared prairie voles, *Microtus ochrogaster*, from Urbana-Champaign, Illinois, were the subjects in these behavioral tests. Individuals were reared under a 14:10 LD light schedule. Lab conditions, including frequency of handling, cage cleaning, and feeding, were constant to all animals. Animals were reared in early social environments that consisted of naturally occurring littermates. Natural litter sizes vary from 1-8, with 3-4 being the average size. Litter sex ratio is also naturally variable and was characterized as the subject having a) no siblings, b) same-sex siblings only, c) opposite sex siblings only, and d) at least one sibling of each sex. All voles were weaned at 21-23 days of age and were housed with littermates, if any, throughout life.

Voles were tested during the light phase of the time cycle between 10:30 -16:00 CST hours. All subjects were tested post-sexual maturity at 55 days of age or older (sexual maturity occurs at 40 days), and were sexually inexperienced. Age variation was minimized whenever possible; however, age at testing did vary. Subjects completed each behavioral test only once so that all individuals remained naïve to each subsequent testing apparatus.

Procedures

Three different exploratory tests were administered. Specific behavioral activities described below were recorded. Each subject, (sample size=168), completed one or more of the tests and the order in which these three tests were presented were randomized.

Fifty-two subjects completed all three tests.

Test 1. Open-field with Novel Objects

Apparatus

The “open-field” arena consists of a black floor, 90 x 90 cm², enclosed by 70 cm high white walls on each side and covered with a clear acrylic sheet. The floor of the arena has a total area of 8100 cm², grid-marked in thirty-six 15 cm² squares. The apparatus was divided into three concentric sections, 1) **edge** which is comprised of the twenty grid squares along the periphery (4500 cm² in area), 2) **intermediate** which is comprised of the twelve grid squares adjacent to the edge (2700 cm² in area), and 3) **center** which is comprised of the four grid squares in the middle of the open-field (900 cm² in area). In each of the corners I arbitrarily placed four distinct novel objects, a) a piece of clear PVC tubing (5 cm long and 2.54 cm diameter), b) 15 cm² of AstroTurf, c) two pebbles of aquarium rock (1.8 cm diameter), and c) a plastic hand mirror (7.6 x 5 cm). Adding novel objects to an open-field allows measurement of a broad range of behaviors in order to capture the complexity inherent in spontaneous exploratory behavior, which would include interactive behavior (Renner & Rosenzweig 1986; Renner 1990; Verbeek *et al.* 1994; Hughes 1997). The novel or stimulus objects were classified as manipulable (Renner & Seltzer 1991) because all were small in size and could provide

kinesthetic feedback when subjects interacted with them. A photo of the open-field with novel objects is presented in Figure 1.

Methods

The subject was placed in a Plexiglas start box, 13.8 (w) x 13.4 (l) x 13.8(h) cm. The start box contained a ventilated Petri dish, 9.5 cm in diameter, made of Nalgene plastic with the two lids attached to one another by a screw and nut with many holes drilled into the upper lid. The Petri dish was filled with scented bedding from the home cage of the subject and mounted to the inside wall of the start box with scotch tape. The bedding-filled Petri dish provided own odor from the subject thus making the start box a location of familiarity (Hughes 1997). Such an experimental set-up is best for examining spontaneous exploratory behavior and is considered ecologically relevant (Hughes 1997), as well as preferred by rats and mice (Russell 1975; Misslin & Ropartz 1981). The start box was placed in the center of the open-field and covered with a black cloth. (The start box remained within the open-field throughout the duration of the test and occupied 185 cm² of the center section). The subject remained in the darkened start box for 5 min to acclimate. Then, the black cloth was removed, the door of the start box was opened with a Solenoid remote control, and the subject was free to leave the start box. The entire test was video recorded and scored from the footage. The following measures were recorded: 1) latency to depart the box (in seconds), up to 10 min; 2) time spent in the novel environment (in seconds); 3) total time spent interacting with novel objects; 4) number of returns to the start box; 5) number of grid squares crossed every 5 seconds during the entire test (5 min long), (methods and measures comparable to McPhee 2003), and 6) number of visits to the edge and center sections of the open-field. The total time of the

test was 5 min after initial exit from the start box. Between tests, the start box, the Petri dish, the novel objects, and the arena were cleaned with soap and water and disinfected with a 15% ethyl alcohol solution to eliminate any odors that might have accumulated from the previous subject.

All behavioral observations were recorded under full illumination with a low-light video camera and video recorder. The video camera was mounted approximately 1 m above the open-field arena. All additional equipment was placed in an adjacent room. For subjects that did not leave the start box after 10 min, the maximum latency time was recorded and zeros were recorded for other measures and included in the data analysis.

Test 2. Two-way Novel Choice

Apparatus

The two-way novel choice apparatus is comprised of a centrally located start box, 153 (w) x 101 (l x h) mm, connected to two runways each made of a long Plexiglas tube, 500 (l) x 7.5 (d) mm. White opaque doors (guillotine-style, made of acrylic plastic) separate the start box from each runway. The terminal of each runway is connected to another box of the same dimensions as the start box. However each runway tube terminates at a screen door (made of opaque acrylic plastic and fine mesh). A schematic of the two-way novel choice apparatus is presented in Figure 2.

Methods

The subject was placed into the start box which contained a ventilated Petri dish, 9.5 cm in diameter, made of Nalgene plastic with the two lids attached to one another by a screw and nut with several holes drilled into the upper lid. The Petri dish was filled with scented bedding from the home cage of the subject and mounted to the inside wall of

the start box with scotch tape. The bedding-filled Petri dish provided odor from the vole thus making the start box a location of familiarity (Hughes 1997). Such an experimental set-up is best for examining spontaneous exploratory behavior and is considered ecologically relevant (Hughes 1997), as well as preferred by rats and mice (Russell 1975; Misslin & Ropartz 1981). The subject remained in the start box for 3 min to acclimate. Then, the doors allowing access to the runways were manually lifted. The following measures were recorded 1) latency to depart the start box (in seconds); 2) time to reach the first terminal after leaving the start box; 3) time to reach the second terminal after the animal visits the first terminal; and 4) total time to complete the test, measured as the time to visit both terminals minus initial latency. A subject was considered to have reached a terminal if its nose came within 3 cm of the screen door of each terminal. This 3 cm region was referred to as the proximity threshold zone.

Each subject completed two trials of this test, once with novel odor stimuli (vanilla and lemon extract) behind each screen door, and once without any novel odors. The order of the trials was counter-balanced. Vanilla and lemon scents were used because the subjects had no previous exposure to them and they were unlikely to be aversive. A drop of vanilla extract and lemon extract was placed on separate filter papers and placed inside of a closed Plexiglas box behind the screened terminal door approximately ten seconds before the start of the trial. A different scented filter paper was randomly placed on each side of the apparatus. The time between the two trials was approximately 30 min. Each trial ended when the subject visited the second terminal. The total time of the test was variable, but if a subject did not leave the start box by 5 min, or became inactive for more than 5 min after initiating the test, then the test was

ended. The maximum time was recorded as the time to complete the test and the remaining measures were left blank, and included in the data analysis. Between tests the start box, the Petri dish, terminal boxes, and the runway tubes were cleaned with soap and water and disinfected with a 15% ethyl alcohol solution to eliminate any odors that might have accumulated from the previous subject.

Test 3. Complex Maze

Apparatus

The maze is a multi-arm labyrinth, 610 (w) x 396 (l) x 12.5 (h) cm, made of a white acrylic base (floor) with black plastic walls with 7.5 cm wide corridors. The maze consists of three arms and five terminals. Each terminal varies in path orientation and distance from the entrance corridor: terminal 1 (15 cm from entrance); terminal 2 (500 cm); terminal 3 (560 cm); terminal 4 (835 cm); and terminal 5 (1095 cm). A schematic of the exploratory maze is presented in Figure 3.

Methods

The subject was placed into a start box, 7.3 (w) x 40 (l) x 6.8 (h) cm, made of white acrylic plastic. The start box opens at the maze entrance corridor. The subject was kept in the start box for 3 min to acclimate. Then, the swinging-hinge access door (made of opaque Plexiglas) was manually pushed open causing the start box space to contract by 2-3 cm. With the swinging door ajar, the subject could choose to proceed into the entry corridor. Once the subject stepped onto the maze floor with all four feet, it was scored as having entered the maze. Once in the maze, the subject could proceed in any of four directions, to the right (arm 1), straight ahead (arm 2), to the left (arm 3) or backwards into the start box. The following data were recorded: 1) latency to depart the

box (in seconds), up to 2 min; 2) number of returns to the start box and 3) number of times each arm was entered. The total time of the test was 3 min after initial exit from the start box; there was no food or other reward in the maze. For subjects that did not leave the start box within the allotted time, the maximum latency time (2 min) was recorded and zeros were assigned for other measures and included in the data analysis.

All behavioral observations were made under reduced illumination (red light) and the observer was standing over the testing apparatus. Infrared wavelengths of light are poorly visible to rodents but still allow researchers to observe behaviors (Finley 1959). Prairie voles are known to be active in both light and dark cycles (Grippo *et al.* 2007) and reduced illumination observations are common for observing dark-cycle activity in rodents (Zurn *et al.* 2005). In this study, reduced illumination was used to mediate the negative effect of having the observer stand over the apparatus during testing. Between tests, the start box and the arena were cleaned with soap and water and disinfected with a 15% ethyl alcohol solution to eliminate any odors that might have accumulated from the previous subject.

Data Analysis

Behavioral parameters followed Viérin & Bouissou (2003). Specifically, data were analyzed using SPSS 15 and 16 statistical packages to identify relationships among multiple variables and to determine the most important measures for determining exploratory profiles. I analyzed measures of exploratory behavior for each test individually using Principal Components Analysis (PCA). PCA separates individuals in a sample in terms of a few independent components that represent the underlying dimensions of the data, and determine which dependent variables best characterize each

component (Math & Anderson 1993). Dependent variables can be interpreted according to their loadings on the most important components, which explain how much of the variability is due to those variables. Variables having a high loading on a component are highly correlated to this component. Only components with an eigenvalue larger than 1 and with dependent variables of a loading of 0.80 or higher were retained for interpretation and cross-test comparisons. Those components accounting for only a small part of the total variability and with dependent variables of low loading values were not further analyzed.

A general PCA was conducted with all the measurements from the three tests. No dominant factors resulted and this analysis yielded no information about the relationship among the variables or tests. The moderately loading factors reflected the nature of the test (open-field with novel objects, two-way novel choice, and exploratory maze); therefore the decision was made to analyze the three tests separately (Viérin & Bouissou 2003).

Exploratory scores were calculated for each subject, and for each test based on the high loading variables. High loading dependent variables were ranked according to their raw values from low to high. Next, the ranks of these variables were averaged to yield an exploratory score for each subject. Exploratory scores are along a continuum creating a gradient ranking system, (see Figure 3). Scores at or below the median value were indicative of subjects having lower values for recorded measures; scores above the median value were indicative a subject having higher values for recorded measures such as time spent in novel environment. High loading dependent variables from each test

were provided a label that subjectively described that dimension of personality (Mather & Anderson 1993) and this was the assigned exploratory profile for that test.

High loading dependent variables from each test were compared using a Pearson Correlation, p -value set at 0.05 to determine if there is a relationship among key dependent variables within each test. I compared the exploratory scores from each exploratory test to determine if there is a relationship among the three exploratory scores from each of the tests. The sample sizes for each test were unequal, so the exploratory scores for each test were re-coded as percentiles to normalize the data before comparing them. I also compared the latency to depart the start box from each exploratory test to determine if there is a relationship among directly comparable behavioral responses from each of the tests.

RESULTS

Principal components analysis identified a primary principal component (PC1) for each test. PC1 is the component that accounted for the highest degree of variability for the dependent variables measured. Those dependent variables with high loadings (0.80 or higher) for PC1 were identified as key dependent variables and an exploratory behavior profile was determined. The relationship of key dependent variables with the factor score, the exploratory profile and the correlation of key dependent variables is reported below.

Open-field with Novel Objects

A principal component analysis was conducted using eight dependent variables. See Table 1a. Two dependent variables had high positive loadings: total visits to the edge of the open-field (0.945) and time spent in the novel environment following initial exit

(0.820); I labeled this factor the degree to which a subject *interacts* with the novel environment. These key dependent variables contributed to the PC1 factor eigenvalue of 3.753. The PC1 factor accounted for 46.9 % of the variance. The within test correlation of these key dependent variables was highly significant. See Table 1b.

Two-way Novel Choice

A principal component analysis was conducted using eight dependent variables. See Table 2a. Three dependent variables had high positive loadings: total time to complete the test in trial one (0.906), the split time in trial one (0.860) and the difference score in trial one (0.821). The presence or absence of novel odor in trial one was inconsequential. I labeled this factor the degree to which a subject *reacts* to the novel environment. These key dependent variables contributed to the PC1 factor eigenvalue of 4.393. The PC1 factor accounted for 54.9% of the variance. The within test correlation of these key dependent variables was highly significant. See Table 2b. No measures from trial two loaded heavily on PC1 and therefore could not be used to explain this exploratory behavior profile.

Complex Maze

A principal component analysis was conducted using six dependent variables. See Table 3a. Three dependent variables had high positive loadings: sum of visits to all three arms of the maze (0.979), number of visits to arm 3 (0.888) and number of visits to arm 2 (0.818). I labeled this factor the degree to which a subject is generally *active* within the novel environment. These key dependent variables contributed to the PC1 factor eigenvalue of 3.482. The PC1 factor accounted for 58.0% of the variance. The within test correlation of these key dependent variables was highly significant. See Table 3b.

Inter-test Correlations

Comparing the exploratory scores from each test of all subjects as percentiles revealed there was no correlation of exploratory scores across the three tests. See Table 4. To further examine the data, I only compared the ranked exploratory scores of subjects that completed all three tests thereby creating equal sample sizes, N= 51. Despite this standardization, there was no correlation of scores. See Table 5a.

There was a significant positive correlation in the latency to depart the start boxes in the open-field and two-way novel choice apparatuses. The correlation of latency to depart start box in either these tests between that of the exploratory maze test was negative, but insignificant. See Table 5b. However, in all three tests latency, failed to load as a significant variable for PC1.

DISCUSSION

The correlation of behavioral responses from the three different exploratory tests failed to demonstrate that an overall exploratory behavioral syndrome exists. The exploratory scores from the three different tests did not correlate with one another. Also, the correlation of the latency to depart start box from the three tests provided mixed results. There was a correlation of measures in the open-field with novel objects test and the two-way novel choice test but no correlation of either of these measures to the latency to depart start box in the complex maze test. The open-field and two-way novel choice tests have similar protocols, which are both quite different than that of the exploratory maze protocol. The open-field and two-way novel choice tests allowed subjects to acclimate in start boxes that contained odors from the subject's home cage and were conducted under full illumination. The maze test start box did not contain home cage

odors from the subject and was conducted under reduced illumination. These procedural differences might account for the relationship of latency to enter the novel environment across these tests. These results with latency measures do call into question the importance of statistically analyzing multiple measures. If all one does is take a single measure arbitrarily from a test, even if it is equivalent across tests, and gets a correlation, what is the relevance of this correlation? A much more rigorous approach of determining important measures is to complete a factor analysis to determine the most significant dependent variables and then correlate those measures. Latency to depart the start box was not a key dependent variable for any of the three novel situation tests, which makes it questionable whether this measure is biologically meaningful. Taking all of my results as a whole, I conclude that no overall exploratory behavioral syndrome was demonstrated.

However, individual differences in behavior were demonstrated with a strong correlation of behaviors within a given test. There was measurable variation in behavior in each testing situation. I discuss the behavioral responses of voles in the different novel environments below.

Open-field with Novel Objects:

The open-field with novel objects test best measured the exploratory behavioral response of *interactivity*. Interactivity is defined as a high interest in novelty, which includes investigating distant or unfamiliar parts of an environment and manipulating novel objects (Renner 1990). As quantified in the results, subjects with high interactivity response scores visited the outer edge of the open-field more than those with lower interactivity scores. They also spent more time sniffing, touching, or in contact with the novel objects in the open-field. These behaviors demonstrate the degree of interaction by

subjects with the novel environment. The more time or increased rate of interaction in novel settings or with novel objects increases the amount and type of information gathered, especially if the novel setting is complex (Drai *et al.* 2001). Gaining more information about the environment is an important component to lifetime fitness because it helps individuals discover and exploit resources opportunistically, e.g., novel food sources, refuges, conspecific scent posts, and mates. This can be beneficial to animals as it relates to recruitment, dispersal, home range and territory size and acquisition in the wild (e.g., great tits, *Parus major*, Dingemanse *et al.* 2003; brown trout, *Salmo trutta*, Adrianenssens & Johnsson 2008).

Two-way novel choice test:

The two-way novel choice best measured the exploratory behavioral response of *proactivity-reactivity*. Proactivity-reactivity is defined according to how quickly or slowly a subject initiates action, and spends its time in a novel situation (Sih *et al.* 2004a, b). Proactive individuals tend to be bold initiators of action, are often observed bolting out into novel environments, and tend to move rather quickly within a novel setting. Reactive individuals tend to move more slowly, seemingly cautiously when introduced to novel settings (Sih *et al.* 2004a, b). In this study, the more proactive subjects reached the first and second terminals in shorter times and completed the entire test faster than subjects labeled as reactive. Depending on the stability of the environment, one behavioral type might be favored over the other. For example, in relatively stable environments, proactive individuals may do better because they quickly procure and utilize resources. However, reactive individuals may do better in variable environments

because they tend to wait and assess situations before taking action, thereby utilizing resources more effectively and avoiding predators.

Complex Maze:

The complex maze test best measured the exploratory behavioral response of *activity*. Activity is defined as the amount of movement within a novel environment. Subjects with high activity scores had higher total number of visits to all arms and to arms 2 and 3, than those with lower activity scores. These animals were moving about the maze more and were often recorded in different locations of the maze over a given period of time. Activity associated with intrinsic exploratory behavior may be of ecological significance because it may indicate the ability of an individual to gather potentially useful information about resources, conspecifics, competitors or predators in the environment (Sih *et al.* 2004a). For a prey species like prairie voles, activity is an ecologically important behavior pattern with competing fitness consequences. Individuals that are highly active encounter resources like food and shelter sites at higher rates and this can positively impact their growth and survival. These same individuals are also likely to encounter predators at higher rates; hence there is a tradeoff (Sih *et al.* 2004a).

Behavioral Syndromes:

Two fundamental components of any definition of behavioral syndrome are 1) there must be a correlation of behavior across situations and 2) behavioral plasticity must be limited between contexts or situations (Sih *et al.* 2004a; Nelson *et al.* 2008). The results of my study failed to meet either of these requirements. Behaviors across tests were not correlated in any way, even though all three tests presumably measured

exploratory tendency. Individuals who were highly exploratory in one test were no more likely to be highly exploratory in either of the other tests. In other words, prairie voles demonstrated strong behavioral plasticity across these three test situations. Only within-test correlations were found, therefore each exploratory test must be independently interpreted. In light of the considerable variation measured for each test and strong within-test correlations we must ask: how does this level of individual variation inform our understanding of behavioral syndromes?

Studying behavioral syndromes is complex and challenging. One complication lies in the discussion as to what is the best evidence of a behavioral syndrome – a broad trait response or a domain-specific trait response (Coleman & Wilson 1998; Sih *et al.* 2004a). Broad behavioral syndromes are certainly more compelling than domain-specific syndromes. However, evidence of both types of behavioral syndromes has yielded mixed results. For example Dingemanse (2008), who works with sticklebacks, *Gasterosteus aculeatus*, a long-lauded animal model of behavioral syndrome research, has found some traits – aggressiveness and exploratory behavior – to be correlated strongly and significantly. However, he has also noted that other traits, such as activity and predator response, were not (Dingemanse 2008). Likewise, Adriaenssens & Johnsson (2008) failed to find evidence of a broad behavioral syndrome in brown trout, *Salmo trutta*. They found no correlation between individual behavioral responses and social dominance measures in brown trout introduced to a stream (Adriaenssens & Johnsson 2008). Similar to my results, there was much individual variation but no measurable effect on overall performance in a novel setting.

Only recently, has there been more research of domain-specific behavior syndromes and these results are also mixed. Within the context of exploratory behavior, Verbeek *et al.* (1994) found a significant correlation between exploration in a novel room and exploration with novel objects in male great tits. Fast novel room explorers were also fast novel object explorers (Verbeek *et al.* 1994). However, in a study with starlings, *Sturnus vulgaris*, Milderman (2008) found no relationship between the exploratory behavior of a subject and its home range size or movement in the wild. Fast explorers or proactive starlings were no more likely to have large or small home range sizes or movement patterns in nature (Milderman 2008).

The initial research efforts that focused on finding individual differences in behavior that may be limiting behavioral plasticity on larger scales (Sih *et al.* 2004a) may have distracted some researchers from examining questions about domain-specific behavioral syndromes. Assuming that behaviors within a context would correlate without testing this assumption may have lead researchers to draw invalid or questionable conclusions about the presence of behavioral syndromes. Nowhere is this problem more clearly identified than in a study of rooster, *Gallus gallus domesticus*, behavior by Nelson *et al.* (2008). They observed calling behavior of roosters in three different contexts: anti-predator, territoriality, and foraging in both a real and a virtual environment. They found statistically significant correlations of behaviors *across* these contexts, confirming the existence of broad behavioral syndromes in these subjects. However, within-context behavior was not correlated. Calling behavior of roosters observed in a real and a virtual situation could not be used to predict or infer behavior of the same subject in a contextually similar situation. Thus, Nelson *et al.* (2008) concluded that these the cross-

contexts correlations were artifacts and dismissed evidence of a broad behavioral syndrome. This study was one of the very first to question not only the validity of the evidence of broad behavioral syndromes, but the biological significance of these cross-context correlations of behavior.

An alternative possibility is that different contexts *and* situations will result in different expressions of behavior. This, in fact, is the definition of a state response – a behavioral response that occurs in a given situation. The calling behavior of roosters in the Nelson *et al.* (2008) study is an example of multiple state responses or situational behavior. Interestingly, psychobiologists who studied animal personality in the first half of the 20th century observed the same thing. Several laboratories researched the maze learning ability of selected lines of “bright” and “dull” rats (Tolman 1924; Searle 1939; Tryon 1940). Tolman and his students (1924) found that no two trials of the exact same test correlated with any degree of reliability. Tryon (1940) and Searle (1949) each independently demonstrated that bright rats performed well and learned one type of maze relatively quickly compared to dull rats. However, when the bright rats were introduced to a different type of maze apparatus the results were the opposite, with the dull rats performing better than the bright rats. Both tests presumably tested for learning ability, yet the two tests yielded contradictory results. Tolman (1924) proposed that the threshold sensitivities of rats to different kinds of stimuli had been affected. The same rats behaved very differently because the testing situations were different and individual differences in performance under such circumstances may be situation-dependent and variation in behavior is a result of an adaptive response to the different situations (Wilson *et al.* 1994 from Nelson *et al.* 2008). Unfortunately, these early biopsychological studies and their

insights into situation-dependent behaviors have been largely overlooked by modern students of behavioral syndromes.

At this juncture it might seem as if it is futile to study behavioral syndromes. After all, failure to find trait responses in multiple contexts (broad) or a single context (domain-specific) leaves us only with state responses to evaluate. However, I believe behavioral syndrome research provides a heuristic framework for studying individual variation in behavior. Thus, I propose a hierarchical approach to studying behavioral syndromes. Any time one sets out to study behavioral syndromes it would seem most appropriate to identify within context-correlations, or domain-specific trait responses, first. This would allow the researcher to determine the existence and stability of a single domain behavioral syndrome before investigating the potential existence of broad behavioral syndromes.

However, as researchers, we must accept the possibility that animal behavior test performance is not always interchangeable (Tolman 1924; Searle 1939). Fox *et al.* (2009) examined exploratory behavior of mountain chickadees, *Poecile gambeli*, in an aviary with multiple perches and novel objects (much like my open-field with novel objects test). They attempted to find evidence of domain-specific trait responses in a single context and were also unsuccessful. For chickadees, the novel room and the novel objects are independent tests for different exploratory traits. Fast novel room explorers were not fast novel object explorers (Fox *et al.* 2009). It would seem that modifying testing situations (for example, this study), or modifying tests in minor ways (e.g. Fox *et al.* 2009), or presenting semi-natural vs. virtual settings (e.g. Nelson *et al.* 2008), or even introducing subjects to the same apparatus multiple times (e.g. Tryon 1940; Searle 1949)

might elicit overwhelmingly different responses in animals. In my study, the strong within-test correlations clearly indicate that prairie voles demonstrate strong exploratory behavior state responses and do not exhibit a behavioral syndrome at all.

Though state responses have the capacity of demonstrating considerable inter-individual variation in behavior, these are not true behavioral syndromes because state responses indicate strong plasticity in behavior. However this plasticity in behavior can still help us understand more about the importance of individual variation in behavior. Although behavioral responses in this study could not be used to predict or infer behavior of the same subject in a different situation, together these different behavioral responses may help us understand how individuals optimally explore novel settings. I found that each of three exploratory tests examined three different exploratory behavioral responses – interactivity, activity, and proactivity-reactivity. Depending on the circumstances, individuals may optimize their ability to gather information by emphasizing different exploratory behavioral responses to the given situation. Some individuals or circumstances might favor varying degrees of activity, interactivity or reactivity in order to best explore a novel environment.

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FIGURES

Figure 1. Photo of the Open-field with Novel Objects Apparatus

Figure 2. Schematic of the Exploratory Maze

Figure 3. Schematic of the Two-Way Novel Choice Apparatus (Odor Tube)

Figure 4. Exploratory Scores Continuum

TABLES

Table 1a. Principal Components Matrix – Open-field with novel objects test

Table 1b. Correlation Analysis - Open-field with novel objects test

Table 2a. Principal Components Matrix – Exploratory maze test

Table 2b. Correlation Analysis – Exploratory maze test

Table 3a. Principal Components Matrix – Two-way novel choice test

Table 3b. Correlation Analysis - Two-way novel choice test

Table 4. Correlation of Exploratory Profile Scores across tests of all subjects

Table 5a. Correlation of Exploratory Profile Scores across tests of common subjects

Table 5b. Correlation of Latency to Depart Start boxes

Figure 1. Photo of the Open-field with novel objects

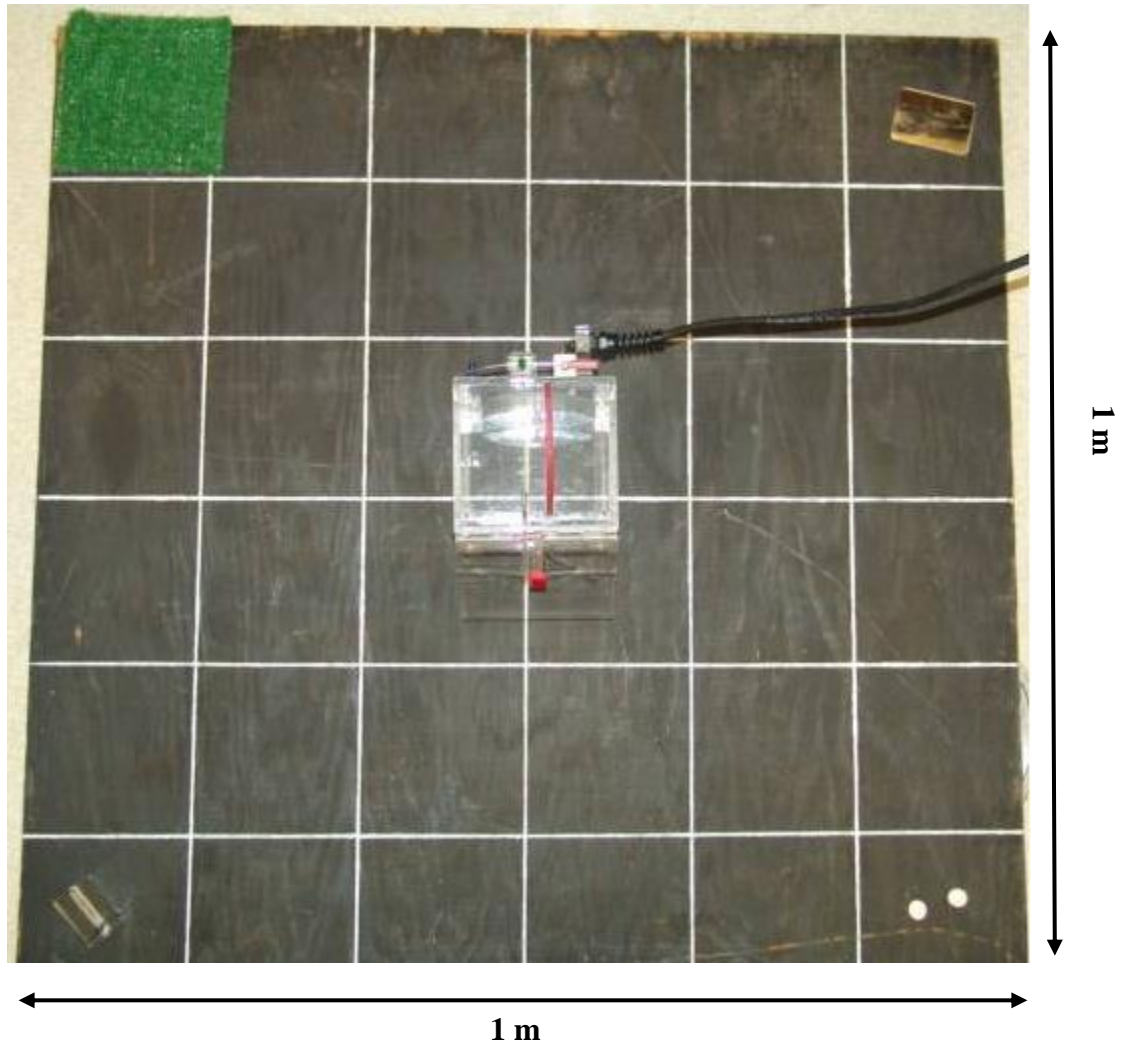


Figure 2. Schematic of the Two-Way Novel Choice Apparatus (Odor Tube)

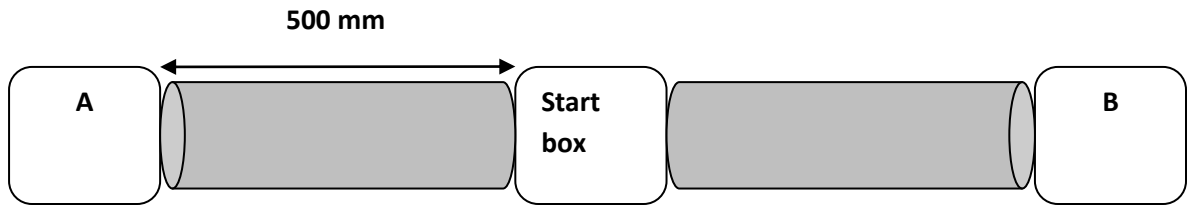


Figure 3. Schematic of the Exploratory Maze

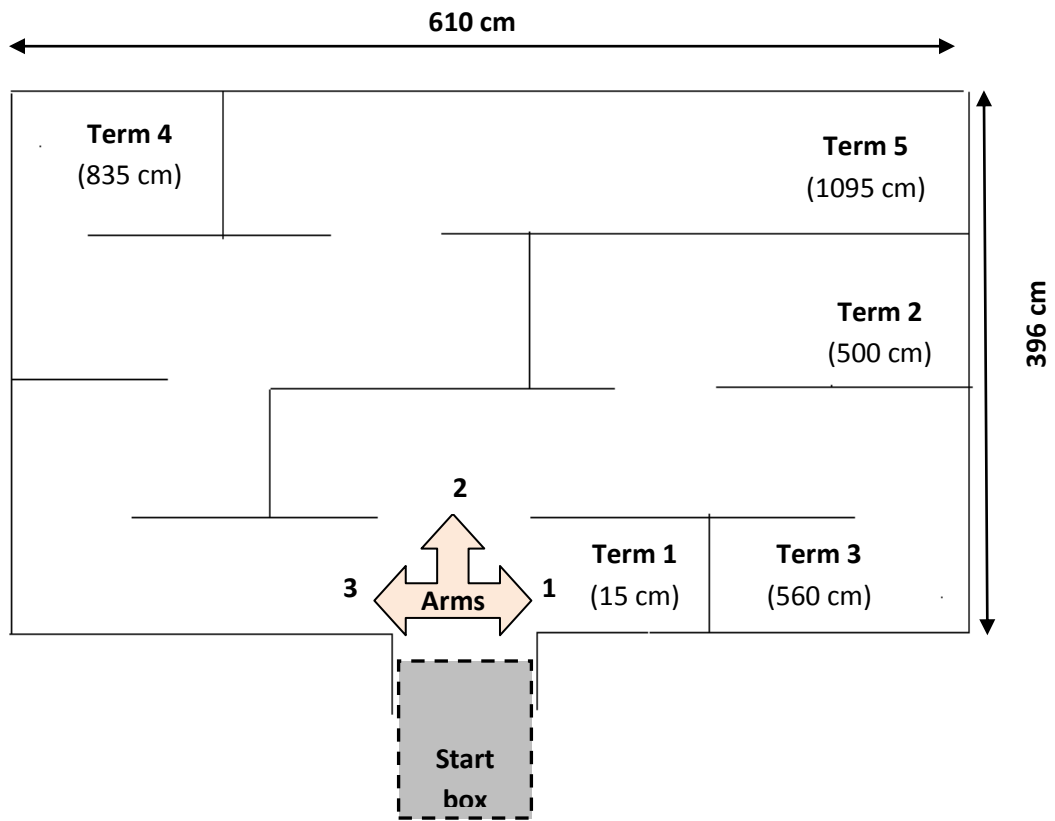


Figure 4. Exploratory Scores Continuum

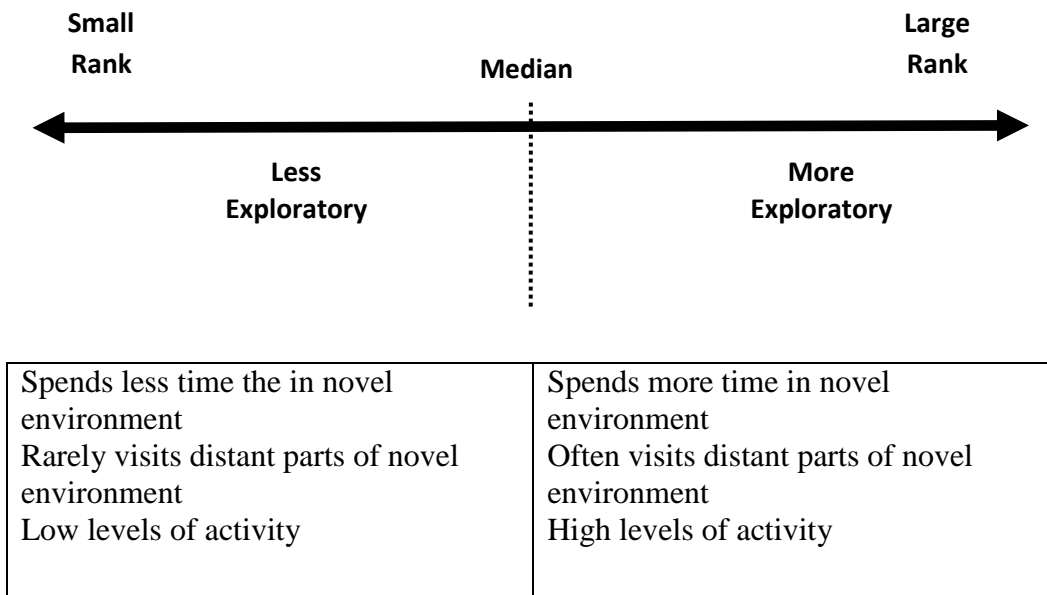


Table 1a. Principal Components Matrix – Open-field with novel objects test

Dependent Variables	Component
	PC1
OF:latency to depart start box	-.353
OF:total squares	.638
OF:returns	-.711
OF:time in novel environment	.820
OF:time w/novelities	.675
OF:total visits to center	-.624
OF:total visits to edge	.945
OF: ratio visits edge to center	.553

Extraction Method: Principal Component Analysis.

Table 1b. Correlation Analysis - Open-field with novel objects test

Correlations of key dependent variables. Data ranked along a continuum.

		Time in novel environment
Visits to edge	Pearson Correlation	.807**
	Sig. (2-tailed)	.000
	N	102

** . Correlation is significant at the 0.01 level (2-tailed).

Table 2a. Principal Components Matrix – Exploratory maze test

Dependent Variables	Component
	PC1
EM:latency to depart start box	-.593
EM:start box returns	.306
EM:arm 1	.787
EM: arm 2	.818
EM:arm 3	.888
EM: sum of visits to all arms	.979

Extraction Method: Principal Component Analysis.

Table 2b. Correlation Analysis – Exploratory maze test

Correlations of key dependent variables. Data ranked along a continuum.

		Entries into Arm #3	Entries into all arms
Entries into Arm #2	Pearson Correlation	.650**	.843**
	Sig. (2-tailed)	.000	.000
	N	97	97
Entries into Arm #3	Pearson Correlation	1.000	.914**
	Sig. (2-tailed)		.000
	N	97.000	97

** . Correlation is significant at the 0.01 level (2-tailed).

Table 3a. Principal Components Matrix – Two-way novel choice test

Dependent Variables	Component
	PC1
OR:T1latency	.753
OR:T1Split time	.860
OR:T1Dscore	.821
T1 total test time (minus latency)	.906
T2latency	.758
T2Split time	.106
T2Dscore	.707
T2 total test time (minus latency)	.711

Extraction Method: Principal Component Analysis.

Table 3b. Correlation Analysis - Two-way novel choice test

Correlations of key dependent variables. Data ranked along a continuum.

		Trial 1 D Score	Trial 1 Total test time (minus initial latency)
Trial 1 Split time	Pearson Correlation	.344**	.631**
	Sig. (2-tailed)	.000	.000
	N	141	141
Trial 1 D Score	Pearson Correlation		.905**
	Sig. (2-tailed)		.000
	N		141

** . Correlation is significant at the 0.01 level (2-tailed).

Table 4. Correlation of Exploratory Scores across tests of all subjects

All subjects, exploratory scores coded as percentiles.

		Pro/Re-activity Score (Two-way Novel Choice test)	Activity Score (Exploratory Maze test)
Interactivity Score (Open-field with Novel Objects test)	Pearson Correlation	-.057	-.094
	Sig. (2-tailed)	.590	.518
	N	90	49
Pro/Re-activity Score (Two-way Novel Choice test)	Pearson Correlation		-.113
	Sig. (2-tailed)		.340
	N		74

No significant pairwise correlations.

Table 5a. Correlation of Exploratory Scores across tests of common subjects

Only common subject, those that completed all three tests.

		Pro/Re-activity Score (Two-way Novel Choice test)	Activity Score (Exploratory Maze test)
Interactivity Score (Open-field with Novel Objects test)	Pearson Correlation	.075	-.052
	Sig. (2-tailed)	.602	.717
	N	51	51
Pro/Re-activity Score (Two-way Novel Choice test)	Pearson Correlation		-.265
	Sig. (2-tailed)		.060
	N		51

No significant pairwise correlations.

Table 5b. Correlation of Latency to Depart Start boxes

		Two-way Novel Choice test	Exploratory Maze test
Open-field with Novel Objects test	Pearson Correlation	.481*	-.035
	Sig. (2-tailed)	.001	.801
	N	90	55
Two-way Novel Choice test	Pearson Correlation		-.100
	Sig. (2-tailed)		.374
	N		81

*. Correlation is significant to the 0.05 level (2-tailed).

CHAPTER TWO

EXPLORATORY BEHAVIOR IN AN OPEN-FIELD AND WITH NOVEL OBJECTS IN PRAIRIE VOLES (*MICROTUS OCHROGASTER*)

ABSTRACT

Though previously used to evaluate neophobia, curiosity, risk-aversion, and risk-prone tendencies of rodents, open-field tests have also been used to evaluate ecologically important behaviors such as exploration. Exploration is a spontaneous behavior that involves investigating novel settings absent of obvious motivating factors such as hunger or risk of predation. In this study, I examined the effects of social complexity and familial relationships, as well as age and sex on exploratory behavior of prairie voles, *Microtus ochrogaster*. Subjects were observed in a 1m² open-field arena containing manipulable novel objects in each corner. Recorded behavioral measures included latency to depart start box, time spent in the novel environment, and interacting with novel objects, as well as number of returns to the start box, and number of visits to each major section of the open-field. Litter size and to a smaller degree, age explained exploratory behavior in the open-field. Subjects from large, socially complex litters and young subjects were less active and less interested in the novel environment than subjects from smaller litters and older subjects. Most subjects across families demonstrated similar behavioral tendencies and it was not possible to determine if exploratory behavior was due to family membership or a part of the general character of this population of prairie voles.

Keywords: open-field behavior, prairie voles, exploratory behavior, novelty-response, interactivity

INTRODUCTION

Exploratory behavior is defined as a response to novel situations (Renner 1987). It is regarded as spontaneous behavior that involves investigating unfamiliar settings or objects absent of obvious motivating factors such as hunger, reproductive drive or escape from danger (Renner 1990; Hughes 1997). Examining spontaneous exploratory behavior helps us to understand how animals react to novel situations in nature; specifically, such exploration may reflect important aspects of an animal's natural behavior including foraging, dispersal, and escape reactions. These behavioral responses allow an individual to gather important information about its environment, such as the location of resources, escape routes, and potential mates (Glickman & Sorges 1966; Archer & Birke 1983; Renner 1990; Verbeek *et al.* 1994; Draai *et al.* 2001). Exploratory behavior is the way an organism covers a given space, processes information, and gathers knowledge about its local environment (Renner 1987; Renner 1990).

There is a long history of observing rodents in novel open-field settings and interpreting their behavioral responses as indicators of the internal state of the animal. Animal behavior researchers, from both the psychological and ethological perspective, regard animal behavior in an open-field as representing an intrinsic factor of an individual; they usually record the amount of movement or activity within a defined, empty, and unfamiliar space (Searle 1939; Russell 1973; Renner 1987; Renner 1990; Nemati & Whishaw 2007). Though researchers from both fields record similar measures, their interpretations of these behaviors are patently different. In psychology, the behavior of an animal in an open-field test has been described as novelty-seeking, a fear-curiosity response, and an anxiety response (e.g., Würbel *et al.* 1996; Maier *et al.* 1998; Kopp *et*

al. 1999). In contrast, ethologists have used open-field tests to examine ecologically relevant behaviors (Verbeek *et al.* 1994; Dingemanse *et al.* 2002) and often describe these behaviors as investigatory or exploratory behavior (Walsh & Cummings 1976).

Spontaneous activity in an open-field was originally studied in rats (Searle 1939) but more recently such tests have been modified and used with many different species (e.g., gold fish, *Carassius auratus*, Kleerekoper *et al.* 1974; Mongolian gerbil, *Meriones unguiculatus*, Laming *et al.* 1989; old fieldmice, *Peromyscus polionotus subgriseus*, McPhee 2003; great tits, *Parus major*, Dingemanse & de Goede 2004). Behavioral measures recorded in these tests include activity, movement, or other locomotor responses. For example, different researchers have measured the lengths of paths traced by animals, the different parts of the novel environment that are occupied, or the number of visits to different sections of an apparatus (Kleerekoper *et al.* 1974; Drai *et al.* 2001). Activity measures have been assumed to be indexes of exploratory behavior (Russell 1983); however, activity alone includes no information about how subjects interact with the environment (Marinelli 2005). Open-field tests that only collect this spatial or movement data are likely to be confounded and incomplete because it does little to explain how animals might gather information or interact with unfamiliar stimuli (Renner 1987, 1990).

A more comprehensive approach to studying exploratory behavior with this apparatus would include quantifying locomotor behavior and quantifying how the subject interacts with the environment (Renner 1990). Recording movement within the apparatus, such as number of visits to different parts of open-field, and providing novel objects could provide researchers more information about the behavioral strategies of

exploration (Renner 1987; Renner 1990; Renner & Seltzer 1991; Hughes 1997). Quantifying the time spent in contact with objects and recording how an animal manipulates objects (Renner 1987), provides the investigator with details about how animals gather information and interact with novel settings, an attribute often referred to as interactivity (Renner & Rosenzweig 1986; Renner 1990; Hughes 1997). Such measures are important because interactivity is essential to information gathering and explains why animals might be intrinsically motivated to explore novel settings.

Exploratory behavior and interactivity in a novel setting are highly variable among individuals. Like all selected traits, individual variation in behavior may be susceptible to natural selection (Fox *et al.* 2009). Individual variation reflects a constraint on the optimization process demonstrated by the animal (Verbeek *et al.* 1994; Clark & Ehlinger 1987). Individual variation in behavioral traits such as exploration may provide the basis of selective differences in fitness traits such as foraging, anti-predator behavior, and dispersal. Examining inter-individual differences in open-field behavior, allows the assignment of behavioral profiles that categorize behavior of subjects in a given test situation (Groothuis & Carere 2005). Behavioral profiles describe behavioral tendencies or 'dispositions' of animals along an axis, such as proactive-reactive or more or less exploratory (Fox *et al.* 2009). These behavioral profiles allow behavioral traits to be examined within the Behavioral Syndrome framework (Bell & Stamps 2004; Bell 2007). This framework not only quantifies individual variation in behavior, but also attempts to explain the development and maintenance of this variation (Sih *et al.* 2004a).

In this study, I examined the environmental influences on the individual variation of exploratory behavior in an open-field test. The objective of the study was to determine

if individual variation of exploratory behavior can be attributed to independent variables such as social environment, developmental factors such as age or sex, and family membership. I tested three hypotheses concerning the development of individual variation of exploratory behavior in a novel choice apparatus test.

Hypothesis 1: Rearing conditions experienced by subjects will influence behavioral responses; thus subjects from similar family compositions are predicted to demonstrate similar behavioral responses. The effects of subtle social differences that may occur normally in the early postnatal environment of mammals living under natural conditions have rarely been studied. The social environment experienced in early life and throughout life may influence the behavioral development of the individual (Carducci & Jakob 2000; Genaro & Schmidek 2002; Neugebauer *et al.* 2004). Being in a small or large litter, and with or without brothers or sisters, may have profound effects on the adult behavior of individuals.

Hypothesis 2: Fundamental biological factors, such as age or sex, are known to be responsible for generating correlations in behavior (Dall *et al.* 2004). This hypothesis addresses how developmental factors, such as sex and age of a subject at time of testing, contribute to individual differences in exploratory behavior. Does the age or sex of the subject influence the exploratory responses more than the other independent factors?

Hypothesis 3: Related individuals demonstrate similar behavioral responses for many behavioral traits. I predict that siblings will demonstrate similar individual behavioral trends when introduced to novel situations. Subjects born to the same parents, which would include litter mates and full siblings from previous or subsequent litters, might share behavioral tendencies due to genomic or non-genomic effects (i.e., culturally

and socially transmitted traits) such as maternal effects. This study does not attempt to disentangle the exact mode of heritability of individual variation in exploratory behavior but does attempt to explore its possibility. See Figure 1, Exploratory Behavior Prediction table.

GENERAL METHODS

1. Animals

Forty-five male and 62 female that were first through third generation, lab-reared prairie voles, *Microtus ochrogaster*, from Urbana-Champaign, Illinois, served as the subjects in this behavioral test. Individuals were reared under a 14:10 LD light schedule. Lab conditions, including frequency of handling, cage cleaning, and feeding, were consistent among all animals.

Animals were reared in early social environments that consisted of naturally occurring littermates and one or two parents. Natural litter sizes vary from 1-8, with 3-4 being the average size. Litter sex ratio is also naturally variable and was characterized as the subject having a) no siblings, b) same-sex siblings only, c) opposite sex siblings only, and d) at least one sibling of each sex. All voles were weaned at 21-23 days of age and were housed with littermates, if any, throughout life.

Voles were tested during the light phase of the time cycle between 10:30 -16:00 CST hours, but voles have been found to be active throughout the day and night (Grippo *et al.* 2007). All subjects were tested post-sexual maturity at 55 days of age or older (sexual maturity occurs by 40 days) (Getz *et al.* 1994), and were sexually inexperienced. Age variation was minimized whenever possible; however, age at testing did vary randomly. Voles were categorized as **young**, 55-120 days of age, **middle age**, 121-349

days, **old**, 350-544 days, and **geriatric**, 545 or more days of age. Though there is no official age designation for prairie voles or other Microtine species, these age groupings are roughly consistent with the relative ages of voles tested in laboratories (Wolff *et al.* 2001; Grippo *et al.* 2007). The mean life span for prairies born and raised in this colony was 345 days \pm 15 (SE) and is consistent with mean life span observed in other studies (Stalling 1990). There were no apparent behavioral, physical, or health disparities among the subjects. Subjects completed the open-field test only one time and were naïve to the apparatus prior to testing. These voles were also used in two other experiments examining exploratory behavior (see Chapters 3 and 4). However, the order in which each of the experiments were completed by subjects was randomized.

2. Apparatus

The open-field arena consists of a black floor, 90 x 90 cm², covered with a clear acrylic sheet, and enclosed by 70 cm high white walls on each side. The floor of the arena has a total area of 8100 cm², grid-marked in thirty-six 15 cm² squares. The arena was divided into three concentric sections, 1) **edge** which is comprised of the twenty grid squares along the periphery (4500 cm² in area), 2) **intermediate** which is comprised of the twelve grid squares adjacent to the edge (2700 cm² in area), and 3) **center** which is comprised of the four grid squares in the middle of the open-field (900 cm² in area). In each of the corners I arbitrarily placed four distinct novel objects, a) a piece of clear PVC tubing (5 cm long and 2.54 cm diameter), b) 15 cm² of Astroturf, c) two pebbles of aquarium rock (1.8 cm diameter), and c) a plastic hand mirror (7.6 x 5 cm). Adding novel objects to an open-field allows measurement of a broad range of behaviors in order to capture the complexity inherent in spontaneous exploratory behavior, which would

include interactive behavior (Renner & Rosenzweig 1986; Renner 1990; Verbeek *et al.* 1994; Hughes 1997). The novel or stimulus objects were classified as manipulable (Renner & Seltzer 1991) because all were small in size and could provide kinesthetic feedback when subjects interacted with them. A photo of the open-field with novel objects is presented in Figure 2.

3. *Methods*

The subject was placed in a Plexiglas start box, 13.8 (w) x 13.4 (l) x 13.8 (h) cm. The start box contained a ventilated Petri dish, 9.5 cm in diameter, made of Nalgene plastic with the two lids attached to one another by a screw and nut with many holes drilled into the upper lid. The Petri dish, filled with scented bedding from the home cage of the vole, was mounted to the inside wall of the start box with scotch tape. The bedding-filled Petri dish provided own odor from the subject thus making the start box a location of familiarity (Hughes 1997). Such an experimental set-up is best for examining spontaneous exploratory behavior and is considered ecologically relevant (Hughes 1997), as well as preferred by rats and mice (Russell 1975; Misslin & Ropartz 1981). The start box was placed in the center of the open-field and covered with a black cloth. (The start box remained within the open-field throughout the duration of the test and occupied 185 cm² of the center section). The subject remained in the darkened start box for 5 min to acclimate. Then, the black cloth was removed, the door of the start box was opened with a Solenoid remote control, and the subject was free to leave the start box. The location of the vole was recorded every 5 seconds according to the grid square occupied at that moment, (methods and measures comparable to McPhee 2003). The entire test was video recorded and scored from the footage. The following measures were recorded: 1) latency

to depart the box (in seconds), up to 10 min; 2) time spent in the novel environment (in seconds); 3) total time spent interacting with novel objects; 4) number of returns to the start box; 5) number of total grid squares visited during the test; and 6) number of visits to center, intermediate, edge. The total time of the test was 5 min after initial exit from the start box. Between tests, the start box, the Petri dish, the novel objects, and the arena were cleaned with soap and water and disinfected with a 15% ethyl alcohol solution to eliminate any odors that might have accumulated from the previous subject.

All behavioral observations were recorded under full illumination with a low-light video camera and video recorder. The video camera was mounted approximately 1 m above the open-field arena. All additional equipment was placed in an adjacent room. For subjects that did not leave the start box after 10 min, the maximum latency time was recorded and zeros were assigned for other measures and included in the data analysis.

4. Data analysis

Data were analyzed using SPSS 17 statistical package to identify relationships among independent variables on the multiple behavioral response measures in an open-field. I completed a General Linear Model – Univariate ANOVA examining the influence of multiple independent variables on each dependent variable, one at a time (litter size x litter sex ratio; age x sex). Tukey's post-hoc test was used to evaluate pair wise relationships. The mean difference in values was evaluated at the alpha = 0.05 level. Parametric statistical test were appropriate for several reasons: 1) reasonably large sample sizes are able to withstand the statistical effects of averaging, 2) parametric tests are less affected by extreme violations of assumptions of models including homogeneity

of variance, normality, small sample sizes, and unequal sample sizes, and 3) parametric tests are generally robust statistical tests (Boneau 1960).

To examine the influence of family membership on the same dependent variables, I completed a Two Step Cluster Analysis of all continuous dependent variables using a BIC Cluster criterion, log-likelihood un-standardized variable method. Individuals were clustered based on each behavioral measure separately. Clusters are based on the mean (central tendency) for all subjects for that measure. Each subject is assigned to the cluster which has a mean closest to its behavioral score. Next, I calculated the proportion of full siblings that fall within the same cluster for each dependent variable.

There were only a few litters raised by the female parent alone due to death of the male parent or adjustment of breeding schedule protocols. However, statistical analysis confirmed that the physical development and behavioral responses of voles raised by one parent were no different than those voles raised by both parents. Therefore, these data were combined.

RESULTS

1. Social environment factors (litter size and litter sex ratio)

Latency: There was a significant difference in the time to depart the start box based on the size of the litter a subject is born into ($df = 5$, $F_{stat} = 3.261$, $p = 0.010$). Tukey's post-hoc tests showed values for subjects from a litter of 6 were greater than those of subjects born to smaller litters ($p < 0.05$, for each comparison). (Figure 3). Litter sex ratio did not have an effect on this measure and there were no interaction effects.

Total squares: There was a significant difference in the number of grid squares visited by voles reared in different size litters ($df = 5$, $F_{stat} = 4.479$, $p = 0.001$). Tukey's post-hoc tests

showed values for subjects from a litter of 6 were less than those for subjects born to all other litters, 1-5, 7 ($p < 0.05$, for each comparison). (Figure 4). Though litter sex ratio did not have a significant effect on this measure, combined with litter size there was a significant interaction effect ($df = 5$, $F_{stat} = 3.914$, $p = 0.003$). Tukey's post-hoc tests showed that subjects with siblings of both sexes visited fewer total squares than subjects with no siblings and those with only opposite sex siblings ($p < 0.05$ for each). By comparing subjects with only opposite sex siblings to subjects with siblings of both sexes (3-6 pups), I could see where the interaction of litter size and litter sex ratio had the most influence. Subjects from litters of five and six appear to be driving the interaction effects. These animals visited a mean of $19.94(\pm 8.28)$ squares whereas, subjects from litters of six with opposite sex siblings visited mean of 20.00 squares (± 2.83 , $n=2$) and those having siblings of both sexes visited a mean of 6.30 squares (± 9.70 , $n=10$). Subjects from litters of five with opposite sex siblings visited a mean of 25.00 squares (± 1.41 , $n=2$) and those having siblings of both sexes visited a mean of 18.38 squares (± 7.68 , $n=13$). However, there were differences between subjects from litters of 4 (mean number of squares visited 18.23 ± 9.42 , $n=13$); subjects from uni-sex litters visited a mean of 10.75 squares (± 12.58 , $n=4$).

Returns: There were no significant differences for either of the independent variables or their interaction on this measure; however litter size suggests a trend, $p = 0.079$. Returns to the start box increase with the number of siblings a subject has. (Figure 5).

Time in novel environment: There was a significant difference in the amount of time spent in the novel environment based on the size of the litter a subject is born into ($df = 5$, $F_{stat} = 4.479$, $p < 0.001$). Mean time spent in the novel environment for subjects from litters

of 6 are significantly less than the time spent for subjects born to all other litters, 1-5, 7 ($p < 0.001$, for each). (Figure 6). Though litter sex ratio did not have a significant effect on this measure, combined with litter size there was a significant interaction effect ($df = 5$, $F_{\text{stat}} = 5.144$, $p = 0.001$). Tukey's post-hoc tests show subjects with siblings of both sexes spent less time in the novel environment than subjects with no siblings and those with opposite sex siblings ($p < 0.05$ for each). Comparing litters that had subjects with only opposite sex siblings and those with siblings of both sexes (3-6 pups), I could again identify where the interaction of litter size and litter sex ratio had the most influence. It appears that subjects from litters of six with both brothers and sisters are driving these interaction effects (mean = $66.96 \text{ s} \pm 106.05$, $n = 2$). The grand mean is 234.98 seconds in the open-field (± 90.90 , $n = 98$). (Table 1).

Time with novel objects: There were no significant differences for either of the independent variables or their interaction on this measure; however in the case of litter size there was a trend, $p = 0.083$. Mean amount of time spent with novel objects decreases with the number of siblings a subject has. (Figure 7).

Visits to center: There was no significant difference in the number of visits to the center section based on either independent variable, but together there was a significant interaction effect ($df = 5$, $F_{\text{stat}} = 2.674$, $p = 0.027$ *note this statistics fails the Levene's statistic, $p = 0.054$). The mean number of visits to the center section of the open-field is greater for voles from larger litters (five and seven, but not six), than those for smaller litters (four or fewer pups). However, subjects from litters of 6 appear to be driving the litter size/litter sex ratio interaction effects: subjects having both brothers and sisters had the lowest mean number of visits to the center, (3.80 ± 4.89 , $n = 10$) and subjects having

only siblings of the opposite sex had the highest mean number of visits to the center (17.50 ± 3.536 , $n=2$). The grand mean was $9.71 (\pm 5.72)$, $n=102$. (Table 2).

Visits to edge: There was a significant difference in the number of visits to the edge section based on the litter size a subject is born into ($df=5$, $F_{stat}= 3.732$ $p=0.004$).

Subjects from smaller litters visited the edge of the open-field significantly more times than subjects from larger litters. (Tukey's post hoc tests show significant differences between the following pairs: litters of 1 vs. 5,6,7; 2 vs. 6; 3 vs. 5,6; 4 vs. 6; 5 vs. 6 – ($p<0.05$ for each). (Figure 8). Though litter sex ratio did not have a significant effect on this measure, combined with litter size there was a significant interaction effect ($df=5$, $F_{stat}= 3.579$, $p=0.005$ *note these statistics fail the Levene's statistic, $p=0.055$). Subjects from uni-sex litters of 4 (16.00 visits ± 20.199 , $n=4$) and subjects from opposite sex litters of 6 (6.50 visits ± 12.250 , $n=10$) stand out because these groups of subjects visited the edge section fewer times than average (30.09 visits ± 15.118 , $n=102$). However, Tukey's post-hoc tests show that subjects with no siblings visited the edge section of the open-field more frequently than did subjects with siblings of both sexes ($p<0.05$). It is more likely that litter size influenced this statistical difference more than litter sex ratio since this comparison is between litters of one and litters of 3 or more.

2. Developmental factors (age and sex)

None of the behavioral measures differed statistically between male and females subjects. Males departed the start box sooner, visited more squares, and spent more time in the novel environment and with the novel objects than females, although all comparisons were non-significant ($p>0.05$).

In general, there were age related differences in behavior. Younger subjects departed the start box later, visited fewer squares, and spent less time in the novel environment and with the novel objects than older voles. There was greater variation in the responses of younger voles than that of older subjects. Variance in behavioral measures decreases step-wise as age increases for all measures except time with novel objects which has a nearly equal standard deviation for each age group.

Latency: There was no significant difference for either independent variable or their interaction on this measure. Though not statistically different, females had a much higher mean to depart to the start box ($90.25s \pm 190.50$, SD, $n=58$) than males, ($47.68s \pm 125.89$, $n=44$). There is a linear relationship between age and mean latency, with younger and middle age voles having higher mean latencies to depart the start box than old or geriatric voles. (Figure 9).

Total squares: There was a significant difference in the number of grid squares visited based on the relative age of the subject ($df = 3$, $F_{stat} = 3.754$, $p=0.013$). Tukey's post hoc tests showed that young subjects visited less of the total area of the open-field than did older subjects ($p=0.005$) or geriatric subjects ($p=0.015$). Middle age subjects visited less of the open-field than older subjects ($p=0.037$). (Figure 10). Though sex did not have a significant effect on this measure, combined with relative age there was a marginal effect ($df = 3$, $F_{stat} = 2.682$, $p=0.051$). However, the difference lies between young female and male subjects. (Table 3).

Returns: There was a significant difference in the number of returns to the start box according to relative age of the subject ($df = 3$, $F_{stat} = 3.852$, $p=0.012$ *note this statistic fails the Levene's statistic, $p=0.061$). Tukey's post hoc tests showed that young subjects

returned to the start box more frequently than old subjects ($p=0.008$) and geriatric subjects ($p=0.016$). (Figure 11). Sex did not have a significant effect on this measure and there were no interactive effects on this measure.

Time in novel environment: There was a significant difference in the amount of time spent in the novel environment according to the relative age of the subject ($df = 3$, $F_{stat} = 3.527$, $p=0.018$). Tukey's post hoc test showed that young subjects spent less time in the novel environment than did old voles ($p=0.003$) and geriatric voles ($p=0.007$). (Figure 12). Though sex did not have a significant effect on this measure, combined with relative age there was a marginal effect ($df = 3$, $F_{stat} = 2.223$, $p=0.091$). However, the difference lies between young female and male subjects. (Table 4).

Time with novel objects: There was a significant difference in the amount of time spent in contact with the novel objects according to the relative age of the subject ($df = 3$, $F_{stat} = 3.148$, $p=0.029$). Tukey's post hoc test showed that geriatric subjects spent more time in contact with novel objects than young voles ($p=0.005$) and middle age voles ($p=0.041$). (Figure 13). Sex had a marginal effect on this measure ($df = 1$, $F_{stat} = 3.609$, $p=0.061$) with females spending less time in contact with novel objects than males. (Figure 14). There were no interactive effects on this measure.

Visits to center: There was no significant difference for either of the independent variables or their interaction on this measure.

Visits to edge: There was a significant difference in the number of visits to the edge section according to the relative age of the subject ($df = 3$, $F_{stat} = 4.448$, $p=0.006$).

Tukey's post hoc test showed that young voles visited the edge of the open-field less

frequently than all other age groups, $p < 0.05$ for each. (Figure 15). Sex did not have a significant effect on this measure and there were no interactive effects for this measure.

3. Family membership

Ninety-two subjects from 15 families, consisting of 2 or more full siblings per family (mean number of subjects per family is 6) were evaluated to determine the similarity of behavioral responses among related individuals. A few families were better represented in the sample than others – for example one male and female pair was responsible for 30% of the full sibling subjects in this test. Individuals were assigned to a cluster based on the mean value for that cluster (high, medium, low). Each behavioral measure was divided into at least two clusters, but no more than three. For most dependent variables, a majority of individuals were assigned to the same cluster as their full siblings. For measures that resulted in some members of a family not being assigned to the same cluster as its other siblings (i.e., the family was split) the proportion of siblings that were in a different cluster than the majority of their family group ranged from 30-45%.

Latency: The general mean was $80.21 \text{ s} \pm 177.55$ (SD, $N=89$) to depart the start box. A majority of subjects (88.8%) were assigned to cluster 1, with a mean latency of $19.05 \text{ s} \pm 31.81$. The remaining subjects in cluster 2 had a mean latency of $563.4 \text{ s} \pm 85.07$. This minority of individuals came from 3 out of 14 families and represented a mean 37% of siblings that clustered differently than their family group.

Total squares: The general mean was $19.31 (\pm 8.546, N=90)$ number of total grid squares visited during the test. A majority of subjects (63.3%) were assigned to cluster 1, with a mean of $24.54 (\pm 2.07)$ total squares visited. 22.2% of the subjects were assigned to

cluster 2, (16.05 \pm 3.67); and 14% of the subjects assigned to cluster 3 (1.38 \pm 2.06). Most families were split (10 out of 15) with a mean representation of 36.1% of siblings that clustered differently than their family group.

Returns: The general mean was 3.51 (\pm 2.91, N=82) returns to the start box during the test. A slight majority of subjects (58%) were assigned to cluster 1 (1.65 \pm 1.11). The remaining 31.7% were assigned to cluster 2 (4.88 \pm 0.86) and 9.8% to cluster 3 (10.38 \pm 1.69). Eight out of 14 families are split on this measure. The mean proportion of siblings that clustered differently than their family group was 40.89%.

Time in novel environment: The general mean was 229.10 s (\pm 94.66, N=87) spent in the novel open-field. A majority of the subjects (80.5%) were assigned to cluster 1 (mean=272.21 \pm 26.52). The remaining individuals were assigned to cluster 2 (51.59 s \pm 59.34), and all came from five out of 15 families. The mean proportion of siblings that clustered differently than their family group was 30.18%.

Time with novel objects: The general mean was 41.43 (\pm 35.487, N=88) for time spent in contact with novel objects. Slightly more than half of the subjects (52.3%) were assigned to cluster 2 (52.39 s \pm 15.86). The remaining 40.9% were assigned to cluster 3 (13.06 s \pm 10.10) and 6.8% to cluster 3 (133.67s \pm 37.53). Thirteen out of 14 families were split on this measure. The mean proportion of siblings that clustered differently than their family group was 44.1%.

Visits to center: The general mean was 9.93 (\pm 5.96, N=90) for number of visits to the center section of the open-field. A slight majority of subjects (56.7%) were assigned to cluster 2 (9.37 \pm 2.06). The remaining 24.4% were assigned to cluster 3 (17.91 \pm 3.19), and

18.9% to cluster 1 (1.29 ± 1.72). Twelve out of 15 families were split on this measure. The mean proportion of disagreement for these split families was 41.93%.

Visits to edge: The general mean was 28.94 (± 15.35 , N=90) for number of visits to the edge section of the open-field. Slightly more than half of the subjects (51.1 %) were assigned to cluster 1 (40.57 ± 6.655). The remaining 31.1 % were assigned to cluster 2 (25.29 ± 4.162) and 17.8 % to cluster 3 (1.94 ± 4.123). Nine out of 15 families were split on this measure. The mean proportion of siblings that clustered differently than their family group was 40%.

DISCUSSION

Open-field tests have been used to examine an “organism’s strategy of covering a given space” (Renner 1990; Nemati & Whishaw 2007). Renner & Seltzer (1991) gathered multiple descriptive measures of exploratory behavior including both movements in space (activity) and details of investigating specific features of the environment when individuals interact with objects (interactivity) (Renner 1990). Activity is defined as the amount of movement within a novel setting (Russell 1973; Kleerekoper *et al.* 1974). Interactivity includes a reaction to the novel environment such as manipulating novel objects or investigating the features of the apparatus, such as the boundaries (Russell 1973; Renner 1990). Measuring both activity and interactivity are necessary in order to capture the complexity inherent in spontaneous exploratory behavior (Renner & Rosenzweig 1986; Renner 1990). By observing how voles occupy different parts of the novel environment, choosing between familiar and unfamiliar areas, I can glean more information about how animals explore (Renner 1990; Drai *et al.* 2001).

This ability to choose between more or less familiar stimuli is an essential design feature in spontaneous exploration tests (Kopp et al. 1999.).

Less exploratory individuals tend to focus their activity on familiar stimuli and more exploratory individuals tend to focus their activity on unfamiliar stimuli. The start box, located in the center of the open-field contained odors from the home cage of each subject. Subjects were acclimated to the box prior to observation. It served as a home base (Drai *et al.* 2001; Nemati & Whishaw 2007; Eilam 2010). Delaying entry into the novel environment, visiting the areas nearest the start box, and returning frequently indicates a low interest in novelty. Subjects are more attracted to the familiar stimuli, one that offers optimal security, and they are generally less curious about the novel stimuli (Whishaw *et al.* 2006; Eilam 2010). These behaviors appear to indicate a low exploratory tendency. Entering the novel environment quickly, spending more time in the open-field, exploring more of the open-field including the outer-most sections, and spending time interacting with novel objects indicates a high interest in novelty. These subjects appear to be less attached to the familiar stimuli and more curious about the novel stimuli. These behaviors are interpreted as high exploratory tendency. The relationship between activity and interactivity are inter-twined. For example, subjects who are reticent to enter or move about novel environments will probably not come into contact with or approach novel objects.

Initially, I thought prairie voles might explore the open-field gradually, spending more time in the center section, near the start box then proceed to the edge as time progressed. In a novel tank goldfish, *Carassius auratus*, move distinctly from one location to another in succession (Kleerekoper *et al.* 1970). This type of behavior is

postulated to be a highly organized pattern of locomotion indicative of appetitive habitat exploration (Kleerekoper *et al.* 1974). Though some domesticated rats and mice have been known to engage in specific patterns of locomotor behavior in novel environments (Drai *et al.* 2001), prairie voles did not appear to explore the open-field in a particular pattern. Like other rodent species, voles move forward and scan, usually while standing still, rearing up, or with the nose to the ground to gather information about their surroundings (Drai *et al.* 2001). However, there was no evidence of a successive exploration pattern in the open-field. The voles explored the novel area all at once, in its entirety with no obvious pattern or approach to investigating the novel setting; and they visited the edge section of the open-field more often. An open-field study with Guenther's social voles, *Microtus socialus guentheri*, found that voles spent more time in the outer-most section and away from the home base in the first 5 min of the test (Eilam 2010). Once, an animal has accumulated a certain amount of presumably new information, it moves on to a different part of the novel environment (Kleerekoper *et al.* 1974). In a homogenous environment, the information can be gathered rather quickly and one would expect animals to move about quickly and perhaps behave indifferently (Kleerekoper *et al.* 1974).

Based on the results from Chapter 1 that examined the range of inter-individual variation of the exploratory behavior in this test, I also wanted to know which, if any, environmental variables influence open-field behavioral responses of subjects. If belonging to a specific treatment group influenced exploratory behavior in a significant way, then subjects from similar social environments or those who are the same age should behave similarly.

The social environment a vole experienced at birth and throughout its captive life seemed to influence how an individual responded to the open-field test. Voles with no siblings demonstrated behavioral responses that were reflective of a high exploratory interest. The converse was true of subjects from larger litters. Singly-raised voles had the lowest latencies to depart the start box, returned to the start box fewer times, and spent more time in the novel environment, than voles from litters of five or more. Subjects from larger litters, delayed entry into the open-field, spent more time in the center of the field nearest the start box, as evidenced by the number of visits to this section, and returned to the start box more frequently. These same subjects also visited the outer-most section of the open-field less frequently, and spent less total time in the open-field and with the novel objects than subjects from smaller litters. The most significant differences in these values were between subjects from smaller litters (of 1 or 2 individuals) and litters of 6 individuals (and sometimes 5 and 7). Subjects from smaller litters were simply 'more exploratory' in the open-field than subjects from larger litters.

There were no significant differences for any of the measures based on litter sex ratio alone. There were significant interactive effects of litter size and litter sex ratio for some behavioral measures, such as time in novel environment, total squares visited, and number of visits to center and edge sections of the open-field. For most measures, the differences were between subjects with no siblings and subjects with both brothers and sisters. This interactive effect is best explained by litter size and reinforces the conclusion that voles with no siblings were more exploratory than voles from larger litters. However, among litters of 2-7, subjects who had both brothers and sisters were the least exploratory in the open-field compared to subjects who were from single-sex

litters or those who had all opposite-sex siblings. Subjects from the most socially complex litters (i.e., large and mixed-sex litters) visited fewer total squares, visited the edge fewer times, and spent less time in the novel environment than subjects from less diverse litters (i.e., small and uni-sex litters).

The number and sex composition of siblings may be influencing individual behavior in some way. Failing to find distinctly different influences of litter size and litter sex ratio makes sense. Litter sex ratio is a function of litter size. Single sex litters were common among smaller litters (2-4 pups) and as litter size increased, mixed-sex litters were common. Among these larger, mixed-sex litters a vole might have all opposite-sex siblings. However, it was rare for that to occur in litters of 5 or more and it never occurred for subjects from litters of 7. Typically, a vole from a larger litter had both brothers and sisters; and these were the subjects who were less exploratory in the open-field. There were dramatic differences in the response to unfamiliar stimuli between subjects with no siblings and those with 4 or more siblings. Perhaps diverse social environments act as an enrichment experience. Much like rats reared in cages enriched with toys and cage mates, voles from complex social groups seemed less interested in novel settings than voles from smaller, less diverse litters (Varty *et al.* 2000). The experimental test appeared to serve as an enrichment opportunity to which these socially 'deprived' individuals responded positively. In this study, subjects without siblings and those from single-sex litters were more engaged and interacted with the novel environment the most. In a study of natal dispersal in wild prairie voles, McGuire *et al.* (1993) found that dispersal was more common among voles from small natal groups rather than those from large natal groups. Individuals from smaller family groups

may have higher exploratory tendencies which may also influence fitness traits like dispersal. I might have expected voles raised in more complex social environments to feel more secure and willing to explore but this is not what I found and neither did McGuire.

Males consistently demonstrated a slightly higher interest in novelty than did females. Males were quicker to leave the start box and enter the open-field, plus they ventured more into the novel environment than did females. They visited the edge section more often and spent more time with the novel objects than did the females. Females made more return trips to the start box, visited the center squares more often, and they visited fewer total squares and the edge of the open-field fewer times than did males. The focus of female activity was in the center of the open-field, near the start box, whereas the focus of male activity was away from the start box and more towards the edges of the open-field. I conclude that males were generally more exploratory than females despite the lack of statistical significance due to large variances.

On the other hand, age did significantly influence exploratory behavior in the open-field. For the most part, younger individuals were less exploratory than older subjects. The very youngest and the oldest subjects behaved distinctively different from each other for most measures. For example, the youngest subjects took more time to enter the novel environment, and spent less time in the open-field and with the novel objects than the geriatric subjects. Young voles visited fewer total squares and returned to the start box more than the geriatric voles. Though sex was not a significant contributor to behavior, the variance in latency to depart the start box was higher among young females than it was for any other set of subjects. However, among geriatric voles

the differences between males and females were less obvious. There were no stark contrasts in responses of males and females to the open-field; however, overall interest in novelty seemed to increase with age.

As individuals age, the need to balance the risk and benefit of high exploratory behavior may become less important. Prairie voles are a short-lived species with hardly an individual living past a year of age under natural conditions (Getz *et al.* 1994, 1997). The average life span of wild prairie voles ranges from 30 – 122 days depending on season, population density, and whether an individual disperses or not (Getz *et al.* 1994). Geriatric voles, those that were more than a year and half old, were the most exploratory set of subjects for most measures. They visited the center fewer times, the edge more, and spent more time in the novel environment and with novel objects compared to younger voles. Perhaps younger voles have more to lose, assuming that exploratory behavior is costly (e.g. due to greater vulnerability to predators), and it may pay for younger voles to be more cautious with regards to exploration. Or it could be that the young voles in this study are behaviorally comparable to natural populations of prairie voles. Most voles, 70% of males and 75% of females, remain at the natal nest until death (McGuire *et al.* 1993; Getz *et al.* 1994). Perhaps what I observed with young voles in this study was a level of exploratory tendency that is common for this species for this age range. On the other hand, older voles that have already reproduced may be able to afford to take more risks and have little to lose from being highly exploratory and bold. Among young and middle age subjects, males were always more exploratory than females, though only marginally so. Nonetheless, these differences in exploratory tendencies disappear in geriatric voles. Again, this leads me to think that as voles age, they may be

willing to take more risks, since they would not be expected to live much longer anyway. However, it would be difficult to test this hypothesis in the wild. Only voles classified as young and some that were classified as middle-age would be encountered in nature (Getz *et al.* 1994). An old or geriatric prairie vole in nature is not at all likely.

My results provided some mixed support for heritability influencing exploratory behavior. Many full siblings, including those from different litters, were assigned to the same cluster for behavioral measures. However, only a few clusters were detected and most subjects were assigned to the same cluster. The clustering method assigned individuals to groups according to mean values that were either significantly higher or lower than the general mean for each behavioral measure. By clustering individuals according to low or high means, I could identify any similarities of behavior among all individuals, including family members. The preliminary findings demonstrated that open-field behavioral responses tended to run in families. However, it was also clear that most subjects across families were assigned to the same cluster. Unfortunately, this result left little room to tease apart if these behavioral tendencies were based on family membership or are just a part of the general character of this population of prairie voles.

Nonetheless, I did find some minor support for family membership influencing open-field behavior. When analyzing data for the influence of litter size or relative age, I found that the same subjects were driving the statistical differences for some behavioral measures: latency, time in novel environment, time with novel objects, total squares visited and visits to edge. These individuals were responsible for skewing the data across analyses; and these individuals all came from four families. For most instances, the entire litter would skew the data in the same direction.

Individual variation in behavioral profiles has been shown to be moderately heritable (Dingemans *et al.* 2002; Cockrem 2007). I did find moderate support for open-field exploratory behavioral tendencies in families. I also found that only a small percentage of individuals from each family reacted to the novel environment differently than the rest of their relatives. In this study, it was not possible to determine if the behavioral similarities among siblings were due to genetics or to a shared social environment. Nonetheless, because so many un-related individuals were assigned to the same cluster, it is unlikely that genetics was the major factor, and environmental effects may be the reason why so many voles clustered together.

Similarity among members of this colony also could be reflective of the natural behavioral variation of this population of prairie voles. My results may simply demonstrate that voles are generally active creatures, which was also observed with Guenther's social voles (Eilam 2010). Some are more exploratory than others, but there is so much variation in behavior that I was only able to demonstrate unambiguous patterns in behavior for a few of the independent variables (e.g. litter size, sexual composition, and relative age). Alternatively, the exploratory tendencies I observed (e.g., young voles and voles from large families being less exploratory) could also be reflective of the natural behavioral tendencies of voles from this source population also studied by others (e.g. Getz *et al.* 1994; McGuire *et al.* 1993). All subjects were F₁₋₃ laboratory raised prairie voles derived from wild parents, F₀, from Urbana-Champaign, Illinois. They were not bred to enhance or reduce specific behavioral tendencies or genetic or physical traits (Tolman 1924; Groothuis & Carere 2005). I wanted to study the natural complexity of prairie vole behavior. By studying behavioral reactions of animals that

have been minimally impacted by captivity I could examine the continuous variation that more likely characterizes natural selection as opposed to artificial selection pressure (Price 1970; McPhee 2003; Groothuis & Carere 2005).

Details of how animals behave in a novel setting could be important for understanding how they gather and process information about its environment (Renner 1987, 1990). The more an individual interacts with novel stimuli, the more information they potentially gain (Glickman & Sorges 1966; Verbeek *et al.* 1994; Renner 1990; Draai *et al.* 2001; Archer & Birke 1983). By exploring more and interacting with the novel environments, animals might gain additional information that might be a benefit to their lifetime fitness, e.g. novel food resources, shelter sites, or finding mates (Glickman & Sorges 1966; Verbeek *et al.* 1994; Renner 1990; Draai *et al.* 2001; Archer & Birke 1983). Less active and exploratory animals are less likely to discover new resources to exploit.

Another possibility is that the animals in this study were pre-selected explorers and not representative of the whole wild population. The more exploratory animals or those likely to travel long distances during dispersal were the one who might have entered the traps during the collection period.

Although increased exploratory behavior also influences an individual's ability to behave adaptively (Renner 1990). Increased activity in a novel environment also increases an individual's exposure to predators (Glickman & Sorges 1966; Glickman & Morrison 1969; e.g. meadow voles, *Microtus pennsylvanicus*, and deer mice, *Peromyscus leucopus* Metzgar 1967, Ambrose 1972; rats, *Rattus norvegicus*, Roeder *et al.* 1980). Thus exploratory behavior may be a high-risk high-gain strategy appropriate for older voles or voles raised in low quality habitats that resulted in small litter size. In

rats, highly active exploring individuals suffer higher death rates because they are more likely to leave the protective shelter to explore territories (Roeder *et al.* 1980). For rodents, the open-field is ecologically akin to an open pasture or land free of cover from aerial predators. Tracking their behavioral responses, for example time spent with novel objects or the amount of activity in different sections of the open-field, helps us learn more about how they balance fitness consequences (Russell 1973; Marinelli 2005).

In this study, what made an individual highly exploratory was its general proclivity to enter the open-field quickly, spend a majority of the observation time in the novel environment, and rarely return to the start box with familiar odor. Moreover, these subjects demonstrated a strong interest in novelty by covering more of the open field, visiting the outer-most boundaries of the open-field more frequently and visiting the center of the open-field (nearest the start box) less often. Subjects judged to be more exploratory were those that were both highly active and interactive in the open-field. They were quick to enter the open-field, spent more time in the open-field, and visited more total squares. They visited the outer edge of the open-field more and spent more time sniffing, touching, or in contact with the novel objects. The more time or increased rate of interaction in novel settings or with novel objects increases the amount and type of information gathered, especially if the novel setting is complex (Drai *et al.* 2001). For example, in an open-field test with novel objects with male great tits, some birds spent more time interacting with each landmark before moving on to the next landmark (Verbeek *et al.* 1994). This is presumably related to how they explored novel situations, including how they gather knowledge in a complex environment. Interactivity is an

essential component to information gathering explanation as to why animals might intrinsically explore a novel setting.

If I were to use this test to profile individuals according the exploratory behavior in an open-field, I would make the following conclusions. Highly active and exploratory voles are likely to be born into small litters where there is little social diversity. Voles from smaller, less diverse families, as well as older individuals were more active in the novel setting than subjects from larger families and those younger in age. The former group of subjects appeared to be more interested in novelty and interacted with the unfamiliar stimuli more than voles from the latter group. Voles from large families experienced a more socially complex rearing environment and seemed to be less interested in the novel stimuli and were less exploratory than individuals from smaller less diverse litters. Similarly, younger subjects seemed to be less exploratory than older subjects. Males were more exploratory than females, though only marginally so. Among the oldest subjects these marginal sex differences in exploratory behavior disappeared. Similarity among siblings appears to be a consequence of a high degree of similarity in behavioral responses among most subjects. To be sure, future studies might include examining exploratory tendencies across generations and expanding the study of full siblings across multiple litters. Comparing parents to offspring, as well as full siblings born to younger and older mothers might provide some additional information about these environmental influences on behavior. I might also prove helpful in learning more about maternal effects over time.

A closer examination of variation in behavioral responses would also help us learn more about the influence of different environmental variables on behavior. High

degrees of variation among subjects from the same treatment group would signal that behavioral responses are very plastic. An overall high level of activity may be necessary for prairie voles to secure resources for survival. However, predicting their overall exploratory tendencies may not be possible (Chapter 1). Similarly, identifying factors that contribute to these different exploratory tendencies may also be challenging. For an r-selected species like the prairie vole, strong behavioral plasticity may be of great adaptive significance. This species is short-lived, has high fecundity, and experiences high predation pressure from both ground and aerial predators. Failure to fully attribute behavioral responses to factors like social environment, age, sex or family membership may signal that this species experiences population-level maintained behavioral heterogeneity.

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FIGURES

Figure 1. Open-field test Exploration Behavior Prediction Continuum

Figure 2. Photo of the Open-field with novel objects

Figure 3. Latency to depart start box by litter size

Figure 4. Number of squares visited by litter size

Figure 5. Returns to start box by litter size

Figure 6. Time in novel environment by litter size

Figure 7. Time with novel objects by litter size

Figure 8. Visits to edge section by litter size

Figure 9. Latency to depart start box by relative age

Figure 10. Number of squares visited by relative age

Figure 11. Number of returns to start box by relative age

Figure 12. Time in novel environment by relative age

Figure 13. Time with novel objects by relative age

Figure 14. Time with novel objects by sex

Figure 15. Visits to edge of open-field by relative age

TABLES

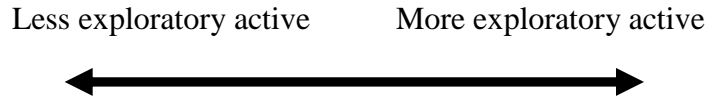
Table 1. Time in novel environment by litter size and litter sex ratio

Table 2. Visits to center section by litter size and sex ratio

Table 3. Total squares visited by subjects according to relative age and sex

Table 4. Time spent in novel environment by subjects according to relative age and sex

Figure 1. Open-field test Exploration Behavior Prediction Continuum



Behavioral Measures	Mean values	
Latency to leave start box	High	Low
# Returns to the start box	High	Low
Time in novel environment	Low	High
Time in contact with novel objects	Low	High
Total squares visited	Low	High
Visits to center section	High	Low
Visits to edge section	Low	High

Figure 2. Photo of the Open-field with novel objects

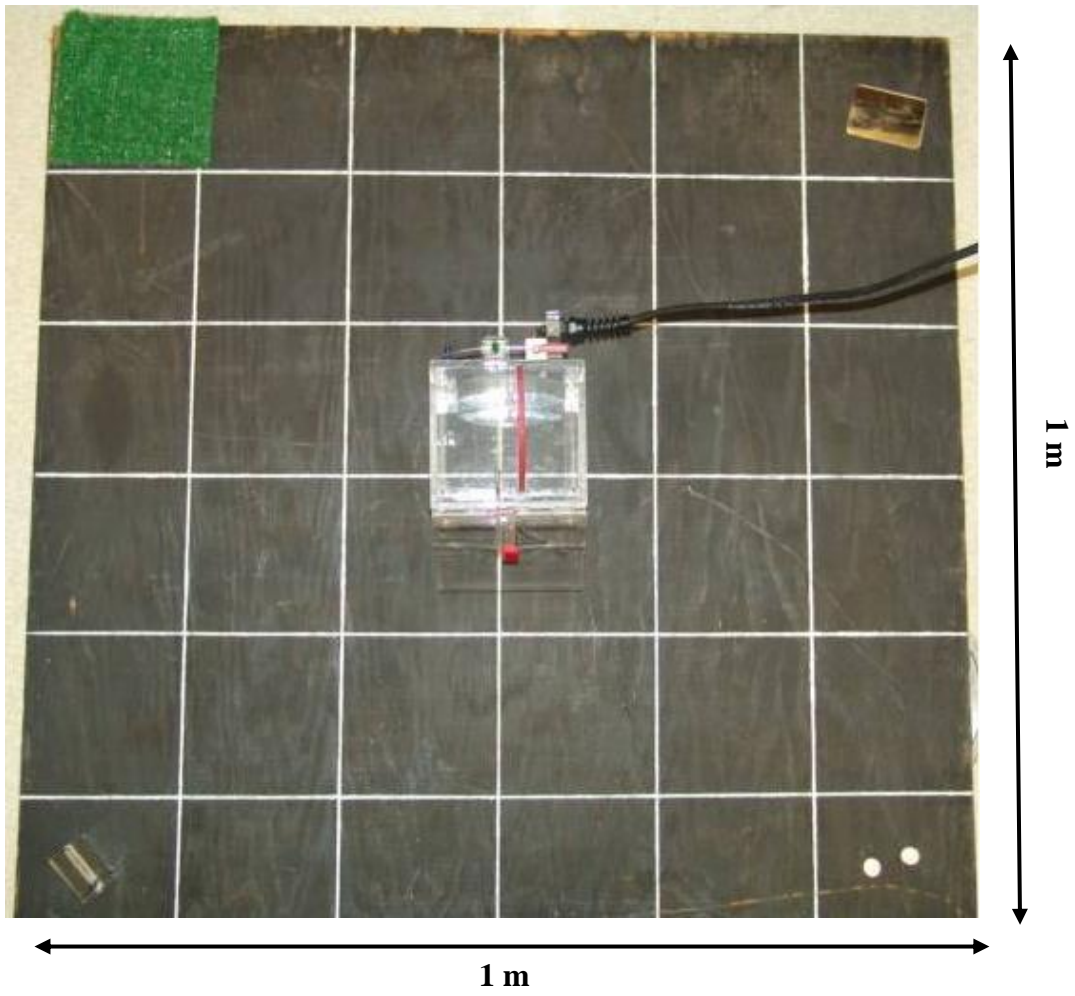
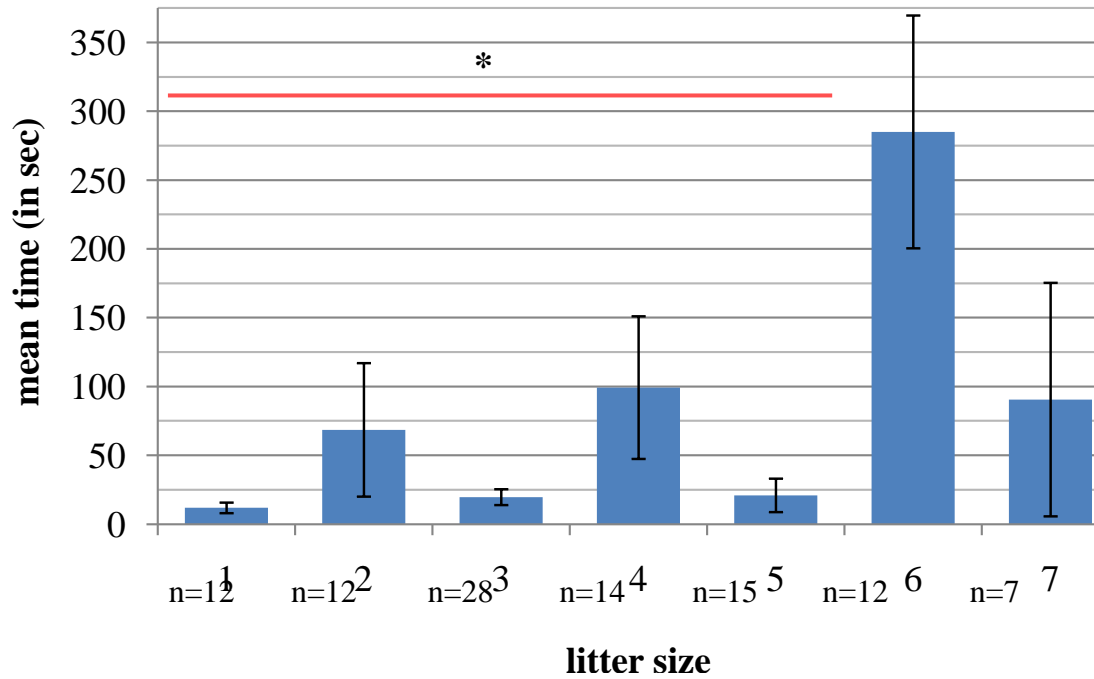


Figure 3. Latency to depart start box by litter size



Mean = 72.76s

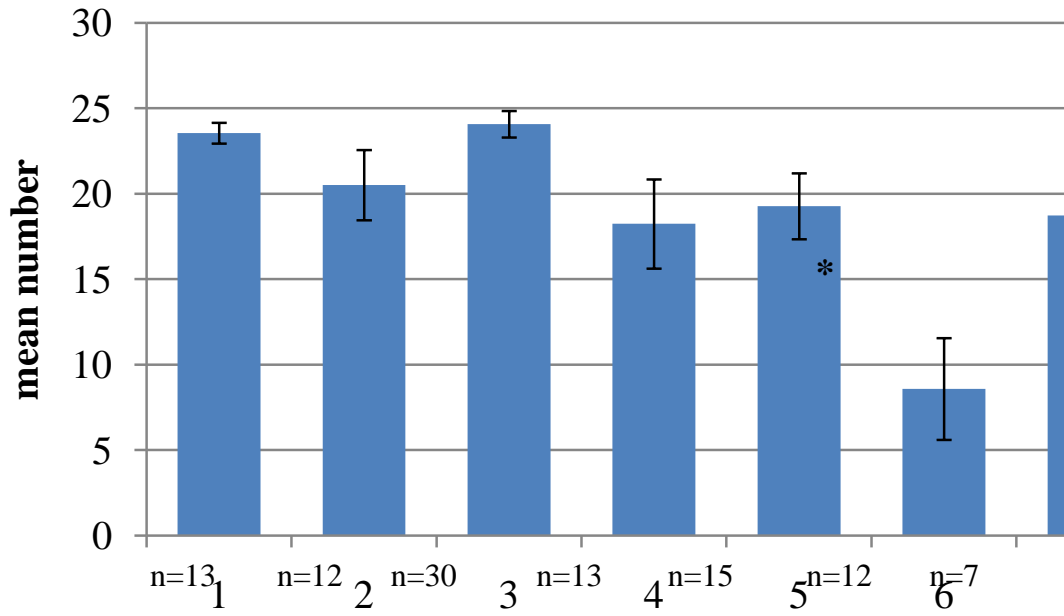
N=100

$p=0.01$

Bars = SEM

Subjects from litters of 6 take longer to depart the start box than subjects from smaller litters, $p<0.05$ for each.

Figure 4. Number of squares visited by litter size



Mean = 19.4

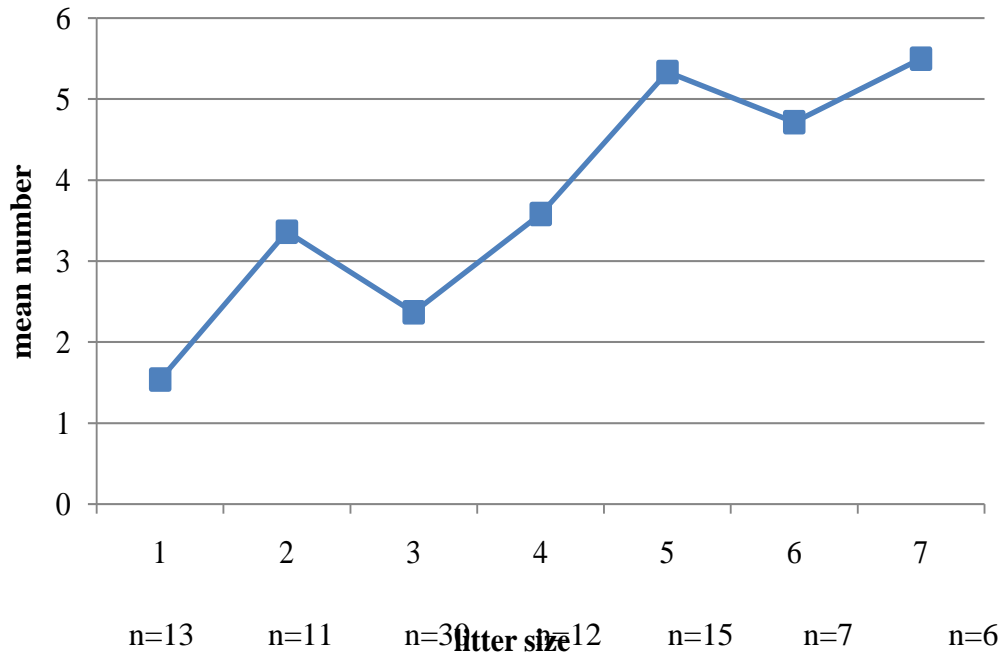
N=102

$p=0.001$

Bars = SEM

Subjects from litters of 6 take longer to depart the start box than subjects from all other litters, $p<0.05$ for each.

Figure 5. Returns to start box by litter size

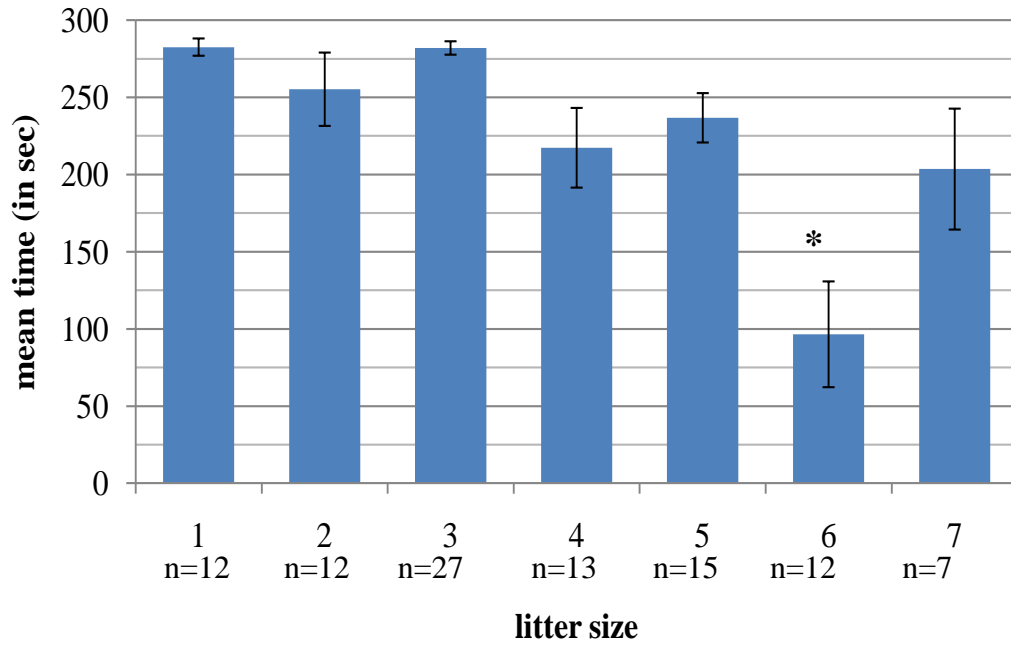


Mean = 3.34

N=94

$p=0.079$

Figure 6. Time in novel environment by litter size



Mean = 234.98s

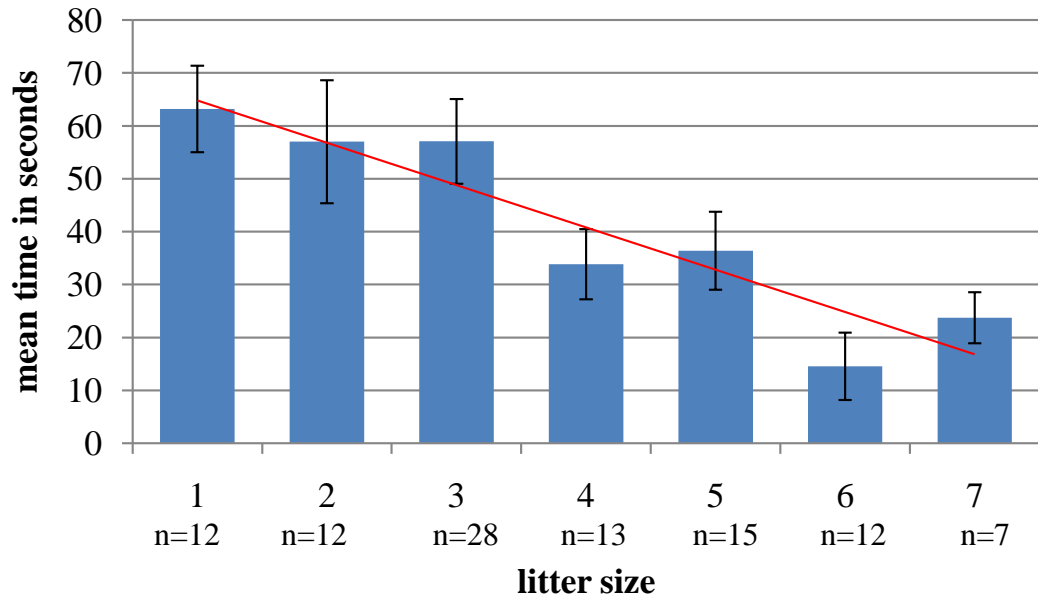
N=98

$p < 0.001$

Bars = SEM

Subjects from litters of 6 spend less time in the open-field field than subjects from all other litters, $p < 0.01$ for each.

Figure 7. Time with novel objects by litter size

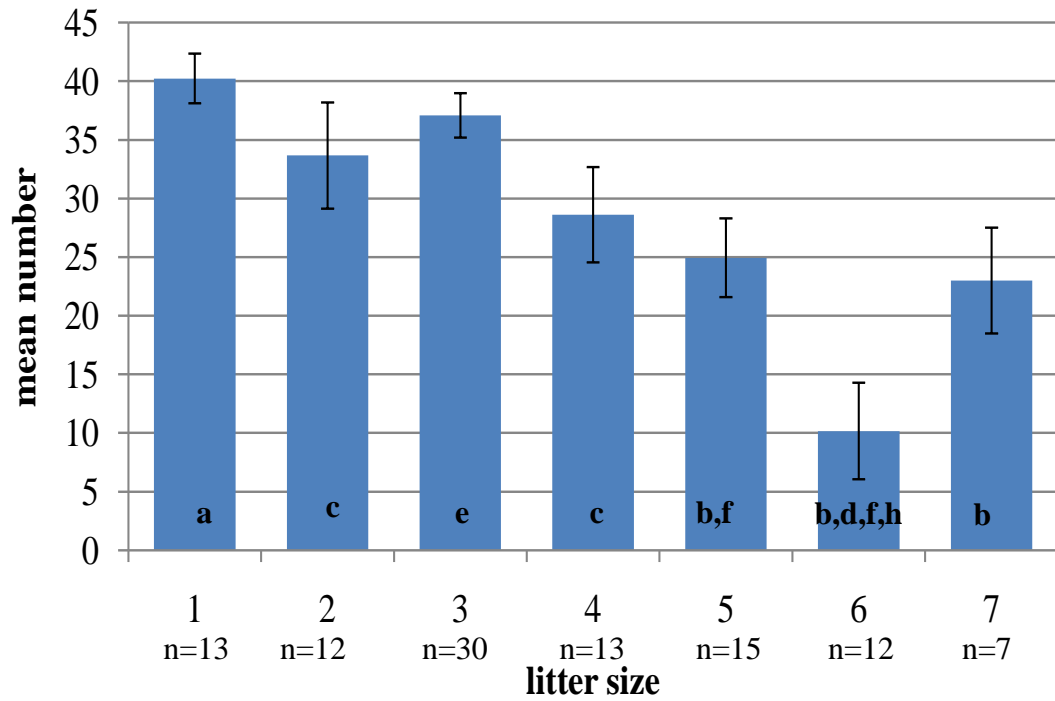


Mean = 44.11s

N=99

$p=0.083$

Figure 8. Visits to edge section by litter size



Mean = 30.09

N=102

$p=0.004$

Subjects from smaller litters visit edge of the open-field more times that subjects from larger litters. Significant pairwise differences indicated with letters.

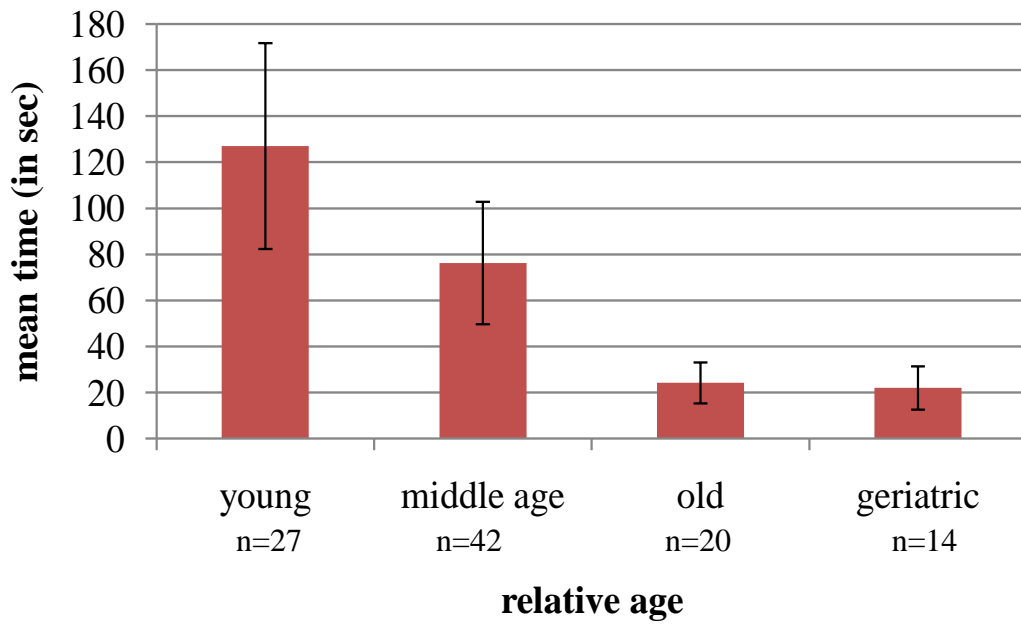
a vs. b, $p<0.05$ (litters of 1 vs. 5, 6, 7)

c vs. d, $p<0.05$ (litters of 2 vs. 6)

e vs. f, $p<0.05$ (litters of 3 vs. 5, 6)

g vs. h, $p<0.05$ (litters of 5 vs. 6)

Figure 9. Latency to depart start box by relative age



Mean = 72.07s

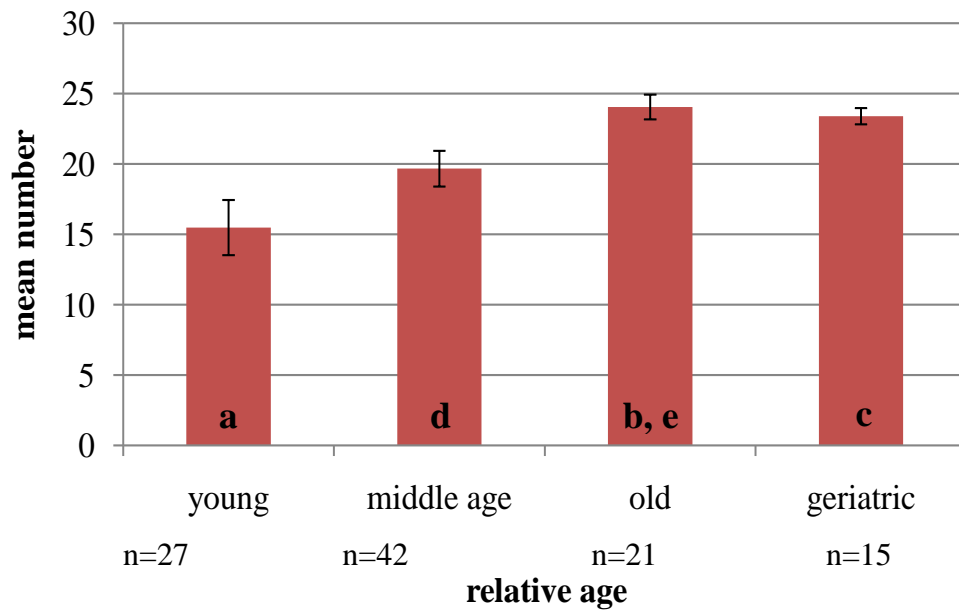
N=103

$p=n.s.$

Bars = SEM

55-120 days -Young
121 – 349 days - Middle age
350 – 544 days - Old
545 + days – Geriatric

Figure 10. Number of squares visited by relative age



Mean = 20.00

N=105

$p=0.013$

Bars = SEM

55-120 days -Young

121 – 349 days - Middle age

350 – 544 days - Old

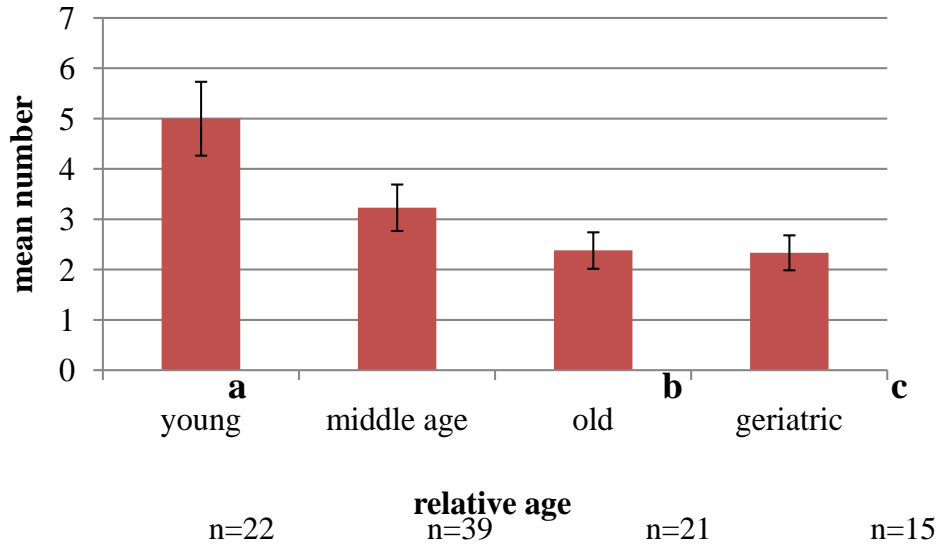
545 + days – Geriatric

a vs. b, $p=0.005$

a vs. c, $p=0.015$

d vs. e, $p=0.037$

Figure 11. Number of returns to start box by relative age



Mean = 3

N=97

$p=0.012$

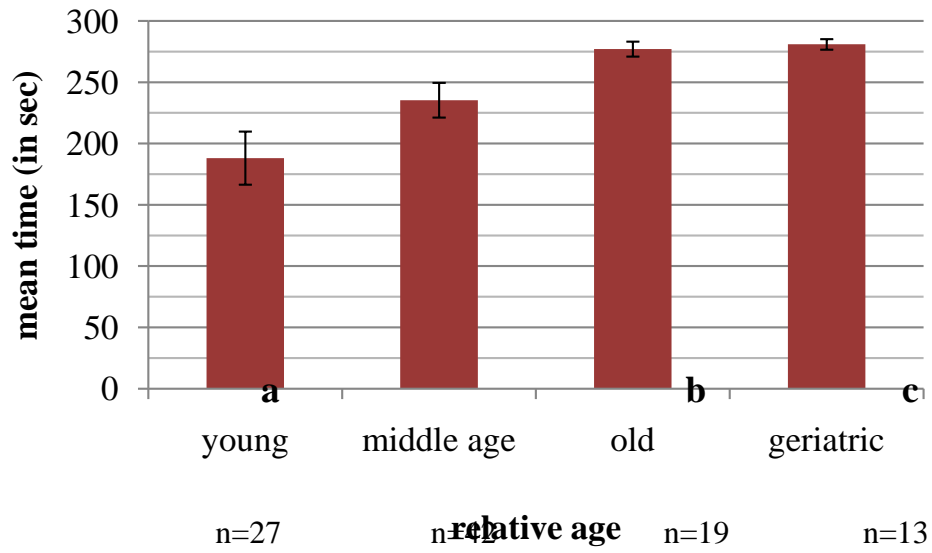
Bars = SEM

55-120 days -Young
121 – 349 days - Middle age
350 – 544 days - Old
545 + days – Geriatric

a vs. b, $p=0.008$

a vs. c, $p=0.016$

Figure 12. Time in novel environment by relative age



Mean = 236.44s

N=101

$p=0.018$

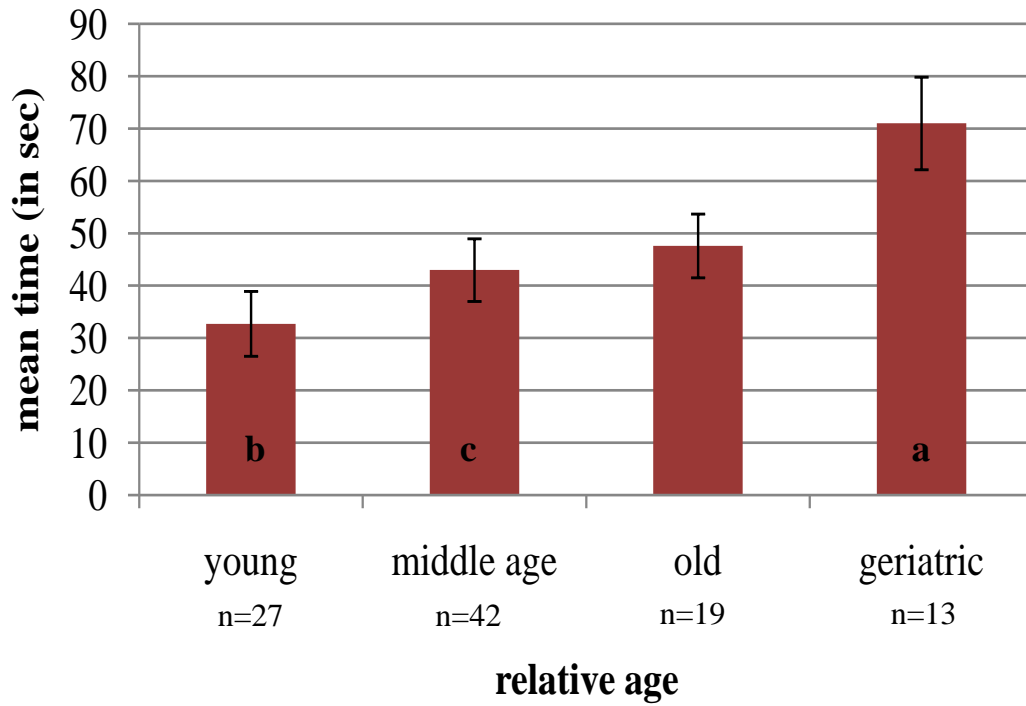
Bars = SEM

55-120 days - Young
121 – 349 days - Middle age
350 – 544 days - Old
545 + days – Geriatric

a vs. b, $p=0.003$

a vs. c, $p=0.007$

Figure 13. Time with novel objects by relative age



Mean = 44.96s

N=102

$p=0.029$

Bars = SEM

55-120 days -Young

121 – 349 days - Middle age

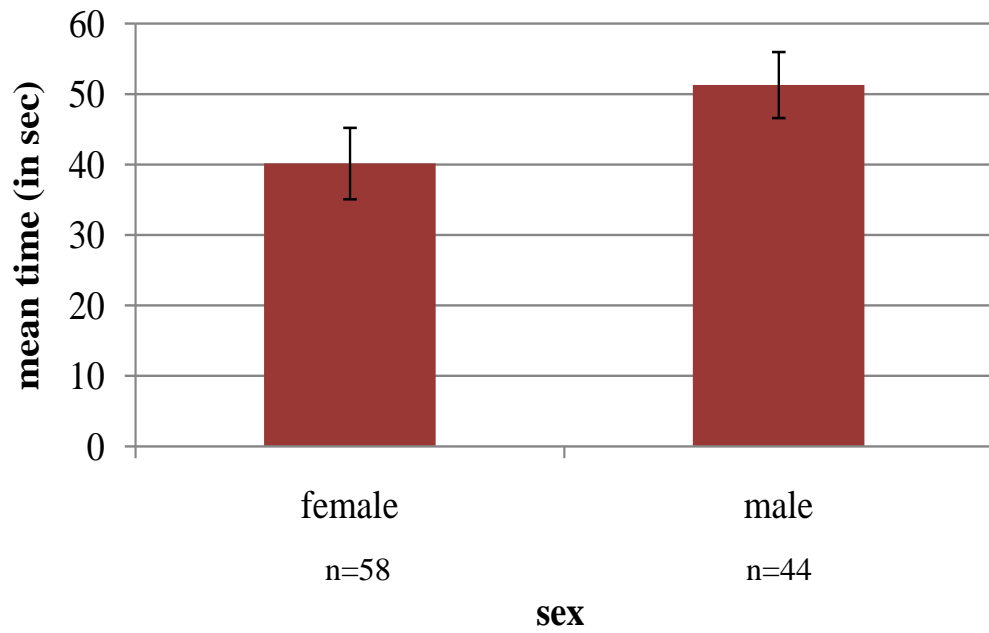
350 – 544 days - Old

545 + days – Geriatric

a vs. b, $p=0.005$

a vs. c, $p=0.041$

Figure 14. Time with novel objects by sex



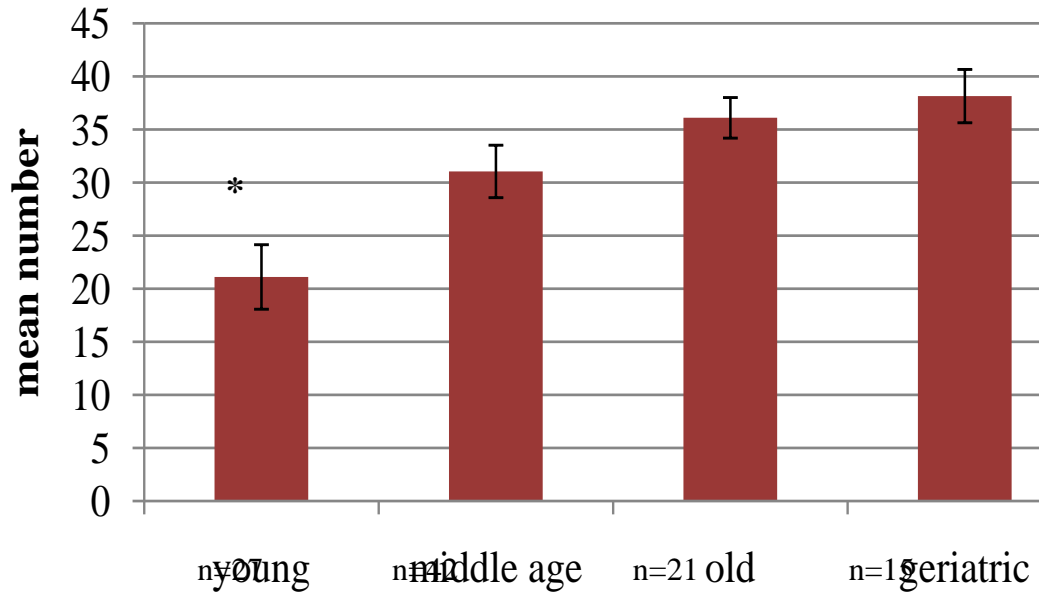
Mean = 44.96s

N=102

$p=0.061$

Bars = SEM

Figure 15. Visits to edge of open-field by relative age



Mean = 30.51

N=105

$p=0.006$

Bars = SEM

55-120 days -Young
 121 – 349 days - Middle age
 350 – 544 days - Old
 545 + days – Geriatric

Young subjects visit the edge section of the open-field fewer times than all other subjects, $p<0.05$ for each.

Table 1. Time in novel environment by litter size and litter sex ratio

Mean in seconds (SD); $\alpha=0.05$, $df=5$

Grand mean = 234.97 s \pm 90.90

N= 98

Litter size	Litter sex ratio	Mean (SD)	N
1	no siblings	282.50 (19.39)	12
	Total	282.50 (19.39)	12
2	sibs same sex	280.71 (20.94)	7
	sibs opposite sex	219.80 (123.64)	5
	Total	255.33 (82.35)	12
3	sibs same sex	269.17 (39.54)	6
	sibs opposite sex	284.22 (13.95)	9
	sibs of both sexes	286.75 (15.71)	12
	Total	282.00 (22.70)	27
4	sibs same sex	127.75 (124.67)	4
	sibs opposite sex	238.67 (54.01)	3
	sibs of both sexes	266.33 (25.54)	6
	Total	217.31 (92.91)	13
5	sibs opposite sex	273.50 (35.54)	2
	sibs of both sexes	231.08 (64.85)	13
	Total	236.73 (61.88)	15
6	sibs opposite sex	245.00 (31.11)	2
	sibs of both sexes	66.90 (106.05)	10
	Total	96.58 (118.72)	12
7	sibs of both sexes	203.57 (103.70)	7
	Total	203.57 (103.70)	7

Table 2. Visits to center section by litter size and sex ratio

Mean (SD); $\alpha=0.05$, $df=5$

N= 102

Litter size	Litter sex ratio	Mean	N
1	no siblings	8.23 (2.77)	13
	Total	8.23 (2.77)	13
2	sibs same sex	9.29 (6.05)	7
	sibs opposite sex	10.40 (7.57)	5
	Total	9.75 (6.41)	12
3	sibs same sex	11.00 (5.60)	7
	sibs opposite sex	9.40 (2.84)	10
	sibs of both sexes	7.77 (5.20)	13
	Total	9.07 (4.68)	30
4	sibs same sex	7.75 (9.29)	4
	sibs opposite sex	8.67 (2.08)	3
	sibs of both sexes	10.00 (3.41)	6
	Total	9.00 (5.31)	13
5	sibs opposite sex	11.50 (0.71)	2
	sibs of both sexes	15.23 (5.33)	13
	Total	14.73 (5.11)	15
6	sibs opposite sex	17.50 (3.54)	2
	sibs of both sexes	3.80 (4.89)	10
	Total	6.08 (7.01)	12
7	sibs of both sexes	11.86 (6.67)	7
	Total	11.86 (6.67)	7

Table 3. Total squares visited by subjects according to relative age and sex

Mean (SD); $\alpha=0.05$, $df=3$

N= 105

p=0.051

Relative age		Mean (SD)	N
young	female	12.79 (10.81)	19
	male	21.88 (4.19)	8
	Total	15.48 (10.18)	27
middle age	female	19.82 (7.86)	22
	male	19.50 (8.79)	20
	Total	19.67 (8.21)	42
old	female	24.38 (3.60)	13
	male	23.50 (4.93)	8
	Total	24.05 (4.06)	21
geriatric	female	24.71 (2.22)	7
	male	22.25 (1.67)	8
	Total	23.40 (2.26)	15
Total	female	19.16 (9.07)	61
	male	21.16 (6.63)	44
	Total	20.00 (8.16)	105

55-120 days -Young

121 – 349 days - Middle age

350 – 544 days - Old

545 + days – Geriatric

Table 4. Time spent in novel environment by subjects according to relative age and sex

Mean (SD); $\alpha=0.05$, $df=3$

N= 101

p=0.091

Relative age		Mean (SD)	N
young	female	160.00 (123.19)	19
	male	254.88 (26.70)	8
	Total	188.11 (112.46)	27
middle age	female	242.64 (81.48)	22
	male	227.30 (103.38)	20
	Total	235.33 (91.72)	42
old	female	268.45 (31.61)	11
	male	289.00 (11.35)	8
	Total	277.11 (26.72)	19
geriatric	female	278.80 (19.06)	5
	male	282.25 (13.76)	8
	Total	280.92 (15.32)	13
Total	female	223.25 (98.76)	57
	male	253.52 (74.82)	44
	Total	236.44 (89.98)	101

55-120 days -Young

121 – 349 days - Middle age

350 – 544 days - Old

545 + days – Geriatric

CHAPTER THREE

EXPLORATORY BEHAVIOR OF PRAIRIE VOLES IN A TWO-WAY NOVEL CHOICE APPARATUS

ABSTRACT

Spontaneous alternation tests examine behavioral responses to novelty as well as memory, learning, and decision-making behavior in pharmacological studies of rodents and other species. The simplicity of these novel choice tests makes them ideal for examining exploratory behavior as a response to novel situations. Exploration is a spontaneous behavior that involves investigating novel settings absent of obvious motivating factors such as hunger or risk of predation. In this study, I examined the effects of multiple social, hereditary, and developmental variables on exploratory behavior of prairie voles, *Microtus ochrogaster*. Social complexity and familial relationships, as well as age and sex of subjects can influence behavioral responses to novel situations such as a modified spontaneous alternation test. Subjects were observed in two runs of a modified T-maze (one time with an odor stimulus, one time without) and their behavioral responses were compared. Recorded behavioral measures included latency to depart start box, initial direction, time to reach the first and second terminal, and total test time. Individual behavioral differences in this test were previously determined to contribute to an exploratory behavior profile continuum labeled as pro-activity/reactivity (Lee, Chapter 1). Proactivity-reactivity explains how subjects respond to novel situations. It is defined according to how quickly or slowly a subject initiates action and spends its time in a novel environment. Proactive individuals tend to be bold initiators of action, are often observed bolting out into novel environments, and tend to

move rather quickly within a novel setting. On the other hand, reactive individuals tend to move more slowly, seemingly cautiously when introduced to novel settings (Sih *et al.* 2004a, b). I found no relationship between behavior in the two-way novel choice apparatus and the independent variables of interest (e.g., litter size, sex, family relationship). Though there was variation in behavior among individuals, it was not statistically different. Most voles traversed the apparatus quickly and there was little change in behavior between trials when the novel stimulus odor was added or removed. This indicated that these subjects were more likely to behave proactively in changing environments, regardless of the dependent variables I examined.

Keywords: prairie voles, exploratory behavior, novelty-response, proactivity, reactivity

INTRODUCTION

Exploratory behavior is defined as a response to novel environments or stimuli (Renner 1987). It includes how an organism moves within a given space, processes information, and gathers knowledge about its local environment (Archer & Birke 1983; Renner 1990; Renner 1987; Verbeek *et al.* 1994; Drai *et al.* 2001). Researchers identify animal exploratory behavior by measuring their responses and investigation of unfamiliar environments (Renner 1987). It is regarded as spontaneous behavior if motivating factors such as hunger, reproductive drive or escape from danger are controlled for (Renner 1990; Hughes 1997). Examining spontaneous exploratory behavior helps us to understand how animals react to novel situations in nature and may reflect important aspects of an animal's behavior including foraging, dispersal, escape reactions, and how an animal responds to dynamic environments. These behavioral responses allow an individual to gather important information about its environment and how it might make decisions crucial to its fitness (Glickman & Sorges 1966; Archer & Birke 1983; Renner 1990; Verbeek *et al.* 1994; Drai *et al.* 2001).

Spontaneous alternation tests, also called T-mazes or Y-boxes because of their shape, have been used as tests of discrimination learning by psychologists since the turn of last century (Dewsbury 1978). These tests provided a choice between relatively familiar arms and relatively novel arms of the apparatus (Hughes 1997). These apparatuses were used to help psychologists understand decision dynamics when an individual faced novel choices. Because of their design, they also were considered to provide a free or spontaneous test of exploration that can be used to examine response to

novelty. Specifically, these tests allow researchers to examine how a subject might respond to a variable environment.

Though exploration is defined as how an animal might interact with and investigate its new surroundings, it also includes how quickly or slowly an animal might explore an unfamiliar setting. Examining exploratory responses as a measure of proactivity or reactivity to novelty would provide insight as to how an individual explores and adjusts to the trade-off of exploration speed and attention to the environment. For example, proactive individuals have been characterized as fast explorers in a novel environment (Dingemanse *et al.* 2002). They respond to situations quickly, formulate routines and are insensitive to external stimuli such that if the environment were to change then these animals would not behave in an obviously different manner (Dingemanse *et al.* 2004). Individuals with proactive strategies cope by actively responding to situations very quickly (Benus *et al.* 1991; Dingemanse *et al.* 2004, Benus *et al.* 2004). They are adapted to behave optimally in stable environments but the same behaviors can be maladaptive in less stable or variable environments (van Oortmersen *et al.* 1985; Benus *et al.* 1987; Clark & Ehlinger 1987; Verbeek *et al.* 1994). On the other hand, reactive individuals have been characterized as slow explorers who are sensitive to external stimuli, and readily adjust behavior to changes in the environment (Dingemanse *et al.* 2004). Individuals with reactive strategies cope by passively responding to situations (Verbeek *et al.* 2004; Dingemanse *et al.* 2002). They are adapted to behave optimally in changing or unstable environments (van Oortmersen *et al.* 1985; Benus *et al.* 1987; Clark & Ehlinger 1987; Verbeek *et al.* 1994).

Exploratory behavior in a novel setting is highly variable among individuals. Like all selected traits, individual variation in behavior may be susceptible to natural selection (Fox *et al.* 2009). Individual variation reflects a constraint on the optimization process demonstrated by the animal (Verbeek *et al.* 1994; Clark & Ehlinger 1987). Variation in proactive and reactive exploratory behavior may provide the basis of selective differences in fitness traits such as foraging, anti-predator behavior, and dispersal. Examining inter-individual differences in novel choice behavior, allows the assignment of behavioral profiles that categorize subjects' behavior in a given test situation (Groothuis & Carere 2005). Behavioral profiles describe behavioral tendencies or 'dispositions' of animals along an axis, such as proactive-reactive or more or less exploratory (Fox *et al.* 2009). These behavioral profiles allow behavioral traits to be examined within the Behavioral Syndrome framework (Bell & Stamps 2004; Bell 2007). This framework not only quantifies individual variation in behavior, but also attempts to explain the development and maintenance of this variation (Sih *et al.* 2004a).

In this study, I examined the environmental influences on the individual variation of exploratory behavior in a two-way novel choice test. The objective of the study was to determine if individual variation in exploratory behavior can be attributed to independent variables such as early social environment, developmental factors such as age or sex, and family membership. I tested three hypotheses concerning the development of individual variation of exploratory behavior in a novel choice apparatus test.

Hypothesis 1: The social environment in which an individual was reared and experiences throughout life may influence the behavioral development of the individual (Carducci & Jakob 2000; Genaro & Schmidek 2002; Neugebauer *et al.* 2004). Subjects

from similar family compositions are expected to demonstrate similar behavioral responses in the two-way novel choice apparatus. Coming from a small or large litter, and with or without brothers or sisters, may have profound effects on the adult behavior of individuals. Moreover, the effects of subtle social differences that may occur normally in the early postnatal environment of mammals living under natural conditions have rarely been studied.

Hypothesis 2: Developmental factors, such as age or sex, are known to affect behavior (Dall *et al.* 2004). This hypothesis addresses if and how developmental factors, such as sex and age of a subject at time of testing, contribute to individual differences in exploratory behavior.

Hypothesis 3: Related individuals often share similar behavioral characteristics. I predict that siblings will demonstrate similar individual behavioral trends when introduced to novel situations. Subjects born to the same parents, which would include litter mates and full siblings from previous or subsequent litters, might share behavioral tendencies due to genomic or non-genomic effects (i.e., culturally and socially transmitted traits) such as maternal effects. This study does not attempt to disentangle the exact mode of heritability of individual variation in exploratory behavior but does attempt to explore its influences. See Figure 1, Exploratory Behavior Prediction table.

GENERAL METHODS

1. Animals

Fifty-three male and 83 female that were first through third generation, lab-reared prairie voles, *Microtus ochrogaster*, from Urbana-Champaign, Illinois, served as the subjects in this behavioral test. Individuals were reared under a 14:10 LD light schedule.

Lab conditions, including frequency of handling, cage cleaning, and feeding, were consistent among all animals.

Animals were reared in early social environments that consisted of naturally occurring littermates and one or two parents. Less than 20% of the voles born in this colony were from litters raised by the female parent alone due to death of the male parent or adjustment of breeding schedule protocols. However, statistical analysis confirmed that the physical development and behavioral responses of voles raised by one parent were no different than those voles raised by both parents. Therefore, these data were pooled. Natural litter sizes vary from 1-8, with 3-4 being the average size. Litter sex ratio is also naturally variable and was characterized as the subject having a) no siblings, b) same-sex siblings only, c) opposite sex siblings only, and d) at least one sibling of each sex. All voles were weaned at 21-23 days of age and were housed with littermates, if any, for the duration of this experiment.

Voles were tested during the light phase of the time cycle between 10:30 -16:00 CST hours. All subjects were tested post-sexual maturity (sexual maturity occurs at 40 days) and were sexually inexperienced (Getz *et al.* 1994). Age variation ranged from 55-1,400 days and was minimized whenever possible; however, age at testing did vary randomly. The mean life span for prairie voles born and raised in this colony was 345 days \pm 15 (SE) and is consistent with mean life span observed in other laboratory studies (Stalling 1990). The average life span of wild prairie voles ranges from 30 – 122 days depending on season, population density, and whether an individual disperses or not (Getz *et al.* 1994). Voles in this study were categorized as **young**, 55-120 days of age, **middle age**, 121-349 days, **old** 350-544 days, and **geriatric** 545 or more days of age.

Though there is no official age designation for prairie voles or other Microtine species, these age groupings are roughly consistent with the relative ages of voles tested in laboratories (Wolff *et al.* 2001; Grippo *et al.* 2007). There were no apparent behavioral, physical, or health disparities among the subjects. Subjects completed the two-way novel choice test only one time and were naïve to the apparatus prior to testing. These voles were also used in two other experiments examining exploratory behavior (see Chapters 2 and 4). However, the order in which each of the experiments were completed by subjects was randomized.

2. Apparatus

The two-way novel choice apparatus is comprised of a centrally located start box, 153 (w) x 101 (l x h) mm, connected to two runways each made of a long Plexiglas tube, 500 (l) x 7.5 (d) mm. White opaque doors (guillotine-style, made of acrylic plastic) separate the start box from each runway. The terminal of each runway is connected to another box of the same dimensions as the start box. However each runway tube terminates at a screen door (made of opaque acrylic plastic and fine mesh). A schematic of the two-way novel choice apparatus is presented in Figure 2.

3. Methods

The subject was placed into the start box which contained a ventilated Petri dish, 9.5 cm in diameter, made of Nalgene plastic with the two lids attached to one another by a screw and nut with several holes drilled into the upper lid. The Petri dish was filled with scented bedding from the home cage of the subject and mounted to the inside wall of the start box with scotch tape. The bedding-filled Petri dish provided odor from the vole thus making the start box a location of familiarity (Hughes 1997). Such an experimental

set-up is best for examining spontaneous exploratory behavior and is considered ecologically relevant (Hughes 1997), as well as preferred by rats and mice (Russell 1975; Misslin & Ropartz 1981). The subject remained in the start box for 3 min to acclimate. Then, the doors allowing access to the runways were manually lifted. The following measures were recorded 1) latency to depart the start box (in seconds); 2) initial direction (left or right) when exiting the start box; 3) time to reach the first terminal after leaving the start box; 4) time to reach the second terminal after the animal visits the first terminal; and 5) total time to complete the test, measured as the time to visit both terminals minus initial latency. A subject was considered to have reached a terminal if its nose came within 3 cm of the screen door of each terminal. This 3 cm region was referred to as the proximity threshold zone.

The ratio of time to reach the first terminal to total time to complete the test (minus initial latency) was calculated. (Equation: $\text{time to first terminal} \div \text{total time to complete the test}$). This ratio represents how subjects explored each side of the tube, specifically the time it took to reach the second terminal relative to the time it took to reach the first terminal. Spending more time in the first arm, revisits to the first terminal, pausing in start box, and/or spending time in the second arm before reaching the second terminal can all result in a longer time to reach the second terminal and a smaller ratio value. Larger ratio values mean that subjects reached the second terminal not much long after reaching the second terminal.

Each subject completed two trials of this test, once with novel odor stimuli (vanilla and lemon extract) behind each screen door, and once without any novel odors. The order of the trials was counter-balanced. Vanilla and lemon scents were used

because the subjects had no previous exposure to them and they were unlikely to be aversive. A drop of vanilla extract and lemon extract was placed on separate filter papers and placed inside of a closed Plexiglas box behind the screened terminal door approximately ten seconds before the start of the trial. A different scented filter paper was randomly placed on each side of the apparatus. The time between the two trials was approximately 30 min. Each trial ended when the subject visited the second terminal. The total time of the test was variable, but if a subject did not leave the start box by 5 min, or became inactive for more than 5 min after initiating the test, then the test was ended. The maximum time was recorded as the time to complete the test and the remaining measures were left blank and included in the data analysis. Between tests the start box, the Petri dish, terminal boxes, and the runway tubes were cleaned with soap and water and disinfected with a 15% ethyl alcohol solution to eliminate any odors that might have accumulated from the previous subject.

4. Data Analysis

Data were analyzed using SPSS 17 statistical package to analyze behavioral response measures. First, I analyzed the data for patterns and trends related to overall response to the testing apparatus. I analyzed the data for side bias in each trial and the influence of order of stimulus odor presented on performance between trials with Chi Square analysis. I analyzed the data for effects of the stimulus odor on performance between trials with a Univariate ANOVA. I also measured the reliability of responses across the two trials. I completed a reliability analysis with a Cronbach's alpha test. Cronbach's alpha is the most common form of internal consistency reliability coefficient and models internal consistency based on the average correlation among items.

Cronbach's alpha score is based on a percent scale. Values that approach one indicate better consistency of scores across two or more trials. By convention, values of 0.7 or higher indicate adequate reliability and many researchers use 0.8 as the cut off for a good scale of reliability. The test yields a Pearson correlation coefficient (ρ) and a p value. The larger the ρ , the more the item contributes to the internal consistency; low inter-item correlation means the item is weakly correlated with the overall scale.

Finally, I completed a General Linear Model – Repeated Measures Analysis examining the influence of multiple independent variables on each set of dependent variables – comparing trials 1 and 2 together, (litter size x litter sex ratio; age x sex). Tukey's post-hoc test was used to evaluate pair-wise relationships. The mean difference in values was evaluated at the $\alpha = 0.05$ level. Parametric statistical test were appropriate for several reasons: 1) reasonably large sample sizes are able to withstand the statistical effects of averaging, 2) parametric tests are less affected by extreme violations of assumptions of models including homogeneity of variance, normality, small sample sizes, and unequal sample sizes, and 3) parametric tests are generally robust statistical tests (Boneau 1960).

To examine the influence of family membership on the same dependent variables, I completed a Two Step Cluster Analysis of all continuous dependent variables using a BIC Cluster criterion, log-likelihood un-standardized variable method. Individuals were clustered based on each behavioral measure separately. Clusters are based on the mean (central tendency) for all subjects for that measure. Each subject was assigned to the cluster which has a mean closest to its behavioral score. Next, I

calculated the proportion of full siblings that fall within the same cluster for each dependent variable.

RESULTS

a. Side bias, stimulus order, and stimulus odor effect

There was no side bias in trial one. Voles were equally likely to exit the start box on the left or right side ($p=.550$, $X^2 = .358$, $df = 1$; see Table 1) and reach either terminal first regardless of the presence of odor stimulus ($p=.881$, $X^2=.022$, $df=1$; see Table 2). However, there was a right side bias in trial 2, $p=0.034$, $X^2=4.496$, $df=1$; see Table 1) but this bias did not carryover to which terminal side was likely to be reached first ($p=.527$, $X^2 = .400$, $df=1$; see Table 2).

Presenting the stimulus odor in the first or second trial did not affect any of the behavioral measures recorded in either trial. However, the X^2 analysis confirmed that voles were more likely to reach the right terminal first in trial 2 when stimulus odor was present (see Table 3); but equally likely to approach either odor in either trial (see Table 4).

Finally, the presence or absence of odor in either trial had no measurably effect on any of the behavioral measures in the test. See Table 5.

b. Reliability Analysis

This analysis tests the consistency or repeatability of behavior across trials within and across individuals. There was strong intra-individual consistency in initial latency to depart the start box (Cronbach's $\alpha=.833$, $\rho= 0.734$, $p=0.01$, 2-tailed, the within individual vs. between individual ANOVA with Friedman's $X^2 = 10.617$, $p=0.001$) and moderate consistency within individuals for the time to reach second terminal (Cronbach's $\alpha =$

.576, $\rho = .405$, $p=0.01$, $X^2 = .000$, $p=.998$) and total test time minus latency (Cronbach's $\alpha=.567$, $\rho = .396$, $p=0.01$, $X^2 = .198$, $p=.656$). There was no consistency for initial direction (Cronbach's $\alpha= -.003$, $\rho =-0.001$, $p= ns$, $X^2 =.941$, $p=.332$), or the remaining measures. See Table 6.

The second introduction to the apparatus resulted in reduced values for all measures. Three measures were significantly different between trials, regardless of the order of the two trials (i.e., odor or no odor stimuli).

Latency to depart the start box is shorter in the second trial. This difference is significant ($p=0.001$, Lower-bound, $F_{stat}=11.40$, Partial $\eta^2=.075$, $df=1$).

Time to reach the first terminal is shorter in the second trial. Although there was a small difference in the mean time to reach the first terminal between trials one and two, this difference is significant ($p=0.035$, Lower-bound $F_{stat}=4.537$, Partial $\eta^2=.033$, $df=1$).

Ratio of time to reach the first terminal to total test time is smaller in the second trial. This difference is significant ($p<0.001$ Lower-bound, $F_{stat}=13.741$, Partial $\eta^2= .094$, $df=1$).

There was no differences of means between trials for remaining measures ($p>0.05$, Partial $\eta^2 <0.05$, $df=1$; See Table 6).

c. Hypotheses testing

1. Social environment factors (litter size and litter sex ratio)

None of the independent variables influenced the behavioral outcomes in the two-way novel choice apparatus. The effect of litter size or litter sex ratio had very little to no effect on the recorded measures (Partial $\eta^2 <0.05$; $p=n.s.$), singly or as an interaction on any of the behavioral measures.

2. Developmental factors (age and sex)

None of the independent variables examined influenced the behavioral outcomes in the two-way novel choice apparatus. The effect of age or sex of subjects had very little to no effect on the recorded measures (Partial $\eta^2 < 0.05$; $p = n.s.$), singly or as an interaction on any of the behavioral measures.

3. Family membership

One hundred-thirty-three subjects from 21 families, consisting of 2 or more full siblings per family (mean=6) were evaluated to determine the similarity of behavioral responses among related individuals. A few families are better represented in the sample than some others – for example one male and female pair was responsible for 27% of the full sibling subjects in this test. Each behavioral measure clustered into two but no more than three clusters (based upon high, medium, and low means). For most dependent variables, a majority of individuals cluster together in the same group as their full siblings. For measures where families were not clustered together, i.e., the family was split, the proportion of siblings that were in a different cluster than the majority of their family group ranged from 15 - 44%.

Initial Latency: Most subjects, 92.5% clustered together in the low mean group (16.67s \pm 19.96; 7.68s \pm 8.10). The remaining 10 individuals were from 6 of 21 families with high mean latency for departing the start box (196.80s \pm 124.04; 159.60s \pm 94.30). The mean disagreement for these families was 16.48%.

Time to reach first terminal: Most voles (96.1%) clustered together in the low mean group. 3 families out of 21 were split, mean disagreement was 15.98%.

Time to reach second terminal: 89.6% of all subjects are in the low mean cluster. Seven out of 21 families were split (a third) mean disagreement was 27.28%

Total test time (minus latency): 91.3% of subjects clustered in the low mean cluster. Five families out of 21 split, mean 30.28%

Ratio time to reach terminal 1: total test time: 52.8% of subjects are in the lowest mean cluster. The remainder are nearly evenly split between the other clusters which are a highT1/mediumT2 mean (25.6%) and a mediumT1/high meanT2 (21.6%) group. Most families were split – 17 out of 21, mean disagreement was 43.56%.

See Table 7.

DISCUSSION

Spontaneous alternation tests have been used primarily for examining discrimination responses in animals (Dewsbury 1978). It allows researchers to measure animal responses to differing degrees of familiarity and novelty (Hughes 1997). Subjects choose between most recently visited (familiar) or unvisited (novel) sections of an apparatus which allows researchers to record orientation and spatial changes, as well as temporal responses to the apparatus (Hughes 1997). For example, visiting left or right sides of an apparatus across multiple introductions or total distance traveled over time are ways of measuring exploratory responses (Berlyne 1960). Temporal responses, such as latency to enter and time spent in a novel environment, are other common measures of exploratory response (Hughes 1997). Together, these different responses to novel environments provide a better understanding of spontaneous exploratory behavior in animals (Renner 1987, 1990).

How quickly or slowly an animal explores a novel environment tells us how animals may gather and process information (Benus *et al.* 1987). In a homogenous environment, the information can be gathered rather quickly (Kleerekoper *et al.* 1974). Once an animal has accumulated a certain amount of presumably new information, then it moves on to a different part of the novel environment (Kleerkoper *et al.* 1974). The more time in a novel setting increases the amount and type of information gathered, especially if the novel setting is complex (Kleerekoper *et al.* 1974; Draï *et al.* 2001) or has changed upon subsequent introductions. Yet proactive individuals, if their behavior is hard-wired or developmentally irreversible, may fail to respond to the changes or complexities in the environment. Proactive individuals are said to be active copers in novel situations (Dingemanse *et al.* 2004). They tend to enter into settings with little delay, quickly form routines, and are insensitive to external stimuli (Sih *et al.* 2004a, b; Dingemanse *et al.* 2004). On the other hand, reactive individuals are regarded as passive copers (Dingemanse *et al.* 2004). They tend to slowly enter novel settings and readily adjust their behavior to changes in the environment (Sih *et al.* 2004a, b; Dingemanse *et al.* 2004). By observing how voles occupy different parts of the novel environment, choosing between familiar and unfamiliar areas, I can glean more information about how animals explore (Renner 1990; Draï *et al.* 2001). The amount of time spent in a novel environment represents exploratory interest in and attention to unfamiliar stimuli.

Proactivity and reactivity to novel settings can be important for fitness and survival. However, being more proactive or more reactive does not mean that an animal is more or less exploratory in a novel situation. In fact, either tendency can be said to be characteristic of highly exploratory individuals. On one side of the continuum,

proactivity speaks to how fast and presumably how far an animal may venture into a new setting. The other character, reactivity, speaks to how thoroughly and completely an animal explores a new setting. For example, in an exploratory test with male great tits, some birds spent more time interacting with each landmark before moving on to the next landmark (Verbeek *et al.* 1994). Some animals were fast but superficial explorers and others were slow, thorough explorers. This is presumably related to how they explored novel situations, including how they gathered knowledge in a complex environment. Increased time with novel stimuli may increase the information gathered about resources, particularly if changes occur (Benus *et al.* 1987). However, taking the time to explore an unfamiliar setting slowly and thoroughly also increases exposure to predators. This presents a trade-off and depending on the stability of the environment, either tendency might be favored over the other.

In this study, reactive individuals would be those with longer time values in the test and across trials, whereas proactive individuals would be those with shorter time values. Values for initial latency to depart start box, time to reach each terminal, and total test time give direct measures of interest in more or less familiar stimuli. The start box contains odors from the home cage of each subject; and each vole was acclimated to the start box prior to each observation. Delaying entry into the novel environment, taking relatively long amounts of time to reach each terminal, and a long time to complete the test would indicate a low interest in novel stimuli. Moreover, subjects would have a smaller ratio value for time to reach the first terminal to total test time. Together, these scores would be indicative of a reactive exploratory response. In contrast, proactive individuals would depart the start box quickly, reach each terminal quickly, and have a

relatively large ratio of time to reach the first terminal to total test time. Highly reactive voles are more attracted to the familiar and presumably safer stimuli, such as home cage odors or the stimulus at terminal one and generally less curious about other novel stimuli (Whishaw *et al.* 2006; Eilam 2010).

Furthermore, if animals were sensitive to the changes in the novel environment, then the time values for the second trial of the test would be equal to or greater than the times for the first trial. Typically, animals respond to subsequent introductions to a novel apparatus with decreased times to enter and complete tests. Such a reaction is often consistent for individuals over time (e.g. male great tits, Verbeek *et al.* 1994). In this study, the latency to enter, time to reach the first terminal and total test time was significantly less in the second trial despite stimulus odor changes to the two-way novel choice apparatus between trials. This indicates that nearly all subjects failed to respond to changes in the external environment and behaved proactively. However, the ratio to reach the first terminal to total test time between trials gives a different account vole exploratory response. The time taken by voles to travel from the first terminal to the second terminal actually increases in trial two, but the difference was not significant. Yet, the ratio of time to reach the first terminal to total test time was significantly different between trials. The ratio is significantly smaller in the second trial, meaning the voles were behaving reactively to the change in the environment. The response to the stimulus change between trials was not initially obvious when measured solely as latency or time to complete the test. The increased time to reach the second terminal and the smaller ratio value between trials presents a nuanced account of vole exploratory behavior in the two-way novel choice apparatus.

Based on the results from Chapter 1 that examined the range of inter-individual variation of the exploratory behavior in this test, I wanted to know which, if any, environmental variables influenced novel choice behavioral responses of subjects. All behavioral measures can be scored along a scaled continuum – from high to low values. If belonging to a specific treatment group influences exploratory behavior in a significant way, then subjects from similar social environments or those who are the same age should behave similarly. However, none of the independent variables explain the differences in behavioral responses across the two trials. The social environment, age, sex, or family membership could not explain these differences in response to the two-way novel choice apparatus.

Though there were no significant differences in behavior according to treatment group, some interesting behavioral patterns did emerge. Statistically, there was no difference in how subjects from large, average, or small litters or those from same-sex or mixed-sex litters behaved. However, subjects from litters of 6 and 7 with both brothers and sisters took more time to enter the novel environment, to reach each of the terminals, and complete the entire test compared to subjects from smaller litters or those having same-sex siblings or opposite-sex siblings. Perhaps diverse social environments act as an enrichment experience. Much like rats reared in cages enriched with toys and cage mates, voles from complex social groups seemed less interested in unfamiliar stimuli than voles from smaller, less diverse litters (Varty *et al.* 2000). In this study, subjects from the larger, mixed-sex litters responded more reactively to the novel environment than subjects from other litter sizes or sex ratio combinations; and subjects from small litters, those having no or few siblings, responded more proactively to the novel environment.

Though fundamental biological factors, such as age or sex, are known to be responsible for generating correlations in behavior (Dall *et al.* 2004), that was not the case in this study. Males took more time to enter the novel environment, to reach each of the terminals, and complete the entire test compared to females, but the differences were not statistically different. Old males had the highest values for latency to enter the apparatus and time to complete the test compared to all other age and sex combinations, but the differences were not statistically significant. They also had higher means for these values in the second trial than in the first trial which is the exact opposite reaction of all other voles to the second introduction to the apparatus. In contrast, both male and female geriatric subjects had some of the lowest mean values across trials. In general, males demonstrated more reactive tendencies than did females, but old males were especially reactive compared to all other subjects. Among geriatric subjects, proactive exploratory behavioral tendencies were more common.

Individual variation in behavioral profiles has been shown to be moderately heritable (Dingemans *et al.* 2002; Cockrem 2007). However, the support for heritability influencing exploratory behavior in this test was inconclusive. The clustering method assigned individuals to groups according to mean values that were either significantly higher or lower than the general mean for each behavioral measure. By clustering individuals according to low or high means, I could identify any similarities of behavior among all individuals, including family members. Initially, the findings demonstrated that novel-choice behavioral responses tended to run in families. However, most individuals in most families were assigned to the same clusters – the low mean clusters. Only a small percentage of individuals from each family reacted to the novel

environment differently than the rest of their relatives. Nonetheless, I did find a small group of related individuals that responded very similarly to the two-way novel choice apparatus. When analyzing the data for the influence of litter size and litter sex ratio I found a group of subjects had very high mean values for all timed measures across both trials. Though these ten subjects, full siblings from two litters, did not alter the data significantly, their behavioral responses were in the same direction. However, because so many un-related individuals were assigned to the same clusters, it is unlikely that genetics was the major factor, and environmental effects may be the reason why so many voles clustered together.

My results may simply demonstrate that voles are generally proactive creatures. Some are slower explorers than others, but there is so much variation in behavior that I was only able to demonstrate somewhat ambiguous patterns in behavior for a few of the independent variables (e.g. litter size, sex composition, and relative age). Alternatively, the exploratory tendencies I observed could also be reflective of the behavioral tendencies of voles from this source population also studied by others (Getz *et al.* 1994; McGuire *et al.* 1993). All subjects were F₁₋₃ laboratory raised prairie voles derived from wild parents, F₀, from Urbana-Champaign, Illinois. They were not bred to enhance or reduce specific behavioral tendencies or genetic or physical traits (Tolman 1924; Groothuis & Carere 2005). I wanted to study the natural complexity of prairie vole behavior. By studying behavioral reactions of animals that have been minimally impacted by captivity I could examine the continuous variation that more likely characterizes natural selection as opposed to artificial selection pressure (Price 1970; McPhee 2003; Groothuis & Carere 2005).

Despite not finding a set of environmental factors that may explain exploratory behavior of prairie voles, as measured in a two-way novel choice apparatus, this study has helped shed light on prairie vole exploratory behavior. In this study, what made an individual proactive was its general proclivity to enter the two-way novel choice apparatus and traverse both sides of the tube very quickly. On the other hand, reactive individuals entered the apparatus more slowly and took more time to traverse both sides of the tube. It seemed that most subjects could be described as proactive or fast explorers because of the decreased times to traverse the apparatus across trials. Most voles seemed to demonstrate little to no sensitivity to change in the apparatus across test trials when the stimulus odors had been added or removed. However, the time to reach the second terminal was increased and the ratio of time to reach the first terminal to total test time was larger in the second trial. These responses indicated that there was a very subtle reactive response to the change in the apparatus. This response was not obvious in most subjects, except with old males whose reactive response was more evident because they took more time to enter the apparatus and complete the test the second time. It seems these subjects were especially sensitive to the changes in the external environment.

Animals with more proactive or reactive exploratory tendencies might both be considered highly exploratory in a novel situation; and either tendency might be selectively favored. It all depends on the stability of the environment in which the animal lives. For example, in relatively stable environments with high predation pressure like the spring or summer, proactive individuals may do better because they quickly procure and utilize resources as well as decrease exposure time to predators. However, reactive individuals may do better in variable environments with lower predation pressure, such as

the autumn when resources are contracting and predators such as snakes begin to hibernate (Getz *et al.* 1997). Reactive individuals tend to wait and assess situations before taking action, thereby utilizing resources more effectively while simultaneously scanning for predators. Voles are a small, short-lived, and heavily predated species. It may be in their best interest to move quickly and not pay very much attention to minor changes in the environment. Or their responses to changes in the environment may be very subtle and hard to detect, as was the case in this study. Details of how small mammals with high population growth capacity behave in novel settings could help ecologists learn more about how voles respond to new or changing details about their environment.

Most voles in this study behaved in a manner that would best be described as proactive or fast explorers. Similarity among littermates, siblings, and individuals of the same age or sex, was a consequence of high degree of similarity in behavioral responses among most subjects. But to be sure, future studies of proactivity-reactivity exploratory tendencies might best be studied with a different testing apparatus. Initially, the simplicity of this test seemed ideal for profiling individuals according to their exploration speed and reaction. However, this test did not allow me to gather enough data to be able to truly differentiate behavioral responses, despite studying a very large sample. I recommend using an open-field apparatus to examine vole exploratory behavior more comprehensively. With the addition of novel stimuli such as odors or objects, a researcher could then record proactive-reactive responses to complex and changing environments. A more complex experimental test would allow a researcher to gather multiple dependent variables to analyze exploratory behavior as a change in temporal,

spatial, and orientation responses to novel environments. Recording latency to enter a novel environment, latency to approach novel objects, distance traveled, and order of novel stimuli approached could provide much more detailed information about multiple dimensions of exploratory behavior, such as activity or interactivity.

A closer examination of variation in behavioral responses would also help us learn more about the influence of different environmental variables on behavior. High degrees of variation among subjects from the same treatment group would signal that behavioral responses are very plastic. However, predicting their overall exploratory tendencies may not be possible (Chapter 1). Similarly, identifying factors that contribute to these different exploratory tendencies may also be challenging. For an r-selected species like the prairie vole, strong behavioral plasticity may be of great adaptive significance. However, an overall high level of proactivity may be necessary for prairie voles to quickly secure resources for survival and help them out-run ground and aerial predators. Failure to fully attribute behavioral responses to factors like social environment, age, sex or family membership may signal that this species experiences population-level maintained behavioral heterogeneity.

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FIGURES

Figure 1. Two-Way Novel Choice test Exploratory Behavior Prediction Continuum

Figure 2. Two-Way Novel Choice Apparatus

TABLES

Table 1. Initial Direction voles entered the apparatus

Table 2. Terminal side reached first, regardless of the presence of stimulus odor

Table 3. Terminal side reached first, when stimulus odor present

Table 4. First stimulus odor reached, regardless of side

Table 5. Effect of odor on behavioral measures in both trials

Table 6. Descriptive statistics and more.

Table 7. Cluster Analysis of families

Figure 1. Schematic of the Two-Way Novel Choice Apparatus

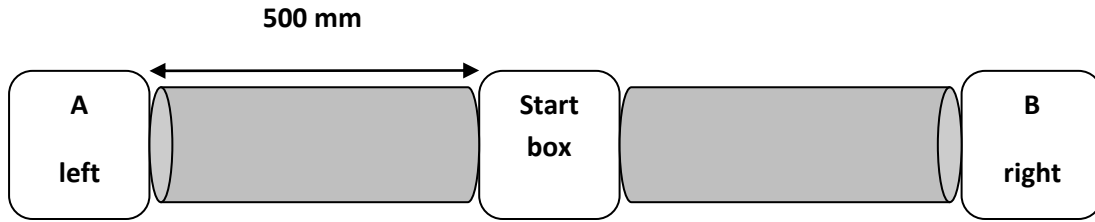


Figure 2. Two-Way Novel Choice test Exploratory Behavior Prediction Continuum

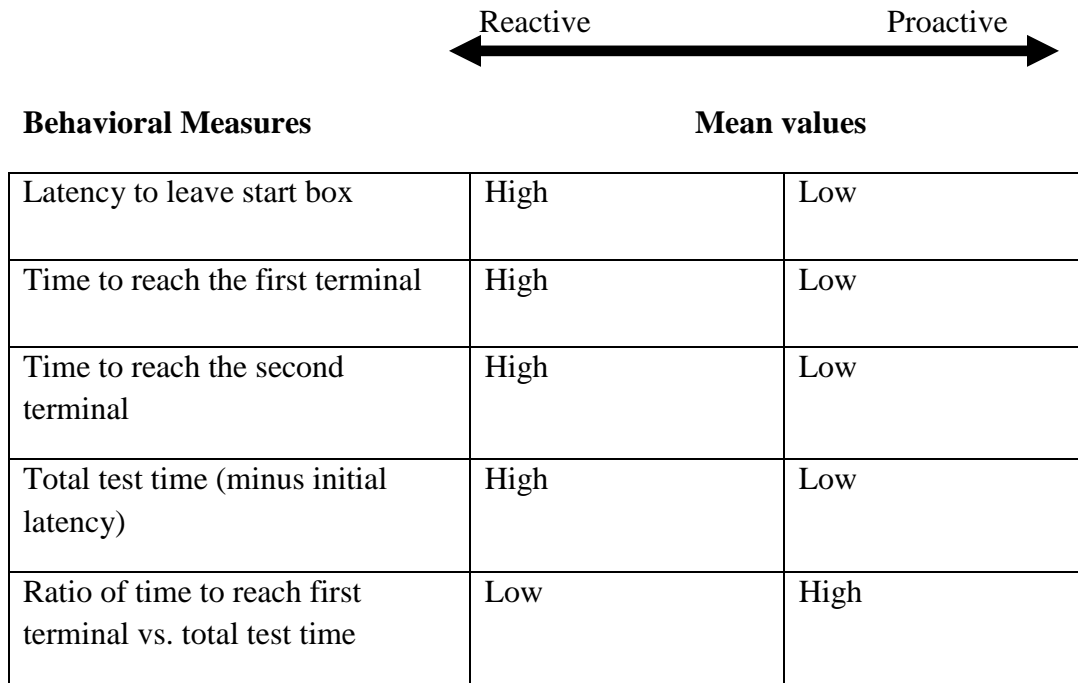


Table 1. Initial Direction voles entered the apparatus

	Trial 1 Observed N	Trial 2 Observed N
left	65	57
right	72	82
X^2 , df=1	.358	4.496
$\alpha=0.05$, $p=$.550	.034
Expected N	68.5	69.5

Table 2. Terminal side reached first, regardless of the presence of stimulus odor

	Trial 1 Observed N	Trial 2 Observed N
left terminal	71	58
right terminal	65	80
X^2 , df=1	.022	.400
$\alpha=0.05$, $p=$.881	.527
Expected N	68.0	69.0

Table 3. Terminal side reached first, when stimulus odor present

	Trial 1 Observed N	Trial 2 Observed N
left terminal	25	36
right terminal	20	54
X^2 , df=1	.556	3.600
$\alpha=0.05$, $p=$.456	.058
Expected N	22.4	45.0

Table 4. First stimulus odor reached, regardless of side

	Trial 1 Observed N	Trial 2 Observed N
lemon	22	42
vanilla	23	48
X^2 , df=1	.022	.400
$\alpha=0.05$, $p=$.881	.527
Expected N	22.5	45.0

Table 5. Effect of odor on behavioral measures in both trials

Mean (SD); $\alpha=0.05$, $df=1$

	Trial 1		ANOVA	Trial 2		ANOVA
	odor	no odor		odor	no odor	
Latency	27.98 s (46.91) N=49	30.95 s (64.80) N=92	$p=.778$ $F_{stat}=.08$ 0	18.78 s (46.20) N=92	17.92 s (47.50) N=49	$p=.917$ $F_{stat}=.011$
Time to 1st terminal	10.21 s (11.071) N=47	9.71 s (24.16) N=89	$p=.892$ $F_{stat}=.01$ 8	7.71 s (16.70) N=91	3.13 s (1.92) N=48	$p=.060$ $F_{stat}=3.590$
Time to 2nd terminal	27.70 s (31.26) N=47	25.81 s (35.25) N=89	$p=.757$ $F_{stat}=.09$ 6	29.34 s (36.43) N=88	23.25 s (28.21) N=48	$p=.317$ $F_{stat}=1.010$
Total test time	48.61 s (63.38) N=49	44.14 s (72.73) N=92	$p=.717$ $F_{stat}=.13$ 2	48.74 s (73.63) N=92	31.96 s (48.01) N=49	$p=.152$ $F_{stat}=1.713$
Ratio time to 1 st terminal: Total test time	.281 (.161) N=47	.234 (.157) N=89	$p=.102$ $F_{stat}=2.7$ 15	.201 (.150) N=88	.169 (.113) N=48	$p=.152$ $F_{stat}=2.072$

Table 6. Descriptive Statistics

 $\alpha=0.05$, $df=1$ Repeatability Analysis: 2-tailed p value, within individual vs. between individual ANOVA with Friedman's X^2 Repeated Measures ANOVA: Lower-bound F statistic and Partial Eta^2 reported.

Measures	Trial 1 Mean (SD) N	Trial 2 Mean (SD) N	Repeatability Analysis (Cronbach's α)	Repeated Measures ANOVA
Latency	29.91 s (59.04) N=141	18.48 s (46.49) N=141	.833; $\rho=.734$ p=0.01 Friedman's X^2 =10.617 p=0.001	p=0.001 $F_{\text{stat}}=11.400$ $\text{Eta}^2=.075$
Time to reach the 1st terminal	9.88 s (20.55) N=136	6.13 s (13.71) N=139	-.051; $\rho=-.029$ p=n.s. Friedman's X^2 =4.422 p=0.035	p=0.035 $F_{\text{stat}}=4.537$ $\text{Eta}^2=.033$
Time to reach 2nd terminal	26.46 s (22.82) N=136	27.19 s (33.78) N=136	.576; $\rho=.405$ p=0.01 Friedman's $X^2=.000$ p=0.998	p=0.998 $F_{\text{stat}}=0.000$ $\text{Eta}^2<.001$
Total test time	45.70 s (69.42) N=141	42.91 s (66.17) N=141	.567; $\rho=.396$ p=0.01 Friedman's $X^2=.198$ p=.656	p=0.658 $F_{\text{stat}}=0.197$ $\text{Eta}^2=.001$
Ratio (time to 1 st terminal: total time)	.2502 (.16) N=136	.1900 (.14) N=136	.178; $\rho=.098$ p=n.s. Friedman's X^2 =12.539 p<0.001	p<0.001 $F_{\text{stat}}=13.741$ $\text{Eta}^2=.094$

Table 7. Cluster Analysis of Families

Mean (SD)

Measures	General Statistics		Low Mean Cluster		High Mean Cluster	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Latency N=133	30.22 s (60.45)	19.11 s (47.79)	16.67 s (18.96)	7.68 s (8.10)	196.80 s (124.04)	159.60 s (94.30)
Time to reach the 1 st terminal N=128	10.07 s (21.14)	5.63 s (12.16)	8.30 s (10.54)	4.11 s (3.29)	57.80 s (88.53)	43.20 s (50.28)
Time to reach 2 nd terminal N=125	27.23 s (5.14)	27.17 s (34.07)	19.47 s (12.36)	19.68 s (11.86)	94.08 s (77.11)	94.31 s (72.39)
Total test time N=127	39.39 s (56.76)	34.91 s (49.13)	28.66 s (19.21)	25.68 s (15.48)	152.55 s (144.67)	132.27 s (127.45)
Ratio (time to 1 st terminal: total time) N=125	.2156 (.16)	.1874 (.14)	.1593 (.073)	.1166 (.061)	.45651 (.138)	.1663 (.104)
					.2156 (.102)	.3854 (.132)

CHAPTER FOUR
EXPLORATORY BEHAVIOR OF PRAIRIE VOLES IN A COMPLEX MAZE
APPARATUS

ABSTRACT

Complex maze tests have been used to examine memory, learning, and decision making behavior in rodents and other species. The design of this test also makes it ideal for examining exploratory behavior as a spontaneous response to novel situations. In this study, I examined the effects of multiple social, hereditary, and developmental variables on exploratory behavior of prairie voles, *Microtus ochrogaster*. Subjects were observed in a multi-arm exploratory maze. Recorded behavioral measures included latency to depart start box, the number of visits to each arm, the number of visits to each terminal, and approximate distance traveled within the maze. Individual behavioral differences in this test were previously determined to contribute to a continuum exploratory behavior profile labeled as activity (Lee, Chapter 1). Activity, defined as the amount of movement within an unfamiliar space, provides information as to how individuals gain input from the environment. Subjects who were the only ones of their sex in a litter entered the maze sooner than subjects from all other litter compositions. There also was a tendency for females to travel longer distances within the maze than males, but this difference was not statistically different.. However, there were very few other differences in behavior of subjects.

Key words: individual differences, behavioral phenotypes, behavioral syndromes, exploratory behavior, exploratory maze, prairie vole

INTRODUCTION

Exploratory behavior is defined as a response to novel environments or stimuli (Renner 1987). It includes how an organism investigates novel stimuli (Hughes 1997; Draai *et al.* 2001; Dingemans *et al.* 2004) as well as how an organism moves within a given space in order to gather knowledge about its local environment (Archer & Birke 1983; Renner 1987; Renner 1990; Verbeek *et al.* 1994; Draai *et al.* 2001). Researchers discern animal exploratory behavior by measuring their responses to, and investigation of, unfamiliar environments (Renner 1987). It is regarded as spontaneous behavior if motivating factors such as hunger, reproductive drive or escape from danger are controlled for (Renner 1990; Hughes 1997). Examining spontaneous exploratory behavior helps us understand how animals react to novel situations in nature and may help us understand how animals respond to dynamic environments in terms of foraging, interacting with conspecifics, dispersing, or reacting to predator cues. These behavioral responses reflect important aspects of an animal's behavior related to information gathering and making fitness decisions (Glickman & Sorges 1966; Archer & Birke 1983; Renner 1990; Verbeek *et al.* 1994; Draai *et al.* 2001;).

Complex mazes or labyrinths have been used to examine learning capacity of rodents for nearly a century (review in Dewsbury 1978). Complex mazes are comprised of multiple corridors with blind alleys. The subject travels along a zig-zag path from a start location to a goal point or terminal (Searle 1939; Dewsbury 1978; Benus *et al.* 1987). The elapsed time, number of turns, and choice directions are typically recorded as dependent variables (Dewsbury 1978). Anxiety responses, measured as the inverse of locomotor activity, have also been examined using complex mazes (Montgomery 1955

from Hughes 1997). Spending more time in the maze, and visiting multiple corridors and terminals many times indicated that a subject was less anxious in unfamiliar environment (Montgomery 1955 from Hughes 1997; Espejo 1997).

Although exploration is defined as how an animal might investigate a new environment, level of activity explains how an animal occupies and moves around in a novel space. Activity provides information as to how an individual gains input from the environment (Hughes 1997; Poucet & Herrmann 2001). It is defined as the amount of movement within a defined empty, and unfamiliar space (Searle 1939; Russell 1973; Renner 1987; Renner 1990; Renner & Seltzer 1991). Recording locomotor responses, such as number of visits to different parts of a novel environment and lengths of paths traced by animals provides information about the behavioral strategies of exploration (Berlyne 1960; Kleerekoper *et al.* 1974; Renner 1987; Hughes 1997; Draï *et al.* 2001). With increased exploratory activity there is a trade-off between the likelihood of learning about the new environment, encountering important resources to exploit, and confronting predators (Glickman & Sorges 1966; Glickman & Morrison 1969; e.g. meadow voles, *Microtus pennsylvanicus*, and deer mice, *Peromyscus leucopus* Metzgar 1967, Ambrose 1972; Roeder *et al.* 1980).

Exploratory behavior in a novel setting is highly variable among individuals. Like all selected traits, individual variation in behavior may be susceptible to natural selection (Fox *et al.* 2009). Individual variation reflects a constraint on the optimization process demonstrated by the animal (Clark & Ehlinger 1987; Verbeek *et al.* 1994). Variation in levels of exploratory activity may provide the basis of selective differences in fitness traits such as foraging, anti-predator behavior, and dispersal. Examining inter-

individual differences in complex maze behavior, allows the assignment of behavioral profiles that categorize the behavior of subjects in a given test situation (Groothuis & Carere 2005). Behavioral profiles describe behavioral tendencies or ‘dispositions’ of animals along an axis, such as more or less active or exploratory (Fox *et al.* 2009). These behavioral profiles allow behavioral traits to be examined within the Behavioral Syndrome framework (Bell & Stamps 2004; Bell 2007). This framework not only quantifies individual variation in behavior, but also attempts to explain the development and maintenance of this variation (Sih *et al.* 2004).

In this study, I examined the environmental influences on the individual variation of exploratory behavior in a complex maze. The objective of the study was to determine if individual variation in exploratory behavior can be attributed to independent variables such as social environment, developmental factors such as age or sex, and family membership. I tested three hypotheses concerning the development of individual variation of exploratory behavior in a novel choice apparatus test.

Hypothesis 1: The social environment in which an individual is reared and experience throughout life may influence the behavioral development (Carducci & Jakob 2000; Genaro & Schmidek 2002; Neugebauer *et al.* 2004). Subjects from similar family compositions are expected to demonstrate similar behavioral responses in the complex maze. Coming from a small or large litter, and with or without brothers or sisters, may have profound effects on the adult behavior of individuals. Moreover, the effects of subtle social differences that may occur normally in the early postnatal environment of mammals living under natural conditions have rarely been studied.

Hypothesis 2: Development factors, such as age or sex, are known to affect behavior (Dall *et al.* 2004). This hypothesis addresses if and how developmental factors, such as sex and age of a subject at time of testing, contribute to individual differences in the exploratory behavior of prairie voles.

Hypothesis 3: Related individuals demonstrate similar behavioral responses for many behavioral traits. I predict that siblings will demonstrate similar behavioral trends when introduced to novel situations. Subjects born to the same parents, which would include litter mates and full siblings from previous or subsequent litters, are expected to share behavioral tendencies due to genomic or non-genomic effects (i.e., culturally and socially transmitted traits) such as maternal effects. This study does not attempt to disentangle the exact origin or source of heritability of individual variation in exploratory behavior but does attempt to explore its influences. See Figure 1, Exploratory Behavior Prediction table.

GENERAL METHODS

1. Animals

Forty-two male and 49 female first through third generation, lab-reared prairie voles, *Microtus ochrogaster*, from Urbana-Champaign, Illinois, served as the subjects in this behavioral test. Individuals were reared under a 14:10 LD light schedule. Lab conditions, including frequency of handling, cage cleaning, and feeding, were consistent among all animals.

Animals were reared in early social environments that consisted of naturally occurring littermates and one or two parents. Less than 20% of the voles born in this colony were from litters raised by the female parent alone due to death of the male parent

or adjustment of breeding schedule protocols. However, the physical development and behavioral responses of voles raised by one parent were no different than those voles raised by both parents. Therefore, these data were pooled. Natural litter sizes vary from 1-8, with 3-4 being the average size. Litter sex ratio is also naturally variable and was characterized as the subject having a) no siblings, b) same-sex siblings only, c) opposite sex siblings only, and d) at least one sibling of each sex. All voles were weaned at 21-23 days of age and were housed with littermates, if any, throughout life.

Voles were tested during the light phase of the time cycle between 10:30 -16:00 CST hours. All subjects were tested post-sexual maturity (sexual maturity occurs at 40 days) and were sexually inexperienced (Getz *et al.* 1994). Age variation ranged from 55-1,400 days and was minimized whenever possible; however, age at testing did vary randomly. The mean life span for prairie voles born and raised in this colony was 345 days \pm 15 (SE) and is consistent with mean life span observed in other laboratory studies (Stalling 1990). The average life span of wild prairie voles ranges from 30 – 122 days depending on season, population density, and whether an individual disperses or not (Getz *et al.* 1994). Voles in this study were categorized as **young**, 55-120 days of age, **middle age**, 121-349 days, **old** 350-544 days, and **geriatric** 545 or more days of age. Though there is no official age designation for prairie voles or other Microtine species, these age groupings are roughly consistent with the relative ages of voles tested in laboratories (Wolff *et al.* 2001; Grippo *et al.* 2007). There were no apparent behavioral, physical, or health disparities among the subjects. Subjects completed the two-way novel choice test only one time and were naïve to the apparatus prior to testing. These voles were also used in two other experiments examining exploratory behavior (see Chapters 2

and 3). However, the order in which each of the experiments were completed by subjects was randomized.

2. Apparatus

Animals were tested in a multi-arm labyrinth, 610 (w) x 396 (l) x 12.5 (h) cm, made of a white acrylic base (floor) with black plastic walls with 7.5 cm wide corridors. The maze consists of three arms and five terminals. Each terminal varies in path orientation and distance from the entrance corridor: terminal 1 (15 cm from entrance); terminal 2 (500 cm); terminal 3 (560 cm); terminal 4 (835 cm); and terminal 5 (1095 cm). A schematic of the exploratory maze is presented in Figure 2.

3. Methods

The subject was placed into a start box, 7.3 (w) x 40 (l) x 6.8 (h) cm, made of white acrylic plastic. The start box opens at the maze entrance corridor. The subject was kept in the start box for 3 min to acclimate. Then, the swinging-hinge access door (made of opaque Plexiglas) was manually pushed open causing the start box space to contract by 2-3 cm. With the swinging door ajar, the subject could choose to proceed into the entry corridor. Once the subject stepped onto the maze floor with all four feet, it was scored as having entered the maze. Once in the maze, the subject could proceed in any of four directions, to the right (arm 1), straight ahead (arm 2), to the left (arm 3) or backwards into the start box. The following data were recorded: 1) latency to depart the box (in seconds), up to 2 min; 2) number of returns to the start box; 3) number of times each arm was entered; and 4) number of times each terminal was reached. The total time of the test was 3 min after initial exit from the start box; there was no food or other reward in the maze. For subjects that did not leave the start box within the allotted time, the

maximum latency time (2 min) was recorded and zeros were assigned for other measures and included in the data analysis.

All behavioral observations were made under reduced illumination (red light) and the observer was standing over the testing apparatus. Infrared wavelengths of light are poorly visible to rodents but still allow researchers to observe behaviors (Finley 1959). Prairie voles are known to be active in both light and dark cycles (Grippio *et al.* 2007) and reduced illumination observations are common for observing dark-cycle activity in rodents (Zurn *et al.* 2005). In this study, reduced illumination was used to mediate the negative effect of having the observer stand over the apparatus during testing. Between tests, the start box and the arena were cleaned with soap and water and disinfected with a 15% ethyl alcohol solution to eliminate any odors that might have accumulated from the previous subject.

4. Data Analysis

First, I calculated the relative distance traveled and minimum distance traveled within the maze. The relative distance traveled was calculated as the number of visits to each terminal times the rank value for that terminal, e.g. 1 for terminal 1, 2 for terminal 2 and so on. It is not an actual distance, but a unit-less value that compares how far each subject traveled. The minimum distance traveled was calculated as sum of the distance to each terminal visited. Multiple visits to the same terminal were not included in the minimum distance traveled calculation.

Data were analyzed using SPSS 17 statistical package to identify relationships among independent variables on the multiple behavioral response measures in the complex maze. All dependent variables except latency to enter the maze, relative

distance traveled, and minimum distance traveled were log-transformed because the values were small and had limited range. Moreover, each of those analyses failed the Levene's test analysis (REF). For all measures including the raw data of latency, relative distance traveled, minimum distance traveled, and log-transformed data for the remaining dependent variables, I completed a General Linear Model – Univariate ANOVA examining the influence of multiple independent variables on each dependent variable, one at a time (litter size x litter sex ratio; age x sex). Tukey's post-hoc test was used to evaluate pair-wise relationships. The mean difference in values was evaluated at the $\alpha = 0.05$ level. Parametric statistical tests were appropriate for several reasons: 1) reasonably large sample sizes are able to withstand the statistical effects of averaging, 2) parametric tests are less affected by extreme violations of assumptions of models including homogeneity of variance, normality, small sample sizes, and unequal sample sizes, and 3) parametric tests are generally robust statistical tests (Boneau 1960).

To examine the influence of family membership on the same dependent variables, I completed a Two Step Cluster Analysis of all continuous dependent variables using a BIC Cluster criterion, log-likelihood un-standardized variable method. Individuals were clustered based on each behavioral measure separately. Clusters are based on the mean (central tendency) for all subjects for that measure. Each subject is assigned to the cluster that has a mean closest to its behavioral score. Next, I calculated the proportion of full siblings that fall within the same cluster for each dependent variable.

RESULTS

1. Social environment factors (litter size and litter sex ratio)

There were no dominant litter size or litter sex ratio-based differences in behavior. Only one statistical analysis yielded a significant difference between groups with different sex compositions.

Latency: There was no significant difference in the time to depart the start box based on the size of the litter a subject is born into. However, there was a significant difference based on litter sex ratio ($df = 2$, $F_{stat} = 3.547$, $p = 0.034$). Voles who only had opposite-sex siblings entered the maze sooner than subjects from all other compositions ($p = 0.036$ vs. voles with same sex siblings and $p = 0.089$ vs. voles with brothers and sisters). This shorter latency to enter the maze was true for all litter sizes 2-7. There were no interaction effects. See Figure 3.

Returns: There were no significant differences for either of the independent variables or their interaction on this measure.

Arms: There were no significant differences for either of the independent variables or their interaction on the number of visits to any of the arms or the sum of visits to all arms.

Terminals: There were no significant differences for either of the independent variables or their interaction on the number of visits to any of the terminals or the sum of visits to all terminals.

Relative distance traveled: There were no significant differences for either of the independent variables or their interaction on this measure.

Minimum distance traveled: There were no significant differences for either of the independent variables or their interaction on this measure.

2. *Developmental factors (age and sex)*

There were no dominant sex or age-related differences in behavior. Only one statistical analysis yielded a significant difference between male and female subjects. For most behavioral measures, young males and geriatric females experienced the lowest mean values and lowest amounts of variance compared to all other subject groups.

Latency: There was no significant difference for either of the independent variables or their interaction on this measure. However, the mean time to enter the maze was similar for young voles and geriatric voles. These similarities are being driven by young males with a mean latency of 22.40s (± 22.50 , n=5) and geriatric females with a mean latency of 10.00 s (± 7.21 , n=3) to enter the maze.

Returns: There was no significant difference for either of the independent variables or their interaction on this measure. However, the mean log-transformed value for this measure was similar for young voles and geriatric voles.

Arms: There were no significant differences for either of the independent variables or their interaction on the number of visits to any of the arms or the sum of visits to all arms. However, number of log transformed visits to the shortest arm of the maze, Arm 1, was highest for geriatric voles.

Terminals: Females had a significantly higher number of log transformed visits to terminal 4 (one of the most distant terminals) than males ($p=0.044$, $F_{\text{stat}}=4.186$, $df=1$) and showed a strong trend towards more visits to terminals 2 ($p=0.091$, $F_{\text{stat}}=2.927$, $df=1$) and 5 ($p=0.087$, $F_{\text{stat}}=3.007$, $df=1$). There were no significant differences for either of the independent variables or their interaction on the remaining dependent variables including sum of visits to all terminals.

Relative distance traveled: There were no significant differences for either of the independent variables or their interaction on this measure. However, there was a marginal difference in relative distance traveled for males versus females ($p=0.094$, $F_{\text{stat}}=2.873$, $df = 1$; did not pass the Levene statistic $p=0.577$). Males had a lower relative distance traveled score than females for all age levels, but not significantly so.

Minimum distance traveled: There were no significant differences for either of the independent variables or their interaction on this measure. However, there was a trend for females to travel longer minimum distances than males ($p=0.064$, $F_{\text{stat}}=3.534$, $df = 1$; did not pass the Levene statistic $p=0.577$). Females traveled longer minimum distances than males for all age levels.

3. *Family membership*

Eighty-six subjects from 15 families, consisting of two or more full siblings per family (mean number of subjects per family is six) were evaluated to determine the similarity of behavioral responses among related individuals. A few families were better represented in the sample than others – for example one male and female pair was responsible for 19% of the full sibling subjects in this test. Individuals were assigned to a cluster based on the mean value for that cluster (high, low). Each behavioral measure was divided into two clusters. For all dependent variables, most families were split, i.e. some members were not assigned to the same cluster as its other siblings. The mean proportion of siblings that were in a different cluster than the majority of their family group ranged from 23-38%.

Latency: The general mean was 45.86 ± 45.13 (SD, $N=86$) to depart the start box. A slight majority of subjects (68.6%) were assigned to cluster 2, with a mean latency of

17.66 s \pm 16.78. The remaining subjects in cluster 1 had a mean latency of 107.83 s \pm 16.83. Most were split for this measure (12 out of 15) with a mean representation of 32.7% of siblings that clustered differently than the rest of their family.

Returns: The log transformed general mean was -0.676 (\pm 1.11, N=86) returns to the start box during the test. A slight majority of subjects (59.3%) were assigned to the high log transformed number of returns cluster with a mean positive value (0.232 \pm 0.21). The remaining individuals were assigned to the low log transformed number of returns cluster with a mean negative value (-2.000 \pm 0.00). All but two families were split for this measure with a mean representation of 37.64% of siblings that clusters differently than the rest of their family.

Arms: The log transformed general mean was -0.359 (\pm 0.98, N=86) for visits to Arm 1 of the maze. A majority of subjects (74.4%) were assigned to the high log transformed number of visits to Arm 1 cluster with a mean positive value (0.205 \pm 0.19). The remaining subjects were assigned to low transformed number of visits to Arm 1 log cluster with a mean negative value (-2.000 \pm 0.00). Most families were split (12 out 15) with a mean representation of 34.2% of siblings that clustered differently than the rest of their family.

The log transformed general mean was -0.178 (\pm 0.96, N=86) for visits to Arm 2 of the maze. A majority of subjects (79.1%) were assigned to the high log transformed number of visits to Arm 2 cluster with a mean positive value (0.304 \pm 0.20). The remaining subjects were assigned to low log transformed number of visits to Arm 2 cluster with a mean negative value (-2.000 \pm 0.00). Most families were split (12 out 15) with a mean representation of 27.8% of siblings that clustered differently than the rest of their family.

The log transformed general mean was $-0.124 (\pm 1.02, N=86)$ for visits to Arm 3 of the maze. A majority of subjects (77.9%) were assigned to the high log transformed number of visits to Arm 3 cluster with a mean positive value (0.409 ± 0.22). The remaining subjects were assigned to low log transformed number of visits to Arm 3 cluster with a mean negative value (-2.000 ± 0.00). Most families were split (11 out 15) with a mean representation of 28.2% of siblings that clustered differently than the rest of their family.

The log transformed general mean was $-0.282 (\pm 1.07, N=86)$ for sum of visits to all arms of the maze. A majority of subjects (82.6%) were assigned to the high log transformed number of visits to all arms cluster with a mean positive value (0.765 ± 0.21). The remaining subjects were assigned to low log transformed number of visits to all arms cluster with a mean negative value (-2.000 ± 0.00). Most families were split (11 out 15) with a mean representation of 25.5% of siblings that clustered differently than the rest of their family.

Terminals: The log transformed general mean was $-0.329 (\pm 0.98, N=86)$ for visits to Terminal 1 of the maze. A majority of subjects (75.6%) were assigned to the high log transformed number of visits to Terminal 1 cluster with a mean positive value (0.211 ± 0.19). The remaining subjects were assigned to low log transformed number of visits to Terminal 1 cluster with a mean negative value (-2.000 ± 0.00). Most families were split (11 out 15) with a mean representation of 30.4% of siblings that clustered differently than the rest of their family.

The log transformed general mean was $-0.594 (\pm 1.05, N=86)$ for visits to Terminal 2 of the maze. A slight majority of subjects (65.1%) were assigned to the high log transformed number of visits to Terminal 2 cluster with a mean positive value ($0.160 \pm$

0.18). The remaining subjects were assigned to low log transformed number of visits to Terminal 2 cluster with a mean negative value (-2.000 ± 0.00). Most families were split (12 out 15) with a mean representation of 38.8% of siblings that clustered differently than the rest of their family.

The log transformed general mean was $-0.461 (\pm 1.00, N=86)$ for visits to Terminal 3 of the maze. A majority of subjects (70.9%) were assigned to the high log transformed number of visits to Terminal 3 cluster with a mean positive value (0.1690 ± 0.18). The remaining subjects were assigned to low log transformed number of visits to Terminal 3 cluster with a mean negative value (-2.000 ± 0.00). Most families were split (11 out 15) with a mean representation of 31.3% of siblings that clustered differently than the rest of their family.

The log transformed general mean was $-0.539 (\pm 1.03, N=86)$ for visits to Terminal 4 of the maze. A slight majority of subjects (67.4%) were assigned to the high log transformed number of visits to Terminal 4 cluster with a mean positive value (0.167 ± 0.19). The remaining subjects were assigned to low log transformed number of visits to Terminal 4 cluster with a mean negative value (-2.000 ± 0.00). Most families were split (12 out 15) with a mean representation of 33.5% of siblings that clustered differently than the rest of their family.

The log transformed general mean was $-0.422 (\pm 1.12, N=86)$ for visits to Terminal 5 of the maze. A slight majority of subjects (67.4%) were assigned to the high log transformed number of visits to Terminal 5 cluster with a mean positive value (0.340 ± 0.22). The remaining subjects were assigned to low log transformed number of visits to Terminal 5 cluster with a mean negative value (-2.000 ± 0.00). Most families were split

(10 out 15) with a mean representation of 29.8% of siblings that clustered differently than their family group.

The log transformed general mean was 0.339 (± 1.1 , N=86) for sum of visits to all Terminals. A majority of subjects (82.6%) were assigned to the high log transformed number of visits to all terminals cluster with a mean positive value (0.833 ± 0.22). The remaining subjects were assigned to low log transformed number of visits to all terminals cluster with a mean negative value (-2.000 ± 0.00). Most families were split (11 out 15) with a mean representation of 25.5% of siblings that clustered differently than the rest of their family.

Relative distance traveled: The general mean was 19.33 (± 13.22 , N=86) for relative distance traveled in the maze. 60.5% of the subjects were assigned to cluster 2 (mean= 28.46 ± 7.51). The remaining individuals were assigned to cluster 1 (5.35 ± 5.482). All but three families were split on this measure. The mean proportion of siblings that clustered differently than their other siblings was 26.58%.

Minimum distance traveled: The general mean was 2035.76 cm (± 1150.41) for minimum distance traveled in the maze. 69.8% of the subjects were assigned to cluster 1 (mean= 2731.00 ± 396.20). The remaining individuals were assigned to cluster 2 (mean= $431.35 \text{cm} \pm 540.44$). All but three families were split on this measure. The mean proportion of siblings that clustered differently than their other siblings was 23.40%.

DISCUSSION

Complex maze tests have allowed researchers to measure the amount of activity in a novel environment. Activity and movement within labyrinths was used to examine memory and learning or interpreted as an anxiety response (Dewsbury 1978;

Montgomery 1955; Espejo 1997). These spatial, orientation, and temporal responses to unfamiliar complex mazes, can also be used to measure exploratory tendency in animals (Hughes 1997; Poucet & Herrmann 2001). For example, visiting particular arms or terminals of a multi-arm maze or total distance traveled over time are ways of measuring exploratory responses (Berlyne 1960; Poucet & Herrmann 2001). Activity is defined as the amount of movement within a novel setting (Russell 1973; Kleerekoper *et al.* 1974). Quantifying movement within the apparatus, such as visits to different parts of the maze, traveling to more distant or proximate parts, and number of visits to each corridor, provides researchers more information about the behavioral strategies of exploration (Renner 1987; Renner 1990; Renner & Seltzer 1991; Hughes 1997). Temporal responses, such as latency to enter a novel environment, are other common measures of exploratory response (Verbeek *et al.* 1994; Genaro & Schmidek 2002). Together, these different responses to novel environments provide a better understanding of spontaneous exploratory behavior in animals (Renner 1987, 1990).

How an animal explores a novel environment, including how far it travels over a period of time, tells us how an animal may gather and process information (Poucet & Herrmann 2001). In a homogenous environment, such as an open-field or sterile runway tube, information can be gathered rather quickly (Kleerekoper *et al.* 1974). However, in more complex environments, animals might require more time to assess their surroundings and gather more information (Kleerekoper *et al.* 1974; Drai *et al.* 2001). The more time an animal spends in a novel setting, the greater the amount and type of information that will be gathered, especially if the novel setting is complex (Kleerekoper *et al.* 1974; Drai *et al.* 2001). Multi-arm mazes, even those without rewards offer a

complex novel situation for animals to explore. Activity has often been treated as an index of exploration (Russell 1983) and with increased activity one would expect subjects to accumulate a significant amount of presumably new information, as it moves on to different parts of the novel environment (Kleerkoper *et al.* 1974).

The focus of activity for less exploratory individuals in a labyrinth would be nearer the start box with relatively little movement within the maze, whereas the focus of activity for more exploratory individuals would be away from the start box with relatively more movement within the maze. Delaying entry into the novel environment, visiting the shorter arms, traveling shorter distances, and returning frequently to the start box would indicate a low interest in novelty. These behaviors are interpreted as representing low exploratory tendency because subjects are not very active and they are generally less curious about the novel stimuli (Poucet & Herrmann 2001; Eilam 2010). Entering the novel environment quickly, visiting the longer arms, traveling longer distances, and seldom returning to the start box indicate a high interest in novelty. These behaviors are interpreted as high exploratory tendency because subjects are less attached to the familiar stimuli and more curious about the novel stimuli (Poucet & Herrmann 2001; Eilam 2010).

Based on the results from Chapter 1 that examined the range of inter-individual variation of the exploratory behavior in this test, I wanted to know which, if any, environmental variables influenced complex maze behavioral responses of subjects. All behavioral measures can be scored along a scaled continuum – from high to low values. If belonging to a specific treatment group influences exploratory behavior in a significant way, then subjects from similar social environments or those who are the same age or sex

should behave similarly. However, none of the independent variables completely explained the differences in behavioral responses in this test.

There were only two significant differences in behavior according to treatment group effects: latency to depart the start box and visits to one of the longer terminals. Latency to depart the start box was significantly shorter for subjects who had all opposite-sex siblings. Subjects with only opposite-sex siblings were significantly faster to enter the maze than subjects with same-sex siblings and marginally different than subjects with both brothers and sisters. This difference in latency according to litter sex ratio was observed in both small and large litters. In other exploratory tests by Lee (Chapters 2 and 3), subjects from larger litters with both brothers and sisters were the most exploratory individuals followed by subjects from litters with opposite-sex siblings.

I suggest that diverse social environments may act as an enrichment experience. Like rats reared in cages enriched with toys and cage mates, voles from complex social groups seemed less interested in unfamiliar stimuli than voles from smaller, less diverse litters (Varty *et al.* 2000). In this study, subjects who were different from their siblings responded more positively to the novel environment than subjects from other sex ratio combinations. It is not readily obvious to me why subjects from the most contrasting social environments would have shorter latencies to enter the novel environment. However, in a study of attack latency in wild house mice, males raised with all sisters had faster attack latencies than males raised with all brothers (Mendl & Paul 1991). Alternatively, the divergent behavior of opposite-sex siblings may be related to their dispersal behavior. Both male and female prairie voles are known to disperse in roughly equal percentages, 30% for males and 25% for females (McGuire *et al.* 1993; Getz *et al.*

1994). Both opposite-sex and same-sex siblings are known to disperse together; and litter mates of the same or opposite sex do tend to settle within 5 m of each other (Getz *et al.* 1994). However, opposite sex siblings never join the same group and only same-sex siblings will join the same breeding group (Getz *et al.* 1994). Perhaps this stark difference in timing to depart a familiar starting point may be related to the differences in dispersal behavior of opposite-sex siblings observed under more natural conditions.

The only other significant difference in maze behavior was based on sex. Females visited terminal 4, one of the more distal terminals, more often than males. Additionally, females also demonstrated trends for traveling longer distances in the maze than males. Though not statistically different, the relative distance traveled and the minimum distance traveled was higher for females at all age levels.

On the other hand, there were no significant differences in behavior according to age of subjects. However, one interesting pattern did emerge: young voles and geriatric voles demonstrated similar mean values for latency to depart and number of returns to the start box.

Individual variation in behavioral profiles has been shown to be moderately heritable (Dingemanse *et al.* 2002; Cockrem 2007). However, the support for heritability influencing exploratory behavior in this test was inconclusive. The clustering method assigned individuals to groups according to mean values that were either significantly higher or lower than the general mean for each behavioral measure. By clustering individuals according to low or high means, I could identify any similarities of behavior among all individuals, including family members. The findings demonstrated a weak tendency for complex maze behavioral responses to run in families. Nonetheless, almost

every family had individuals who reacted to the novel environment differently than the rest of their relatives. Nearly every family was split for every behavioral measure; on average one-fourth of all subjects was assigned to a different cluster than the rest of its siblings. This minority of individuals entered the maze much later than other subjects and did not move around very much. High incidences of behavioral heterogeneity within families eliminate the possibility of any hereditary effects including environmental ones such as maternal effects. Furthermore, it is unlikely that genetics can be used to explain exploratory behavior tendencies because so many un-related individuals were assigned to the same clusters.

The behavioral responses of these voles could be reflective of the natural behavioral variation of this population of prairie voles. My results may simply demonstrate that voles are generally active creatures. Some are less active than others, but there is so much variation in behavior that I was not able to demonstrate unambiguous patterns in behavior. Alternatively, the exploratory tendencies I observed could also be reflective of the behavioral tendencies of voles from this source population, which also has been studied by others (McGuire *et al.* 1993; Getz *et al.* 1994). All subjects were F₁₋₃ laboratory raised prairie voles derived from wild parents, F₀, from Urbana-Champaign, Illinois. They were not bred to enhance or reduce specific behavioral tendencies or genetic or physical traits (Tolman 1924; Groothuis & Carere 2005). I wanted to study the natural complexity of prairie vole behavior. By studying behavioral reactions of animals that have been minimally impacted by captivity I could examine the continuous variation that more likely characterizes natural selection as

opposed to artificial selection pressure (Price 1970; McPhee 2003; Groothuis & Carere 2005).

Despite not finding a set of environmental factors that may explain exploratory behavior of prairie voles in a maze apparatus, this study has helped shed light on prairie vole exploratory behavior. In this study, what made an individual more active was its general proclivity to enter the complex maze very quickly, seldom return to the start box, and have a high number of visits to all arms and terminals. Additionally, more active individuals were more likely to have traveled longer distances during the observation period. On the other hand, less active individuals entered the apparatus more slowly, frequently returned to the start box, and had a low number of visits to all arms and terminals. Moreover, less active individuals were more likely to have traveled shorter distances during the observation period. Overall, however, most voles in this study behaved in a manner that would best be described as highly active explorers. Similarity among littermates, siblings, and individuals of the same age or sex, was a consequence of high degree of similarity in behavioral responses among most subjects.

Level of activity within a novel setting can be important for fitness and survival. In this test, level of movement within the apparatus also corresponded to traveling longer distances within the maze over a period of time. Typically, traveling longer distances is considered a highly exploratory trait. However, traveling shorter or longer distances is not the definitive way to measure exploratory tendency in animals. Yet, long distance exploration might be advantageous. For example, prairie voles that disperse very long distances, more than 30 m from the natal nest, survived longer than voles that disperse shorter distances and those that do not disperse at all (Getz *et al.* 1994).

However, one short-coming of this test was how much data could be reliably gathered in real-time. With the use of video recording equipment, I could have gathered more detailed accounts of exploratory behavior such as paths taken and exact distance traveled. Measuring exploratory behavior in animals is very complicated and activity or distance traveled is not a sufficient indicator of this behavioral tendency. Although using the maze provided some useful information, I recommend using a different apparatus in order to examine a vole exploratory behavior more comprehensively. The open-field apparatus has been successfully used to characterize level of exploratory activity in rodents and other animals (e.g. Lee, Chapter 2; Dingemans *et al.* 2002). With the addition of novel stimuli such as odors or objects, a researcher could measure multiple dependent variables to analyze exploratory behavior as a change in temporal, spatial, and orientation responses to novel environments. Recording latency to enter a novel environment, latency to approach novel objects, distance traveled, and order of novel stimuli approached could provide much more detailed information about multiple dimensions of exploratory behavior, including activity, interactivity, and proactivity-reactivity responses.

A closer examination of variation in behavioral responses would also help us learn more about the influence of different environmental variables on behavior. High degrees of variation among subjects from the same treatment group would signal that behavioral responses are very plastic. However, predicting their overall exploratory tendencies may not be possible (Chapter 1). Similarly, identifying factors that contribute to these different exploratory tendencies may also be challenging. For an r-selected species such as the prairie vole, strong behavioral plasticity may be of great adaptive

significance. However, an overall high level of exploratory activity may be necessary for prairie voles to quickly disperse long distances, secure resources for survival, and help them out-run ground and aerial predators. Failure to fully attribute behavioral responses to factors like social environment, age, sex or family membership may signal that this species experiences behavioral heterogeneity that is maintained at the population level.

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Chapter 4. Figures

FIGURES

Figure 1. Complex Maze test Exploration Behavior Prediction Continuum

Figure 2. Schematic of the Exploratory Maze

Figure 3. Latency to depart start box by litter sex ratio

Figure 1. Complex Maze test Exploration Behavior Prediction Continuum

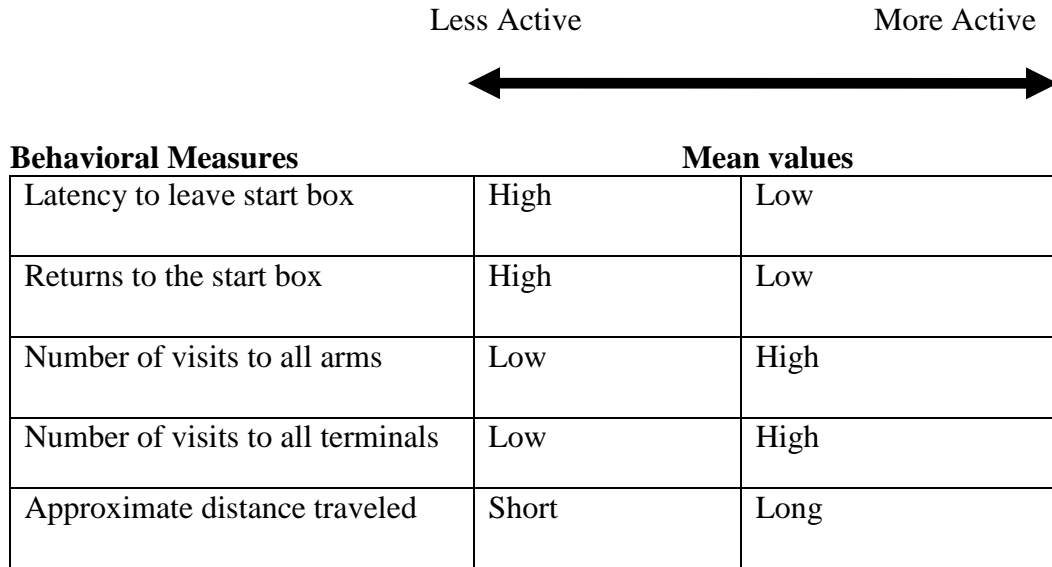


Figure 2. Schematic of the Exploratory Maze

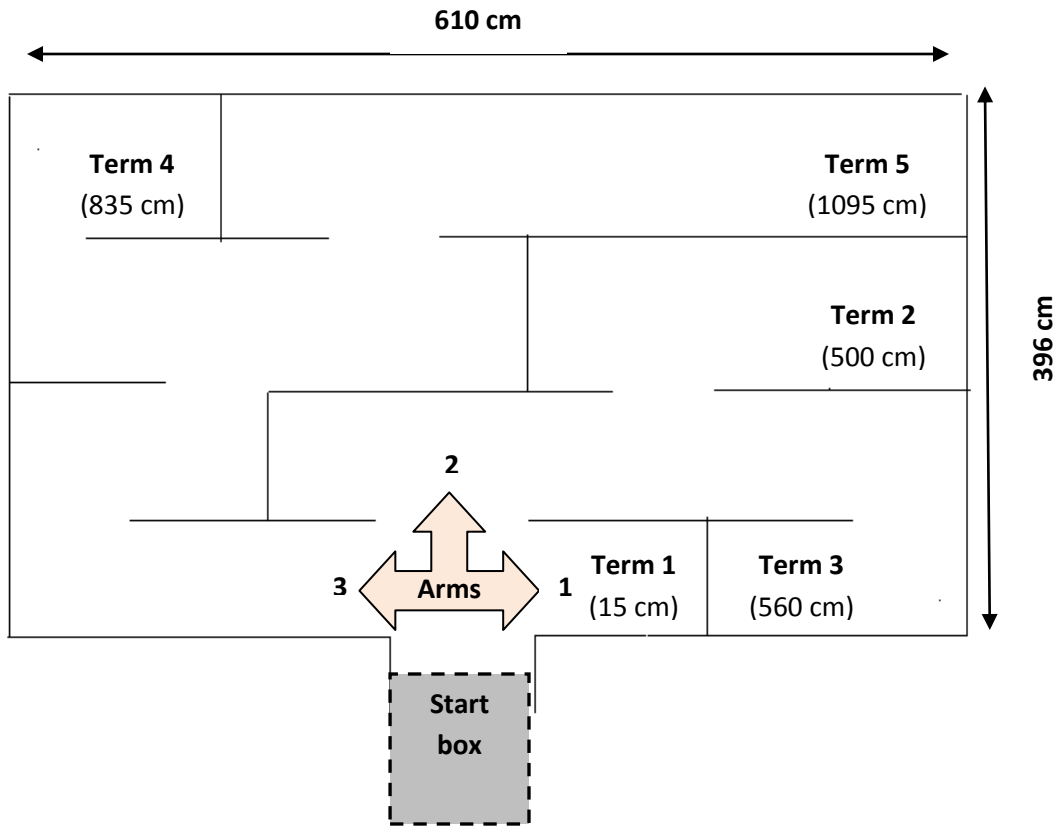
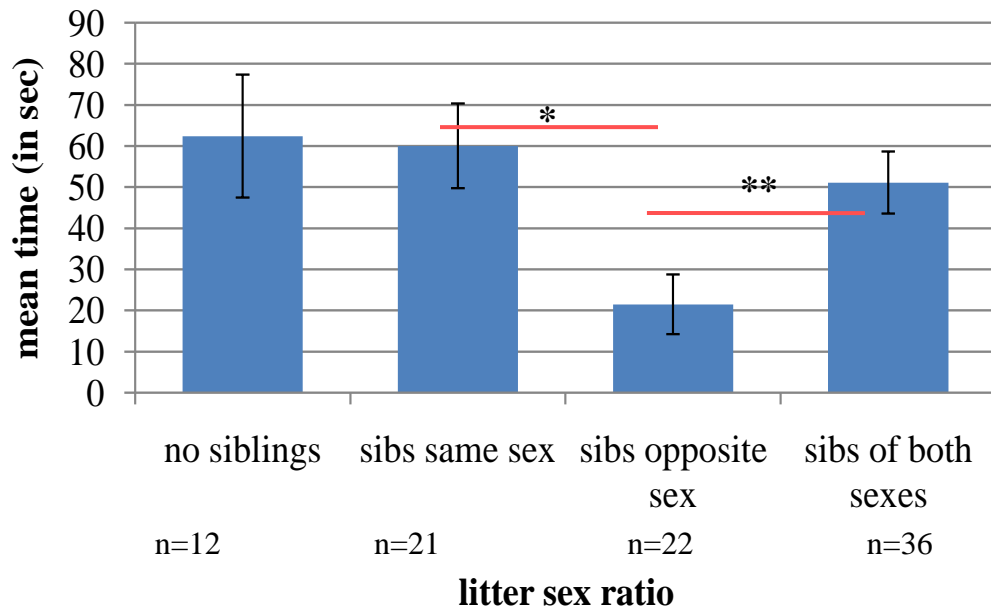


Figure 3. Latency to depart start box by litter sex ratio

$\alpha=0.05$, $df=2$
 ANOVA, $F_{stat}= 3.547$



Mean = 47.51s

N=91

$p=0.034$

Bars = SEM

* $p=0.036$ Subjects with opposite siblings vs. subjects with same sex siblings

** $p=0.089$ Subjects with opposite siblings vs. subjects with brothers and sisters

APPENDIX

Photo of a prairie vole, *Microtus ochrogaster*

Photo credit: Danielle N. Lee

