

ABSTRACT

Title of Document: DEVELOPING A METHODOLOGY FOR THE ASSESSMENT OF FREEDOM OF MOVEMENT: THE INFLUENCE OF SPATIAL PARAMETERS ON MOVEMENT AND SPACE USE IN MICE (*Mus musculus*)

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Despite recent progress in developing housing conditions that more effectively meet the needs of managed species, there remains a lack of detailed information regarding the influence of a space on animal health and well-being. If improvements are to be made to current guidelines so that they accurately reflect the needs of the animals, this information and a reliable method for collecting it, is critical. The objective of this project was to systematically examine the effects of spatial parameters on movement and space use of mice (*Mus musculus*) in open-field arenas. This approach may provide researchers with a means to describe what factors are most important for maintaining an adequate welfare state, and may help address questions regarding why (and how) these factors function as they do. To accomplish this, a standardized method was used across three experiments to test the effects of floor area, arena shape and structural complexity on movement and space use of mice. In each experiment 40

female Swiss Webster mice were observed in four arenas differing in spatial characteristics. In Experiment One, mice were individually observed in four square arenas that differed in floor area (0.5, 1, 2 and 4m²). The results from this experiment demonstrated an effect of both floor area and perimeter length on movement and space use of mice, as measured by track length, area traversed and mean distance to the nearest wall. In Experiment Two, the same variables were used to describe the behavior of mice in four arenas of equal floor area (1.5m²) that differed in shape (circle, square, 2:3 rectangle or 1:3 rectangle). Observation of animals in a circular arena demonstrated the impact of corner space on continuity of movement. In the final experiment, partitions were added in pre-determined configurations to examine the influence of partition location on animals demonstrating thigmotaxis. While the addition of partitions affected the distribution of movements, the specific location of partitions had little impact on movement or space use. With some refinement, this methodology could be used across species to identify specific needs and determine how effective housing systems are at meeting them.

DEVELOPING A METHODOLOGY FOR THE ASSESSMENT OF FREEDOM OF
MOVEMENT: THE INFLUENCE OF SPATIAL PARAMETERS ON
MOVEMENT AND SPACE USE IN MICE (*Mus musculus*)

By

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Dedication

I dedicate my dissertation to my father, Raymond Matthew Brooks Sr., who was always my biggest supporter, through the best and worst of times, and without whom I would have been lost. Though the powers that be did not allow him to see its completion, I am confident that he was right behind me every step of the way. He always told me that I can do anything I set my mind to, and though I spent years doubting the truth of those words, I'm finally a believer.

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Chapter 1: Introduction

All animals used in food production are social animals and exhibit some degree of social organization within groups if allowed to do so. In a modern production setting, these animals will experience some degree of confinement, whether within a relatively large pasture (e.g. sheep paddocks) or an isolated indoor individual enclosure (e.g. gestating sow stalls). There is some concern about how adequately these housing conditions meet the behavioral and physiological needs of the animals, as confinement affects feeding behavior and intake (Grant and Albright, 2001), social contact, physical comfort, movement freedom and freedom from disease or infection (Bartussek, 1999). Behavior associated with these behavioral and physiological needs typically results in some characteristic pattern of movement, preference for spatial location and preference for orientation relative to physical boundaries or other individuals (Stricklin et al., 1979). It is assumed that these persist even when movement is restricted (Thompson, 2004). How the animals arrange themselves in and make use of a given space should influence how they are housed and handled, such that health and well-being can be efficiently and sufficiently maintained within a production setting.

Practical information about social and spacing behavior, gathered primarily through simple observation, has been used to guide husbandry for as long as animals have been selectively bred by humans. However, it is unclear how well this knowledge is being incorporated into the guidelines for “space” allowances in modern production systems. These production systems are becoming more intensive,

and are focusing more heavily on achieving high productivity at minimal cost to consumers. Despite this effort, consumers are also becoming more aware of and more concerned about what may be considered shortcomings of these larger production systems, especially relative to animal welfare. Therefore, it is becoming increasingly important to answer questions regarding the social and spacing behavior of domestic animals, the special needs associated with behavior, and how to best meet these needs within the modern production environment. In addition to this, it is becoming increasingly important that we develop methods for improving welfare that can be implemented in a cost-effective manner. The overall objective is to improve the health and well-being of the animals without failing to pay close attention to the well-being of producers and the agricultural industries as a whole. Ultimately, the information gathered from the following research and the methodology used to collect it is relevant to not only the agricultural or research environments, but also to any circumstance in which it is necessary for animals to be confined.

As the public, governmental and commercial awareness of and concern for the animal welfare issues surrounding the housing and handling of food animals increases, more questions will arise about the nature of an animal's experience within the production system. The current method of making recommendations for housing and handling, while acknowledging the importance of animal well-being, may fail to approach the subject from the animal's viewpoint. Focusing more on ease and efficiency of production, current guidelines may fail to determine what is best for the animal. The clearest example of this failure is in the allowances for close confinement and isolated housing of species that are known to be social grazers. It may be

impossible to minimize stress experienced by animals when the environment in which they spend the majority of their life is likely to be the primary stressor.

Space availability and quality are two of the most noticeable and easily altered aspects of animal housing and handling. In many cases, simple changes to stocking density can have a significant impact on overall well-being and may affect its subjective experience. In order to examine the spatial needs of an animal or group of animals, it is imperative to have objective measures of an animal's ability to move and achieve the goal(s) for which the movement was initiated and a better understanding of how animals perceive their surroundings.

The overall objective of this dissertation is to compile information from planned experiments and information found in past experiments to develop a practical and universally applicable methodology for evaluating the housing of production and research animals (i.e., a "Freedom of Movement Index") based on principles presented originally by Stricklin et al. (1979). In doing so, I plan to work towards a less ambiguous definition of movement freedom that takes into consideration the causation for movement and provides a basis for which "adequacy" can be expressed in quantifiable terms. I hypothesize that arena size, arena shape and arena complexity will affect space use and thus amount of movement freedom of the animals housed within.

Chapter 2: Review of Literature

The majority of behaviors performed by living organisms require space. In fact, space use provides a commonality of needs of all organisms across the five kingdoms, affecting access to resources, rate of growth, survival and reproductive success in all species. In this review, the term “space” is inclusive of all areas in which an organism may dwell, from large-scale complex habitats to microscopic cellular environments. The structure of a habitat often determines which species thrive. The ability to take greater advantage of the properties of a given habitat provides the groundwork upon which the evolutionary process is built, and the evaluation of qualitative and quantitative properties of an environment is integral for survival. In wild populations, these properties influence the ability to readily access resources, locate nesting sites and maintain predator vigilance. The factors that influence space use and the subsequent behavior of animals have been documented for most domestic livestock species (Newberry and Hall, 1990; Wiegand et al., 1994; Sibbald, et al. 2000), research model species (Latham and Mason, 2004) and many wild captive species (Korhonen et al., 2000).

2.1 Principles of Animal Spacing (after Stricklin)

To date, there is no singular method for evaluating the quality of an environment that seamlessly incorporates the specific needs of various species of animal or even strains (or breeds) within a given species. Those parties setting regulations for the space allowances for agricultural and laboratory animals have

tended to approach the subject from the top down, by setting absolute minimum values for floor area per individual with little to no mention of what exactly can be accomplished (behaviors, social interactions, etc.) within that given amount of area. This simplification of the process is almost a necessity, as there is a general lack of basic, detailed information regarding animal responses to environmental stimuli and how a space affects the animals occupying it. This understanding is fundamental for determining, and ultimately meeting, an animal's needs. In any case, this top down approach may not be the proper approach. In nutrition, diet formulations are based not on the number of meals or feedings, but based on the nutritional components of those meals. Considerations are made for necessary vitamins, minerals, amino acids. This same approach should be applied when examining the needs of animals as they relate to a given space (Stricklin and Gonyou, 1995).

Stricklin and Gonyou (1995) suggested that animals have no specific spatial needs, only behavioral needs that, in order to be met, require certain quantities and qualities of space. For this philosophy to be applied in a bottom up approach, efforts must be made to determine the behavioral needs of the animal and how the characteristics of a space can be manipulated to meet those needs. Thus, I contend that guidelines and recommendations can be more effectively implemented by first understanding how relevant spatial parameters influence behavior. Design parameters for animal housing may include (but are in no way limited to) floor area, perimeter length, perimeter length to floor area ratio, length to width ratio, and placement of resources. The ultimate goal of the following research is to examine these and other parameters more closely, and in doing so, work towards a more appropriate

methodology for evaluating the housing and handling of animals. The following concepts are critical for a comprehensive assessment of the behavioral needs of confined animals.

2.2 Freedom of Movement

Almost all welfare standards associated with space mention a need for “adequate freedom of movement” (Committee, 1999). However, these standards lack a clear and unequivocal definition of movement “freedom” and a practically applicable method for quantifying it. It is difficult to define freedom of movement completely and in a manner that facilitates the development of regulations or guidelines for housing, handling and husbandry. One reason for this difficulty is that attempts at an unambiguous definition of ‘freedom of movement’ should inevitably give rise to other important questions. Some of these questions are of a scientific nature:

- How much space is needed to perform certain behaviors?
- How will animals use the space in which they are housed?
- How do changes in the environment affect the freedom allowed by a given space?

Others are question of ethics:

- What behaviors do we want these animals to be able to perform?
- How much freedom is adequate?

What is known is that movement, for the most part, is goal oriented. Rarely do animals move without some purpose for doing so. Instead, most movement is a

consequence of a need to approach something that is beneficial, like an essential resource, or to avoid something that is detrimental, such as a predator (Bernstein, 2001). Gonyou (1996) suggested that ‘freedom of movement’ is dependent upon the level of control an individual can exhibit over a space, such that it can readily access resources, avoid aggressive encounters, or merely make changes to their physical, physiological, behavioral and cognitive states. Any definition of movement freedom, or discussion of the degree to which it can be provided by a given space, must include simple, objective measures of space use, and must extend beyond merely measuring the relative ability for straight-line movement within a given space. However, it should be noted that while there may be some motivation to move, the process itself could at times be considered random (Gonyou, 1996). It should be noted that, while some patterns of movement or space use have a discernable proximate causation, this does not imply that all aspects of goal-oriented movement is deterministic.

The current regulations for the housing of beef cattle (Committee, 1999) mention a need again for “adequate space to move freely,” there is no attempt to define how much space that is. The primary basis for these regulations is instead the management of water and waste, such that provisions are made to compensate for different types of flooring. Thus, we find that the basis for the regulations of housing animals is not on the properties of the animals housed within (with the exception of their size) but on the properties of the enclosure itself.

The primary application of the following research is in the revision of housing standards and regulations for not only agricultural and laboratory animals, but all animals housed in confinement. My objective is to begin formulating a “Freedom of

Movement Index,” applicable to all housing enclosures, that is based on how animals make use of, or respond to, the properties of a space.

It is likely that freedom of movement varies depending on several variables, including age, sex, and social status of the animals. This introduces some of the previously mentioned difficulty in defining it and basing guidelines on that definition. For instance, while it may seem obvious that animals of a similar age and size will have similar space needs, the social dynamics of a group or herd will introduce great variation, such that a dominant animal is likely to utilize space much differently than a subordinate one (Stricklin and Keeling, 2000), and may have more freedom of movement as a result.

Studying movement freedom

Under most circumstances, there seems to be a continuum between a complete lack of space, thus a lack of freedom of movement, and infinite unbounded space (which may provide infinite freedom of movement). Following this, an animal confined within a space only slightly bigger than its body exists at the low end of the spectrum, and an animal in a completely unbounded environment exists at the upper end, with animals housed under current regulations lying somewhere between the two extremes. It is a near impossibility to carry out a study that provides an animal, especially a large livestock animal, with near-infinite unbounded space. However, it is possible, and maybe more practical, to study a small animal model (e.g. a rodent) in an environment that provides “functionally-infinite” bounded space. In using this term, it is proposed that there may be a limit to which animals will use the space

provided to them, depending on the behavioral needs of the individuals in question. Therefore, it is possible that a small animal will explore, and make use of, a 5m x 5m bounded space, a 30m x 30m bounded space and a completely unbounded space equally. However, below this maximum enclosure size, some size (or shape) of enclosure should exist in which use of space can be optimized, if not maximized. By observing the behavior of several species independently, it may be possible, with the inclusion of species-specific parameters, to develop a model for the estimation (and eventually prediction) of how much movement freedom a given enclosure provides.

2.3 Patterns of spacing (unbounded space)

It is difficult to evaluate the spatial needs of a confined animal without first understanding basic information about animal movement and how animals space themselves in the wild. There is an obvious connection between social and spacing behavior, such that you rarely find studies that analyze one and not the other. For example, social and competitive interactions have been described as the primary factors in determining how animals are distributed throughout a habitat (Brown, 1975). In turn, patterns of spacing can affect several aspects of population dynamics, the development of social behavior, and, in some species, may have placed restrictions on the influence natural selection has had on behavior (Brown, 1975).

As discussed by Wilson (1975), patterns of spacing or dispersal observed in natural environments are often retained in captive environments, including those environments devoid of the social conditions under which the tendencies may have evolved. The following is a discussion of three primary forms by which animals may

‘claim’ a given space, their individual space, a larger territory and a more their home range.

Home Range

A home range is defined as “that area traversed by the individual in its normal activities of food gathering, mating and caring for young” (Burt, 1943). This range exists within a fixed area, but is usually defended less vigorously (or not defended at all) than a fixed territory. In fact, home ranges of individuals may overlap considerably. There are several methods available for quantifying an individuals’ home range, including minimum convex polygons (Mohr, 1947), bivariate normal home ranges (Jennrich and Turner, 1969), kernel home ranges (Worton, 1989) and core areas (White & Garrott, 1990). In bounded spaces, such as those found in modern production facilities, some measurements of home range sizes (and the behaviors associated with the maintenance of these areas) may not be as accurate as estimates made in unbounded spaces. Therefore, these estimates may less accurately represent the needs of the animal. However, in confinement, core area estimation as a measure of relative space use within a field can provide some useful information.

Territory

Territorial behavior generally involves the defense of a fixed area through overt aggression or use of signals. An animal’s territory exists within its home range. The primary function of territory defense is increased breeding success through increased access to resources and mates (Brown, 1975). Because of the significant

influence it has on fitness, territorial behavior may be expressed even when the maintenance of a formal territory is impossible (i.e. in confinement) (Anderson and Hill, 1965).

Individual Field

The individual (portable) field is the space immediately surrounding the animal. In this discussion, the term ‘individual space,’ which in the literature is often used synonymously with ‘personal space,’ will be used to describe the area within an individual field. McBride (1971) defined individual spacing as the maintenance of an area free of other animals; most commonly, individuals of the same species. Intrusions into the individual field often lead to social conflicts between individuals.

The individual field can be described as an abstract area composed of a collection of attractive and repulsive forces, the strength of which is determined by several factors, including age, sex, hormonal state (especially during reproductive activity), social status, relatedness (genetic or perceived), and environment . In fact, the individual field was initially termed the social force field (from McBride, 1971). Descriptions of this phenomenon have even made use of Newton’s second law, describing the behavior (motion) of an object when the forces acting upon it are not equal (Okubo, 1986). According to Gueron et al. (1996) these forces may include locomotory forces (fluid viscosity), social forces (attraction or repulsion of neighbors), arrayal forces (tendency to match neighbors), deterministic environmental factors (obstructions) and random forces (probabilistic changes in behavior or environment). In gregarious animals, animals that tend to group together (e.g. sheep),

it can be expected that the attractive forces are relatively high; therefore, fields are relatively small. Animals that may be described as solitary (e.g. bears) can be expected to have larger repulsive pressure, and thus tend to maintain larger fields. While the approximate size of these fields can be determined through close observation of social interactions, an exact demarcation is extremely difficult if not impossible.

In captive environments where the ability for dispersal without individual field intrusions is limited, subtle signals and interactions between group mates may increase the 'tolerance' of intrusions and limit the need for aggressive behavior (Jensen, 1982; Hand, 1986). Despite this, knowledge of the individual field should continue to be applied to housing and husbandry practices, as ineffectiveness of appeasement behaviors under housing conditions in which escape is impossible can result in aggressive encounters that significantly reduce well-being. Within modern housing facilities, the increase in individual field intrusions caused by confinement is likely to increase the amount of social stress experienced by the animals housed within and may lead to encounters that are costly to both the producer and the animals. Therefore, this knowledge should be implemented in the housing guidelines for all managed animals, such that the improvement of animal well-being exists as a goal instead of a cost or side-effect. Furthermore, discussions of the individual field and its influence on social spacing should be a component of any attempt to clearly define movement freedom.

2.4 Social Grouping

Though the following research focuses on the responses of individual animals, the importance of social grouping cannot be overlooked. The evolutionary significance of grouping behavior is apparent by its influences such as a relative decrease in risk of predation (Hamilton, 1971; Pulliam, 1973), an increased ability to compete with heterospecifics or unrelated conspecifics for resources (Wrangham, 1980), feeding efficiency, modification of environment and reproductive efficiency/success (Ward and Zahavi, 1973; Mangel, 1990). Despite physical and physiological changes that occurred in domestic animals as a result of selective breeding, most, if not all, of their behavioral repertoire remains (Hughes, 1980; Kiley-Worthington, 1983; van Rooijen, 1983; Price, 1984). Included in the repertoire are those behavioral responses that are relevant to those benefits that make grouping a successful strategy. In fact, these pressures still greatly influence the needs of the animals and are still a part of our attempts to meet these needs. Thus when creating guidelines for the housing, handling and general husbandry of animals we should consider how social grouping and social behavior in general affects the needs of the animals and how these needs may change if the social environment changes. It is my intention to observe the interaction of social rank and space use given a change in the space, specifically available surface area.

A distinction should be made between stable social groups and aggregations. Wilson (1975) defines a society as a group of conspecifics that is organized in a cooperative manner that extends beyond sexually-motivated activity. He defines an aggregation as a group of conspecifics gathered in the same location, commonly

around some resource, but not engaged in internally-organized behavior. This distinction is important in modern agricultural systems. In instances where continual mixing of unfamiliar animals is standard, groups of animals may be more accurately described as an aggregation than a social group (Stricklin, 2001). Furthermore, under these conditions, “rules” for spacing may also differ.

The characteristics that define a social grouping are dependent upon the maintenance of a stable social system. The stability of groups, which can be measured by relatively unchanging relationships between individuals in the group and a resulting reduction in possibly detrimental agonistic encounters, is dependent upon continual social interactions, ranging from simple postures displays to overt aggression in the form of bites, bunts and kicks. Over time, these interactions typically result in a hierarchy of social ranks. The formation of hierarchies, originally described as “pecking orders” by Schjelderup-Ebbe (1922), has been well documented in all livestock species and most laboratory species. Under resource-limited conditions, dominant animals in a linear social hierarchy may have relatively greater “freedom” to access resources than will animals occupying a lower (subordinate) position (Banks et al., 1979). Injury that may be incurred during the establishment of these ranks and the energy expenditure associated with the entire process make continuous social conflict a costly endeavor (Reichert, 1988). In some species, where agonistic bouts are especially intense, researchers have found that injuries and deaths that are a direct result of these conflicts account for a greater percentage of the mortality rate than do predation, disease and resource shortages combined. In fact, one of the more convincing explanations for the evolution of

hierarchies is that they are a mechanism for limiting the cost (either in the form of physical damage or energy use) of agonistic encounters within a group of social animals, especially where resources are limited. In laboratory animals, where the effects of agonistic encounters are exacerbated by close-confinement, social aggression often results in injury or death. Under these circumstances, an understanding of animal's spatial needs is paramount.

2.5 Factors affecting availability and use of a bounded space

Descriptions of space in wild populations go beyond the amount of floor area available to each individual. In modern production and research settings, where movement is usually restricted in some manner, space is most commonly measured by available floor/pasture area. However, "a space" is not so easily defined. A space has both quantitative and qualitative properties, and both may be of equal importance in how they affect the behavior and welfare of animals. The quality of a space may change as a result of changes in population density/composition, environmental conditions and individual perception. Current housing guidelines are based primarily on quantitative measures. The adequacy of a given space is typically measured only by the amount of floor area per animal; however, other important quantitative measures exist, including, but not limited to, perimeter length, maximum distance of separation and the length-to-width ratio (Table 2-1). As these values all influence space use in their own way, doubling floor area does not result in twice the amount of space. In fact, when area is doubled for a square, perimeter length, which should be

included in the measurement of space, is only increased by a factor of $\sqrt{2}$ (Figure 2-1). Guidelines for space allowances should also be inclusive of qualitative properties.

Pen shape

Characteristics of an enclosure clearly affect the behavior of animals housed within (Wiegand et al., 1994; Cornetto and Estevez, 2001; Jensen et al., 2003). Changes in design may provide an individual an increased opportunity to hide, escape aggression, or find a comfortable distance from other individuals. In a group setting, where individuals are enclosed not only by the pen walls, but also by other individuals, it is important to understand how geometric characteristics, such as perimeter, maximum distance two animals can separate (D_{max}), and the number of corners, may affect the individual animal and thus the group as a whole, and how this effect changes as group structure is altered. Wiegand et al. (1994) found that pen shape had a significant impact on how group-housed animals partitioned themselves into smaller sub-groups. In square pens, pigs tended to form large social groups (13-15 pigs) more often and small social groups (1-3 pigs) less often than did animals in pens of other shapes. The size of these sub-groups may be relevant to the distribution of resources, the number of social interactions, and the perception of spatial freedom for individuals within these groups, though the effects may be confounded by the influences of the space itself.

Interestingly, by adding artificial corners to the circular pen, and thus introducing four corners, experimenters Wiegand et al. (1994) were able to remove the difference in aggression patterns between the circular and square pens, supporting

the findings of McGlone and Curtis (1985) that within the confines of common housing systems, corners provide an important means of escape from aggression. Shape may influence relative movement along the perimeter and as a result changes in grazing patterns on pasture (Sevi et al., 2001) or laying position within a pen. Changing the shape of a pen while keeping the floor area constant may also result in differences in the amount of space available to each individual and the number of resulting intrusions of individual space, as illustrated in Figure 2-2. In some instances this may lead to a subsequent increase in aggressive encounters.

It is unclear if this relation persists with larger group sizes. It is possible to test this relation further, not only in live animals (by using solid partitions to increase the number of corners), but also with the aid of computer simulation. Using animats (computer-simulated animals), Zhou (1991) found a direct relation between pen shape and group size with respect to the number of violations of individual personal fields. These violations influence the degree of crowding, the amount of aggressive encounters, and thus the amount of social stress incurred by animals within a given space. Zhou found a lower frequency of violations when the number of animals matched the number of corners for the simulated enclosure. Accuracy in analysis of the effect of pen parameters on the movement, behavior and welfare of a group of animals is dependent upon a clear understanding of the effects of the space (devoid of social intricacies) on the individual.

Table 2-1

Quantitative properties of pens equal in area but differing in shape. D_{\max} is the maximum straight line distance within the shape.

Shape	Comers	Area (m2)	Perimeter (m)	D_{\max} (m)
Rectangle (3:1)	4	10	14.61	5.77
Triangle (1:1:1)	3	10	14.43	4.81
Square (1:1)	4	10	12.64	4.47
Circle	0	10	11.21	3.56

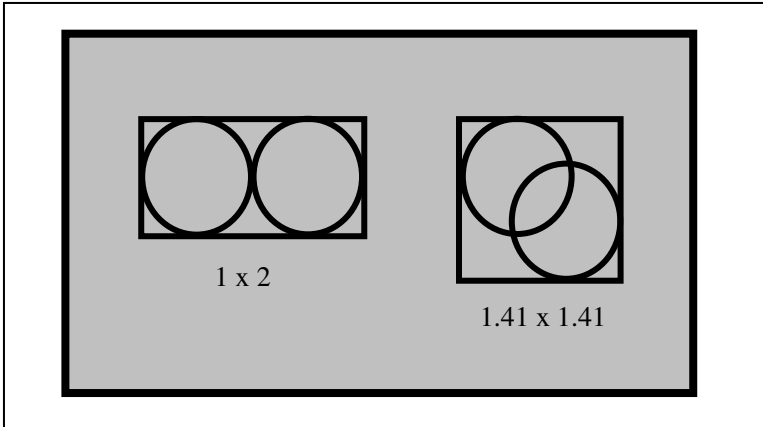


Figure 2-1

The effect of pen shape on likelihood of intrusions. Circles represent space allowance per animal and possibly the animal's individual field.

Perimeter

Stricklin et al. (1979) reported that cattle used relatively more peripheral area than central area. This has since been demonstrated in several other species. While the final result of this spacing trend is a disproportionate use of vertical surfaces, the causative factors are likely to differ across species. These factors may be critical in accurately describing the movement and spacing behavior of confined animals. For example, mice exhibit significant thigmotaxic, or wall-following, behavior when released into an open field (Buhot et al., 2001). It is widely accepted that this tendency to remain in close contact with walls is a result of the increased risk of predation associated with open areas. It is unclear if any alterations can be made to a space to reduce this tendency and encourage more efficient use of that space. These concerns are equally relevant in a production setting, where construction and maintenance costs are significantly higher.

Discussions of the influence of available perimeter on spacing behavior are tied closely to the previously mentioned pen-shape effects. The tendency to occupy areas near the perimeter of a bounded space is prevalent in many animal species (Fraser and Broom, 1990; Stricklin et al., 1979). Therefore, it should follow that, in situations where an animal clearly prefers the space close to the perimeter of a space, an increase in perimeter length relative to available surface area will result in more efficient use of available space and more “comfort” or “freedom” for those animals attempting to rest or move along the pen walls. However, before making this conclusion, it is important that we clarify if it is in fact the perimeter area, the existence of a vertical surface, or both, that is preferred. By adding central partitions

to an enclosure, Cornetto & Estevez, 2001 observed a significant change in movement patterns, including dispersal from the perimeter of the space into more central locations. In this case, observations indicate that a preference for perimeter space is based on vertical wall space and the protection it provides over open areas within the pen. However, in large livestock production facilities, the partitions alone may result in additional maintenance costs, and in small cages commonly used for research animals it is unclear what, if any, influence additional perimeter space would have on movement, behavior and well-being. In the following research, I will examine movement patterns within various environments and how they are influenced by the amount and quality of perimeter space.

Group Size, Crowding and Density

The effects of crowding on an animal's behavior have been observed and documented since the early days of ethology as a discipline (Calhoun, 1962; McBride, 1971; Russell and Russell, 1985). One of the primary reasons for this attention within the research community is that crowding has an immediate and relatively overt impact on animals, especially agricultural species, housed as groups in confinement. Depending on the properties of the space (e.g. opportunity to escape aggression or access to resources), and the characteristics of the animals, a densely packed space is not necessarily a crowded space. Stocking density is defined as the number of animals per unit area. Though often used interchangeably with the "degree of crowding," in measuring the behavior and spatial distribution of animals, the two differ. When used in objective behavioral research, the term density should represent

an absolute measurement, and thus make no inferences about the subjective state (i.e. comfort or discomfort) of the animal. Crowding, while density-dependent, is heavily dependent upon the subjective experience (physical or perceptual) of the animal(s) in question. McBride (1971) suggested that crowding only occurs when animals are forced into the individual space (defined above) of their neighbors. Modern welfare certification organizations rely on both objective measures (e.g. housing size and stocking density) and a judgment of an animal's subjective experience.

Tolerance of intrusions and the degree to which intrusions become a problem differ both across and within species, and are strongly influenced by social status. For higher ranking individuals, extremely dense conditions are needed for crowding to be apparent, as their social rank allows for almost unhindered movement (McBride et al., 1963; Hall and Fedigan, 1997). Animals at the other end of the social spectrum may be faced with a more crowded environment, if they are forced out of preferred locations and into more densely packed sections of an enclosure in order to avoid or escape dominant individuals. It is possible that these different microenvironments across social ranks can be overcome by alterations in pen design, while keeping density constant, thus allowing the same number of animals to make better use of the space provided for them.

In any examination of the effects of group size and density on well-being, there are likely to be several inferences about the impact on individual animals within the group. In these studies, it may be difficult, if not impossible, to determine if any changes on an individual level are a result of pressures imposed by the space, by group mates, or by both. If the group in question is composed of relatively unfamiliar

animals, these inferences may be even more difficult. Thus, my goal will be to approach the problem from the perspective of the individual animal. It is my belief that this is a necessary starting point in any investigation of the effects of confinement on a group (social or aggregate) of animals.

2.6 Measures of animal movement and spacing behavior in a bounded space

Track length (distance traveled)

The track length, or the total distance an individual travels, is the sum of the linear distances between successive animal locations. It is still unclear how track length relates specifically to freedom of movement within a bounded space. It is possible that more movement is indicative of a general lack of fear or anxiety. Primary examples of fear responses in mice include defecation, immobility, and hiding, all of which should reduce the amount of movement in a given period of time. Stress could also promote increased movement if, as a result, the confined animals continue to actively seek a location of relative safety or a means of escape. Concurrent investigations of movement data and other relevant measures of movement freedom are needed to clarify the relation of track length to freedom of movement.

Angular dispersion (tortuosity)

Angular dispersion, which measures the complexity of a given path of movement, can be used to describe the degree to which an individual's movement is restricted. Because space is often defined as expansion from a point, and because

movement freedom is often defined relative to the opportunity for unhindered travel, one may eventually come to the logical conclusion that as the angular dispersion increases, the ability to move freely within the space is diminished.

Mean distance to nearest wall

As mentioned above, almost all animals used in agriculture and research exhibit some tendency to maintain positions near the pen perimeter. Following the premise that forms of thigmotaxy or wall-following behavior developed in order to reduce risks of predation or even avoid social ‘conflict’ with conspecifics, an individual’s proximity to vertical wall space over an established period of time may provide us with important information. It may be helpful in understanding how an individual perceives a space and the risks associated with moving within that space. Average distance to the nearest wall can be used to describe an individual’s “willingness” to enter central pen areas.

Floor area coverage

While home range analysis is often used to describe an animal’s use of space, it is often inaccurate when describing movement in a bounded space. By using kernel smoothing techniques, this limitation can be somewhat overcome (Wand and Jones, 1995). With advances in object-tracking software, it has become possible to base use of space measurements on a continuous and complete collection of animal locations instead of an estimate or extrapolation of movement between sampled points. Estevez and Christman (2006) demonstrated that sampling effort has significant impact on

measures of space use. With a high enough sampling rate, estimation or extrapolation of animal location becomes unnecessary, though the calculation of the total floor area an animal covers is necessarily an estimate. The tracked animal locations are most easily sampled as a collection of single-pixel coordinate points, centered on the body of the animal; therefore, they fail to represent the total amount of space that is occupied by that animal at the time the location is sampled. However, on average, this underestimation of occupied area should be consistent across all animal observations. After sampling, the relative floor area that is traversed by an individual can be calculated as $\sum_{i=1}^N x_i / N$, where N represents the total number of possible locations for individual i and x represents a single, non-repeated observed location.

Net displacement

Net displacement is defined as the Euclidean distance between the first and last points in an animal's movement track or in observations of that movement. Jeanson et al. (2003) suggested that net displacement can be used to describe an individual's ability to disperse from its initial location. It is a simple measure of the degree to which an individual is constrained and is likely to be one of the responses most heavily influenced by the size of an enclosure, as its maximum possible value is equal to the D Max of the enclosure.

Immobility (Path count)

The relative degree of immobility in an animal's movement can be expressed quantitatively as time spent in an immobile state or as the number of bouts of

immobility, where the time required to categorize the subject's state as immobile has been previously determined. The following research will make use of the latter, by separating an individual animal's complete track of movement into paths. In this work, a path is defined as the course of travel between inactive states of greater than or equal to two seconds. This inactivity may be simply that; a pause in the individual movement. However, as mice are generally very active within the confines of an open field, especially during short-term observation, this inactivity has also been described in terms of expression of displacement behavior, during which an animal has a conflict between two or more drives (Maestripieri et al., 1992; Espejo, 1997), or of risk-assessment behavior, during which the individual determines the relative risk associated with moving in a given direction (Shepard et al., 1994).

2.7 Animal Models: Social and Spacing Behavior

The house mouse (*Mus musculus*) is the most common research animal, reaching a world population of approximately 30 million (Latham and Mason, 2004). Its benefits as a research model include short generation intervals, small size and superior adaptability. Mice are social animals and in commensal populations, those relying on humans for food and shelter, groups may consist of family units called demes. These demes typically include a dominant male, breeding females and their litters, and adolescent males and females that have yet to disperse. Mice are territorial animals, though how vigorously they defend a given space is strongly influenced by food availability, population density and habitat structure. The home range of an animal in a wild or feral state often changes in size, shape and location as food

availability and overall habitat quality changes. For wild mice, this home range may be up to 4100m² for females and 7200m² for males (Mikesic and Drickamer, 1992); however, the territory size is typically only 1-2m² in size (Torre and Bosch, 1999). Mice living in commensal populations tend to divide all of the available space and maintain strict boundaries. Intrusions by nomads, animals from other demes and even siblings typically results in aggressive behavior until the intruder has left the territory or the original occupant is displaced (Brain and Parmigiani, 1990). If adequate space is not available for escape or the appropriate submissive response, aggressive encounters can be, and often are, fatal.

As their small size allows for observation in a wide range of specifically-designed environments, mice are an excellent species for the study of movement and space use. Open field observation has long been the standard method comparing the responses (behavioral, cognitive or locomotive) of rodents under various chemical influences or rodents having certain known physical or genetics differences. Consequently, there is a wealth of information regarding the movement of mice within an open, bounded space of a consistent size and shape. For instance, open-field experiments using rats and mice have shown that, within a range of field sizes, subjects will explore a relatively small portion of the total area. As mice are highly preyed-upon, caution dictates a slow, repetitive and methodic approach to movement and exploration. This pattern of movement typically involves initial movement to a wall or corner, then laterally reciprocating movement along the wall, with increasing displacement from the starting, central point and increasing distance from the wall (Tchernichovski et al., 1998). With this pattern of movement a relatively large portion

of the area, depending on the size of the enclosure, remains unexplored, and thus unused.

This pattern is likely the product of a tendency to express thigmotaxis, during which the animal maintains bodily contact with solid objects (Mackintosh, 1981). Common in most small mammals, and apparent in most open-field observations of laboratory-reared mice (Bronikowski et al., 2001), this propensity is important in the avoidance of their many predators (Elton, 1942; Berry, 1970; Berry and Bronson, 1992).

In comparing the predator avoidance strategies of mice and other small rodents to that of larger prey species, many obvious differences exist. As discussed, rodents rely heavily on environmental cover and maintaining paths of travel that lead them back to an area of relative safety. Larger herbivores, including the domestic and wild ungulates, may rely on environmental cover when young, but switch to the “safety in numbers” strategies (e.g. group maintenance) when hiding becomes impractical or impossible. Although obvious differences exist, several important similarities also exist. For example, much of the social and spacing tendencies of both cattle, as representative of larger prey species, and mice are strongly influenced by the need to optimize access to resources while maintaining relative safety from predation and are strongly influenced by social interactions. It follows that this should be considered when determining the spatial “needs” of the animals.

For animals in social groups within a modern confinement-based agricultural setting, where resources are usually available in abundance, the importance of social mechanisms (i.e. behavior that reinforces the hierarchical system) to reduce the costs

of social interactions still remains; however, in groups of intensively housed animals the effectiveness may be limited. Some display of submission by the subordinate individual seems to be an integral part of this system. Therefore, we tend to expect an increase in aggressive encounters and a subsequent decrease in animal well-being when the closeness of pen walls or other animals limits or eliminates any ability to escape aggression (Wiegand et al., 1994). For example, during interactions with a dominant animal, one that may have greater claim over a given resource (e.g. food, water, or space), the primary response to aggression is avoidance. Within a bounded space, a subordinate animal's ability to escape aggression, and thus avoid injury, is limited by both the walls of the enclosure and by other group members. In fact, an animal's freedom of movement (inclusive of freedom to escape aggression) may also be limited, in some cases quite severely. Thus, in making recommendations for the design of animal housing, the ideal pen design for a group of animals is one that addresses the need for a means of escape from aggressive interactions that could result in injury to the subordinate animal and increased energy expenditure by all animals in the group. In a setting where these effects cannot be overcome, the welfare of the animals is compromised.

Chapter 3: The Influence of Spatial Parameters on Movement and Space Use in Mice (*Mus musculus*) - Floor Area.

Abstract

Current guidelines for the housing of domestic animals suggest that intensive housing systems should provide animals with an adequate freedom of movement. The majority of recommendations for movement freedom, or space in general, are stated solely in terms of floor area provided per animal. While the size of the pen, or the associated density of animals housed within, will have a major impact on movement and space use, the nature of that impact is not yet fully understood. If floor area alone adequately equates to movement freedom or available space, then increasing that floor area should result in a proportionate increase in measure of movement and space use. In this first of three studies, I investigated the effect of increasing pen size (floor area) on the movement of mice (*Mus musculus*). Forty female Swiss Webster (CFW) mice were observed in four square experimental arenas that differed in floor area: (A) 4 m², (B) 2 m², (C) 1 m² and (D) 0.5 m². Track length ($F_{3, 8} = 69.6, P < 0.0001$) and net displacement ($F_{3, 8} = 84.18, P < 0.001$) both differed across the four arena sizes, increasing as floor area increased. Neither the number of paths per track nor the angular dispersion of the observed paths differed significantly across floor areas.

Of the measures used to determine the effects of floor area on behavior and use of space, only in the proportion of the area traversed were any significant differences observed. These results suggest that floor area has a greater impact on movement of mice than on their use of the space. Patterns of movement were consistent across

treatments and seem to be relatively resistant to change, despite large differences in 'available' space.

3.1 Introduction

Current guidelines for the housing of animals used in agriculture, teaching and research are based largely on the assumption that floor area is equivalent to both movement freedom and the availability of “space”. Recommendations for housing animals in intensive production systems include statements about providing “sufficient” space or movement freedom (Committee, 1999); however, these guidelines do not specifically define freedom relative to movement and do not define space in terms of the requirements of animals. Furthermore, there is no clear indication of how floor area alone can be used to determine the amount of space or freedom that is adequate, such that we can be confident that the individual animals’ needs are being met.

Several studies have evaluated the influence of pen size on production-related performance measures in animal agriculture, including growth rate, feed intake, and feed efficiency (Wiegand et al., 1994; Edmonds et al., 1998; Hamilton et al., 2003; Brumm, 2004). Wiegand et al. (1994) found no effects of pen size on performance traits in growing pigs, but a clear effect of pen size on behavior and social interactions. Similarly, Brumm (2004) found no effects of space allowance on feed efficiency. Few studies have fully addressed the effect floor area has on behavior (Wiegand et al., 1994; Biensen et al., 1996; Sibbald et al., 2000). Even fewer research efforts have explored the effect of floor area on movement and use of space in animals, and, as a result, it is less clear if previously mentioned effects are due solely to changes in floor area.

Stricklin (1995) suggested that doubling floor area may not result in a proportionate increase in the amount of space available to the animal, and in doing so, stated the importance of the animal's subjective assessment of space in regards to setting standards for animal care. The idea that floor area alone is not a sufficient indicator of space and movement freedom is acknowledged by some housing guidelines (Committee, 1999); however, there is still a general lack of detailed information about the relative importance of floor area in the behavior of a confined individual. One of the difficulties in studying the effect of floor area (pen size) on movement in larger species is that it is often impractical to construct experimental arenas to test this effect over a wide enough range of successively larger sizes that maximize potential for movement. To accomplish this, I have turned to a smaller animal model: the laboratory (house) mouse (*Mus musculus*). The mouse demonstrates several characteristics that make it ideal for studying the movement of individual animals: its small size makes it possible to observe movement in significantly larger environments, its activity level provides the opportunity to collect large amounts of movement data during relatively short observation times, and open-field movement and spacing behavior in mice is both more exaggerated, as compared to larger animals, and better documented, resulting in easily identified and measured responses that can be compared to an ever-increasing database of existing information.

Mice and rats are highly valued as animal models for human health, and their behavior in open-field tests has been used extensively in pharmacological, toxicological and neurophysiologic research (Kaster et al., 2007; Bellum et al., 2007;

Pan et al., 2006; Fuke et al., 2006). These studies rely on the broad base of available rodent data for comparative analysis of deviations from ‘normal’ open-field behavior that may arise from genetic manipulation (Stearns et al., 2007) or from an array of chemically-induced physiological or psychological states. The aim for the majority of these studies is to gather information that can be indirectly applied to address concerns for human health and well-being. However, the standard measures of fear and ‘emotionality’ in open-field research (i.e. ambulation, ‘freezing,’ and elimination) that are often used to represent analogous human psychological states may be used just as effectively to evaluate, and possibly improve, captive animal environments. In examining the effects of space allowance on behavior, Sherwin (2004) successfully demonstrated the motivation of mice to gain access to additional space, but observed no differential preference for the amount of additional space provided. In this study, the author noted an inability of the experiment in question and previous investigation (Sherwin and Nicol, 1997) to determine why there was no differentiation between different sizes of additional space. In doing so, it was also noted that for a better understanding of this phenomenon, it is critical that studies consider the properties of the additional space. I contend that it is equally important to understand how, and ultimately why, these properties impact biological and behavioral responses.

The following study is the first in a series of experiments aimed at providing detailed information concerning the effects of various spatial parameters on the movement and space use of mice, and in doing so, help to gain a better understanding of how an individual animal perceives its surroundings and how characteristics of environment affect this perception. The primary objective of this first experiment is to

describe the effect of incremental increases in floor area, with minimal changes in perimeter length and maximum internal distance, on movement and space use.

3.2 Animals, Methods and Materials

This experiment was conducted in the University of Maryland, College Park Animal Research Wing with forty 7-8 week old pair-housed female Swiss Webster (CFW) mice obtained from Charles Rivers Laboratories, Inc. An albino strain of mouse was used for this study to facilitate both marking and tracking of the animals. Forty mice were chosen for observation at random from an original pool of 50 animals, and the ten remaining animals were kept as replacements.

Each pair of mice was assigned a cage number (1 through 25) and then the cage numbers were randomly ordered using a random number generator that excluded duplicate integers (Haahr, 2002). From the generated cage order, two observation groups of 10 pairs of animals were created. The AM group (n=20) was observed in the morning, starting approximately at times between 900 and 1100 hours. The PM group (n=20) was observed in the afternoon, starting approximately at times between 1300 and 1500 hours.

3.2.1 Open field arena and observation procedure.

The floor of the test arena was a 0.32 x 240 x 240cm (depth x width x length) sheet of composite hardboard. The entire surface was supported from below and leveled to minimize environmental inconsistencies. The walls of the arena were constructed from 0.32 x 31cm sheets of composite hardboard of various lengths to

create a dynamic testing arena, the size of which could be easily altered between observation sessions. The floor and wall panels were painted with a dark gray, low odor, no-VOC semi-gloss latex paint (Sherwin-Williams Harmony® Web Gray), which allowed for more efficient video-tracking of the animals. A 28 cm (diameter) cylindrical opaque plastic container was attached to a pulley system and placed in the center of the field, serving as the starting point for all observation trials. Prior to the start of this experiment, all mice were individually placed in a 200 x 200cm test arena in three separate 3 minute sessions to familiarize them with the apparatus.

The mice were observed in four square-shaped experimental arenas. The dimensions for the arenas were as follows: 200 x 200cm, 141 x 141cm, 100 x 100cm and 71 x 71cm. All observations were made in a dimly lit room (approximately 8-10 lux). Bronikowski et al. (2001) suggested 5 lux as the optimal light level for both mouse activity and video-capture of this activity; however, a slightly higher light level was required to create an evenly illuminated environment. The schedule of presentation for these pen designs (Table 3-1) was adjusted to control for possible confounding effects, including time and order effects, and treatments were randomly assigned to the schedule of presentation prior to the start of the experiment. Mice assigned to the morning (AM) and afternoon (PM) groups were observed an equal number of times. The order of observation within a housed pair was also determined prior to the start of the experiment. Each mouse was marked with a non-toxic food coloring to identify its within-cage observation order, and that order was used throughout the extent of the experiment. Re-marking of individuals was done

Table 3-1

Experiment Schedule. Letters denote the different pen treatments.

		DAY OF TEST											
		1	2	3	4	5	6	7	8	9	10	11	12
SESSION	AM	A	C	B	D	C	A	D	B	C	D	B	A
	PM	B	D	C	A	B	D	C	A	A	B	D	C

periodically throughout the extent of the experiment to ensure that individual animal identification and observation order was maintained.

Two sessions of observations (morning and afternoon), approximately 4 hours apart (from the start of observations), were completed per day of test. Each mouse was individually observed during one of the two daily sessions for a total of 40 observations per day, and every mouse encountered each arena design three times. The observations were completed in 6 four-treatment blocks, each block requiring two days to complete.

3.2.2 Data collection and measurement variables

Total track analysis

For a given trial, a single mouse was placed at the starting point. The trial began when the mouse was released into the field. Trials were 3 minutes in duration, after which the mouse was caught and returned to its cage and the surface of the arena was cleaned with an acetic acid/water solution to remove odor cues, as suggested by McGivern et al. (1997), and allowed to dry. Each observation was captured using a Panasonic closed-circuit color video camera, mounted approximately 2.5m above the arena floor surface. Trials were recorded to an uncompressed AVI video format and later processed using Viewer II tracking software (v. 2.0.0.31 by BIOOBSERVE, Fort Lee, New Jersey; See Appendix for tracking procedure) to translate the tracks of movement into a series of Cartesian coordinates. From these coordinates, several measures of space use and spacing behavior were summarized for each subject. The dependent variables summarized for the total track were as follows: Track length

(total distance traveled), net displacement, mean distance to the nearest wall (MWD), proportion of movement in the peripheral zone, floor area used (area traversed), and angular dispersion (path tortuosity or complexity).

To calculate the total distance an animal traveled, the Euclidean distances between successive coordinate points were summed. Net displacement was calculated as the Euclidean distance between the first and last tracked locations. Two measures were used to describe the animals' use of space along the arena periphery. The first, mean distance to the nearest wall (MWD), was calculated for each observed track as the mean straight-line distance from each sampled coordinate point to its nearest vertical surface. To further elucidate the wall-following behavior previously described in mice (Bronikowski et al., 2001), the captured coordinate points were used to partition each digitized experimental arena into two zones (central and peripheral) that were equal in area, and the proportion of tracked locations that fell within the peripheral zone was determined.

The relative floor area that is traversed by an individual can be calculated as $\sum_{i=1}^N x_i / N$, where N represents the total number of possible locations for individual i and x represents a single, non-repeated observed location. With the advent of advanced contrast-based animal tracking technologies, it is now possible to capture a continuous stream of animal location coordinates from previously-recorded (or live feed) video data. Estevez and Christman (2006) demonstrated that in tracking animal movement by hand, and thus limiting the frequency at which locations can be sampled, there is a risk of dramatically underestimating the movement. Processes that use continuous tracking, as was used in this experiment, result in a more accurate

representation of animal movement by eliminating the need to estimate movement that occurred between sampled points. To calculate total floor area traversed, I expressed the total number of unique coordinate point locations in each individual's movement track as a proportion of the total number of coordinate points in the arena. The resolution of tracking was set to 1cm, such that the total number of coordinate points in an arena was equal to the area of that arena.

Tortuosity, or angular dispersion, has been used to describe the path complexity of a range of objects, from blood vessels (Hart et al., 1999) to rivers (Seker et al., 2005). In studies of animal movement, tortuosity can be used to describe the spatial restriction imposed by a given space. Though, to do so, we must assume that it is these restrictions (either physical or perceived) that cause animals to alter the direction of their movement. This departure is used to calculate the concentration of tracked points $r_k = \sqrt{\bar{x}_k^2 + \bar{y}_k^2}$, where r is the correlation of angular departures across k total points. Angular dispersion is represented as $1-r$ and ranges from 0 to 1, such that a path in which all angles of movement are perfectly correlated (straight line) maintains a dispersion value of 0 (no tortuosity) and a track in which departures from the previous vector occur at random maintain a value that approaches 1 (high tortuosity) (for a full description see Estevez and Christman, 2006).

Two classical measures of fearfulness and anxiety in mice, immobility and fecal score (number of fecal deposits) (Augustsson and Meyerson, 2004), were also analyzed in this experiment. Immobility, measured in this study as a path count, was quantified by parsing an individual's complete track of movement into distinct paths.

A path ended, and a new path began, when an animal's location remained unchanged for approximately 2 seconds.

3.2.3 *Statistical analysis*

For each dependent variable measured, the mean was taken across all tested individuals within a single session. Session means were analyzed using a repeated measures mixed model procedure for analysis of variance with treatment and day of experiment (1 through 12) modeled as the fixed effects and session (AM and PM) modeled as a random effect (SAS, v. 9.1). Compound symmetry and autoregressive covariance structures were examined for each model. The structure that fit best, as determined by AICC goodness of fit statistic, was chosen for each model. Assumptions of normality and homogeneity of residual variances for each parameter were examined. Track length data failed to meet the assumptions for normality and was modeled with a Poisson distribution using generalized linear mixed models to satisfy those assumptions.

3.3 **Results**

3.3.1 *Movement*

The results from this experiment suggest a positive relation between arena floor area and total track length. The total distance mice traveled in a single observation period increased significantly as the arena floor area increased ($F_{3, 8} = 69.6$, $P < 0.0001$) (Figure 3-1). The mean distance traveled ranged from 31.8m in the smallest arena to 48.4m in the largest. It is most interesting to note that, in increasing

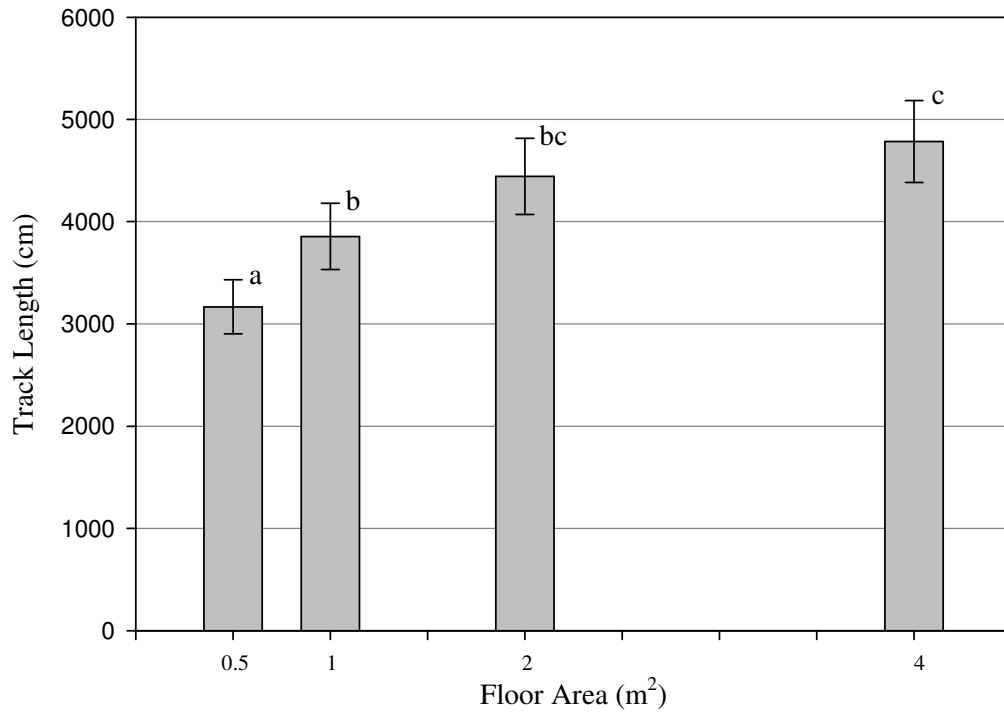


Figure 3-1

Effects of floor area on track length. Means sharing any common letters are not significantly different ($P>0.05$).

the floor area by a factor of 2, the resulting total movement only increased, at a decreasing rate, by a factor of approximately 1.2, from 0.5m² to 1.0m², and approximately 1.1 for the remaining increases. The distance mice traveled did not differ significantly across experiment days ($F_{11, 8} = 3.24, P > 0.05$).

Net displacement, the distance between a mouse's first and last sampled positions, differed significantly across floor areas ($F_{3, 8} = 84.18, P < 0.001$), but did not differ across days of the experiment ($F_{11, 6} = 1.26, P > 0.05$). This distance tended to increase as floor area increased (Figure 3-2).

The angular dispersion, or tortuosity of the observed paths, was not significantly influenced by floor area ($F_{3, 8} = 0.38, P > 0.05$) and did not change significantly across the duration of the experiment ($F_{11, 8} = 1.64, P > 0.05$). The dispersion was approximately 0.90 for all arena sizes. The path count did not differ significantly between the tested floor areas ($F_{3, 7} = 0.71, P > 0.05$) or across the days of the experiment ($F_{11, 7} = 3.13, P > 0.05$).

3.2 Behavior and space use

The increase in arena floor area resulted in an increase in the mean distance to the nearest wall (MWD) (Figure 3-3), though this effect was not statistically significant ($F_{3, 8} = 3.29, P = 0.08$). MWD was also not significantly different across experiment days ($F_{11, 8} = 1.72, P > 0.05$). MWD was also calculated relative to the area of the experiment arenas, as a means of controlling for differences in the distance from the wall to the arena center. No significant differences in relative MWD were

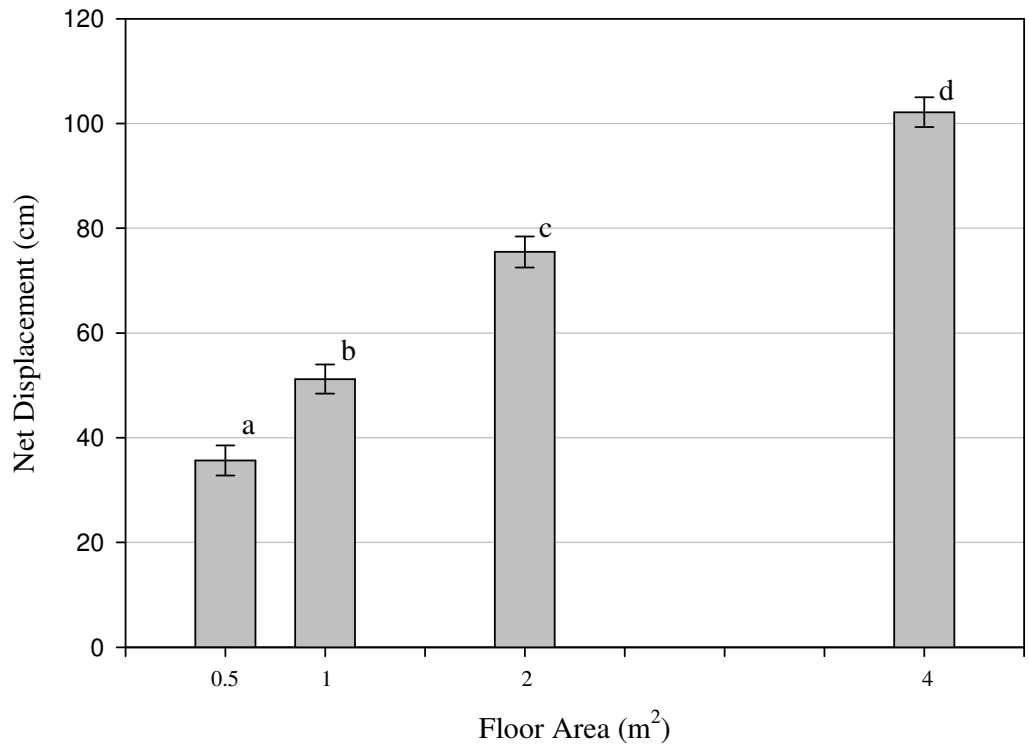


Figure 3-2

Effects of floor area on net displacement. Means sharing any common letters are not significantly different ($P>0.05$).

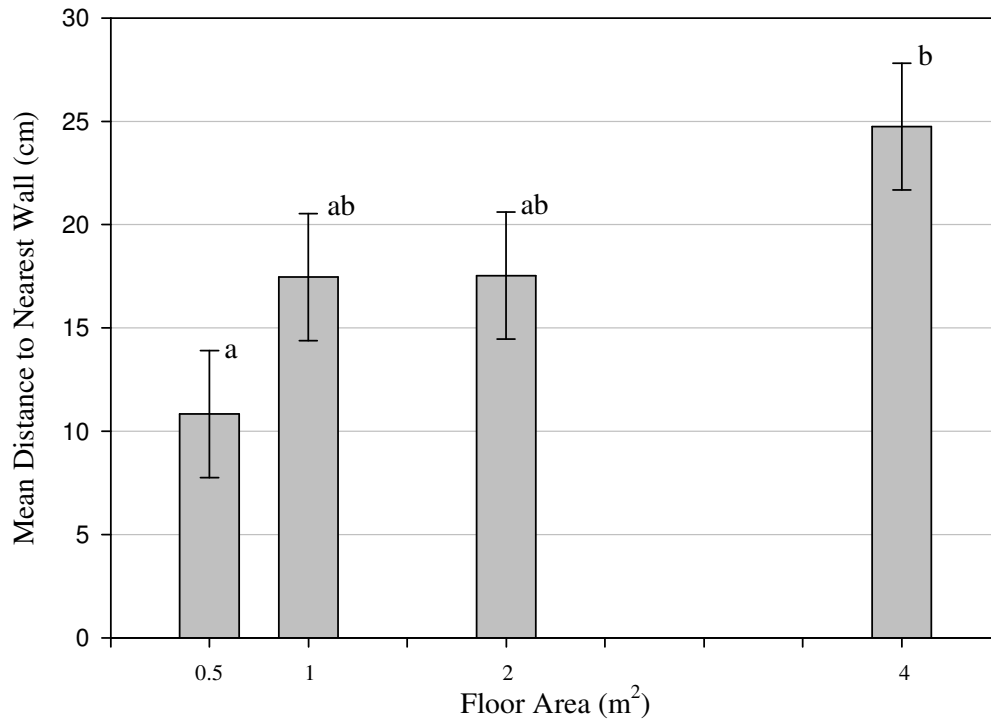


Figure 3-3

Mean distance to the nearest wall (Effects of floor area on mean distance to the nearest wall. Means sharing any common letters are not significantly different ($P>0.05$)).

observed between the tested floor areas ($F_{3,8} = 0.34, P > 0.05$) or across experiment days ($F_{11,8} = 2.17, P > 0.05$). In all treatments, mice spent more time in the peripheral zone than in the central zone (71, 66, 72 and 63% for 0.5, 1, 2 and 4m² arenas respectively), though this tendency did not differ significantly across floor area treatments ($F_{3,8} = 0.66, P > 0.05$) or across experiment days ($F_{11,8} = 1.62, P > 0.05$).

The proportion of the area traversed differed significantly between floor areas ($F_{3,8} = 10.52, P < 0.01$), but did not differ across experiment days ($F_{11,8} = 2.61, P > 0.05$). As expected, this proportion was highest in the smallest arena size (0.5m²) and decreased as floor area increased (Figure 3-4). However, the proportion did not differ significantly between arenas that measured 1, 2 and 4m². Fecal scores differed significantly across the four floor areas ($F_{3,8} = 6.15, P < 0.05$) but did not differ across experiment days ($F_{11,8} = 1.63, P > 0.05$). Fecal scores were highest in the arena with the smallest floor area and lowest in the arena with the greatest floor area.

3.4 Discussion

To date, there is no clear, unambiguous, and uniformly accepted definition for either space or freedom of movement. Turvey (2004) described several mathematical, physical, biological, and psychological concepts that have historically been used to define space. Current housing guidelines most often make use of mathematical, physical and biological concepts to evaluate a space in terms of waste management or ease of access to resources (Committee, 1999). However, a definition that combines these concepts with an understanding of how animals perceive space may be more appropriate for use in recommended or regulated standards of animal care (Stricklin,

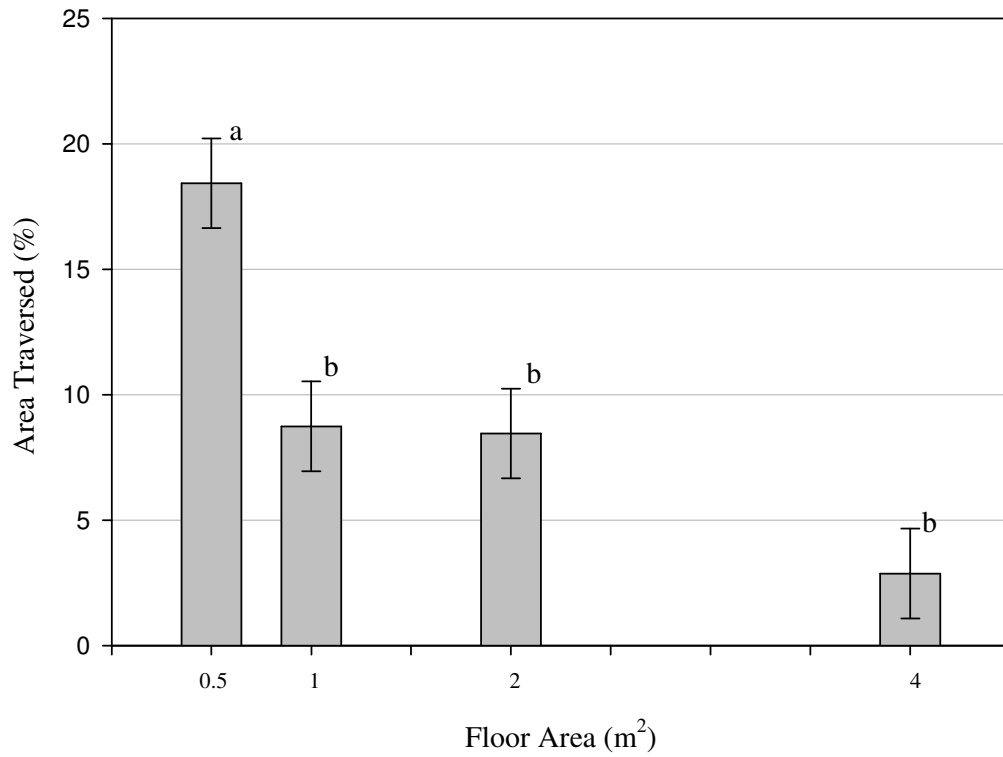


Figure 3-4

Effects of floor area on area traversed. Means sharing any common letters are not significantly different ($P>0.05$).

1995). Examples for criterion that would constitute sufficient movement freedom include the ability for an animal to control its environment (Gonyou, 1996) or a reduction in fear and anxiety experienced within that environment. One reason for the lack of consensus on the definition of space or freedom of movement is an inability to assess the subjective experience of confined animals. One solution to this problem is to measure patterns that are reliable indicators of an animal's subjective state.

The primary objective of this experiment was to describe the movement, space use and behavioral responses of mice allowed to travel freely within arenas of the same shape that differed in size (floor area). In doing so, the hope was to gain useful information regarding the influences of floor area on movement and use of space, as well as to further understand the relative importance of area when compared to other spatial parameters that define a given space. A second objective of this experiment was to address the common use of floor area as a measure of available space or allowed "freedom of movement" when discussing housing and welfare of confined animals.

The majority of mice observed in the experiment moved almost continuously throughout the 3-minute observation periods. Despite their small size (approximately 11cm from nose to base of tail), their high levels of activity resulted in a mean total distance traveled (track length) of approximately 32 meters over 3 minutes within the smallest arena (Figure 3-1). This track length continued to increase as floor area increased, reaching a mean of almost 48 meters within the largest arena (4m²). While consistent with previous observations of mice, rats and gerbils (Walsh and Cummins, 1976), these results were inconsistent with recent investigations of open field activity

in similar rodent species. In studies by Eilam et al. (2003) and Eilam (2003), no differences in locomotion were reported across pens of different sizes, though results in both experiments were based on relatively small sample sizes.

At first glance, the increase in ambulation reported above may be interpreted as conclusive evidence that an increase in floor area is indicative of, and possibly equivalent to, an increase in movement freedom or available space. However, without mention of causation or comparisons across multiple environments/experiments, the quantitative measures of activity tell us little about the animals' subjective experience. Archer (1973) observed that increases in open-field activity may result from elevated expression of active escape behavior, during which time the animal is constantly seeking a location of safety. It is also widely accepted that fear or anxiety in rodents result in an overall suppression of open-field locomotion (Espejo, 1997; Blanchard et al., 2001). These different, and somewhat contradictory, explanations for the causation of open-field movement patterns demonstrate that measuring total movement alone may not be fully indicative of the animal's subjective experience. In this experiment, total distance traveled increased at a decreasing rate, and is expected to asymptote when movement becomes continuous (no pauses) and/or animals travel at their maximum average run speed. Furthermore, these results demonstrate that increasing floor area does not result in a proportionate increase in total movement. In fact, instead of doubling as floor area was doubled, total distance traveled for the 3 minute observation period increased only by a factor of approximately 1.2 when area was increased from 0.5 to 1m² and approximately 1.1 for the remaining increases.

As another common measure of emotionality in mice (Bronikowski et al., 2001), the fecal scores observed in this experiment tended to support the results for track length described above, assuming that open-field activity is indeed negatively correlated with fear or anxiety. In early studies of emotional reactivity of rodents in an open field, Hall (1934) suggested that a greater number of fecal boli deposited by individuals was indicative of an elevated level of fearfulness. In the current experiment, mean fecal score was highest in the smallest arena (0.5m²) and lowest in the arena with the greatest floor area (4m²). This is consistent with track length data, and provides some evidence that mice may experience less stress while traveling within the larger arena. Some caution should be taken when interpreting defecation data, as several authors have questioned the validity of the measurement (Archer, 1973; Walsh and Cummins, 1976) for the assessment of emotional states.

The majority of experiments that describe patterns of movement for mice and rats within an open field report repeated excursions, of increasing distances, from a “home base” (Zadicario et al., 2005; Wallace et al., 2006). Following this, it was expected that the values for net displacement would fluctuate unpredictably; resulting in a mean value that approached half of the internal diagonal (maximum net displacement). This is because the net displacement measurement is dependant upon the point at which the final sample was taken. However, this original prediction was based on previous studies in which subjects were released into the arena through one of the outer walls. In the current experiment, mice were released into the test arena at a central location, which resulted in mean net displacement values that approached the mean distance between the arena bisect and the boundary walls (Table 3-2).

Table 3-2

Experiment Results. Means sharing any common letters are not significantly different (P>0.05).

	0.5	1	2	4
Floor area (m²)				
Perimeter (cm)	284	400	564	800
Diagonal (cm)	100	141	200	283
Mean Distance from Center to Wall (cm)	40.9	57.5	81.1	114.9
Track Length (cm)	3167 ± 266 ^a	3854 ± 323 ^b	4443 ± 373 ^{bc}	4784 ± 401 ^c
Path Count	11.31 ± 1.28	11.69 ± 1.25	9.45 ± 1.39	10.82 ± 1.29
Angular Dispersion	0.902 ± 0.007	0.890 ± 0.007	0.899 ± 0.007	0.899 ± 0.007
Net Displacement (cm)	35.23 ± 2.87 ^a	51.21 ± 2.77 ^b	75.47 ± 2.98 ^c	102.17 ± 2.85 ^d
Area Traversed (%)	18.43 ± 1.79 ^a	8.74 ± 1.79 ^b	8.46 ± 1.79 ^b	2.87 ± 1.79 ^b
Mean Distance to Nearest Wall (cm)	10.83 ± 3.07 ^a	17.46 ± 3.07 ^{ab}	17.53 ± 3.07 ^{ab}	24.74 ± 3.07 ^b
Relative Mean Distance to Nearest Wall (%)	26.0 ± 3.0	26.5 ± 3.0	23.3 ± 3.0	24.5 ± 3.0
Points in Perimeter	70.5 ± 6.4	64.4 ± 6.4	72 ± 6.4	63.5 ± 6.4
Fecal	1.19 ± 0.24 ^a	0.37 ± 0.24 ^{bc}	0.61 ± 0.24 ^{ac}	0.10 ± 0.24 ^c

Thus, net displacement increased proportionately with diagonal length and distance to outer wall (Figure 3-5), at a rate of approximately $\sqrt{2}$ for each doubling of floor area. Previous work by Jeanson et al. (2003) suggested that net displacement is indicative of an animal's ability to disperse from its initial location. Results from this experiment demonstrate that, because of their tendency to adhere to the walls, this response is more dependent upon the length-to-width ratio (the perimeter-to-area ratio) of the enclosure rather than the absolute floor area. This relation will be further examined in the second of three experiments.

It was hypothesized that angular dispersion (path complexity) would decrease with increases in floor area. The reason for this predication is that an individual traveling within a small, enclosed space will likely be forced to alter its trajectory more frequently than an animal moving within a larger space. This shorter distance between obstacles (walls) should have a greater influence on the individual's pattern of movement, resulting in greater overall path complexity. According to previous work, the complexity of an animal's path of movement should be indicative of its ability to move freely within a space (Estevez and Christman, 2006). Following this, it was rather unexpected and somewhat disappointing that no significant differences in the tortuosity of movement were observed across the four floor areas. However, after taking into consideration the typical movement pattern of rodents in an open field, mentioned above, we find that this result may be a factor of the similarity in arena shape, the typical movement pattern of confined mice and the nature of measurement. The magnitude of the tortuosity measurement is dependent upon the

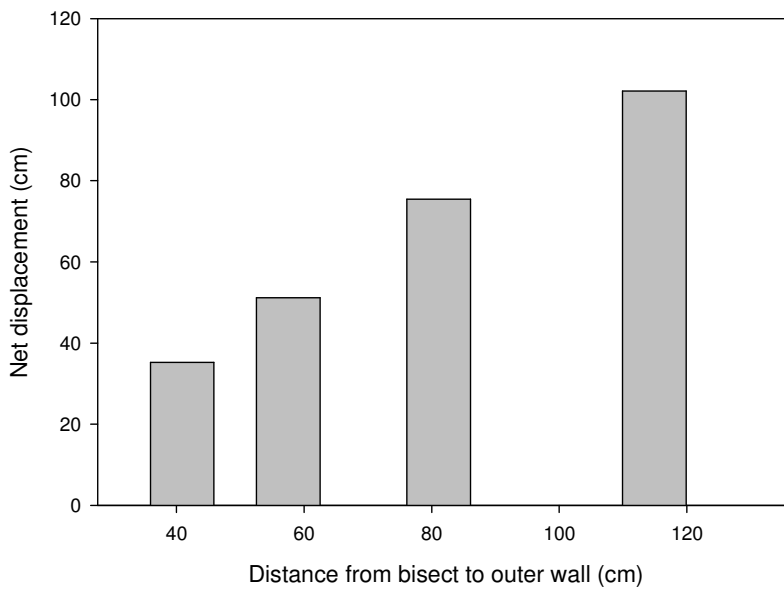
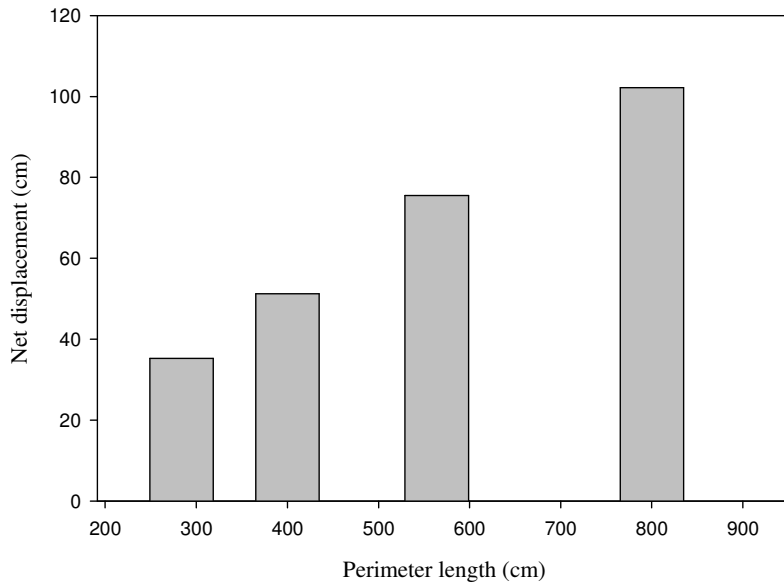


Figure 3-5

The relationship of net displacement to perimeter length and bisect-to-boundary distance. Net displacement appears to increase proportionately with both measure.

distribution of orientation angles in the object's path, thus imposing a "trapping effect" (Erlandsson et al., 1999) on the individual's movement. Thus for a path in which all angles are the same, such as a straight line or a circle, we find zero tortuosity. For the four arenas tested, the distance the animals moved may have differed, but the general pattern of movement remained the same. That is to say the majority of movement was still along the arena perimeter, with frequent changes in direction.

Currently, floor area alone is the most common measurement used to describe the amount of physical space (or movement freedom) available to a confined animal. If it is accepted that floor area is equivalent to space, and if an increase in such space will presumably result in a subsequent increase in an animal's use of that space, then a logical conclusion is that increasing floor area will result in a consequent increase in space use. In this experiment, contrast-based tracking software made it possible to base the primary measure of space use, percentage of area traversed, on a series of continuously-sampled animal locations. This method lacks any dependence on the estimation of animal locations that fall between sampled points, and may provide a value with more accuracy and of greater relevance than would be obtained by core area or kernel methods.

Despite it being a more accurate technique for measuring use of space, the amount of pertinent information that can be gathered from percentage of area traversed alone may be limited in this experiment without some level of correction for the differences in floor area. The results for this experiment demonstrated a

phenomenon that, after the fact, seems like an inevitable response. As floor area increased, the total percentage of area being used decreased, which is to say that as the size of the arena increased, mice used relatively less of the total area. Within the smallest arena (0.5m²), mice demonstrated greatest use of space (18%), and within the largest arena (4m²) area traversed was the lowest (2%). However, when these values were corrected for the differences in floor area, by multiplying the response by the floor area, the differences in area traversed were no longer significant ($F_{3,8} = 2.41, P > 0.05$). The relative area traversed was lowest for the 0.5m² arena, but this could have been the result of the area traversed calculation using only unique coordinate points. The smallest arena may have had more locations that were repeatedly visited. With reliable evidence of how much of the space is being used, it is also important to note where an individual animal spends the majority of its time during the observations, as described below.

It is widely accepted that mice tend to maintain close contact with vertical surfaces because, by doing so, they are less vulnerable to predation (Dickman, 1992; Jensen et al., 2003) and aggression from conspecifics (Gray et al., 2000). This tendency is also well-documented in a number of other species (Stricklin et al., 1979; Newberry and Hall, 1990; Cornetto and Estevez, 2001). Subjects that venture farther from the protection of a vertical surface are seemingly less constrained by the relative risk associated with doing so, and may be expressing an increased ‘freedom’ to explore, or make use of, their surroundings. Despite the assumption that floor area and movement freedom have a positive relation, it was hypothesized that as floor area is increased, the average distance mice are willing to travel from the arena walls will

decrease. Therefore, in smaller arenas, where the mean distances between areas of relative safety are minimized, one would expect the greatest observed mean distance to the nearest wall (MWD), and greater movement freedom, as it relates to their willingness to enter central areas

In this experiment, mice demonstrated an increase in the absolute MWD as floor area increased, though this difference was only statistically significant between the floor areas of 0.5m² and 4m². While these results differed from what was expected, they do provide support for previous assumptions mentioned above. Namely, if greater MWD is indicative of greater freedom to move within a space, then animals observed in the larger arenas experienced greater movement freedom. Interestingly, when MWD was analyzed relative to the size of the arenas, no significant differences were observed. This relative MWD was expressed as a percentage of the distance between the wall and the center of the arena. It may be that the tendency to travel along the pen perimeter is closely tied to the size of the space, such that the average distance a mouse is willing to travel from the perimeter is equal to some percentage of the distance between the outer wall and the pen center regardless of the magnitude.

As an additional means for describing the tendency to maintain contact with a vertical surface, each sampled animal location was designated as falling in either the central or peripheral regions of the arena, which were equal in floor area. While there were no significant differences between the treatments, on average, mice spent the majority of their time in the arena periphery. If, in fact, the space along the boundary is preferable, it is possible that enclosures that minimize the area to perimeter ratio

may provide the animals with a more 'comfortable' space and one with a greater perceived level of safety and control. This possible disparity between the importance of floor area and perimeter length will be examined in the second of three planned experiments.

It is evident that floor area influences the quality and quantity of space, depending on the species in question; however, results from this experiment are consistent with the prediction that area alone does not necessarily define a space or the movement freedom it provides an animal (Stricklin et al., 1979; Stricklin, 1995). None of the variables measured showed a response that was proportionate to the incremental increases in floor area tested, though some of these effects were in fact proportionate to the change in perimeter and internal diagonal lengths. It is difficult to determine if changes in movement and space use were a result of the increase in floor area, the increase in perimeter length (PL) and maximum internal diagonal (MID), or all of these factors.

Using only floor area measurements to describe the amount of space and movement freedom an enclosure provides may oversimplify the concept of space. Furthermore, by not addressing the animal's perception of its environment and by overlooking the importance of other features (i.e. perimeter length and internal diagonal) there is a risk that guidelines for animal care will not effectively address the needs of the animals.

Estimates of proximity to vertical surfaces and the frequency at which animals are found in this proximity may provide useful information concerning the spatial freedom provided by an enclosed area, in terms of how "comfortably" animals can

travel within the enclosed space. In mice, it is likely that there is a point at which increasing floor area may result in an actual decrease in movement freedom. Data from this experiment demonstrate a possible asymptote in track length as floor area is increased. These data also demonstrate the influence that the simple geometric parameters of a space have on movement. It is unlikely that the similarity between the patterns of increase in net displacement and perimeter length/diagonal length as floor area is doubled is purely coincidental. In order to make accurate assessments of the spatial needs of animal, more empirical evidence must be obtained regarding how specific characteristics of an animal's housing, including floor area, influence movement freedom and space. This information is vital in not only the assessment of current housing systems and the welfare of animals housed within, but also in the design of new environments that may better attend to the needs of the animals.

In modern agricultural and research systems, the amount of space provided for individual animals is one of the leading concerns. From primates to gestating sows to laying hens, the debate over what constitutes adequate availability and quality of space has dominated the majority of discussions regarding the housing and handling of animals, and, specifically, how best to meet the needs of the confined animals. It seems the primary obstacle in determining what housing designs is that there are clear economic advantages to square pens, because maximizing the area to perimeter ratio reduces the amount of building materials and, consequently, building costs. Future experiments will look to determine the relative importance of spatial parameters other than floor area, and if it is possible to optimize the costs effectiveness in favor of greater spatial freedom.

Chapter 4: The Influence of Spatial Parameters on Movement and Space Use in Mice (*Mus musculus*) - Pen Shape

Abstract

Following previous work that demonstrated a significant effect of floor area, perimeter length and internal diagonal length on movement and space use, 40 female Swiss Webster mice were observed in four arenas that differed in shape in an attempt to differentiate between the effect of floor area and perimeter/internal-diagonal length. The dimensions for the arenas in which each mouse was observed in three 3-minute sessions were: (A) a 122cm x 122cm square, (B) a 212cm x 71cm rectangle, (C) a 150cm x 100cm rectangle and (D) a 138cm (diameter) circle. Measures of movement (track length, net displacement and path count) were significantly influenced by arena shape. In rectangular arenas, the observed mean track lengths ($F_{3, 6} = 19.54, P < 0.01$) and net displacements ($F_{3, 7} = 7.26, P = 0.05$) were higher in arenas with greater perimeter length to floor area ratios. Circular arenas differed consistently from rectangular arena in both total movement and bouts of immobility. These results demonstrate the importance of qualitative and quantitative properties of perimeter space on the movement of mice. This is made especially clear by the apparent differences between the circular arena and all rectangular treatments. However, there is little indication that the species-specific patterns of movement and space use are significantly influenced by arena shape.

4.1 Introduction

It is known that the quality and quantity of space in a given enclosure are influenced by several characteristics of the space, including, but not limited to, the total perimeter length (PL), the number of corners, and the length to width ratio (Stricklin et al. 1979). Stricklin et al. (1979) reported that cattle use a disproportionate amount of perimeter locations as compared to more central locations in the pen. This tendency has since been reported in sheep (Sevi et al., 2001), chickens (Cornetto and Estévez, 2001; Leone et al., 2007), insects (Jeanson et al., 2003), and in rodents (Tchernichovski et al, 1998; Tchernichovski and Benjamini, 1998; Jensen et al., 2003). In the first part of this study, in which I determined the effect of floor area on movement in mice, I obtained similar results, suggesting that floor area, though often used as the primary measure of space and movement freedom, may not be as important as the ratio of perimeter space to open floor space when describing the quality of a space and predicting the subjective experience of animals housed within it. Few research efforts have been made to explore the relation of pen shape to movement and space use. Wiegand et al. (1994) found that pigs housed in triangular, circular and square pens spent more time in areas that contained walls, while pigs in rectangular pens demonstrated no difference in the relative use of open areas and areas containing walls. Experiments in animal production and applied ethology have demonstrated a marked effect of varying the shape of an enclosure on the behavior of animals observed within (Wiegand et al., 1994; Sevi et al., 2001). While there is limited work devoted to determining how pen shape influences movement and use of space in live animals, Stricklin et al.(1979), using randomly positioned, mobile

animats, found that varying pen shape while keeping floor area constant resulted in differential spatial and movement patterns. Additionally, recent mathematical calculations and computer simulations by Perlitz (unpublished) have indicated that certain pen shapes theoretically add more to the movement freedom of the “individuals” housed within than do others. In this work, a 2:3 ratio rectangle should provide the greatest “freedom of movement” (for all four-sided enclosures), as measured by internal minimum distance to the borders. However, it has yet to be determined if this perceived benefit to movement freedom occurs in live animals. In the present study I aim to determine if these mathematical predictions of greater freedom of movement exist in actual animal housing situations.

Recommendations for housing and handling of animals tend to focus heavily on floor area as a means of measuring space availability and movement freedom. By ignoring other properties of that enclosure, they may overlook not only the behavioral needs of the animal but also the actual amount of space required to meet those needs. By pooling information about the behavior of animals observed in pens that vary in size (floor area) with observations of the same animals in pens varying in other characteristics, we may gain a better understanding of how the animal perceives the space. By adopting this bottom up approach to describing behavior, in which we focus on acquiring detailed basic information about movement, I hope to remove some of the assumptions and guesswork that become necessary when extrapolating individual animal information from observations of a social group or an aggregation. Furthermore, once this baseline information is collected and catalogued, observations of social groups can be made under similar experimental conditions, and comparisons

of these groups to individual animal observations may paint a clearer picture of how the additional social interactions affect movement and space use.

This experiment is the second in a three-part study aimed at describing the effects of spatial parameters on movement and space use in mice. In a previous experiment, it was determined that floor area does in fact influence movement and behavior in an open-field. On no variable measured was this effect proportionate to the incremental increases in floor area tested, though some of these effects were in fact proportionate to the change in perimeter and internal diagonal lengths. It was thus difficult to determine if changes in movement and space use were a result of the increase in floor area, the increase in perimeter length (PL) and maximum internal diagonal (MID), or all of these factors. The purpose of the following experiment was to further explore how differences in PL, MID and the existence of corners affect animal movement and behavior. This will be accomplished by evaluating the responses of animals in arenas of equal floor area but of different shapes.

4.2 Animals, Methods and Materials

4.2.1 Experimental animals

In this experiment, conducted in the University of Maryland, College Park Animal Research Wing, I observed the movement and space use of forty 16-20 week old pair-housed female Swiss Webster (CFW) mice, obtained from a commercial animal model supplier (Charles Rivers Laboratories, Inc.). Each pair of mice was assigned a cage number (1 through 25) and then the cage numbers were randomly ordered using a random number generator that excluded duplicate integers (Haahr,

2002). From the generated cage order, two observation groups of 10 pairs of animals were created. Animals in the AM group (n=20) were observed for 3 minutes between the hours of 0900 and 1100. Animals in the PM (n=20) group were observed for 3 minutes were observed between the hours of 1300 and 1500. The order of observation within each cage was determined prior to the start of the experiment, and every cage was observed once daily for 12 consecutive days.

4.2.2 Open-field arena and observation procedure.

The walls of the three rectangular arenas were constructed using 30.5cm wide composite hardboard sheets. The wall of the circular arena was constructed using a 0.32 x 30.5 x 434cm plastic sheet, attached at two ends to form a circle. The arena floor surface, common across all arenas, was constructed from a 240 x 240cm composite hardboard panel. All surfaces were painted with a dark gray, low odor, no-VOC semi-gloss latex paint. The gray color was chosen to maximize contrast for the video-tracking of the small animal models. A 28cm (diameter) cylindrical opaque plastic container attached to a pulley system and placed in the center of the field was used as the starting point for all observations. In addition, non-reflective white paper was hung to a height of approximately 2.1m in the area surrounding the arena. These steps were taken to ensure that all mice were released from the same location, the researcher was not visible immediately prior to, or during any portion of, the recorded observations, and the space was devoid of spatial cues that could influence movement or space use.

Four pen shapes of equal floor area were tested in this experiment: a 122cm x 122cm square (square), a 212 x 71cm rectangle (long), a 150 x 100cm rectangle (short) and a 138cm diameter circle. All observations were made in a dimly lit room (approximately 8-10 lux) to optimize mouse activity (Bronikowski et al., 2001). The experimental design resembled a Latin Square, in which schedule of presentation for these pen designs was adjusted to control for possible confounding effects, including time and order effects (Table 4-1), and treatments were randomly assigned to the schedule of presentation prior to the start of the experiment. Two sessions of observations, approximately 4 hours apart (from the start of observations), were completed per day of test. Every mouse encountered each experimental pen design three times. Each mouse was individually observed during one of the two daily sessions for a total of 80 mice per day. The observations were made in 6 four-treatment blocks, each of which required two days to complete. The schedule of testing for pen designs was adjusted such that each pen design was tested an equal number of times in both the morning and afternoon sessions.

For a given observation period, two mice (pair-housed) were transported to the test room, individually observed, and then returned to their home cage. The order of observation within a cage was determined prior to the start of the experiment and that order was maintained for the extent of the experiment.

Table 4-1

Experiment Schedule. Letters denote the different pen treatments.

SESSION	DAY OF TEST											
	1	2	3	4	5	6	7	8	9	10	11	12
AM	A	C	B	D	C	A	D	B	C	D	B	A
PM	B	D	C	A	B	D	C	A	A	B	D	C

4.2.3 Data collection and measurement variables

Total track analysis

For each experiment trial, an individual mouse was placed under the cylindrical container in the center of the arena. The trial, which was 3 minutes in length, began when the mouse was released into the field. After each trial the mouse was caught and returned to its cage and the surface of the arena was cleaned with an acetic acid/water solution to remove odor cues, as suggested by McGivern et al. (1997), and allowed to dry. Each observation was recorded using a Panasonic closed-circuit color video camera, mounted approximately 2.5m above the arena floor surface. Trials were captured in an uncompressed Audio-Video Interlaced (AVI) file and later processed using Viewer II tracking software (v. 2.0.0.31 by BIOBSERVE, Fort Lee, New Jersey) to acquire central x and y coordinates. From these coordinates, several measures of space use and spacing behavior were summarized for each subject. The dependent variables summarized for the total track were as follows: Track length (total distance traveled), net displacement, mean distance to the nearest wall, percentage of total points that occurred in the periphery, fecal score, floor area used, number of independent coordinates, and angular dispersion (tortuosity or movement complexity), path count and the number of paths per total track length.

To measure the total distance an animal traveled, I calculated the sum of the Euclidean distances between each successive coordinate point. Net displacement was measured as the Euclidean distance between the first and last tracked locations. Two measures were used to describe the tendency of animals to spend a disproportionate amount of time at peripheral locations within an enclosed space. First, I calculated

each individual's mean distance from the nearest wall (MWD). To calculate this value, I determined the straight-line distance to the nearest wall at each coordinate point within a track and then determined the mean of these distances for each observation. To calculate the second estimate of wall-following behavior, I used the captured coordinates to partition each experimental arena into two zones (central and peripheral) that were equal in area, and then determined the proportion of tracked locations in the two zones (only peripheral proportions are reported).

Angular dispersion, or tortuosity, has been used to describe the path complexity of a range of objects (Hart et al., 1999; Seker et al., 2005; Estevez and Christman, 2006). If we assume that restrictions on animal movement (physical obstacles or perceived threats) result in aberrations from a straight-line path of travel, the angular dispersion provides a useful measure for quantifying the restrictions a particular environment imposes. In this experiment, angular dispersion is calculated by measuring the average angular departure of a tracked location from the animal's previous location. This departure is used to calculate the concentration of tracked points $r_k = \sqrt{\bar{x}_k^2 + \bar{y}_k^2}$, where r is the correlation of angular departures across k total points. Angular dispersion is represented as $1-r$ and ranges from 0 to 1, such that a path in which all angles of movement are perfectly correlated (straight line) maintains a dispersion value of 0 (no tortuosity) and a track in which departures from the previous vector occur at random maintain a value that approaches 1 (high tortuosity) (for a full description see Estevez and Christman, 2006).

To calculate relative floor area used (area traversed) by an individual, the total number of unique coordinate point locations in each individual's movement track was

expressed as a proportion of the total number of coordinate points in the arena. The value was obtained using the equation $\sum_{i=1}^N x_i / N$, where N represents the total number of possible locations for individual i and x represents a single independent observed coordinate point. The resolution for tracked coordinates was 1cm, such that the total number of coordinate points in an arena was equal to the area of that arena.

Immobility, measured in this study as a path count, was quantified by parsing an individual's complete track of movement into distinct paths. A path ended when an animal's location remained unchanged for approximately 2 seconds. To determine the effect of the number of pauses in movement on the total distance an individual traveled, the count was also calculated per total track length. Immobility, total distance traveled, perimeter adhesion and fecal scores have all been used to describe relative states of fearfulness or anxiety in mice (Augustsson and Meyerson, 2004).

Statistical analysis

For each dependent variable measured, the mean was taken across all tested individuals within a single session. Session means were analyzed using a repeated measures mixed model procedure for analysis of variance with treatment and day of experiment (1 through 12) modeled as the fixed effects and session (AM and PM) modeled as a random effect (SAS, v. 9.1). Compound symmetry and autoregressive covariance structures were examined for each model. The structure that fit best, as determined by AICC goodness of fit statistic, was chosen for each model. Assumptions of normality and homogeneity of residual variances for each parameter were examined. Track length data failed to meet the assumptions for normality and

was modeled with a Poisson distribution using generalized linear mixed models to satisfy those assumptions.

4.3 Results

4.3.1 Movement

The track length differed significantly between the tested arena shapes ($F_{3,6} = 19.54, P < 0.01$). The results from this experiment suggest a tendency for track length to increase as perimeter length (PL) increases. However, this only holds true for rectangular pens (Figure 4-1). Mice demonstrated the greatest mean distance traveled (4549cm) in circular arenas, which had the shortest PL (433cm). Track length did not differ significantly across experiment days ($F_{11,6} = 3.58, P > 0.05$).

The angular dispersion, or path tortuosity, was not significantly influenced by arena shape ($F_{3,6} = 1.64, P > 0.05$); however, dispersion did change significantly across experiment days ($F_{11,4} = 7.78, P < 0.05$).

The net displacement differed significantly across arena shapes ($F_{3,7} = 7.26, P = 0.05$). Net displacement in the Long (3:1) rectangular arena, which had the greatest maximum internal diagonal (MID) length (224cm), differed significantly from all other arena shapes (Figure 4-2). The net displacement did not differ significantly across experiment days ($F_{11,4} = 1.55, P > 0.05$).

The number of distinct paths within a total track differed significantly between the experimental arenas ($F_{3,6} = 6.84, P < 0.05$) and across experiment days ($F_{11,6} = 4.84, P < 0.05$). Among the rectangular arenas, no differences were observed in the number of individual paths. However, least squared means procedures showed significant

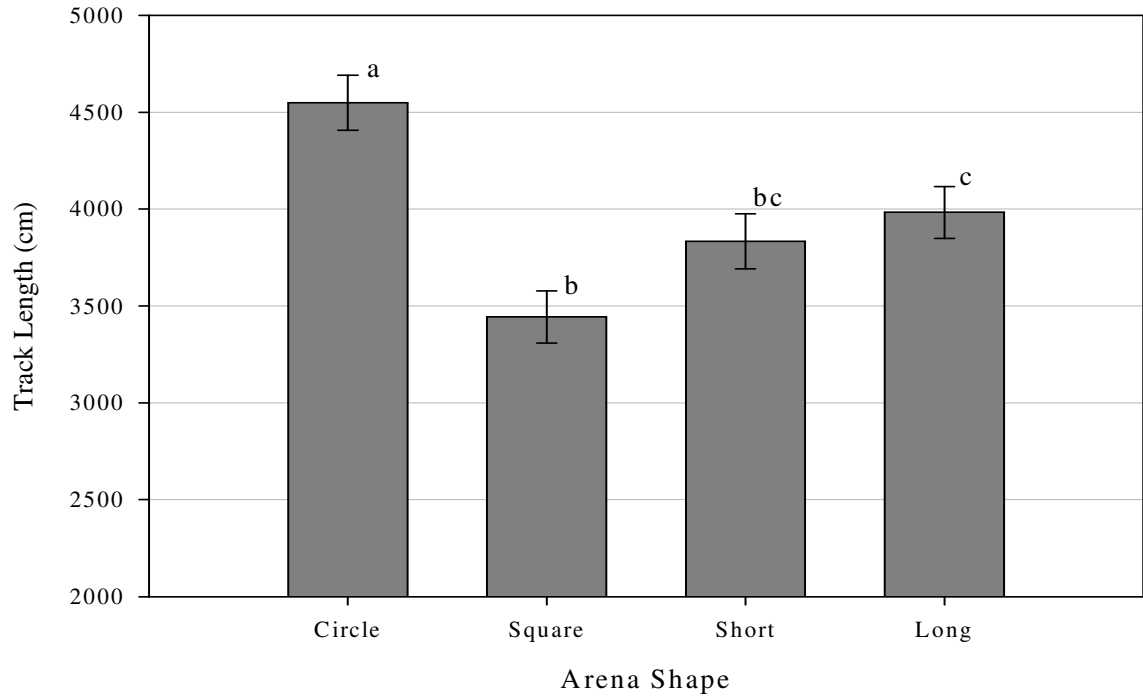


Figure 4-1

Effects of arena shape on track length. Means sharing any common letters are not significantly different ($P>0.05$).

differences in path counts between the circular arena and all other arena shapes (Figure 4-3). Animals had significantly more bouts of immobility, as measured by the mean number of paths per track, when observed in pens with corners (4.6, 5.8 and 6.2 paths for the square, long and short arenas, respectively). Path count was then adjusted for differences in observed track lengths to determine if track length and path count were independent. The effects of arena shape ($F_{3,5} = 38.67, P < 0.01$) and experiment day ($F_{11,5} = 18.58, P < 0.01$) were still apparent, even after path count was adjusted for track length, suggesting a relationship between the two values.

4.3.2 Behavior and space use

The mean distance to the nearest wall (MWD) did not differ significantly between arena treatments ($F_{3,4} = 1.43, P > 0.05$) or across days of the experiment ($F_{11,4} = 0.87, P > 0.05$). The MWD was approximately 11cm in all arenas except the short (2:3) arena, in which MWD was approximately 15cm (Figure 4-4). Though differences in MWD between the short arena and other arenas tested were not statistically significant, they may provide support for previous findings on the maximizing available space in quadrilaterals, discussed in greater detail below.

In all treatments, mice spent more time in the peripheral zone than in the central zone (81, 78, 75 and 82% for circular, square, short and long arenas respectively). This tendency did not differ significantly between arena shapes ($F_{3,6} = 0.5, P > 0.05$) or across experiment days ($F_{11,6} = 1.89, P > 0.05$). The proportion of the area traversed did not differ significantly between floor areas ($F_{3,6} = 0.31, P > 0.05$) or across experiment days ($F_{11,4} = 5.44, P > 0.05$). There was no significant

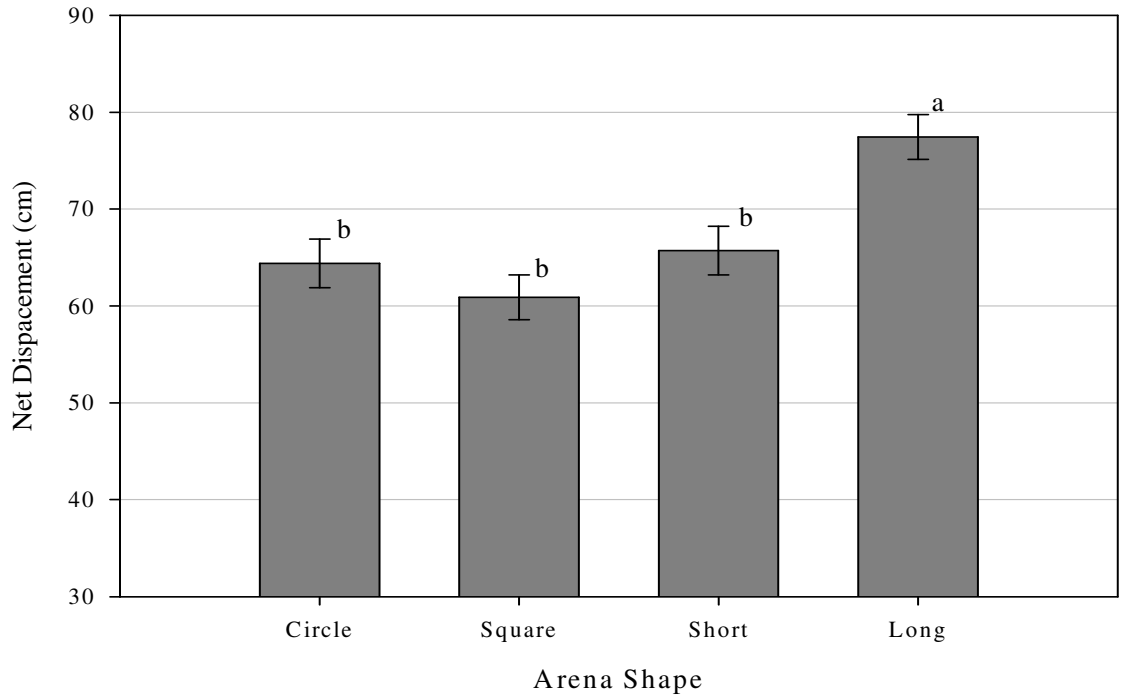


Figure 4-2

Effects of arena shape on net displacement. Means sharing any common letters are not significantly different ($P>0.05$).

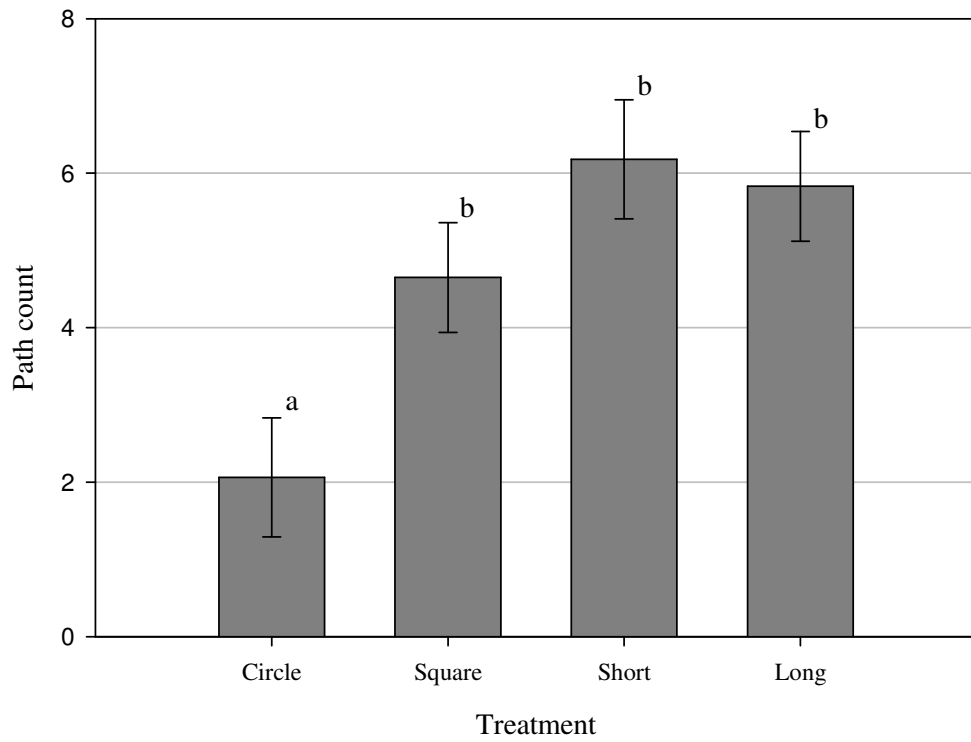


Figure 4-3

Effects of arena shape on area traversed. Means sharing any common letters are not significantly different ($P>0.05$).

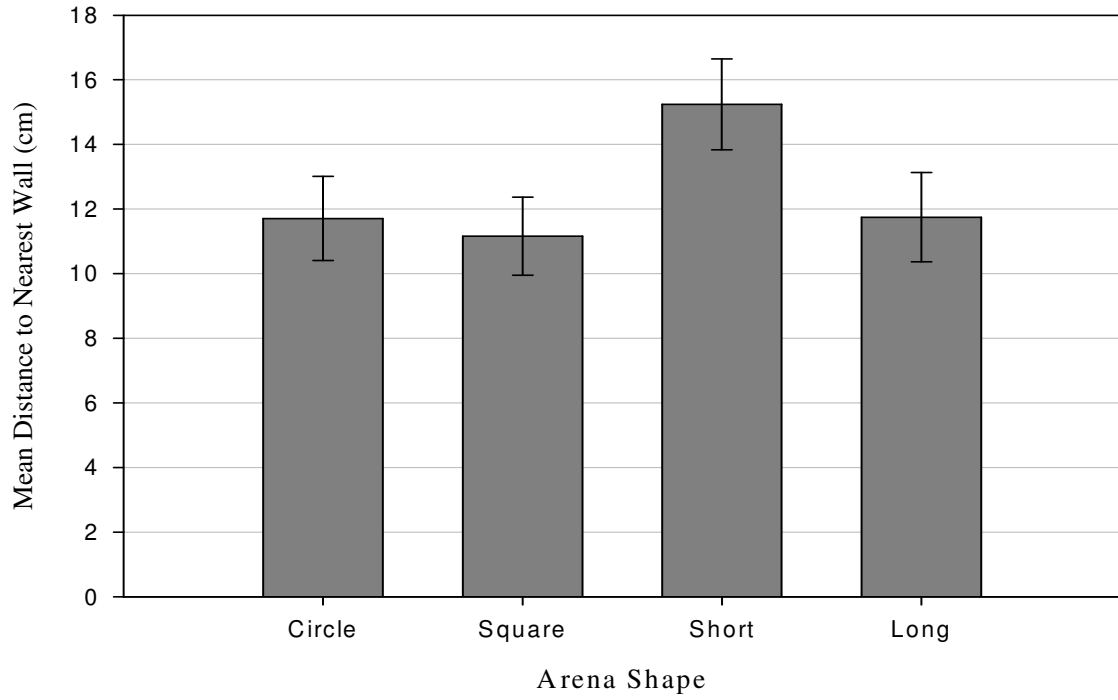


Figure 4-4

Effects of arena shape on mean distance to the nearest wall.

effect of arena shape ($F_{3, 7} = 0.57, P > 0.05$) or experiment day ($F_{11, 6} = 1.78, P > 0.05$) on fecal scores.

4.4 Discussion

Consistent with what was observed in Experiment One, mice moved almost continuously within all treatment arenas. The results from the current experiment demonstrate a positive relation between mean total distance traveled (track length) and arena perimeter length (PL). However, this relation was only apparent in rectangular arenas, where track lengths measured 34m, 38m and 40m in the arenas with progressively longer PL (488, 500, and 566cm, respectively). Mice traveled significantly longer distances in the circular arena, which had the shortest PL (434cm). This inconsistency was likely the result of the lack of corners in the circular arena.

Seeking the safety of corners in order to avoid stressful encounters has been well documented (Wiegand et al., 1994). In a barren enclosure, the range of angles from which an individual animal can be approached is lowest in corners and highest in open areas. This decrease in accessibility has an obvious impact on the relative safety of an animal's location, and will inevitably influence its movement. Open-field mouse movement typically involves repeated excursions, with occasional pauses, between corner spaces (Zadicario et al., 2005). As a result, a reduction in the number of corners per unit area, or the removal of corners altogether, should promote more continuous movement. Alternately, arenas with equal numbers of corners should promote equally-continuous movement. This was observed in the first experiment of

this study in which the effect of floor area on movement and space use was tested and no differences in path counts were found between the four rectangular arenas (Chapter 3), despite differences in total track length. Though this experiment was not designed to provide detailed information about the effect of corner space, the differences in track length and path count between the circular arena and all rectangular arenas support the idea that corner space is important in the subjects' perception of space and is likely to influence movement freedom, especially as defined by Gonyou (1996). As such, this effect will be further evaluated in future experiments.

In this experiment, path counts differed between animals observed in the circular arena and those observed in all other arenas. Immobility in open-field observations is most often noted as indicative of an elevated level of fearfulness or anxiety (Blanchard et al., 2001). Following this, it could be concluded that animals in this experiment exhibited a greater fear response, and are thus experiencing more stress, in rectangular pens. However, in pens that are not geometrically similar, this measure may be more representative of a differential ability to express coping behaviors associated with a fear response than of the fear responses themselves. In fact, the degree of immobility expressed by the subjects is closely linked to the previous discussion concerning the role of corner space in open-field movement patterns. Therefore, it is likely that individuals in enclosures with corners seek that space because it may provide a greater perception of control over the immediate environment and, according to Gonyou's (1996) definition, may provide greater movement freedom.

Pen shape had little effect on path complexity. In fact, dispersion values for this experiment, though direct statistical comparison is not possible, did not differ from dispersion values observed in Experiment One of this study. Given the predictable nature of mouse movement in open-field tests, it seems likely that the measured angular dispersion of a mouse's track within a barren enclosure will vary little despite dramatic differences in spatial parameters.

Net displacement values in this experiment were consistent with what was observed in the previous experiment. As was expected, the net displacement increased as the perimeter length and internal diagonal length (IDL) increased, with the exception of the circular arena. As the mice spent the majority of the time in close proximity to the walls of the arena, the mean net displacement for each treatment approached one half of the mean distance between the center of the arena and the walls ($\pm 10\text{cm}$). Consequently, as the perimeter to area ratio and internal diagonal length increased, the measured net displacement increased (Table 4-2). This is consistent with the previous experiment's results.

The reluctance of mice to enter open areas is well documented (Podhorna and Brown, 2002). This reluctance was evident in the current experiment, despite a reduction in the area to perimeter ratio that was intended to decrease the perceived risk of crossing the unprotected areas of the open-field, and thus provide a perception of increased safety. The primary measure of wall-following behavior in this experiment, mean distance to the nearest wall, should provide some indication of the subjects' willingness to leave the relative safety of the arena perimeter. No significant differences were observed in the mean distance to the nearest wall (MWD), as mice

Table 4-2

Experiment Results. Means sharing any common letters are not significantly different (P>0.05).

Shape	Circle	Square	Short	Long
Floor area (m²)	1.5	1.5	1.5	1.5
Perimeter (cm)	434	488	500	566
D_{max} (cm)	138	173	180	224
Length:Width	na	1:1	3:2	3:1
Mean Distance from Center to Wall (cm)	69	70.1	65.1	66.9
Track length (cm)	4549 ± 142 ^a	3443 ± 134 ^b	3834 ± 142 ^{bc}	3983 ± 134 ^c
Path count	2.06 ± 0.77 ^a	4.65 ± 0.71 ^b	6.18 ± 0.77 ^b	5.83 ± 0.71 ^b
Angular Dispersion	0.906 ± 0.006	0.896 ± 0.005	0.889 ± 0.006	0.891 ± 0.005
Net Displacement (cm)	64.40 ± 2.52 ^b	60.88 ± 2.31 ^b	65.71 ± 2.52 ^b	77.43 ± 2.31 ^a
Area Traversed (%)	7.31 ± 0.78	8.2 ± 0.72	8.14 ± 0.78	8.21 ± 0.72
Mean Distance to Nearest Wall (cm)	11.71 ± 1.3	11.16 ± 1.2	15.24 ± 1.4	11.75 ± 1.4
Points in perimeter (%)	81 ± 4.8	78.6 ± 4.4	75.3 ± 4.8	82.1 ± 4.4
Fecal score	0.72 ± 0.20	0.75 ± 0.18	0.35 ± 0.20	0.52 ± 0.18

maintained an MWD of approximately 11cm for the circular, long (1:3 ratio) and square arenas and 15cm for the short (2:3 ratio) arena. It is interesting to note that the short (2:3 ratio) rectangle was chosen for this experiment because previous work (Perlitz, unpublished) had indicated that, among other quadrilaterals, this ratio maximized 'available space.' In this previous work, available space was defined by the minimum distance from the boundary of a polygon from within. In this work, the researcher suggested that if a bounded space is designed such that individuals within will inevitably be in close proximity to the walls, then that individual has less space than would be available if the average distance from the wall is greater. Though not statistically significant, the differences in observed MWD between the short arena and all other treatments approached significance and provided concrete support for the previous abstract results.

To further quantify wall-following behavior, tracked coordinate points were separated into those falling within two zones, central and peripheral, that were equal in floor area. As expected, based on results from Experiment One of this project, the majority of tracked mouse locations were within the peripheral zone. Though no significant differences were observed in the periphery use measure across the four arena shapes, it is interesting to note that animals in the long arena, which was assumed to provide the lowest average perceived risk in its central area, demonstrated the most disproportionate use of space (82% of movement occurred in the periphery). The simplest explanation for the elevated use of peripheral space in the long arena is that the arena's PL to area ratio is the highest of the four arena shapes, thus increasing the probability of an observation occurring along the perimeter. However, if such is

the case, it should be reflected in the remaining treatments, with a tendency toward elevated peripheral space use as PL to area ratio increased. Such a trend did not occur in the results (Table 4-2). Though the relative similarity in periphery use makes it difficult to ascribe causation for the unexpected results, a mean difference of approximately 4% may warrant further investigation.

No significant differences were observed in the overall use of floor space between the four treatments. The percentage of area traversed differed between the arenas by an average of only 0.5% (Table 4-2), and ranged from 7.3% in the circular arena to 8.2% in the square arena. Taken in combination with the results from Experiment One, the results from this experiment seem to demonstrate that the amount of space a mouse uses in the open-field is more heavily influenced by the size of the field than other spatial parameters. Finally, there were no significant differences in fecal scores among the four treatments. The utility of fecal scores as an indicator of diminished welfare is unclear for this study. However, the scores must be included in the analysis and will be calculated in the final experiment of the project, as further analysis may reveal information that is not apparent in the initial examination of results.

Chapter 5: The Influence of Pen Complexity on Movement and Space Use in Mice (*Mus musculus*) – Path Complexity

Abstract

It is known that most animals in confinement spend the majority of their time in close proximity to the walls of their enclosure. This was observed in the two previous experiments of this project. The primary goal for this experiment was to determine how pen complexity and the location of added complexity, as measured by the addition and selective placement of partitions, affect movement freedom and space use. To determine this effect, four square arenas of equal floor area were designed with varying partition configurations: (A) Ccon, with 2 centrally-placed connected pairs of partitions, (B) Csep, with 4 centrally-placed partitions, (C) Pcon, with 4 equally-sized partitions bisecting and connected to the arena perimeter, and (D) Psep, with 4 equally-sized partitions, two of which were connected to the arena perimeter. Experiment arenas differed in the number of available corners, with 4, 6, 8 and 12 for Ccon, Csep, Pcon and Psep respectively. Among the variables measured in this experiment, only path count (degree of immobility) differed significantly between complexity treatments ($F_{3, 10} = 4.31, P < 0.05$). The remaining variables were largely unaffected by the placement of partitions or the number of available corners. Comparisons to data from previous experiments suggest that the addition of partition may increase in the proportion of total arena space used; however, this cannot be statistically verified. While differences in corner space may have some influence behavioral responses, there is little indication from these results that, in

relatively large spaces, location of additional vertical wall space will influence movement patterns or general use of space.

5.1 Introduction

In two previous experiments it was demonstrated that the length of an enclosure's perimeter significantly influences movement and use of space of mice. This is generally consistent with descriptions of mouse thigmotaxic, or wall-following, behavior (Buhot et al., 2001), in which the majority of movements occur in close proximity to some vertical surface. Movement of mice in any environment, including the experimental open field, involves continuous assessment of risk (Augustsson and Meyerson, 2004). The disparity between the levels of risk associated with the central and peripheral regions within an enclosure is expected to result in differences in space use and general activity. The obvious explanation for this differential risk, and subsequent effect on behavior, is the relative protection provided by the walls of the enclosure. The influence of vertical surfaces on movement patterns has been observed in several species, including cattle (Stricklin et al., 1979), sheep (Sibbald et al., 2000), chickens (Cornetto and Estevez, 2001), rodents (Gray et al., 2000; Jensen et al., 2003) and insects (Jeanson et al., 2003). In many species that exhibit a distinct wall-following behavior, it is clear that the pressures associated with the tendency to stay in close contact with a wall, and, in the case of mice and rats, establish a "home base" from which excursions can be safely made (Wallace et al., 2006), limit the animals' ability to move freely within the space. Cornetto and Estevez (2001) demonstrated that introducing vertical wall space at central locations within a pen resulted in a more uniform use of space by domestic fowl. One could conclude that this increase in distribution was indicative of an increase in overall movement freedom. In the current experiment, vertical surfaces were added to both

the central area and the periphery to determine if, given an expected increase in overall movement per unit increase in perimeter length, differences in the use of central and peripheral space will persist or if the nature or severity of this disparity will change.

The previous experiment in this series, which evaluated the effect of arena shape on movement, provided evidence that corners also significantly influence aspects of animal movement and spacing. Wiegand et al. (1994) demonstrated that the number of corners in an environment clearly influences behavior, such that the addition of corners to a circular pen resulted in the extinction of some of the behavioral differences observed between square and circular pens. However, by artificially adding corners to a circular pen the length of vertical wall space was also increased. In the following experiment, I examined the effects of adding corners to a bounded space when the total length of accessible vertical wall space was held constant.

The overall objective of this experiment was to further explore the extent to which the spatial parameters most commonly used to define an enclosed environment's level of complexity effect an animal's movement and space use. The information to be obtained from this research has potential in the evaluation and improvement of housing systems for agricultural animals; indeed, for all animals kept in confinement. The hypothesis for this experiment was that the complexity of the enclosed environment, including the number of corners and location of additional vertical wall space, would affect movement and space use of mice. It was predicted that, as was observed in other species, placement of vertical panels would influence

spatial distribution and the number of available corners would affect movement, resulting specifically in a reduction in total movement as the number of corners increases.

5.2 Animals, Methods and Materials

5.2.1 Experimental animals

In this experiment, conducted in the University of Maryland, College Park Animal Research Wing, I observed the movement and space use of forty 24-28 week old pair-housed female Swiss Webster (CFW) mice, obtained from a commercial animal model supplier (Charles Rivers Laboratories, Inc.). All mice were housed in 20.3 x 48.3 x 26.7 cm cages. Each pair of mice was assigned a cage number (1 through 25) and then the cage numbers were randomly ordered using a random number generator that excluded duplicate integers (Haahr, 2002). From the generated cage order, two observation groups of 10 pairs of animals were created (AM and PM). Experimental trials, involving 3 minutes of continuous observation, were completed between the hours of 0900 and 1100 for animals in the AM group (n=20) and between the hours of 1300 and 1500 for animals in the PM (n=20). The order of observation within each cage was determined prior to the start of the experiment, and every cage was observed once daily for 12 consecutive days.

5.2.2 Open field arena and observation procedure.

The walls of the arena were constructed using 30.5cm wide composite hardboard sheets. The arena floor surface was constructed from a 240 x 240cm

composite hardboard panel. All surfaces were painted with a dark gray, low odor, no-VOC semi-gloss latex paint, which was chosen to maximize contrast for the video-tracking of the small animal models. The starting point for this experiment was a 28cm (diameter) cylindrical opaque plastic container attached to a pulley system and placed in the center of the field. In addition, non-reflective white paper was hung to a height of approximately 2.1m in the area surrounding the arena. These steps were taken to ensure that all mice were released from the same location, the researcher was not visible immediately prior to, or during any portion of, the recorded observations, and the space was devoid of spatial cues that could influence movement or space use.

In this final experiment the movement and space use of mice were observed in a 122 x 122cm arena. Opaque hardboard panels were placed vertically within the interior of the arena to create four treatment configurations (Figure 5-1). These configurations differed in the number of corners and the location of vertical panels, but the combined length of the added panels was equal across the four treatments. In two of the treatments, panels were added in central locations and in the two other arenas panels were added near the periphery. For the two centrally-complex arenas, four panels (2 x 18cm and 2 x 28cm) were either completely separate (Csep), and held in place by transparent plexiglass pieces that attached the upper corners of the panels, or connected at the edges in pairs to create 2 L-shaped structures (Ccon). For the peripherally-complex arenas, four panels of equal size (23 cm) bisected the four arena walls. In one treatment, all panels abutted the wall surface (Pcon) and in final treatment (Psep), two panels abutted the outer walls while the remaining two panels were placed 5cm from the wall surface. A transparent strip of plexiglass bridged the

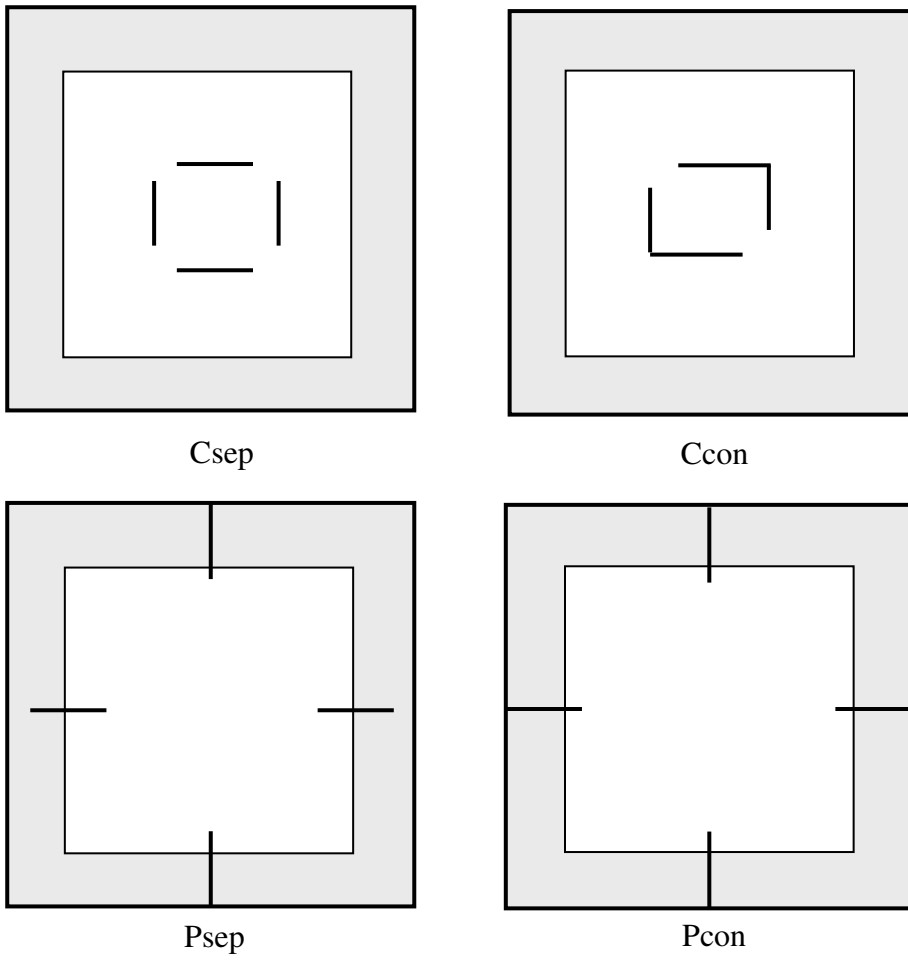


Figure 5-1

Treatment arena configurations. Shaded area represents peripheral zone.

gap between the wall and the panels to keep them upright and stable. The Csep, Ccon, Psep and Pcon configurations had 4, 6, 8 and 12 corners, respectively.

All observations were made in a dimly lit room (approximately 8-10 lux) to optimize mouse activity (Bronikowski et al., 2001) and minimize shadows. The experimental design resembled a Latin Square, in which schedule of presentation for these pen designs was adjusted to control for possible confounding effects, including time and order effects (Table 5-1), and treatments were randomly assigned to the schedule of presentation prior to the start of the experiment. Two sessions of observations, approximately 4 hours apart (from the start of observations), were completed per day of test. Each mouse was individually observed during one of the two daily sessions for a total of 40 mice per day, and every mouse encountered the four experimental pen designs three times. The observations were made in 6 four-treatment blocks, each of which required two days to complete.

For a given observation period, two mice (pair-housed) were transported to the test room, individually observed, and then returned to their home cage. The order of observation within a cage was determined prior to the start of the experiment and that order was maintained for the extent of that experiment.

5.2.3 Data collection and measurement variables

Total track analysis

For each experiment trial, an individual mouse was placed under the cylindrical cover in the center of the arena. Each trial, which was 3 minutes in length,

Table 5-1

Experiment Schedule. Letters denote the different pen treatments.

SESSION	DAY OF TEST											
	1	2	3	4	5	6	7	8	9	10	11	12
AM	A	C	B	D	C	A	D	B	C	D	B	A
PM	B	D	C	A	B	D	C	A	A	B	D	C

began when the mouse was released into the field. After each trial the mouse was caught and returned to its cage and the surface of the arena was cleaned with an acetic acid/water solution to remove odor cues, as suggested by McGivern et al. (1997). Each observation was recorded using a Panasonic closed-circuit color video camera, mounted approximately 2.5m above the arena floor surface. Trials were captured in an analog format, digitized into an uncompressed Audio-Video Interlaced (AVI) file and later processed using Viewer II tracking software (v. 2.0.0.31 by BIOBSERVE, Fort Lee, New Jersey) to acquire x and y coordinates (See Appendix for a complete description of the tracking process). From these coordinates, several measures of space use and spacing behavior were summarized for each subject. The dependent variables summarized for the total track were as follows: Track length (total distance traveled), net displacement, mean distance to the nearest wall, percentage of total points that occurred in the periphery (percent in periphery), fecal score, floor area use (area traversed), number of independent coordinates, angular dispersion (tortuosity or movement complexity), path count and the number of paths per traveled distance (paths per distance).

To measure the total distance an animal traveled, I calculated the sum of the Euclidean distances between each successive coordinate point. Net displacement was measured as the Euclidean distance between the first and last tracked locations. Two measures were used to describe the tendency of animals to spend a disproportionate amount of time at peripheral locations within an enclosed space. First, I calculated each individual's mean distance from the nearest vertical surface (MWD). To calculate this value, I measured the straight-line distance to the nearest vertical

surface, including partitions, at each coordinate point within a track. I then determined the mean of these distances for each observation. To calculate the second estimate of wall-following behavior, percent in periphery, I used the captured coordinate points to partition each experimental arena into two zones (central and peripheral) that were equal in area, and then determined the proportion of tracked locations in the two zones (only peripheral proportions are reported).

Angular dispersion, or tortuosity, has been used to describe the path complexity of a range of objects (Hart et al., 1999; Seker et al., 2005; Estevez and Christman, 2006). Assuming the restrictions on animal movement (physical obstacles or perceived threats) result in aberrations from a straight-line path of travel, the angular dispersion may provide useful information regarding the restrictions imposed by a particular environment. In this experiment, angular dispersion is calculated by measuring the average angular departure of a tracked location from the animal's previous location. This departure is used to calculate the concentration of tracked points using $r_k = \sqrt{\bar{x}_k^2 + \bar{y}_k^2}$, where r is the correlation of angular departures across k total points. Angular dispersion is represented as $1-r$ and ranges from 0 to 1, such that a path in which all angles of movement are perfectly correlated (straight line) maintains a dispersion value of 0 (no tortuosity) and a track in which departures from the previous vector occur at random maintain a value that approaches 1 (high tortuosity) (for a full description see Estevez and Christman, 2006).

To calculate relative floor area use (area traversed) by an individual, the total number of unique coordinate point locations in each individual's movement track was expressed as a proportion of the total number of coordinate points in the arena. The

value was obtained using the equation $\sum_{i=1}^N x_i / N$, where N represents the total number of possible locations for individual i and x represents a single independent observed coordinate point. The resolution for tracked coordinates was 1cm, such that the total number of coordinate points in an arena was equal to the area of that arena.

Immobility, measured in this study as a path count, was quantified by parsing an individual's complete track of movement into distinct paths. A path ended and a new path began when an animal's location remained unchanged for 2 seconds of immobility (based on observations made during a preliminary experiment). To determine the effect that bouts of immobility had on the total distance an individual traveled, path count was also calculated in relation to the total track length (paths per distance). Immobility, total distance traveled, perimeter adhesion and fecal scores have all been used to describe relative states of fearfulness or anxiety in mice (Augustsson and Meyerson, 2004).

Statistical analysis

For each dependent variable measured, the mean was taken across all tested individuals within a single session. Session means were analyzed using a repeated measures mixed model procedure for analysis of variance with treatment and day of experiment (1 through 12) modeled as the fixed effects and session (AM and PM) modeled as a random effect (SAS, v. 9.1). Compound symmetry and autoregressive covariance structures were examined for each model. The structure that fit best, as determined by AICC goodness of fit statistic, was chosen for each model. Assumptions of normality and homogeneity of residual variances for each parameter

were examined. Track length data failed to meet the assumptions for normality and was modeled with a Poisson distribution using generalized linear mixed models to satisfy those assumptions.

5.3 **Results**

The results for measured variables and the spatial parameters of all treatment arenas are listed in Table 5-2.

5.3.1 *Movement*

The total distance traveled (Figure 5-2) did not differ significantly between the tested arenas ($F_{3, 5} = 0.77, P > 0.05$) or across experiment days ($F_{11,5} = 0.82, P > 0.05$). The angular dispersion, or path tortuosity, was not significantly influenced by arena shape ($F_{3, 6} = 1.64, P > 0.05$); however, dispersion did change significantly across experiment days ($F_{11, 4} = 7.78, P < 0.05$). The net displacement (Figure 5-3) did not differ significantly across arena shapes ($F_{3, 10} = 1.12, P > 0.05$) or across experiment days ($F_{11, 10} = 1.04, P > 0.05$).

The number of distinct paths within a total track (Figure 5-4) differed significantly between the experimental arenas ($F_{3, 10} = 4.31, P < 0.05$), though no differences were observed across experiment days ($F_{11, 10} = 1.63, P > 0.05$).

5.3.2 *Behavior and space use*

The mean distance to the nearest wall (MWD) (Figure 5-5) did not differ significantly between arena treatments ($F_{3, 4} = 1.03, P > 0.05$) or across days of the experiment ($F_{11, 1} = 0.31, P > 0.05$). In all treatments, mice spent more time in the

Table 5-2

Experiment Results. Means sharing any common letters are not significantly different (P>0.05).

Treatment	Csep	Ccon	Psep	Pcon
Floor area (m²)	1.5	1.5	1.5	1.5
Outer perimeter length (cm)	488	488	488	488
Total wall length (cm)	672	672	672	672
Wall length in periphery (cm)	488	488	600	640
D_{max} (cm)	173	173	173	173
Corners	4	6	8	12
Track Length (cm)	3970 ± 163	4033 ± 164	3875 ± 173	3756 ± 165
Path Count	1.41 ± 1.29 ^{ac}	4.61 ± 1.29 ^{ab}	5.72 ± 1.23 ^b	0.54 ± 1.27 ^c
Angular Dispersion	0.932 ± 0.006	0.917 ± 0.005	0.931 ± 0.006	0.935 ± 0.006
Net Displacement (cm)	61.06 ± 2.71	58.87 ± 2.69	54.17 ± 2.61	56.73 ± 2.71
Area Traversed (%)	11.57 ± 0.86	11.22 ± 0.82	10.67 ± 0.82	12.50 ± 0.81
Mean Distance to Nearest Vertical Surface (cm)	6.57 ± 0.68	8.90 ± 1.21	7.80 ± 0.66	8.41 ± 0.74
Mean Distance to Nearest Outer Wall (cm)	13.35 ± 0.92	13.24 ± 0.89	12.56 ± 0.87	13.70 ± 0.88
Points in periphery (%)	76.0 ± 3.3	73.7 ± 3.3	75.5 ± 3.2	72.3 ± 3.3
Fecal	0.55 ± 0.17	0.51 ± 0.17	0.34 ± 0.15	0.41 ± 0.16

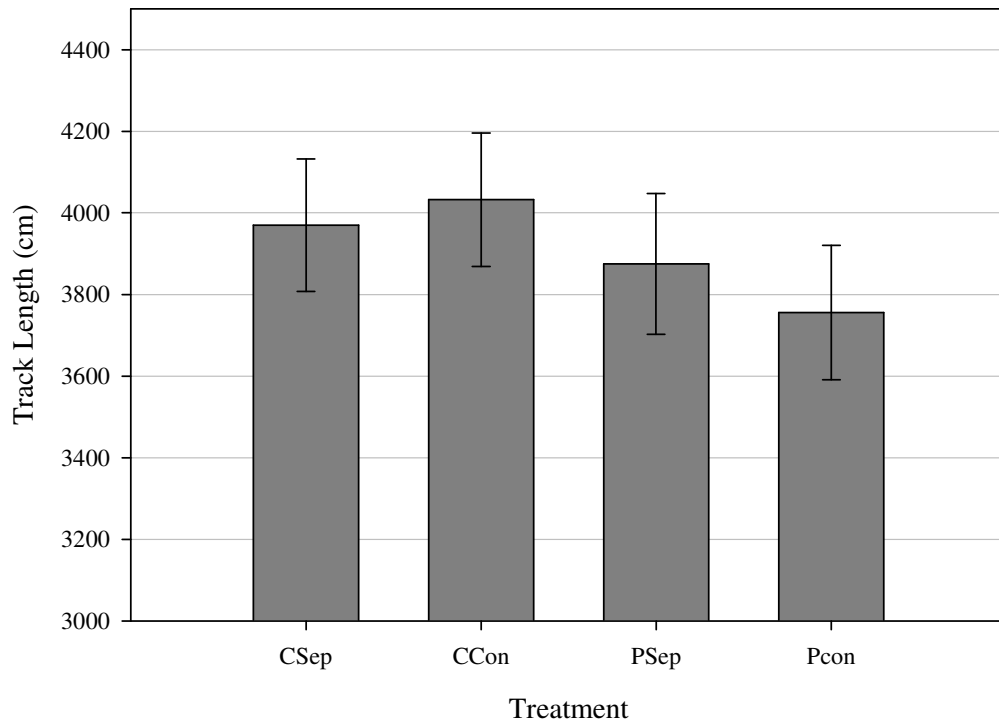


Figure 5-2

Track length (LSM ± SEM) across the four arena configurations.

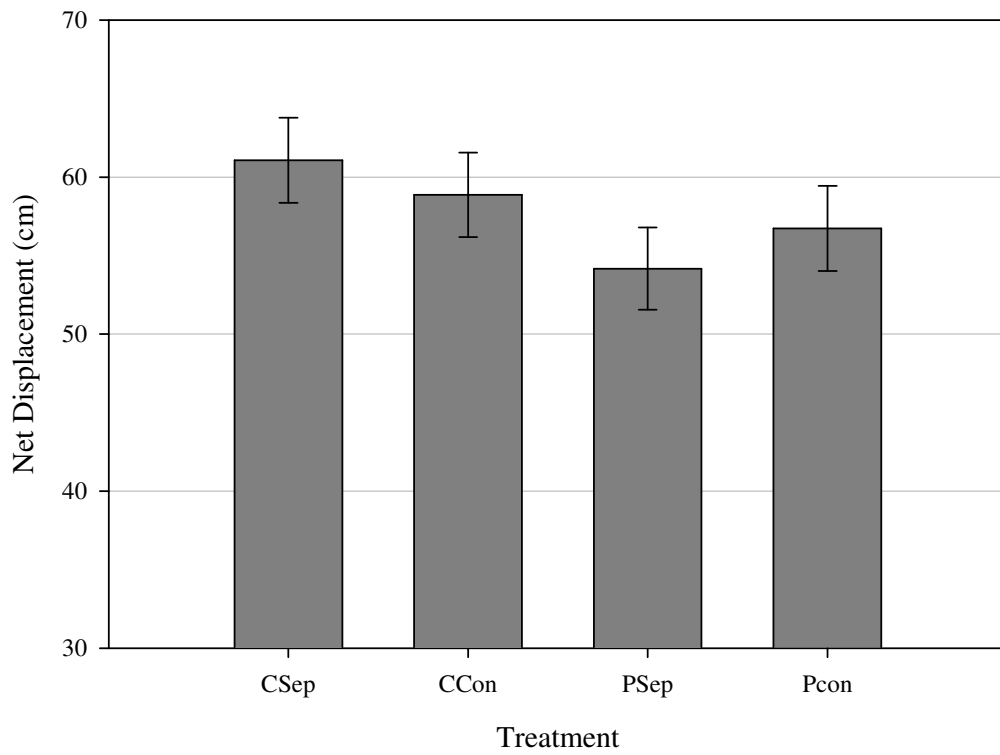


Figure 5-3

Net displacement (LSM ± SEM) across the four arena configurations.

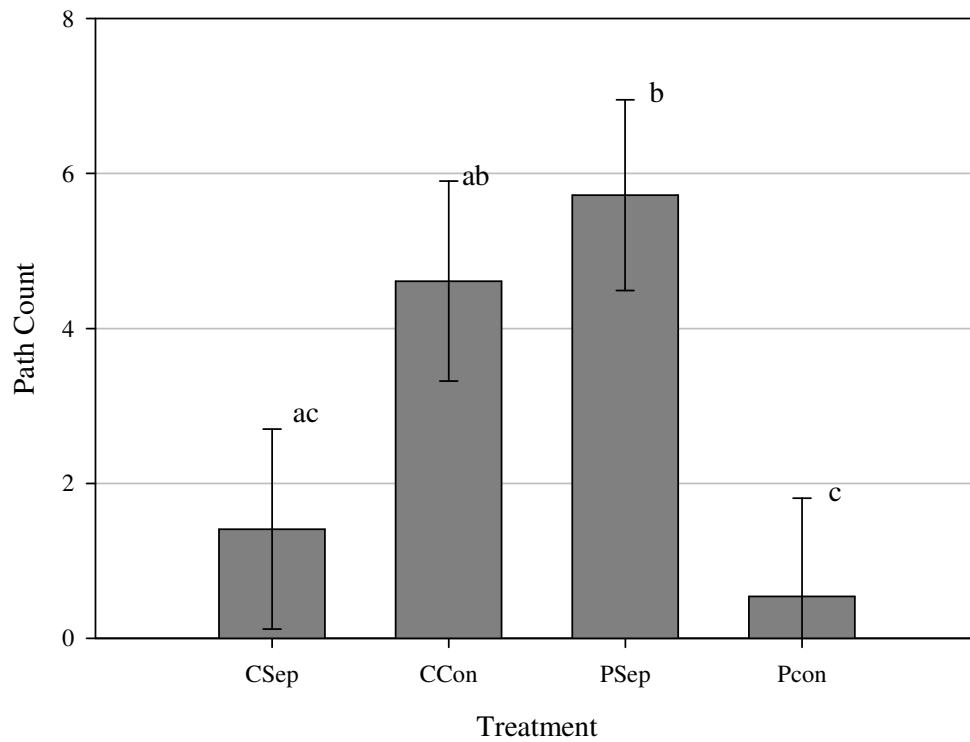


Figure 5-4

Path count (LSM ± SEM), used to indicate relative immobility, across the four arena configurations. Means sharing any common letters are not significantly different ($P > 0.05$).

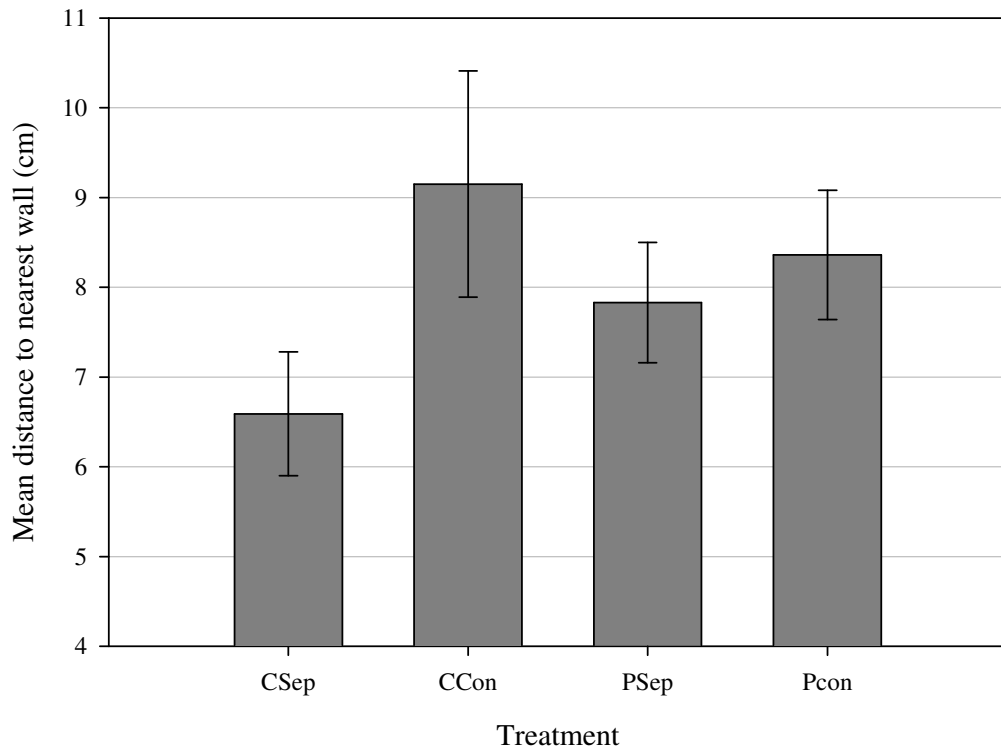


Figure 5-5

Mean distance to the nearest wall (LSM \pm SEM) across the four arena configurations.

peripheral zone than in the central zone (75, 74, 73 and 71% for Csep, Ccon, Psep and Pcon arenas respectively). This tendency did not differ significantly between arenas ($F_{3, 10} = 0.25, P > 0.05$) or across experiment days ($F_{11, 10} = 1.37, P > 0.05$). The proportion of the area traversed did not differ significantly between floor areas ($F_{3, 10} = 0.93, P > 0.05$) or across experiment days ($F_{11, 9} = 1.49, P > 0.05$). There was no significant effect of vertical panel location ($F_{3, 8} = 0.58, P > 0.05$) or experiment day ($F_{11, 8} = 7.63, P > 0.05$) on fecal scores.

5.4 Discussion

This experiment was the third in a series of experiments designed to determine the specific influence that certain spatial parameters have on movement and space use of mice. The objective of the study discussed herein was to determine how environmental complexity affects movement and space use when floor area and arena shape are held constant. Results from previous experiments, in which I determined the effects of floor area and arena shape, provided some evidence of differential spatial patterns relative to availability of corner space. In this experiment, vertical panels were used to alter the number of corners, provide additional vertical wall space vary the general location of additional wall space. It should be noted that comparisons between the results of the current experiment and those of experiments One and Two of the project cannot be made in terms of statistical significance, but may provide useful information regarding application of collected information and development of future studies. Several differences were found both within experiment treatments and

relative to previous findings; however, few of the differences observed showed any statistical significance.

Despite the introduction of vertical panels in the central area of the arena, no difference were observed between the four treatments in either the overall percentage of space used (11.3% and 11.6% for the central and peripheral treatments respectively) or in the proportional use of peripheral space (89.6% and 87.7% for the centrally complex and peripherally complex treatments, respectively). These results are not consistent with the previous demonstration with chickens (Cornetto and Estevez, 2001) that providing vertical surfaces positively influences dispersion in an enclosed area; however, the results are consistent with results from the previous experiments in this project. It seems clear that the stronger wall following tendencies of mice suppress aberrations in movement along the perimeter once an individual animal comes in contact with the outer vertical surface. This bias in directional movement after initial wall contact has also been observed in insects (Jeanson et al., 2003) However, this does not explain why, in treatments that contain central vertical panels, mice made the initial trek to the outer wall early in the session. As suggested by Chapman and Webb (2006), it is possible that subjects in open field tests may travel along the periphery not only because of the decreased risk of predation, but also because of the increased likelihood of discovering a means of escape. Thus, even in treatments where the release location for all trials was surrounded by central vertical panels, mice traveled from the central region to the outer wall. The centrally-paneled treatments were designed to encourage central space use by providing a 'safe zone' approximately the same area as the animals' permanent housing, but this design

did little to alter the typical movement patterns of the mice. It is predicted that any release location other than the center of the arena would result in even greater movement bias.

As further indication of a general hesitance to enter central areas of the arena, areas assumed to be relatively less safe; mice in all treatments maintained a short mean minimum distance to the nearest wall. This distance did not differ significantly between the four treatments, and the range of mean MWDs was approximately only 3cm. The increased environmental complexity of arenas used in Experiment Three, makes even superficial comparisons to previous experiments difficult. Therefore, the mean minimum distance to the nearest outermost wall was also calculated. While wall distance relative to all vertical surfaces was lower in the third experiment than what was observed in the previous two, distances to the outer walls were consistent with previous results and remained non-significant ($F_{3,9} = 0.32, P > 0.05$).

The mean number of stops mice made in a single track was significantly influenced by panel placement (Figure 5-4), though the general pattern of the differences is somewhat difficult to interpret. Mice demonstrated the fewest number of stops per track (0.54) in the Pcon treatment, which had the greatest number of corners (12). At first glance, this result is somewhat counterintuitive, as one would expect corners to provide areas of greater safety and thus sites for frequent stops. However, previous authors have suggested that mice in the open field demonstrate more frequent stops in movement when in a fearful or anxious state (Bronikowski et al., 2001). Results from this experiment demonstrate that, by providing the greatest length of continuous wall space, the Pcon treatment positively influenced movement.

As the wall-following tendencies are such an important component in the mouse's behavioral repertoire, it could be argued that this treatment may provide the most spatial freedom. This negative relation between the number of corners and the number of stops was not consistent across all treatments. In fact, there appears to be a clear positive relation in treatments with breaks in the added vertical space (Csep, Ccon and Psep treatments). These differences persisted when immobility was expressed per unit distance traveled, as expected, given no observed significant differences in total movement between the treatments. Interestingly, the Pcon treatment had the shortest mean distance traveled (37.56m), despite also having the fewest number of stops. This discrepancy could result from generally lower travel velocities that could, in turn, be indicative of lower relative stress, fear or anxiety.

The placement of vertical panels had relatively little impact on angular dispersion within this experiment. This general lack of effect was consistent with previous experiments, where path complexity was neither affected by floor area nor length-to-width ratio. However, average dispersion values for all treatments in this experiment were generally higher than those observed in previous experiments (Chapters 3 and 4). Though direct statistical comparisons cannot be made between experiments, the mean dispersion value across all treatments in this experiment (approximately 0.938 ± 0.032) is lower than that observed in experiments testing effects of floor area (approximately 0.894 ± 0.041) and arena shape (approximately 0.898 ± 0.038). This difference is somewhat expected, given that an increase in environmental complexity should result in a general increase in path complexity (dispersion).

Fecal scores in Experiment Three were generally lower than those observed in previous locations. This value is typically used to describe the level of stress or anxiety experienced by the subjects, especially within unfamiliar settings. Therefore, it may be that the lower scores are more representative of general decrease in novelty, since the same subjects were observed in all 3 experiments.

Chapter 6: General Discussion

The objectives of this project were to gather specific information regarding the effects of spatial parameters on movement and space use, and to apply this information to the development of a standardized methodology for evaluating the handling and housing of confined animals. While the results from this work have not provided an answer to the occasional inadequacies of current guidelines for animal care, information was gathered that will be useful in developing tests for determining what is truly best for the animals. The goal was to approach the problem from the ground level, in terms of both the experiment subjects (observation of individuals vs. the group) and the relative simplicity of the measures. *Entia non sunt multiplicanda praeter necessitatem*. "Entities should not be multiplied unnecessarily" (Thorburn, 1918; Marcora, 2007).

In discussing the results of this project, it should be noted that direct comparisons between responses across the three separate experiments are not statistically sound. However, given that the same subjects were used for all three experiments, and that there were very few procedural differences between the experiments, it may be possible to make some general statements about the effects of pen parameters. Comparison between Experiment 1 and the remaining two experiments is also hindered by the use of 1.5m² arenas in Experiments Two and Three, a floor area that was not used in the first experiment. Following a trial experiment, the decision was made to use an arena size for the second and third experiments that bridges the gap between previously observed sizes. The hope was that it would slightly increase the resolution of the information gathered. However,

this change in floor area prevents easy comparison to observations in Experiment One. Observations in which only floor area is altered are logically the best choice as baselines for comparisons when other parameters are later altered. As such, maintaining some consistent size across all experiments would have added even more utility to the information gathered in Experiment One.

It appears that floor area is in fact of some importance, but it is also clear that it is not the only spatial parameter that influences the movement and behavior of animals. In fact, based on results from this project, it is quite possible that floor area has less impact than the amount of available perimeter space, the maximum separation distance and the number of corners within a bounded space.

Results from experiment one, in which the effects of spatial parameters on movement and space use were examined, provided evidence that floor area may in fact have an impact on behavior and well-being. In this experiment mice were observed in arenas that differed in floor area. This comparison was important, as the majority of recommendations for the housing of animals rely on expectations and assumptions about floor area as the prominent spatial characteristic determining an animal's freedom of movement. Definition of this movement freedom most often allude to some overall ability to move and turn freely within the space. As such, the simplest example for expression of this freedom is an individual's ability to travel within a space without its path of movement being interrupted or blocked by a physical obstacle. In typical production and research settings, the boundaries of an enclosure and the presence of other individuals are most often credited with impeding movement or limiting an animal's 'freedom' within a space.

Track length and angular dispersion were the two measures in this project that were chosen to represent the individual's ability to disperse freely and thus their movement freedom. This decision was based largely on the premise that movement freedom is defined, in the classic sense, as a freedom from hindrance (physical or mental) in their movement and even the degree to which an object or entity can move in a straight-line path before it is forced to alter that path. This definition may lead one to assume that the easiest way to increase spatial freedom or 'available' space is to increase floor area. The original thought regarding track length was that movement freedom would be reflected in the amount of total movement during the time allowed, since this should help demonstrate the level of path obstruction. A longer track would therefore indicate greater freedom to move in the allowed 3-minute observation period. This assumption also allowed for the testing of the notion that, if floor area is equivalent to space and spatial freedom, that a doubling of that space should result in a similar increase in total movement.

The results from Experiment One supported the idea that floor area does in fact lend significantly to the amount of available space, as measured by the ambulation of observed individuals. Track length increased significantly as floor area was increased. However, in plots of the data that initially appeared to demonstrate some linear relation between floor area and track length, the track length began to increase with floor area at a decreasing rate, to the point of inferring an eventual asymptote. Upon further analysis, it was determined that, instead of doubling as a result of a two-fold increase in floor area, the increase in track length was closer to a factor of $\sqrt{2}$. Interestingly, the value represents the increase in both perimeter length

and internal diagonal as the area of a square is doubled. This supported the hypothesis that that floor area is not the only parameter that influences movement in a bounded space. Experiments Two and Three provided further support by demonstrating that constant floor area with various perimeter lengths results in significantly different track lengths (Experiment Two) and that constant floor area with constant outer perimeter length results in no significant differences in track length (Experiment Three) (Figure 6-1).

The results for net displacement, mean distance to the nearest wall and percent in periphery across the three experiments demonstrate the importance of the perimeter length in the subjects' movement patterns and possibly their 'comfort' within a space. Prior to the start of the experiment, the relative value of the net displacement measure in providing information about movement and behavior was somewhat underestimated; however, this parameter may have provided the clearest example of how the structure of the environment can influence behavior. This variable did not differ significantly when the length of the outermost vertical surface was held constant, but increased predictably as perimeter length increased. In fact, in both Experiment One and Experiment Two, the rate of increase in mean net displacement almost mirrored the increase in perimeter length between treatments.

The explanation for these results is quite simple. As mentioned above, the typical movement pattern of mice in confinement involves rather intense wall-following behavior, which may both decrease their vulnerability to predation or conspecific aggression and increase the likelihood of finding an escape route (in an environment that is not completely enclosed). This adherence to the arena perimeter

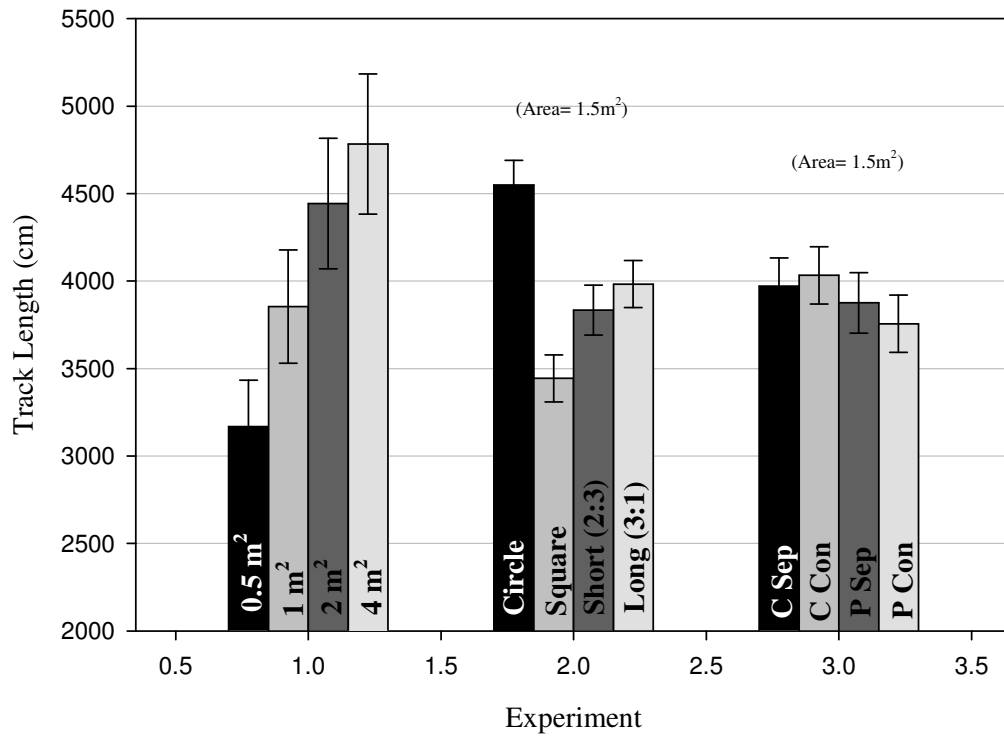


Figure 6-1

Track length (LSM ± SEM) across all experiment treatments.

was observed rather consistently throughout the three experiments and increased the probability that the observed net displacement values would be similar to the average distance from the center of the arena, where the subjects were released into the arena, to the walls of the arena. In this, it is apparent that net displacement is influenced most by that perimeter length or the area to perimeter ratio. If net displacement can be used as an indicator of an animal's ability to disperse from its original location (Jeanson et al., 2003), then dispersal is seemingly optimized by maximizing the perimeter length to floor area ratio.

Mean distance to the nearest wall was the primary means of quantifying the subjects' wall-following tendencies. By measuring an individual's average distance from a structure that provides protection, and the individual's willingness to enter areas where no such protection exists, this variable was intended to help describe the individual's subjective movement freedom. Mean distance to the nearest wall was not influenced by the size, shape, or structural complexity of the test arenas (Figure 6-2). This suggests that the perceived risk of predation that drives mice to maintain contact with the wall persists, regardless of changes to the enclosure. Responses from Experiment One showed that this distance is not influenced greatly by the amount of absolute (vs. perceived) space available to the individual. Differences between responses across the four arena sizes, though not statistically significant, seemed to initially provide some support for the importance of floor area (or perimeter length to area ratio) in the subjects' use of space. However, closer examination revealed some very interesting information. When MWD was expressed relative to the size of the arena, it was discovered that, while absolute distance from the wall differed, distances

as a percentage of the distance from the bisecting plane of the arena to the outer wall were almost equivalent (23-26%).

In Experiment Two of this study, arena designs were chosen that were deemed most likely to influence use of space. Specifically, the perimeter-length to area ratio was increased in hopes that the decrease in distance between the walls would encourage more trips into the central space and thus increase the mean distance to the nearest wall. This was based on the premise that the primary reason for wall-following behavior was the perceived risk associated with traveling into open, unprotected areas. This premise was supported by the findings of Perlitz (unpublished) that space, as measured by the minimum distance from the boundary of a polygon, was maximized in a polygon with the length-to-width ratio of 2 to 3. While no main-effect significant differences in MWD were observed between arenas that differed in shape, the mean response within the short (2:3) arena treatment, as evident in Figure 6-2, was slightly higher (15.24cm) than the mean responses in all other treatments (~11cm). This finding was quite exciting, as it provides concrete evidence for a phenomenon that was previously observed in abstract mathematical calculations. This, together with the results from Experiment One above, also illustrates that behavioral responses and state of well-being are extremely dependent upon the individual's environment.

Results from Experiment Three were similar to those observed in other experiments. It was anticipated that treatments in this experiment would continue to promote wall-following behavior. Treatments were chosen in Experiment Three that would promote the use of central space. Mean distance to the nearest vertical surface,

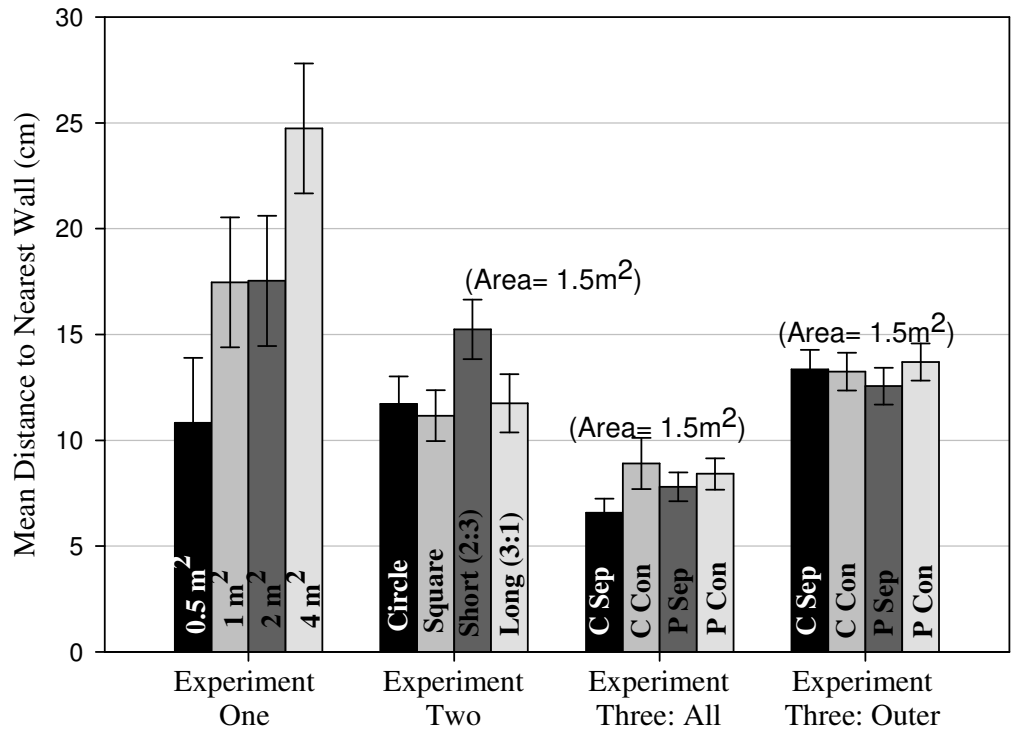


Figure 6-2

Mean distance to nearest wall (LSM ± SEM) across all experiment treatments.

which includes the added partitions, was slightly higher than was observed in other experiments. However, mean distance to the outermost walls, which more closely resembled the calculation in the two previous experiments, was more similar to the previous results. It is clear that the addition of partitions adjacent to, and abutting, the perimeter of a bounded space can be used as a means of encouraging more extensive use of that space, even if that use will continue to be along some vertical surface.

As a result of the strong wall-following tendencies, the majority of mouse movements occurred within the peripheral zone. This was the case for all three experiments (Figure 6-3). It was initially expected that certain arena designs would result in greater use of the central areas of the arena; however, this was not the case. There appeared to be no significant statistical difference in zone usage within experiments and only slight differences across experiments. The mean percentage of points in the periphery across all treatments in Experiment Three (73.2%) was marginally lower than the mean value across all treatments of Experiment Two (79.3). It is possible that this slight reduction was caused by the increase in the complexity of the central space. If such is the case, then these results are consistent with the findings of Cornetto and Estevez (2001) that increasing environmental complexity will result in more uniform usage of a space.

In none of the three separate experiments was angular dispersion significantly influenced by the treatments (Figure 6-4). These results were initially somewhat disappointing, especially for the second and third experiments in the series. Treatments in the latter experiments were designed, in part, to focus on the parameter combinations most likely to influence movement freedom. The usefulness on the

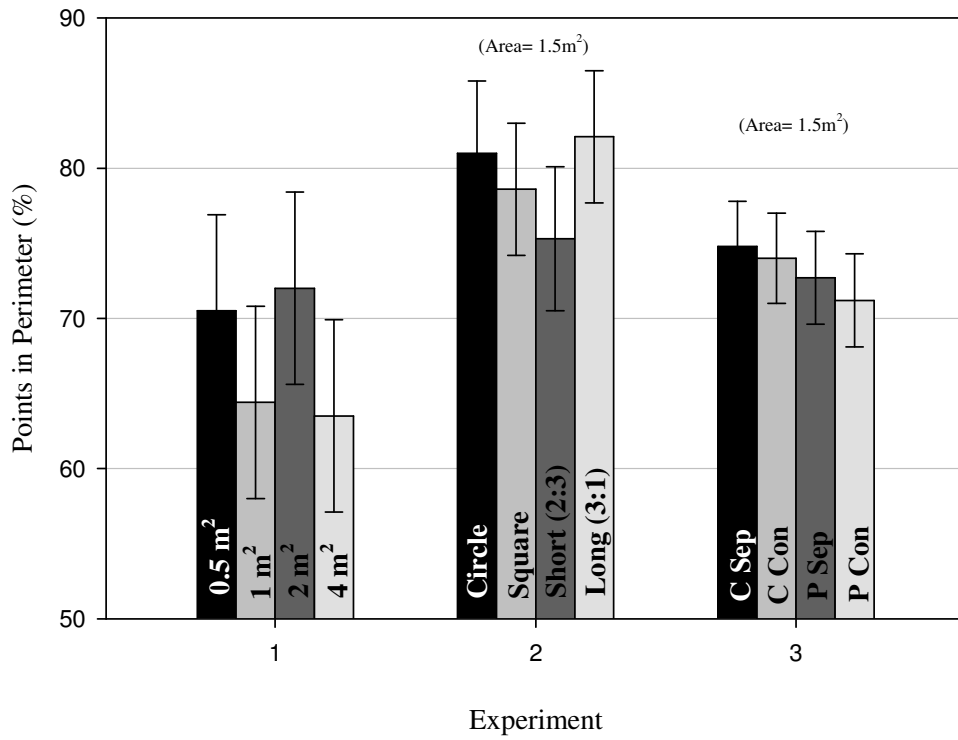


Figure 6-3

Points in periphery (LSM ± SEM) across all experiment treatments.

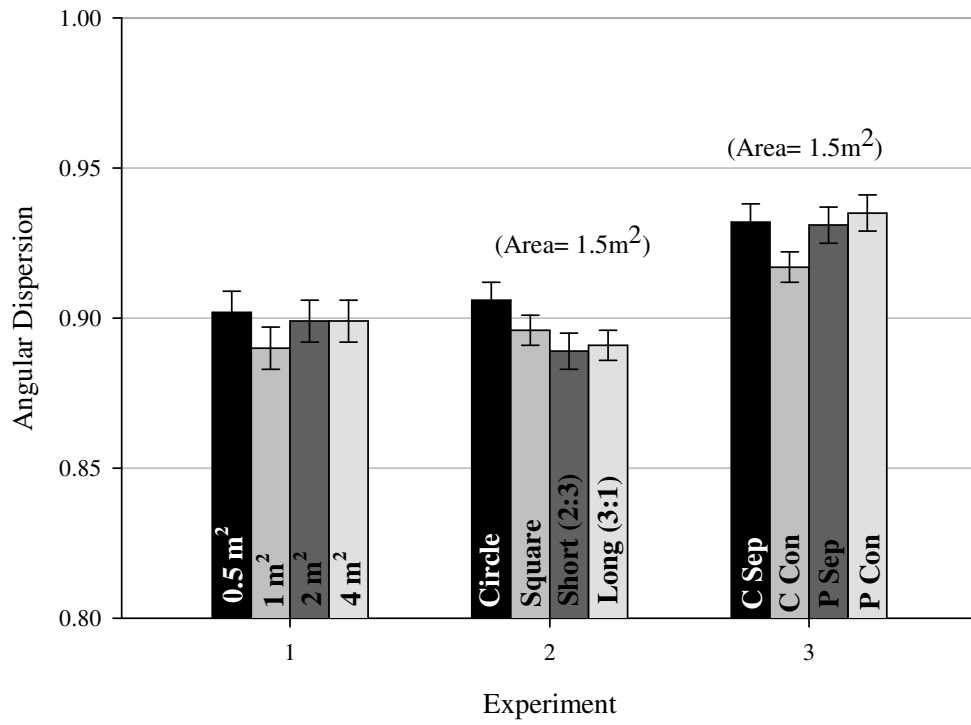


Figure 6-4

Angular dispersion (LSM ± SEM) across all experiment treatments.

dispersion responses alone is limited by what seem to be extremely persistent and predictable patterns of movement in mice. However, this limitation provides support for the inclusion of contextual information in the development on tools for evaluating animal housing, especially with regard to the definition of movement freedom. It is clear that the previously stated definition of movement freedom as the relative freedom from path hindrance (i.e. ability to travel in a straight line) may only be applicable to a completely barren environment. The introduction of environmental complexities, which are likely to enhance the individual's welfare state, will inevitably influence an individuals overall use of a space and its pattern of movement during that time of use. This is evident in the slightly higher dispersion values observed in Experiment Three. While statistical comparison across the three experiments is not impossible, it appears that the introduction of added floor space may result in a more complex path. One could argue that, by encouraging more frequent changes in direction, the vertical partitions positively influenced space use, as measured by the total area traversed.

For the path-count variable, which provided information about the number of stops the subjects made during observations, there were significant within-experiment differences and apparent differences across the three experiments (Figure 6-5). As mentioned above, immobility is thought to be positively correlated with the level of fear or anxiety experienced by individuals in an open field. The primary explanation seems to be that the pause in movement is an example of some type of displacement behavior, during which the individuals' motivations are in conflict (e.g. the need to explore versus fear of the environment). This conflict is often manifested as grooming

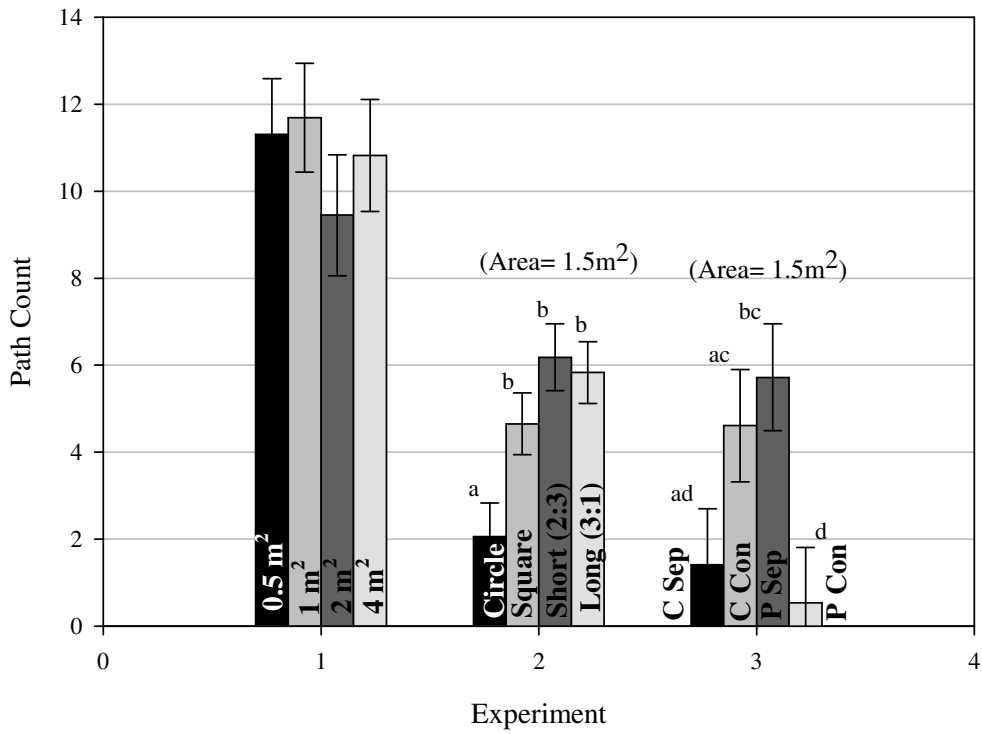


Figure 6-5

Path count (LSM ± SEM) across all experiment treatments. Means sharing any common letters are not significantly different ($P > 0.05$).

or scratching bouts that require a pause in movement. The average path count was highest in Experiment One of this project. It was in this experiment that one would expect the stress or anxiety associated with a novel environment to be highest. Tracks in Experiment Two consisted of much fewer paths. In addition, observations in the circular arena tended to consist of significantly fewer paths than observations within arenas that had corners.

It is probable that this difference is less a result of differences in stress and more a product of the means for coping with that stress. By providing an area with relatively more protection from perceived threats, arenas with corners may have encouraged more breaks in exploration or general locomotion.

It is somewhat difficult to interpret the path-count results from Experiment Three. Similar to what was observed in Experiment Two, it appears that the treatment responses differed based on the type of movement patterns they encouraged. The Csep and Pcon treatments seemed to encourage more continuous movement than the other two treatments. A possible reason for the lower path count in the Csep treatment is that, in most cases, the subjects would immediately move to outer walls after being released into the arena. Once in the periphery, the wall space along which they were most comfortable traveling was unbroken and probably provided greater motivation for continuous travel. This also seems to be the case for subjects in the Pcon treatment, where vertical wall space was continuous, reducing the time needed to decide in which direction their next move should be. Within the Ccon treatment, where central wall space was more continuous, the central corner space may have

provided an added amount of safety, which may have made it more likely that they would travel along those walls.

In reviewing the video data for the Psep treatment, it was clear that several of the subjects paused at the gaps between added vertical partitions and the arena wall. It appears that at this point a decision was made between continuing to travel along the wall, and thus through the gap, or traveling along the length of the partition and further into the central area of the arena. As such, these results may provide relatively little information about how indicative immobility is of the individuals welfare state, but may provide some added insight about the animal's decision making process and about what properties of the space are more important, in terms of the difficulty in choosing between movement along a protected space or one further out in the open field. The behavioral responses of subjects to the Psep treatment also provide some important information about the design of the arena. When preparing to travel through the gaps in the partitions, behavior was often similar to what one would expect when an individual is preparing to enter a new area through a small opening. The design of the treatment resulted in a rather large blind spot on the opposite side of the partition that may have reduced the perceived safety of continued movement along the periphery. Increasing the space between the outer wall and the partition would have decreased the size of this blind spot. This apparent flaw in design demonstrates the importance of including even the simplest examples of an animal's behavioral processes in the design and evaluation of animal housing.

In this project, area traversed was the primary means by which total use of space was measured. It was determined along the course of the experiments that this

method is somewhat flawed in that it underestimates, though consistently, the magnitude of the space through which the subjects travel. The consistency of this measurement is important because the similarity in animal models and the use of the same subjects for the three consecutive experiments makes it possible to make accurate comparisons, despite the relatively low usage values. With the collected coordinate data, it is possible, though tedious, to take the size of the individual into consideration when calculating the amount of space it covered.

In Experiment One, least squared means analysis showed a significant difference in space use between the smallest arena and all other arenas ($18.43 \pm 1.79\%$ for 0.5m^2 arena; $8.74 \pm 1.79\%$, $8.46 \pm 1.79\%$ and $2.87 \pm 1.79\%$ for 1, 2 and 4m^2 arenas respectively). In addition, the difference in area traversed between the 1 and 4m^2 arenas approached significance ($P = 0.07$). However, while percentage of the area that was used differed significantly, the actual amounts of space that the percentages represented were almost equivalent. On average, mice used approximately 0.09, 0.09, 0.17 and 0.11m^2 of the 0.5, 1, 2 and 4m^2 arenas, respectively. The amount of space used actually decreased with floor area, relative to track length (total movement), supporting the idea that increasing the floor area alone may not be enough in terms of the individuals use of, and freedom to move within, a space (Figure 6-6).

No significant differences were observed for percentage of space used in Experiment Two. These results were similar to those observed in the 1 and 2m^2 arenas of Experiment One (Figure 6-7). The amount of space used was also consistent with Experiment Two, ranging from 0.10 to 0.12m^2 for the four treatments. Observations from Experiment Three showed a slight increase ($\sim 3.5\%$) in area

traversed over the mean for observations in Experiment Two. It is possible that this difference is the result of the addition of partitions that encouraged movement into and across the central portion of the arena. If this is the case, it is consistent with results of previous work by Cornetto and Estevez (2001) and Leone and Estevez (2008) that demonstrated a positive influence of additional panels on the distribution of broilers in a pen. Furthermore, these results demonstrate the similarities between mice and other 'prey' species, in terms of the importance of vertical wall space.

Unfortunately, fecal scores provided little information regarding the subjects' behavioral responses to any of the treatments in the project, as there were no apparent patterns in the number of boli deposited. Locations of fecal boli were collected but not used for this project, and may provide more information in any future analyses of the data.

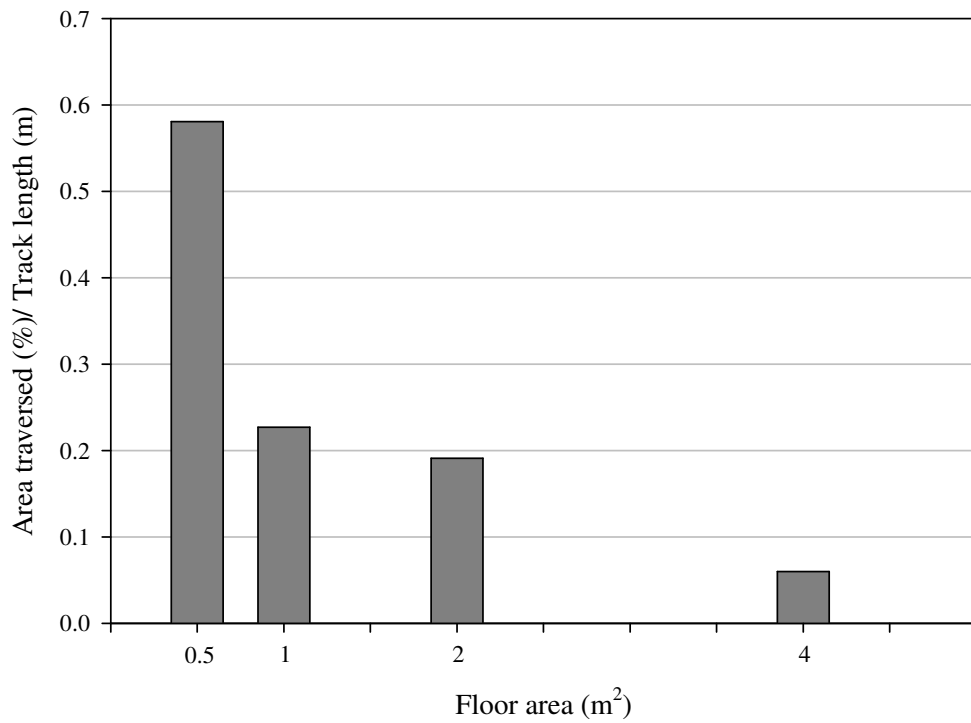


Figure 6-6

Area traversed per unit track length across floor areas 0.5, 1, 2 and 4 (Experiment One)

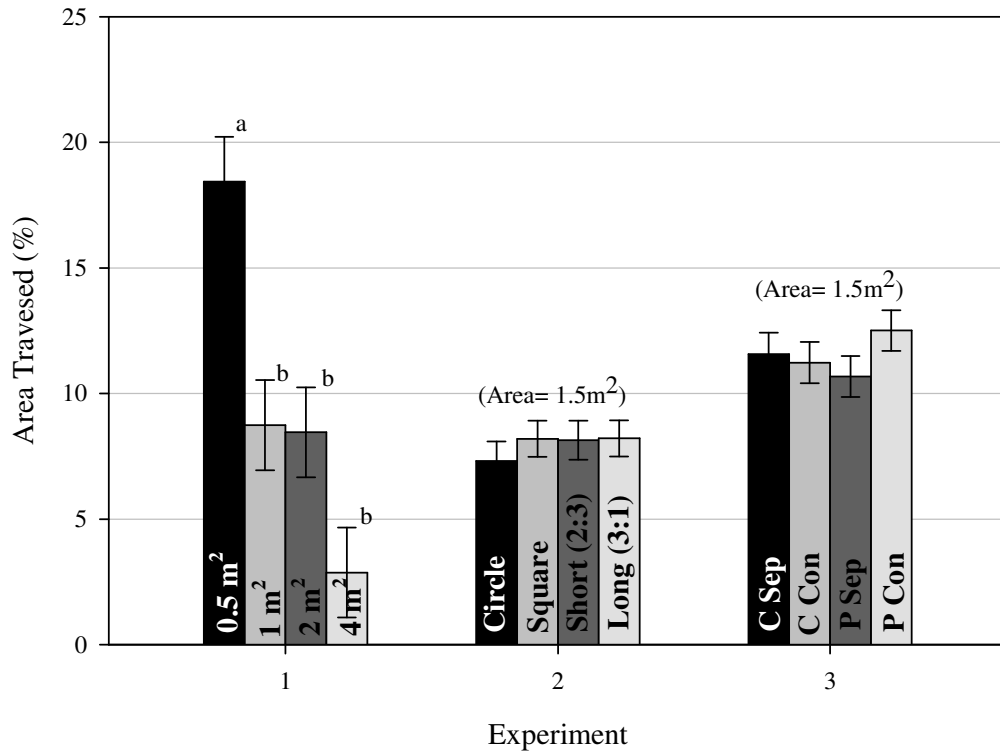


Figure 6-7

Area traversed (LSM ± SEM) across all experiment treatments.

Appendix I: Tracking and Analysis

A. Tracking Procedure

- Captured using Panasonic closed-circuit color camera
 - Digitized to uncompressed AVI format
 - Tracked using Biobserve Viewer II
1. Load AVI video file into Viewer 2 software.
 2. Established tracking zone, using cursor to highlight the area in which movement will be tracked
 3. Calibrate
 - a. Play video until the subject to be tracked is visible. Information input by user includes:
 - i. Conversion from pixel dimensions to actual experimental dimensions (e.g. 4.3 pixels = 1cm). This conversion factor is logged for use in calculations and statistical analysis. Once conversion is complete, it is good practice to go back and check the converted dimensions of the tracking zone, to ensure that zone size matches size of actual test area.
 - ii. Sensitivity of tracking/resolution of the tracked pixel
 - iii. Properties of image/video being tracked (e.g. light subject on a dark background vs. dark subject on a light background)
 4. Adjust contrast threshold, so that only the locations of the intended subject is logged.

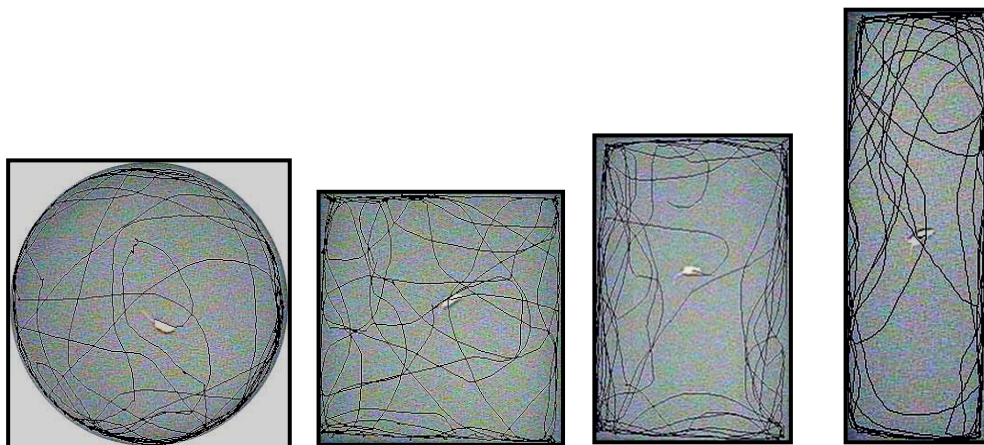
note This is an extremely important step, as any noise in the video can potentially be mistakenly logged as an animal location. See example of complete track below.

5. Reset video. Choose option to show tracked path. Start tracking manually, when cover of starting point is removed and subject is exposed to the maze.
6. Monitor video throughout the tracking process, noting any aberrations or artifacts in the tracking process.
 - a. If an error occurs in the tracking process, make any necessary adjustments (most often to the contrast threshold), and restart tracking until the error no longer occurs.
7. Save file containing with complete list x coordinate, y coordinate, time and date using a pre-determined naming system.
 - a. Naming system for this project:

Day of Test-Session-Cage-Animal

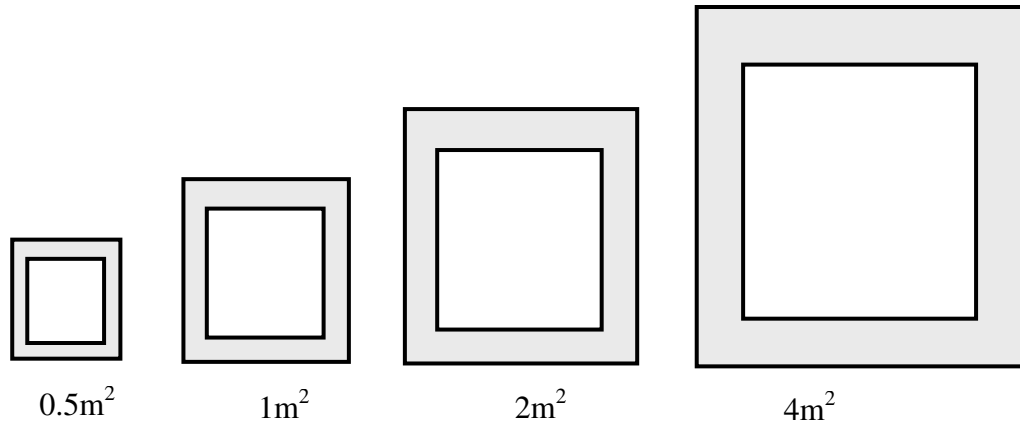
Example: File 122202.xls contains data for mouse 2 of cage 20, observed in the second session of the 12th day of the experiment.
8. Export image of animal path using same naming system (e.g. 122202.bmp)
9. Repeat steps 1-8 for each animal observation.

B. Sample Images of tracked data.

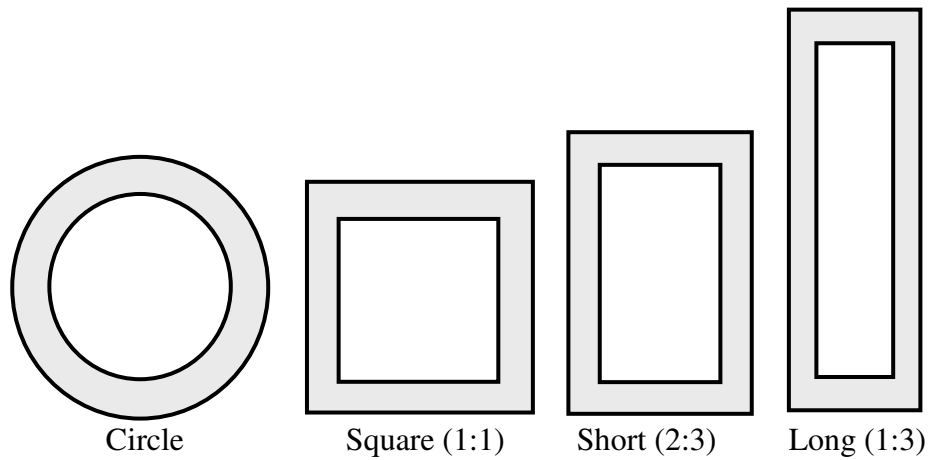


Appendix II: Experimental Arenas

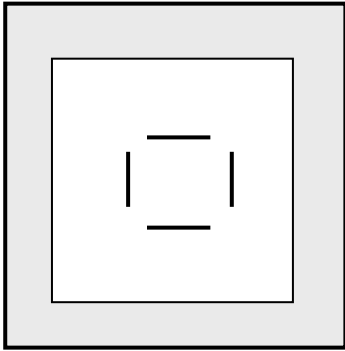
A. Test Arenas: Experiment One. Shaded area represents peripheral zone.



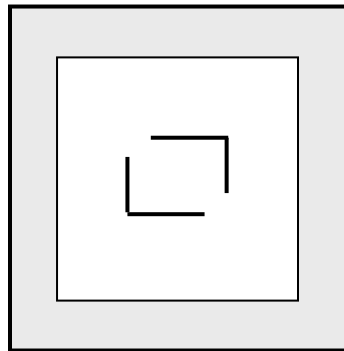
B. Test Arenas: Experiment Two. Shaded area represents peripheral zone.



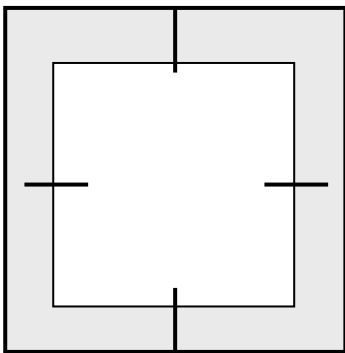
C. Test Arenas: Experiment Three. Shaded area represents peripheral zone.



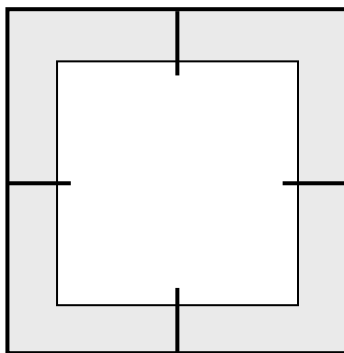
Csep (4 corners)



Ccon (6 corners)



Psep (8 corners)



Pcon (12 corners)

Glossary of Terms

Aggregation: A group of conspecifics gathered in the same location, commonly around some resource, but not engaged in internally-organized behavior (Wilson, 1975).

Angular dispersion: A measure of the spread of a set of angles (Mardia, 1972), used here to describe the complexity (or tortuosity) of a series of movements.

Anxiety: Anticipation of a loss of the sense of security of the self (Sullivan, 1949): A continuous fear of low intensity (English and English, 1958).

Crowding: A density-dependent behavioral state that often leads to discomfort for some or all animals in a group or aggregation. Crowding is a subjective term and is strongly influenced by the perceptual ability of the individual(s) in question. Crowding is a property “of the animal.”

Conspecific: Pertaining to individuals belonging to the same species (Hurnik, et al., 1995).

Density: Quantity per unit area or volume. Density is an absolute measurement and should carry no implications of discomfort to animals. Density, though dependent on objects in a given space, is a property “of the space of the space.”

D_{max} : The maximum straight line distance in a given space. The maximum distance two individuals can separate.

Fear: An emotional state in the presence... of a dangerous or noxious stimulus (Reber, 1985) that typically enhances one’s ability to remove, destroy, or escape from the source (Sullivan, 1949).

Freedom of Movement: The relative ability to move in a given space without movement being hindered by physical (walls), behavioral (social interactions) or mental (fear of predation) barriers. (As defining this term is a goal of my dissertation, this definition will evolve as my work progresses).

Net displacement: The Euclidean distance between the first and last points in an animal’s movement track.

Path: The course of travel between inactive states of greater than or equal to two seconds.

Pen area: Measurement of the surface included within the walls of a pen.

Pen size: Three-dimensional measurement of a bounded space that includes surface area and height (depth) of the enclosure.

Pen space: Measurement of a bounded enclosure that includes both quantitative (area, size, perimeter length) and qualitative (shape) properties.

Social behavior: Activities directed toward and influenced by other members of the social unit (Hurnik et al., 1995)

Social group (society): A group of conspecifics that are organized in a cooperative manner that extends beyond sexually-motivated activity (Wilson, 1975)

Socio-spatial behavior

Space: Expansion from a point (Powers, 1973). The distance from other (people) or things that a person needs in order to remain comfortable (Space, 2008).

Spacing behavior: Behavioral activities by which organisms establish and maintain appropriate distances among group members or between adjoining groups (Hurnik et al., 1995).

Thigmotaxy: Tendency for an organism to orient itself in space by mechanical contact (Fraenkel and Gunn, 1961).

(Complete) Track: A mark or succession of marks left by something that has passed through a space. In the following work, each track may consist of several paths.

Wall-following behavior: Trend to move along edges (Creed and Miller, 1990).

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