SEX DIFFERENCES IN MOVEMENT ORGANIZATION - II
The organization of sex differences in movement during food protection, contact righting, skilled reaching and vertical exploration in the rat: The role of gonadal steroids, body morphology, and the central nervous system

EVELYN F. FIELD

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DEDICATION

This thesis is dedicated to my wonderful daughter Emilie, my husband Rodger, and all my family. I couldn't have done it without any of you.

ABSTRACT

Whether there are sex differences in the kinematic organization of non-reproductive behaviors is rarely addressed. In this thesis, evidence is presented that male and female rats organize their posture and stepping differently during a food protection task, contact righting, skilled reaching, and vertical rearing. Neonatal gonadal steroid exposure can alter sex-typical patterns of movement organization. Whether these differences are due to sex differences in body morphology or the central nervous system (CNS) was also addressed using gravid females and *tfm* males. The results reveal that sex differences in movement organization are CNS based. Furthermore, the expression and choice of sex-typical patterns of movement can be altered by CNS injury. Finally, evidence is presented that sex differences in movement organization are also present in marsupials and insects. The implications of these results for our understanding of the evolution of sex differences in CNS anatomy and behavior will be discussed.

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What thou seest in me is a body exhausted by the labours of the mind. I have found in Dame Nature not indeed an unkind, but a coy mistress: Watchful nights, anxious days, slender meals, and endless labours must be the lot of all who pursue Her, through her labyrinths and meanders.

Memoirs of the extraordinary life, works
and discoveries of Martinus Scriblerus
A. Pope and J. Arbuthnot

CHAPTER 1 - INTRODUCTION

> There are two major classes of living organisms - male and female. In many cases, they are so different in form and habit that one might well be excused the thought that males and females are different species.
>
> (Kelley, 1986, p. 499)

1.0 General Introduction

In this thesis, I will present evidence that male and female rats are fundamentally different in how they organize their movements. Evidence will also be presented showing that sex differences in movement organization can be modified by gonadal steroid exposure neonatally, are not due to sex differences in body morphology, can be altered by CNS injury and are present in a diverse selection of species including mammals, marsupials and insects. The relevance of these findings for the study of sex differences in the organization of movement in humans will be discussed. A theoretical perspective regarding the evolutionary origins of sex differences in movement and their implications for the current state of nervous system will also be proposed.

Aristotle (384-322 B.C.), who many consider to be the father of natural philosophy and the first experimental biologist, was one of the first scholars to speculate with regards to how and why males and females differed (Cosans, 1998). Aristotle thought that inquiry into the question of why we come in two sexes, male and female, would ultimately lead to an understanding of what is "naturally more knowable" (Cosans, 1998). Only by observation would we be able to understand the organization of the natural world. Aristotle's speculations about how males and females differed were driven primarily by his observations that it takes both a male and a female to produce offspring.

> Male and female differ in their essence by each having a separate ability or faculty, and anatomically by certain parts...for union of parents and production of offspring. And these must differ from each other, so that consequently the male will differ from the female. (Even though we speak of the animal as a whole as male or female, yet it is not male or female in virtue of the whole of itself, but only in virtue of a certain faculty and a certain part...) (Aristotle, 2004b, p.3)

His analysis of what made males and females distinct from one another was driven by how males and females differed in external physical form; that is by what he could see. Aristotle's analysis however, did not end with his observations of external form. He also speculated with regards to why males and females develop differences in body morphology and behavior. For example:

> ...he supports his belief that males have their characteristic voice due to "strength in the sinews" with the fact that castrated males have feminine voices. Aristotle reasons that the testes exist for the purpose of weighing down sinewy channels.
>
> ...When boys grow into men, their testes weigh down and tighten their sinewy reproductive systems.... The consequences of castration follow from this common mechanism. ...when the animal's testes are cut off the

> channels are pulled back into the body thereby slackening the whole sinew system. The resulting "loosening of sinewy strength" causes the voice to change over to the more feminine tone.
>
> (Cosans, 1998, p. 313-14)

While speculation about how testes are related to physical form and behavior began with the deductive reasoning of Aristotle, it wasn't until the middle of the 19th century with the work of A. A. Berthold, in 1849 at the University of Göttingen (Forbes, 1949), that the role of the testes and their link to the development of the male form and male-typical behavior were causally tied together.

A. A. Berthold is considered by many to be the father of behavioral endocrinology and was the first scientist to conduct an experiment that is "proof of endocrine function as we know it" (Forbes, 1949). Berthold castrated six pre-adult roosters, and in four of them, re-implanted one testis back into their abdomen. Berthold's description of the subsequent behavior and appearance of the roosters, with the re-implanted testis, is the first documented evidence that the removal and subsequent replacement of a hormone could affect the appearance and behavioral expression of an animal. He describes the behavior of the roosters with the re-implanted testis as follows:

> So far as voice, sexual urge, belligerence, and growth of combs and wattles are concerned, such birds remain true cockerels. Since, however, transplanted testes are no longer connected with their original innervation, and since...no specific secretory nerves are present, it follows that the results in question are determined by the productive functions of the testes. i.e., by their action on the blood stream, and then by the

> corresponding reaction of the blood on the entire organism… of which… the nervous system represents a considerable part.
>
> (Beach, 1981, p. 328-9)

Upon dissection of these animals, it was noted that the testicular grafts, placed near the intestines, had become vascularized but no reconnection of the nerve supply had been established. It is interesting to note that even at this time, when no direct evidence was available, Berthold came to the conclusion that the development of what we consider the male-typical phenotype was due to a "contribution of the testes to the blood and then to the action of the added substance throughout the entire body" (Forbes, 1949, p. 264). While a connection had now been made between the gonads, their secretory substance, sex-typical behavior and putatively, the nervous system, it would take many decades for proof to be found that linked the effects of gonadal steroids to nervous system development (Gorski, Gordon, Shryne, & Southam, 1978; Raisman & Field, 1973) and subsequently behavior (Baum, 2003; McCarthy & Konkle, 2005).

The initial thought experiment by Aristotle and the work by Berthold focused on behaviors not linked directly to sexual behavior. However most studies have historically focused on the study of male and female differences in the development of sexual behavior.

> Growth [in a scientific discipline] is not balanced or synchronized…. A definite unevenness in the advance of behavioral endocrinology is reflected in the excessively large portion of research to date which has concentrated upon sexual behavior and gonadal hormones. This state of imbalance undoubtedly will be corrected gradually as more effort is

> channeled into the study of hormonal contributions to learning, to non sexual social behavior, and to a variety of homeostatic behaviors.
>
> (Beach, 1975, p.186)

Frank Beach, one of the founders of the field of behavioral neuroendocrinology, has referred to the field as an emerging discipline that will continue to expand (Beach, 1981). A recent Pub Med search (December 1, 2005) for the following search parameters (sex OR gender) AND differen*[1] yielded 141 332 hits. A graphical representation by decade shows that the growth of research related to gender or sex differences has continued to increase as predicted by Frank Beach. (Fig. 1).

The advent of new genetic and molecular techniques has, in part, driven the expansion of research conducted concerning the relationship of gender, or sex, to physiology and behavior. Contemporary experimental questions, regarding how males and females develop have been driven by questions such as: (a) How are gonadal steroids related to the development of female and male external genitalia (Jost, 1960; Jost, 1983; Voutilainen, 1992)? (b) How are alterations in the physical form of males and females related to the expression of sex-typical sexual behavior (Beach, 1947; Beach, 1984; Beach & Holtz, 1946)? (c) When, during development, is gonadal steroid exposure required for males and females to develop sex-typical patterns of sexual behavior in adulthood (Baum, 2003; Phoenix, Goy, Gerall, & Young, 1959)? and (d) How do gonadal steroids affect the development and composition of the CNS (Gorski et al., 1978; McCarthy, 1994; Raisman & Field, 1973)?

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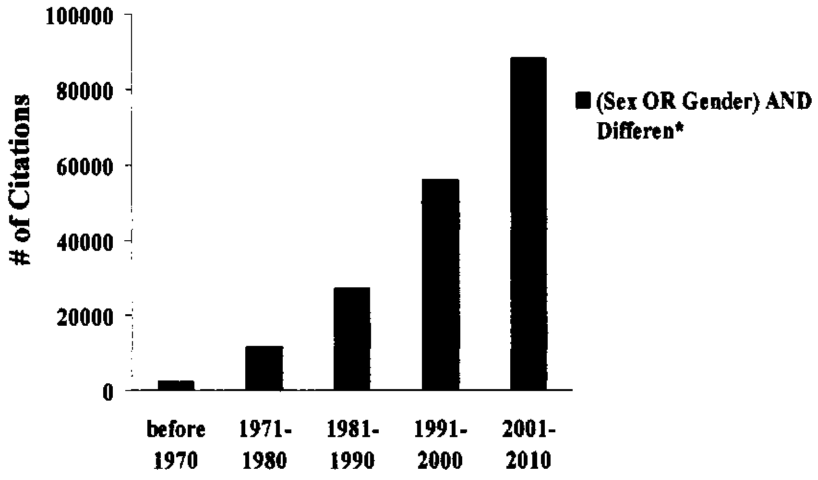
[1] The use of the search term differen* gives Pub Med the command to search for any word that begins with the string of letters 'differen' and ends with multiple endings. For example, the above search will find references with the terms (sex OR gender) AND differences, (sex OR gender) AND differentiation etc.

Figure 1

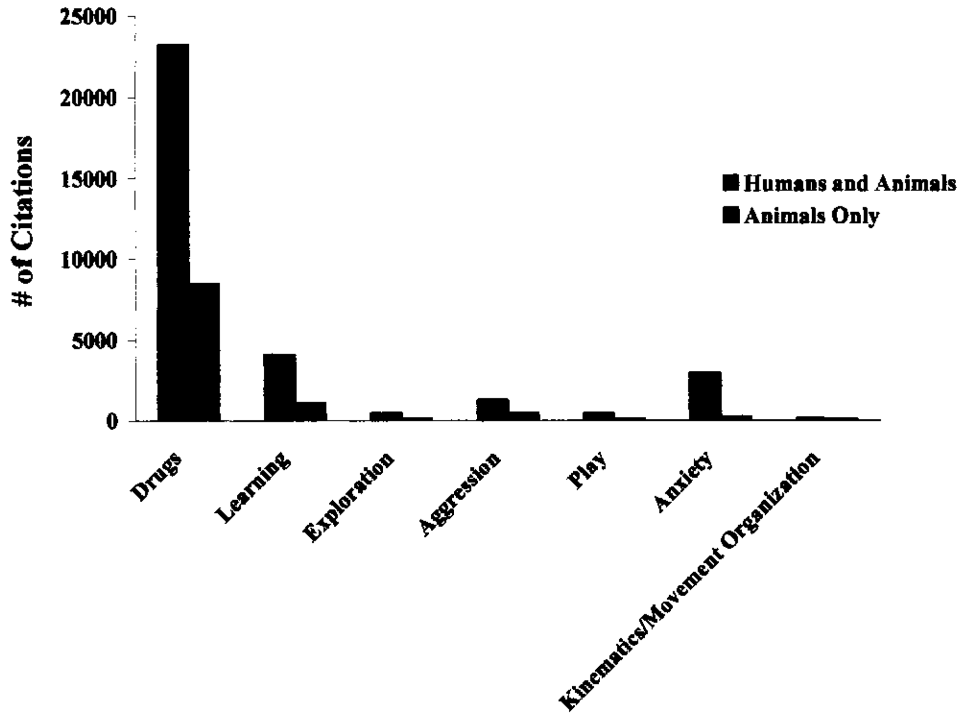


The number of citations found in PubMed prior to 1970, and subsequently by decade, for (sex OR gender) AND differen* is depicted. It is clear from the graph that the number of studies conducted that are related to sex or gender differences has increased with each decade. (The data for 2001 to 2010 has been extrapolated from the data available for 2001 to 2005).

Contemporary questions, regarding the sex-typical development of mammals, have focused on the contribution of sex differences in gene expression, in addition to gonadal hormone exposure, to subsequent development. For example, can the sex chromosome complement of XX or XY, in a male or female mouse, directly affect the development of a male and a female phenotype independently of gonadal steroid exposure (Arnold, 1996; Arnold, 2003; Carruth, Reisert, & Arnold, 2002; De Vries et al., 2002)? Even with the increase of research regarding questions relating to sex or gender differences there are still many questions that have not yet been answered, especially with regards to how and why sex-differences in non-reproductive behaviors develop and are expressed in adulthood.

As Frank Beach (1975) stated, the majority of initial research regarding sex differences in behavior was related to understanding how males and females differed during sexual behavior. Subsequent research has continued to focus on this area of inquiry, but other non-sexual behaviors have also been studied. PubMed listings (December 5, 2005) for sex differences in behaviors using the following search parameters (sex OR gender) AND differen* AND drugs, learning, exploration, aggression, play, anxiety, or (kinematic OR movement organization) returned 23 226, 4079, 471, 1269, 501, 2972 and 130 hits respectively (Fig. 2).

Figure 2



The total number of citations found in PubMed for (sex OR gender) AND differen* AND respectively drugs, learning, exploration, aggression, play, anxiety, (kinematics OR movement organization) is depicted. Citations restricted to non-human animals are shown in blue for comparison to the overall number of citations found. See text for details.

While research in areas regarding the sexual differentiation of non-sexual behavior is being done with increasing frequency, it is still only between 20 and 25% of the overall amount of research conducted with regards to the study of sex or gender differences[2,3]. The reason for the relatively low number of studies addressing questions regarding the sexual differentiation of behavior likely has its roots in the historical development of the field of behavioral endocrinology and its 'parent' discipline endocrinology.

By exploring the history of behavioral endocrinology, an appreciation can be gained for the events that underlie the development and present state of the discipline. A brief overview of this history will be presented in the next few sections. In addition to the history of behavioral endocrinology, a brief history of the discipline of kinematic analysis, and its application to biological motion, will also be presented. As mentioned previously, the number of studies pertaining to sex differences and kinematic or movement organization is very small. Less than 0.001% of the studies returned by PubMed, regarding the study of sex differences, were related to the study of sex differences in kinematics. The lack of studies in this area may be due, in part, to the theoretical perspective that has evolved with the historical development of kinematic research. In general, movement is often considered to be determined primarily by an animal's form. Questions pertaining to whether sex differences in movement organization can be generated by the nervous system have received minimal attention. It is only through an understanding of the history of both the discipline of behavioral

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[2] Many use the term sex or gender interchangeably to describe differences between males and females. For simplicity I will only use the term sex differences from this point forward.

[3] See Footnote # 1 for details of search parameters. The value of 20 -25% was calculated by dividing the total number of studies for non-reproductive behaviors (32 648) by the number of studies obtained from the search for (sex OR gender) AND different* (141 332) mentioned on page 5.

endocrinology and kinematics that one can understand the historical context within which the current thesis fits.

1.1 The History of Behavioral Endocrinology and the Study of Sex Differences

In many aspects, the study of the relationship between hormones and behavior, now known as behavioral endocrinology, has developed along a similar trajectory to that of its parent discipline endocrinology (Beach, 1981; Turner & Bagnara, 1976). Three stages have been defined: (a) *the predisciplinary era* that encompassed the late 1800's; when A. A. Berthold did his seminal work, (b) *the formative period* that lasted from the early 1900's until the mid 1950's - a time when the study of hormones and their effects on behavior became accepted as a separate and identifiable area of research, and (c) *the contemporary or modern era*, that began with the seminal paper by Phoenix and colleagues (Phoenix et al., 1959). The modern era can be characterized as a time when scientists began to specialize and exclusively study the relationship of hormones to behavioral and neural development (Beach, 1981).

The Predisciplinary Era of Behavioral Endocrinology

Following the work of Berthold there was flurry of scientific activity in Europe and Russia, to determine how the removal or addition of the sex glands affected the behavior of an animal. While there appeared to be agreement that gonadal secretions had effects on the developing organism, and the CNS which governed behavior, the exact mechanism and the effects of hormones on the expression of sex-typical behavior was one of continued debate (Beach, 1981).

Although research related to the effects of gonadal steroids on the development and behavior of an organism continued, research was also being conducted regarding how secretions from other endocrine glands, such as the thyroid, liver, pancreas, or adrenals, affected the function of an organism. Claude Bernard, in 1855, coined the term "internal secretion" to refer to the physiological effects of endocrine gland secretions on an organism (Beach, 1981). Bayliss and Starling in 1905, after their discovery of secretin in 1902, introduced the word hormone, from the Greek word hormao, to refer to a substance that "urges on, initiates, irritates and stimulates" (Hendriksen & Schaffalitzky de Muckadell, 2000, p. 464). They also introduced the "hormone concept" that began to represent "the name of all the active principles found in one part of the body, distributed with the circulating blood to perform its function, regulation, stimulation and growth in other parts of the body" (Hendriksen & Schaffalitzky de Muckadell, 2000, p. 464). The study of the effects of glandular secretions on the physiological workings of the body was well underway.

During this time, questions about the effects of glandular or testicular secretions on the development of human behavior were also being scrutinized. It had been known for centuries that the castration of human males led to an altered development of the male-typical phenotype (Nelson, 2005). One of the first experiments conducted with regards to the effects of gonadal secretions on human well being, and thus behavior, was by Claude Bernard's successor Charles Edward Brown-Sequard. In 1889, in his early 70's, Brown-Sequard conducted an experiment to determine whether a mixture of blood, semen, and fluid from the crushed testes of dogs and guinea pigs could have a rejuvenating effect on his health. After ten days of intra-muscular injections, Brown-

Sequard reported an increase in mental alertness, resistance to fatigue and improved bladder and bowel function. His suggestions to colleagues however, to try it on themselves was met with ridicule and was not likely repeated (openly) by others (Beach, 1981).

The Formative Years

Brown-Sequard was a dedicated scientist, with an excellent scientific reputation who published over five hundred papers during his career on adrenal function. Possibly through a loss of objectivity, he likely misinterpreted the results of his personal experiment (Price, 1975). It was this type of error that led to the belief that the correlation of the action of hormones to behavior and subsequently, to the nervous system, would be too difficult. "Even the behavior of lower animals is too labile and unpredictable to serve as a useful measure in endocrinological experimentation" (Beach, 1981, p. 332). Thus, the study of hormone and behavior relationships became infrequent. In the early 1900's, research endeavors concerning endocrine function began to focus increasingly on the study of the physiology of the endocrine glands and the secretions they produced. A new scientist in endocrinology was not likely to study the relationship of hormones and behavior. The following quote by Frank Beach (1981) summarizes the thinking of the late 40's and early 50's:

> Any bright young endocrinologist receiving his training around that time could clearly see where his professional future lay so that he would scarcely waste time in experiments on behavior when there was so much to be learned about biochemistry and cellular biology. (p.336)

While the study of endocrinology was beginning to mature into a discipline in its own right, there were still a few individuals who continued to try and explore the relationship of hormones to behavior - Frank Lillie was one of these individuals. Lillie was the first to describe the masculinized development of a female twin, or freemartin, of a normal male calf (Lillie, 1917). His work suggested that a hormone secreted by the male's testes could influence the development of the twin animal's sex-typical characteristics. Thus, not only were adult behaviors, such as those shown by Berthold, modifiable by gonadal steroids, but the development of males and females may also be influenced by exposure to gonadal steroids during early development. Lillie, in 1923, suggested that the study of sexual development should focus on the following questions:

> 1. How is sex determined? Can sex determination be controlled? How?
> 2. What are the factors active in the development of sexual characteristics, whether anatomical, physiological, or psychological?
> 3. The problems of sex relationships; their nature, their control.
>
> Practically all problems concerning sex come under these three. The specific problems are innumerable.
>
> (Aberle & Corner, 1953, p. 17)

Although Lillie had the laid the foundation for future research regarding the role of hormones in development and behavior, little progress was made during his time. Carl Moore, a student of Lillie's, summed up the reason for the lack of progress in behavioral research as one that was driven by the unreliability of behavioral responses to hormone manipulations.

> After the stage of puberty, or masculine maturity, the loss of the hormone is less evident. It is clear in many specific cases, but less clear in others, that the instinct of mating is conditioned by male hormones, either directly or indirectly. ...We have often employed male guinea pigs, castrated at 30 days of age (prepubertal) as a means of detecting females in heat when actual mating is undesired; the mating instinct persists and male behavior persists for many months despite the early castration.... Castration, therefore, does not immediately and permanently destroy sexual inclinations and behavior, even in lower animals.
>
> (Moore, 1931, p. 520)

Thus, the lack of interest regarding the study of the relationship between behavior and gonadal steroids was due to two factors: (a) the increased focus on determining the molecular structure of hormones and how they are produced physiologically, and (b) the apparent variability, or lack of change in behavioral responsiveness after the removal of gonadal steroids. It was not until the work of one of Lillie's academic grandchildren and Carl Moore's students, William Young, that the role of gonadal steroids with regards to the expression of male-and female-typical behavior began to be elucidated. The seminal paper of Phoenix and colleagues in 1959 marks the beginning of the modern era and our current understanding of how gonadal steroids affect the development of sex-typical patterns of behavior.

The Modern Era

The landmark study of Phoenix, Goy, Gerall and Young (1959) heralded the re-entry of the study of behavior, with respect to its organization or modification by gonadal steroids, into mainstream scientific investigation. They demonstrated that the potential for hormonally induced masculine or feminine sexual behavior, in adult guinea pigs, is dependent on gonadal steroid exposure early in development. The organizational/activational hypothesis of hormone action, which has driven a great deal of contemporary research, was also presented with this work. In general, they postulated that there is a critical period, early in development, when gonadal steroids can organize the CNS as masculine or feminine. In adulthood, exposure to gonadal steroids can subsequently activate a masculine or feminine CNS to drive the expression of male- or female-typical behavior.

Since this seminal work, a number of studies have demonstrated the validity of the organizational/activational hypothesis of gonadal steroid effects for sex-typical sexual behavior in mammals (Adkins-Reagan, Mansukhani, Thompson, & Yang, 1997; Baum, 2003)[4]. Research has also revealed a role for gonadal steroids in the development and expression of non-sexual behaviors such as aggression (Blanchard, Sheperd, Carobrez, & Blanchard, 1991; Blanchard & Blanchard, 1990), play (Pellis, 2002; Pellis, Field, Smith, & Pellis, 1997; Pellis & Pellis, 1990), spatial behavior (Kanit et al., 2000; Kanit et al., 2000; Roof & Stein, 1999), spontaneous and exploratory behavior (Mead, Hargreaves, & Galea, 1996; Quadagno, Shryne, Anderson, & Gorski, 1972; Swanson, 1966), and

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[4] For a discussion of exceptions to this hypothesis however see: Arnold, A. P. (1996). Genetically triggered sexual differentiation of brain and behavior. Hormones and Behavior, 30, 495-505; Arnold, A. P., & Breedlove, S. M. (1985). Organizational and activational effects of sex steroids on brain and behavior: A re-analysis. Hormones and Behavior, 19, 469-98.

anxiety (Beck & Luine, 2002; Papaioannou, Gerozissis, Prokopiou, Bolaris, & Stylianopoulou, 2002).

As mentioned previously, one research question that has received little attention is whether males and females organize their movements differently to complete a behavior that is functionally equivalent or equally successful. Furthermore, the possible contributions, in both males and females, of gonadal steroids, and the CNS to possible sex differences in movement organization have not been addressed. A brief survey of the history of the kinematic analysis of behavior will highlight why this area of research has likely been neglected.

1.2 The Historical Development of Kinematic Behavioral Analysis

Historically, studies of sex differences in sexual and non-sexual behaviors were restricted to what could be seen in real time with the naked eye. Lordosis and mounting during rat sexual behavior could be discriminated as they occurred. The ability to learn and solve a spatial task could be timed with a stop watch. The number of aggressive or playful bouts that occurred in a social encounter could be scored as they took place. Many of the initial behaviors that were studied, with regards to sex differences in behavior, were determined by what could be seen and tracked in real time. With advances in technology, however, the details of how behaviors are organized could be observed, and quantified, in the time scale of milliseconds. The detailed analysis of movement organization, in vertebrates, is commonly referred to as the study of functional vertebrate morphology; or kinematics (Ashley-Ross & Gillis, 2002). Research in this discipline is generally focused on how the expression of a behavior, such as locomotion, is generated by the overall morphology and skeletomusculature of the organism being studied.

The advent of the field of kinematic analysis or the study of how the form of an animal relates to its function, like the study of how males and females differ, can also be traced back to Aristotle:

> We have now to consider the parts which are useful to animals for movement...why each part is such as it is and to what end they possess them.... First then let us lay down how many questions we have to consider.... What are the fewest points of motion necessary to animal progression...why some animals are footless, others bipeds, others quadrupeds, others polypods, and why all have even number of feet, if they have feet at all.
>
> (Aristotle, 2004a, p. 4)

The historical study of how animals' movements are generated or constrained by their physical form continued with the scientific work of Galen (129-201A.D.), who realized that muscles were the driving force behind animal movement, and Leonardo da Vinci (1452-1519), who realized that it was the intrinsic properties of muscles which allowed an organism to move. This was followed by the work of Borelli (1608-1679) in the 1600's, the acknowledged father of biomechanics, who established that the movement of a limb could be determined mechanistically and could be reduced to a matter of "mechanics" (Ashley-Ross & Gillis, 2002).

The description of movement based purely on mechanical principles, coupled with the work of scientists such as Cuvier (1769-1832) and Geoffroy St.-Hilaire (1772-

1844)[5], who attempted to explain the development of animal form, led to an increased emphasis on the concept that the form of an animal was what dictated its behavior (Ashley-Ross & Gillis, 2002; Panchen, 2001). The study of morphology and kinematics continued to grow in the 19th and 20th centuries. However initially, like the study of hormone and behavior relations, researchers had to focus on what could be seen with the naked eye (Russell, 1982).

> Lacking the means by which to manipulate experimentally or measure animal movements, early natural philosophers were limited to inferring function from their observations of the structure and behavior of animals.
>
> (Ashley-Ross & Gillis, 2002, p. 183)

The analysis of the fine details of movement organization, and how it relates to animal form, was dramatically advanced with the development of photographic techniques. Eadweard Muybridge (1830-1904), who after a near fatal stagecoach accident decided to follow the recuperative advice of his physician and take up photography as a 'natural therapy', became the first person to document the fine details of animal locomotion (Ashley-Ross & Gillis, 2002). Muybridge is best known for providing the first photographic evidence that horses, when galloping, have all four of their hooves off the ground simultaneously (Ashley-Ross & Gillis, 2002). His interests soon went beyond horses, and he carried out numerous studies of animal and human locomotion while walking, running, or during various tasks such as throwing a ball or

---

[5] Cuvier and Geoffroy St.-Hilaire are noted for their debate regarding how various animal forms came into existence. Cuvier championed the view that all differences in animal form represented adaptations to the environment, thus each organism is defined by its functional characteristics. Geoffroy St.-Hilaire, in contrast, argued that how structures of the body are connected to one another and how these patterns of connection are similar, or homologous, between species was the most important determinant of the development of animal form. Panchen, A. L. (2001). Étienne Geoffroy St.-Hillaire: Father of "evo-devo"? Evolution & Development, 3, 41-46.

shoeing a horse (Muybridge, 1979). The work of Muybridge provided the impetus for the work of Etienne-Jules Marey (1830-1904) who developed the initial photo/video techniques used to see the details of motion in animal or human behavior. Marey's work laid the foundation for the subsequent development of the contemporary videographic techniques that are currently used for the kinematic analysis of both animal and human behavior (Braun, 1992).

The development of specialized techniques for the study of animal locomotion, coupled with the advances in techniques such as electromyelography, allowed researchers to tie muscle activation patterns to changes in movement. The contributions of specific muscles to the generation of behaviors, such as locomotion, could now be studied in great detail (Shumway-Cook & Woollacott, 2001). Today, the focus of a large majority of kinematic studies is to describe movement using paradigms such as postural sway, which does not involve a voluntary movement component, or forward locomotion, a voluntary repetitive behavior. A current review article by Biewener (2002) critiques this approach:

> Moving from steady-state locomotion to more varied locomotor behaviors represents a critical area in which future study is greatly needed. Animals must contend with varying environmental conditions, requiring irregular and non-steady locomotor behaviors (p. 49). In the face of developing cellular and molecular approaches for studying animal form and function, there remains a critical need for experimental studies of naturally behaving animals. (p. 40)

In addition to the general trend of applying kinematic analysis primarily to regular, repetitive behavior, many kinesiologists and functional morphologists, either implicitly or explicitly, focus on how an animal's peripheral form (i.e. number of limbs), determines its kinematic profile. The relationship between the CNS that ultimately controls an organism's movement, and the detailed expression of movement, is rarely considered. Consequently, the common, often unspoken, consensus is that differences in the kinematic performance of a behavior, between two organisms or between two sexes, are due to differences in an animal's form, not to differences in the CNS commands that govern the expression of a select behavior (Smith, 1994). It is possible, however, that the organization of movement is not determined entirely by the form of the animal. Thus, the organization of a pattern of movement may be determined by the CNS in spite of variations in animal morphology.

1.3 An Introduction to the Current Research Questions

As mentioned previously, sex differences in mammals exist in both reproductive (Ward, 1992) and non-reproductive behaviors (Beatty, 1992). The study of most sexually dimorphic behaviors has, however, focused on the study of endpoint measures; behaviors are dimorphic only in the sense that one sex is more likely to perform them than the other (Aron, Chateau, Schaeffer, & Roos, 1991; Goy & Roy, 1991). Thus, the study of sex differences in behavior has usually focused on how males differ from females, not in what they do, but in how likely they are to perform particular behavior patterns.

Evidence will be provided in this thesis that when males and females perform a particular pattern of behavior, specifically during non-reproductive behaviors, it cannot be assumed that the execution of that behavior pattern is similar in males and females. Furthermore, gonadal steroid exposure may affect the expression of sex-typical patterns of movement organization. This idea is not without precedent. Frank Beach showed that the execution of a movement pattern can be different in males and females dogs and that these differences can be influenced by gonadal hormones. Prior to the onset of sexual maturity, both male and female dogs squat during micturition. After puberty while females continue to squat, males often raise one hind leg. The sex difference in adult micturition patterns is modifiable by neonatal androgen exposure (Beach, 1974).

Research has also been conducted in females, revealing that the successful performance of a movement can be influenced by the stage of the estrous cycle of a rat. The ability of a female rat to traverse a beam, and accurately place their feet on that beam, is significantly better at behavioral estrus than proestrus or diestrus (Becker, Snyder, Miller, Westgate, & Jenuwine, 1987). This finding has been linked directly to 17β-estradiol levels in the striatum (Becker et al., 1987). A correlation between sensorimotor performance and whether an animal is in behavioral estrus or diestrus, has also been shown for female rats that are placed on a treadmill with unpredictable changes in speed. Females in diestrus compensate for the changes in speed by varying the swing phase of the step cycle. In contrast, females in estrus compensate for changes in treadmill speed by changing their stride length (Smith, 1998; Smith & Chapin, 1996; Smith et al., 2000). Research using human subjects has also shown that motoric performance can change across the menstrual cycle (Hampson & Kimura, 1988). Thus, there is evidence that the organization of movement can be sex-typical and that the execution of a movement can be modified by gonadal steroid exposure either acutely or during development.

Sex Differences in the Kinematic Organization of Play Behavior

The initial description of sex differences in the kinematic organization of a non-sexual behavior was conducted for evasion during play. Play-fighting, or rough and tumble play, is an activity common to the juveniles of many mammalian species (Fagen, 1981). It has been stated that "(s)ex differences in social play are quantitative and not qualitative, referring to the frequency and not the form of behaviors" (Meaney, 1989, p. 247). The frequency of play fighting in juvenile rats is sexually dimorphic (Pellis, 2002). In general, males initiate more attacks to the nape, the area of preferred contact during social play, than do females (Meaney, 1989; Pellis & Pellis, 1990; Thor & Holloway, 1983). Males and females also differ in their response to a playful attack. Females are more likely to evade an approaching conspecific than are males. Males, in contrast, are more likely to roll over to a supine position (Pellis & McKenna, 1992; Pellis & Pellis, 1990). While males and females differ in their frequency of play behavior and the type of defense they use in response to a playful attack, they also differ in how they organize their movements (Pellis et al., 1997).

Females, when swerving away laterally from an approaching conspecific pivot around a point close to the pelvis and thus their bodies move unidirectionally, in a forward, cephalocaudal direction away from the conspecific. Males, in contrast, are more likely to couple evasive tactics with a movement of their pelvis back towards the conspecific that is attempting to make nape contact. Thus, males move their napes away from the attacker and use the lower body to block the approach of the opponent (Pellis et al., 1997; Pellis & Pellis, 1987). One difficulty, however, with the analysis of sex differences in the organization of movement during play fighting, is that sex differences in the organization of a behavior such as evasion may be due to the males' attempts to enhance their opportunity for counterattack by placing themselves in closer proximity to their attacker after the completion of an evasive maneuver. The motoric behaviors during play fighting are also often composed of movements in different planes of movement (Fig. 3). The defending animal, while evading an approaching conspecific during a play bout by turning laterally, may also simultaneously leap vertically or incorporate rotation around the longitudinal access to move the nape away from the conspecific.

Therefore, while the initial study of sex differences in the kinematic organization of behavior was done by analyzing the evasive movements performed during play, the complexity of the movements led to the selection of a different behavioral paradigm. The behavior that was chosen for analysis, to determine whether males and females differed in their organization of lateral evasive maneuvers, was dodging to protect a food item from an approaching conspecific (Whishaw, 1988; Whishaw & Tomie, 1987; Whishaw & Tomie, 1988).

Figure 3

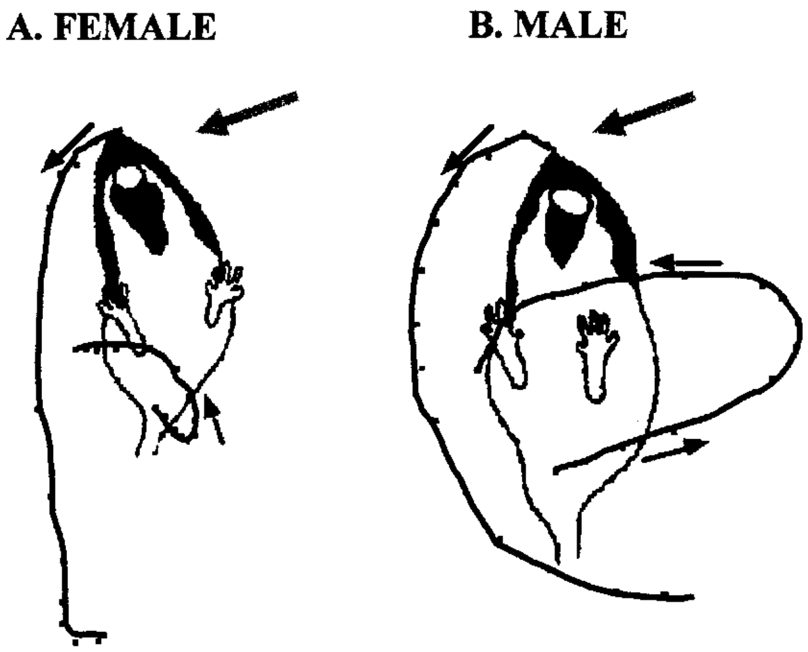
When analyzing behavior, it is useful to consider the different directions, or planes of movement, within which a behavior can occur within. In general movements can be categorized as occurring in: (a) the anterior/posterior plane, e.g. forward locomotion, (A), (b) the lateral plane, e.g. turning on a horizontal surface (B), or the vertical plane, e.g. rearing (C). In addition to linear movements, rotation can occur around any body axes, e.g. the longitudinal axis, such as when an animal rotates over from supine to prone (D) (Golani, Bronchti, Moualem & Teitelbaum, 1981).

Sex Differences in the Kinematics of Dodging to Protect a Food Item

Dodging to protect a food item from an approaching conspecific was subsequently chosen for the analysis of possible sex differences in movement organization since the magnitude of the dodge is approximately 180° away from an approaching conspecific and its beginning and end are easily identified. It had also been previously documented that males and females were equally successful in the completion of this task and in its overall characteristics (Whishaw, 1988; Whishaw & Gorny, 1994; Whishaw & Tomie, 1987; Whishaw & Tomie, 1988). The third advantage of this task was that it occurs primarily in the horizontal plane and does not normally involve a vertical displacement of the body or rotatory movements around the long axis to complete the behavior (Field, Whishaw, & Pellis, 1996a). The final advantage was that dodging is a behavior that rats express naturally; it first appears just after weaning (Bolles & Woods, 1964). Thus, the early onset of this behavior makes it amenable to analysis during development and in adulthood.

A kinematic analysis of dodging in males and females revealed that they do use different patterns of movement and postural adjustments to complete this task even though their overall success in completing the task was equivalent (Field et al., 1996a). Males make a significantly larger excursion with the pelvis than do females. This difference is due to a larger backwards and sideways movement, in the opposite direction to the movement of the snout, of the pelvis (Fig. 4). These differences are accompanied by differences in the stepping patterns of the hindpaws (See Chapter 2 Fig. 6 for details).

Figure 4



The trajectories of movement of the snout and pelvis, for a typical dodge by a Long-Evans female and male, are depicted (4A & B) (Adapted from Field et al., 1997a). The black arrows indicate the direction of movement. The black dots underneath the blue and red lines represent two frames of video. The green arrows represent the approaching robber. Note that unlike the female, the male makes a large outward swing of the pelvis.

No effect of either the robber's behavior or sex, with respect to the sex-typical expression of dodge tactics, was found This suggested that the differences in the tactics used by males and females to protect a food item are intrinsic to the organization of the movements themselves (Field et al., 1996a; Field, Whishaw, & Pellis, 1997b).

Subsequent to the initial description of the sex differences in dodging behavior, it was found that the presence or absence of testicular hormones during development could influence the development of male-typical patterns of dodging. Male were castrated at three different ages: (a) within the first 24 hours of birth, (b) just prior to puberty, and (c) in adulthood. Females were treated with 200 µl of testosterone propionate (TP) on postnatal days 1 and 2. All animals were tested as adults. It was found that males castrated at birth were more similar to intact females than to intact males. Castration prior to puberty, or in adulthood, had no effect. In contrast TP-treated females, were more like intact males than intact females. Thus, the development and expression of the male-typical pattern of dodging is likely dependent on the organizational, but not activational, effects of testicular hormones (Field, Whishaw, & Pellis, 1997a).

A confound of these results is that the manipulation of gonadal steroids in males and females also affected the overall body mass of these animals. Males castrated at birth were significantly smaller than intact males and conversely, females that were injected with TP at birth were significantly larger that normal females (Field et al., 1997a). Thus, the research described above led to a number of new hypotheses regarding the study of sex differences with respect to the organization of movement.

1.4 The Hypotheses Addressed in the Current Thesis

In this thesis, by analyzing sex differences in the kinematic organization of non-sexual behaviors, the following hypotheses were tested:

1) Sex differences in the kinematic organization of behavior are dependent on exposure to gonadal steroids, in females, for their organization.

2) Sex differences in movement organization are due to sex differences in body mass or skeletomusculature.

3) Sex differences are present in the kinematic organization of behavior, in the laboratory rat, in all planes of movement (see Fig. 3).

4) Sex-typical kinematic organization of behavior can be altered by CNS injury.

1.5 The Current Research Questions

The Role of Ovarian Steroids

Previous evidence has shown that testicular hormones are necessary for the development of male-typical patterns of movement organization. The question of whether there is a role for ovarian steroids in the development of female-typical patterns of dodging to protect a food item has not been addressed. The classical model of gonadal hormone effects on the sexual differentiation of males and females categorizes females as the default condition, and thus testicular hormones are required to divert males away from a female-typical pattern of development (MacLusky & Naftolin, 1981; McCarthy & Konkle, 2005). There is evidence, however, suggesting that ovarian steroids are necessary for the organization of female-typical patterns of behavior (Forgie & Stewart, 1994; Pellis, 2002). The question of whether female-typical patterns of dodging are influenced by the removal of ovarian steroids was conducted by analyzing the dodge patterns of females that were ovariectomized within the first 24 hours after birth, prior to puberty, or in adulthood. The results of this experiment are presented in Chapter 2.

The Role of Body Morphology

The results of the study showing that testicular hormones can influence the development of male-typical patterns of dodging also suggests that sex differences in movement organization may be neurally based (Field et al., 1997a). It is possible, however, that the overall body mass or skeletomusculature of an animal is a relevant variable with regards to the development and expression of male- or female-typical patterns of movement. Two experiments were conducted to address this issue. In the first experiment, the kinematic organization of dodging to protect a food item was compared for gravid female, at gestational (G) day 19-20, nulliparous female and intact male rats. Gravid females, at G19-20, are indistinguishable from normal males with respect to body mass. If adult body mass is the determining factor, with regards to the sex-typical expression of dodging tactics, then gravid females should be more like intact males than nulliparous females. The results from this experiment are presented in Chapter 3A.

The second approach used to determine whether skeletomusculature was relevant to the type of dodge pattern performed was to analyze the behavior of the testicular feminized mutation or *tfm*-affected male (*tfm*) rat. The *tfm* rat (Stanley, Gumbreck, Allison, & Easley, 1973), as a consequence of a point mutation (*tfm*) in the androgen receptor gene (Yarbrough et al., 1990), is insensitive to the direct effect of androgens which are necessary to masculinize the skeletomusculature (Vanderschueren et al., 1994). Thus, the *tfm* males express a feminine peripheral phenotype, including a blind end vagina, a nipple line (Stanley et al., 1973), an unandrogenized skeletomusculature and a smaller overall body mass than intact males (McGinnis, Marcelli, & Lamb, 2002; Vanderschueren et al., 1994).

Masculinization of the rodent CNS, in contrast to the periphery, is thought to be accomplished primarily by the aromatization of testosterone to estrogen (McCarthy, 1994; Olsen, 1992). *tfm* males have functional secretory testes located in the abdomen, male-like levels of circulating testosterone (Purvis, Haug, Clausen, Naess, & Hansson, 1977), and possess a nuclear estrogen binding system that is similar to control males (Purvis et al., 1977). It has been demonstrated that *tfm* males can exhibit male sexual behavior as adults (Olsen, 1979b; Shapiro, Levine, & Adler, 1980). In addition, it has been shown that castration during the neonatal period, prior to postnatal day ten (Olsen, 1979a), in contrast to castration after postnatal day ten (Olsen & Whalen, 1981), is necessary to induce lordosis with estrogen treatment in adulthood. Therefore, it is thought that the CNS of the *tfm* male is largely masculinized and defeminized via the aromatization of testosterone to estrogen during the perinatal period. The results of the analysis of whether male- or female-typical patterns of dodging to protect a food item are used by *tfm* males, as compared to their wild-type male and female controls, is presented in Chapter 3B.

The study of the hormonal mechanisms that underlie the development of sex-typical patterns of dodging are interesting and worthy of further study. This direction of study would not address the question of whether sex-typical patterns of movement are present solely in the behavior of dodging to protect a food item or whether sex differences in the organization of movement are a more general characteristic of rat behavior. To address this question the experiments described in the following sections were conducted.

Are Sex Differences in Movement Organization Generalizable across Tasks?

When considering the question of what behaviors to select for kinematic analysis and whether sex differences in the kinematic organization of movement are generalizable across behaviors, it is useful to consider all of the possible dimensions within which movements can occur. It has previously been shown that movements primarily occur within three planes of movement: forward, lateral or vertical (Eilam & Golani, 1988; Golani, et al., 1981). Movement can also involve rotations around various axes of the body, for example, the longitudinal axis of the body that, in the rat, runs from the head to the tail (Pellis, Pellis, & Teitelbaum, 1991) (see Fig. 3). It is possible that the sex differences described for dodging are due to sex differences in the organization of behaviors that occur primarily within the lateral plane and that these differences are not generalizable to other planes of movement. If sex differences in postural adjustments and movement organization are present in behaviors that occur within other planes of movement, this would suggest that there are general differences between males and females in how they organize their movements.

Contact Righting

An analysis of whether sex differences are present in how males and females organize their movement during contact righting, or the rotation from supine to prone on a solid surface, was conducted. Contact righting requires that an animal integrate rotatory movements around the longitudinal axis, of the upper body or forequarters, with the lower body or hindquarters (Pellis, Pellis, & Nelson, 1992; Pellis, Pellis, & Whishaw, 1996; Pellis et al., 1991). Thus, to test whether the differences in the integration of fore- and hindquarter movements are sex- versus task-typical, contact righting was chosen for analysis. In addition, to determine whether sex-differences in this task were due to sex differences in body morphology the behavior of Long-Evans males and females was compared to *tfm* animals and their wild-type controls. The results of this experiment are presented in Chapter 4.

Skilled Reaching

The documentation of sex differences in the integration of movements during dodging to protect a food item and contact righting provide evidence that males and females integrate their movements differently during tasks that occur in the lateral and rotatory planes of movement. To address further whether sex differences in movement organization exist in other planes of movement, such as forward movement, a new task was chosen - skilled reaching (Whishaw & Pellis, 1990). This task was chosen for three reasons: (a) males and females when reaching in the skilled reaching task are trained to move to the back of the test enclosure and only move forward to approach the food, and position themselves to reach for the food item, after it has been placed in the food well,

(b) this task requires the integration of fore- and hindlimb movements, with postural shifts and forward movement, to enable the animal to lift its forepaw up from the substrate to attain the food item, and (c) the analysis of sex differences in the postural adjustments of the fore- and hindquarters could be contrasted to the presence, or not, of sex differences in a skilled forelimb movement - the reach. The question of whether all the movement components of skilled reaching are sexually dimorphic or whether sex differences are restricted to the integration of fore- and hindquarter movements provides a basis for speculation regarding where in the CNS sex differences in movement organization may originate. The results of this experiment are presented in Chapter 5.

Does CNS Injury affect Sex Differences in Movement Organization?

The final experimental work formally presented in this thesis was done to address the question of whether sex differences are present in naturally occurring vertical exploratory behaviors (Gharbawie, Whishaw, & Whishaw, 2004). This allows for an exploration of possible sex differences in the last plane of movement - vertical movement. In addition to addressing the question of whether sex differences are present in vertical rearing and exploration the question of whether sex differences in the organization of movement can be altered by CNS injury, using an animal model of Parkinson's disease (PD), was addressed.

Parkinson's disease is characterized by a loss of dopaminergic neurons in the substantia nigra which leads to difficulties in movement initiation. In general, PD individuals show impairments in movement initiation and gait. They tend to move at a slower walking speed than non PD individuals and have difficulties coordinating vertical posture and locomotion (Ebersbach et al., 1999; Hariz, Lindberg, Hariz, & Bergenheim, 2003; Nieuwboer et al., 1999). While sex differences in the occurrence and progression of PD have been reported (Bower, Maraganore, McDonnell, & Rocca, 2000; Carey, Deskin, Josephson, & Wichmann, 2002; Kimura et al., 2002; Lyons, Hubble, Troster, Pahwa, & Koller, 1998; Scott, Borgman, Engler, Johnels, & Aquilonius, 2000), whether male and female Parkinsonian patients use different movement strategies to compensate for the difficulties that occur during movement initiation and maintenance has not been adequately addressed.

In order to address whether sex differences in the kinematics of vertical behavior are present after CNS injury, the behavior of male and female rats that received a unilateral 6-hydroxydopamine (6-OHDA) nigrostriatal bundle lesion, a rodent model of PD (Schallert, Fleming, Leasure, Tillerson, & Bland, 2000; Tillerson et al., 2002; Tillerson et al., 2001; Whishaw, Coles, Pellis, & Miklyaeva, 1997; Whishaw, Li, Whishaw, Gorny, & Metz, 2003; Whishaw et al., 2002; Woodlee & Schallert, 2004) were compared to sham-operated males and females. Whether sex differences in the postural adjustments made during vertical behavior are altered by CNS injury and whether there are differences in how males and females adjust their posture and movements to compensate for CNS injury was assessed. The results of this study are presented in Chapter 6.

1.6 A Discussion of the Findings Reported in this Thesis

In Chapter 7, the findings presented in this thesis, in conjunction with the findings that have been previously reported describing sex differences in movement organization, will be integrated and discussed. These findings will be discussed in relation to: (a) gonadal steroids, (b) body morphology, (c) the CNS, (d) developmental processes, (e) in relation to other species, (f) genetics, and (g) sex differences in the kinematic organization of human behavior. This discussion will be also include speculations for further research and a theoretical discussion of what sex differences in movement organization may tell us about the evolution of the CNS and its current function in the control of both normal and pathological behavior.

Please Note:

The individual chapter discussions have been kept short. This was done to avoid redundancy with the general discussion that concludes this thesis.

CHAPTER 2*

Neonatal and Pubertal, but not Adult, Ovarian Steroids are Necessary for the Development of Female-Typical Patterns of Dodging to Protect a Food Item

ABSTRACT

Rats protect food by dodging horizontally away from a conspecific. Females and males use different movement and stepping patterns to execute a dodge. An unresolved question is whether exposure to ovarian steroids, in females, is necessary for the development of the female-typical pattern. Females ovariectomized as adults used female-typical patterns of dodging to protect a food item. Females ovariectomized prior to puberty, use a combination of both male and female tactics. Females ovariectomized just after birth used male-typical tactics. Thus, the contribution of ovarian steroids, to the development of female-typical patterns of behavior, needs to be studied further at both a behavioral and neural level with regards to the organization of movement.

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Field, E. F., Whishaw, I. Q., Forgie, M. L., Pellis, S. M. (2004). Neonatal and pubertal, but not adult, ovarian steroids are necessary for the development of female-typical patterns of dodging to protect a food item. Behavioral Neuroscience, 118, 1293-1304.

2.0 Introduction

It has been shown previously that manipulation of testicular hormones immediately after birth can modify the organization of movements used during dodging tactics by male and female rats in adulthood. Females treated with testosterone propionate were more male-like, whereas males who were gonadectomized on postnatal day one were more female-like. In addition, it has been shown that for males, the expression of the male-typical pattern is not dependent on the presence of androgens during the pubertal or adult period (Field et al., 1997b). These results support the classical view that it is the presence or absence of gonadal steroids perinatally (Baum, 1987), that is important for the neural organization of the CNS systems that underlie the expression of sex-typical patterns of behavior.

One question that was not previously addressed however was whether the presence of ovarian steroids in females is necessary for the development of female-typical patterns of dodging. It has been shown that fluctuations in circulating ovarian steroid levels in adults are correlated with changes in their performance on various motoric tasks (Becker et al., 1987; Hampson & Kimura, 1988; Smith, 1998). Further, it has been suggested that the presence of gonadal androgens during puberty can defeminize endocrine function and behavior in female rats (Bloch, Mills, & Gale, 1995; Pellis, 2002). Thus, gonadal steroids during the pubertal period may influence the organization of female-typical patterns of behavior. Given the possible organizational role of gonadal hormones in behavior during puberty in males (Romeo, Rhichardson, & Sisk, 2002) and the evidence that development of female-typical non-reproductive behaviors can be influenced by the removal of ovarian steroids neonatally (Field, Whishaw, & Pellis, 2000; Smith, Forgie, & Pellis, 1998), it is possible that the presence of ovarian steroids either neonatally, during puberty, or in adulthood, may be necessary for the expression of female-typical patterns of dodging to protect a food item.

In this experiment the question of whether the presence of ovarian steroids is necessary for the development of female-typical patterns of dodging to protect a food item, was addressed using females whose ovaries were removed just after birth, just prior to the onset of puberty, (Brown, Hochberg, Naftolin, & MacLusky, 1994) or in adulthood. A number of the behavioral aspects of the social interaction of the dodging animal with the conspecific were also examined.

It has been shown previously that the orientation of the dodging animal to the conspecific at the end of the dodge is sexually dimorphic; males generally align their pelvis to the head of the robber, whereas females generally align their pelvis to its midbody (Field et al., 1997b; Pellis, Field, & Whishaw, 1999). Furthermore, the sex difference in the final alignment of the dodging animal to the robber is independent of the dodge pattern used (Pellis et al., 1999). The dynamics of the interaction between the animals were analyzed to determine whether the social aspects of the dodger-robber interaction were dissociable from possible motoric changes in female-typical patterns of dodging following the absence of pre- and post-pubertal ovarian steroids.

2.1 Methods

2.1.1 Subjects

Thirty two female and eight male Long-Evans rats were used. The animals were housed in same condition pairs, in clear Plexiglas cages (17x25x20 cm), from weaning, in accordance with the Canadian Council on Animal Care (1984) guidelines. They were maintained in the main colony room on a 12-hr light-dark cycle until adulthood, and were tested between 6 and 8 months of age. Subjects were maintained on a limited feeding schedule of approximately 20-30g/day (Purina Rodent Chow) during testing, to maintain their body weight at about 80-85% of their free feeding weight.

2.1.2 Surgery

The females were divided into four groups of eight animals each. The neonatal ovariectomized group (NOVX) was ovariectomized within 24 hours of birth. The pre-pubertal (POVX) group was ovariectomized between postnatal days 38 and 40, just after vaginal opening, which generally coincides with the initial preovulatory gonadotrophin expression that precedes regular ovulatory cycles and thus the onset of puberty (Brown et al., 1994). The female control group received a sham surgery at this time. The adult (AOVX) group was ovariectomized on postnatal day 90.

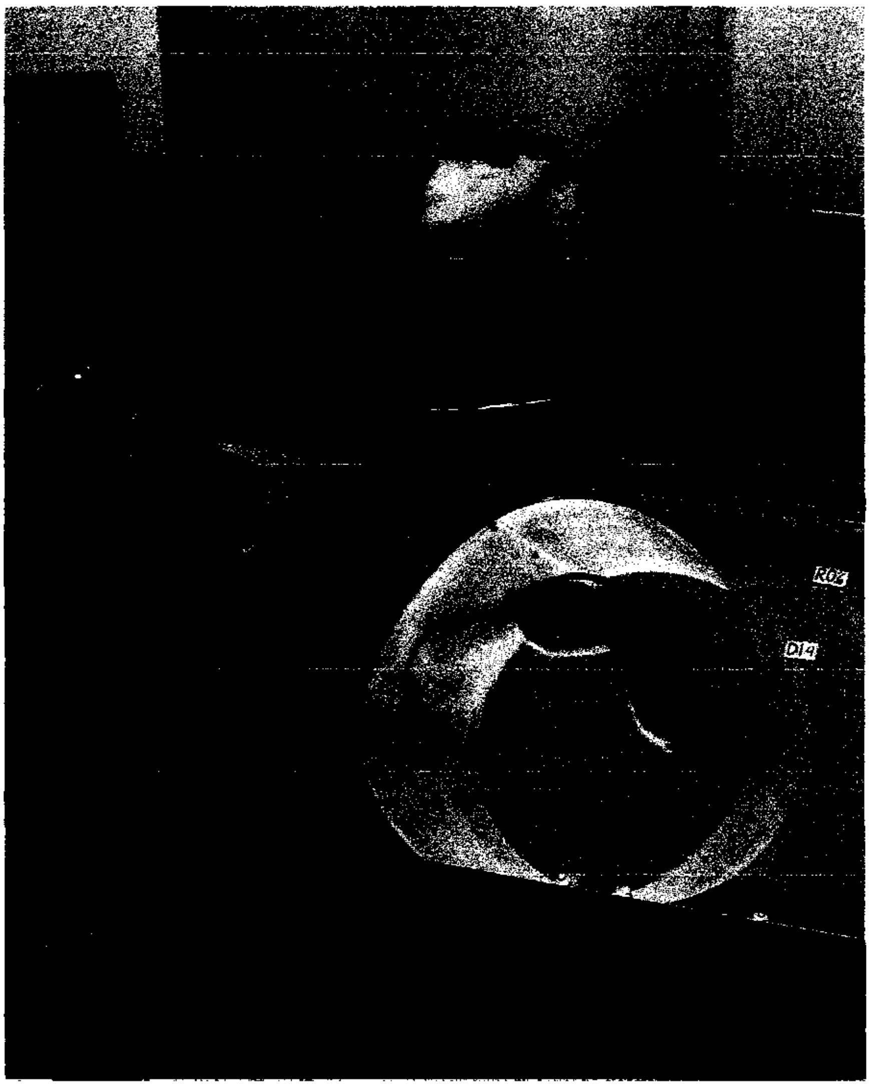
For the NOVX group, the animals were anesthetized with hypothermic anesthesia by placing them on ice until they no longer responded to a foot or tail pinch. All animals in the control, POVX and AOVX groups were anaesthetized using Isofluorane anesthesia (isoflurene USP, Bimeda MTC, Animal Health, Cambridge, Ontario, Canada). Once the animals were anaesthetized, they were shaved (POVX, AOVX and controls) and cleaned with antiseptic soap. Two incisions, each approximately 1-2 cm long, lateral to the spinal cord, were used to expose the ovaries. At this point the abdominal and dermal incision of the females receiving sham surgeries was closed using a series of single, interrupted silk sutures. For the NOVX group the ovaries were removed by blunt dissection, for both the POVX and AOVX groups the uterine tube and ovarian ligaments were ligated and transected and the ovaries removed by blunt dissection. The abdominal wall and dermal incision were then closed as in the sham surgery.

2.1.3 Testing and Video Recording

All animals were tested between postnatal days 120 and 140. Trials were conducted between 0900 and 1200 hours. Each pair was placed in a thin Plexiglas container, 40 cm in diameter and 45 cm high. The cylinder was placed on a table with a clear glass top. Under the table was a mirror inclined at 45 degrees from which the ventral side of the animals could be viewed and videotaped (Pinel, Hilton Jones, & Whishaw, 1992) (Fig. 5).

For both habituation and testing, a single food pellet, weighing approximately 2.5g, was placed in the testing apparatus with two-same condition cage mates. Habituation was complete when shortly after being placed in the cylinder, one of the rats began to eat and the other attempted to steal the food. Once one of the rats had completed at least 10 dodges of 135 degrees or more, the remaining food was removed and a new piece was given to the rat that had previously attempted to rob the food pellet. The trial continued until 10 dodges were obtained from both animals of each pair. Video recordings were made of the dodging trials with a Sony Hi-8 Camcorder at a shutter speed of 1/250s. Additional light was provided by two 150-W spotlights on the ceiling 125 cm above the glass table. The Hi-8 video was subsequently transferred to a Sony VHS tape and a digital time code was added using a TRG-50 Horita Micro Window.

Figure 5



The testing apparatus and filming setup are depicted for the dodging to protect a food item task. The rats are placed, in pairs of two, in a Plexiglas cylinder on a clear table top. An incline mirror is positioned at 45 degrees beneath the table top. The ventral view of the rats is then videotaped, from the mirror. Tapes of the behavioral sequences are then later analyzed frame-by-frame.

2.1.4 Behavioral Analysis

Analysis of a dodge began on the video frame where the first movement of the snout was visible. Analysis continued until the rat had realigned its fore- and hindquarters and had resumed eating. For each animal, the trajectories of the snout and pelvis were traced onto transparencies, and the distance of each trajectory was calculated, for the first five dodges of 135 degrees or greater, for each rat. In addition, the stepping pattern and direction of the hindpaw steps were recorded and the final orientation of the pelvis of the dodging animal to the robber's body was noted. Finally, the angle of approach and the distance between the snout of the dodging animal and the robber were recorded at the frame at which the dodge was initiated.

Quantification of the Distance Traveled by the Pelvis in Relation to the Snout: The distance traveled by the base of the tail (pelvis; y) was divided by the distance traveled by the snout (x). The resulting value was subtracted from 1 and the result was multiplied by 100 to obtain the percentage difference in distance traveled by the snout relative to the pelvis. The larger the percent difference, the more distance traveled by the snout as compared to the distance traveled by the pelvis (See Field et al., 1996a for more details).

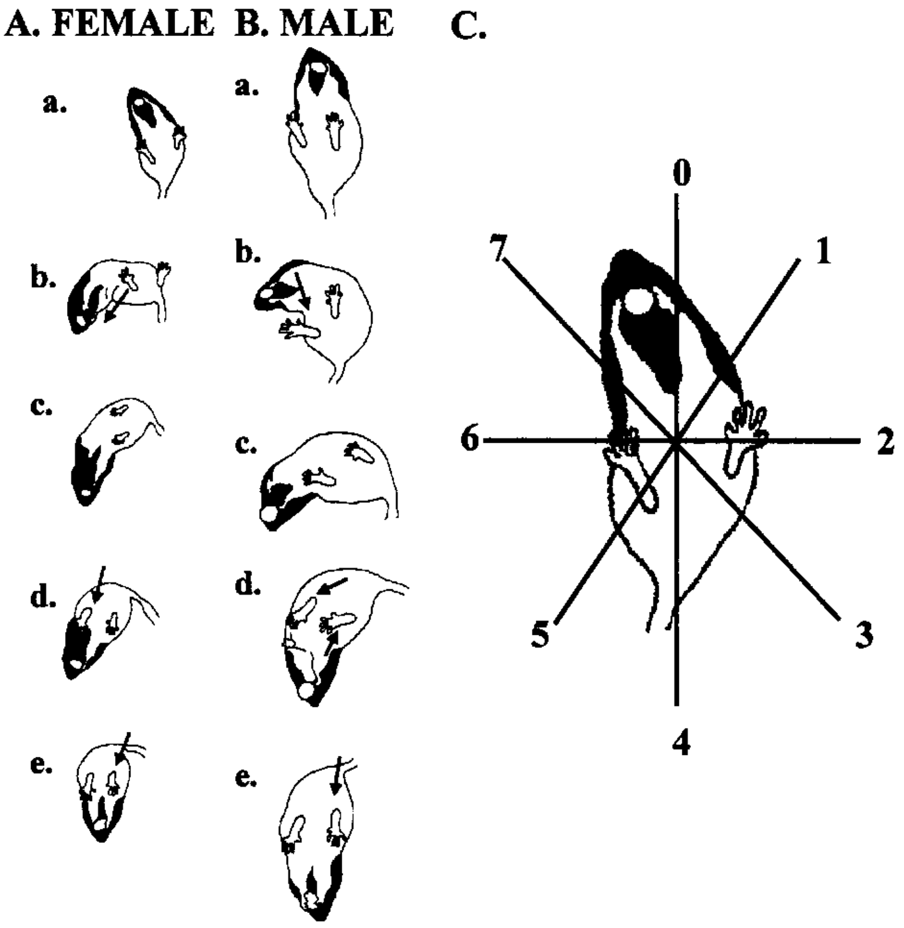
Analysis of the Hindpaw Stepping Patterns: It has previously been reported that male and female rats differ in both the number of hindpaw steps and the pattern of these steps when dodging away from a conspecific (Field et al., 1996a). Furthermore, it has been shown that the pattern of hindpaw stepping is modifiable by postnatal androgens (Field et al., 1997a). The difference in the stepping patterns is illustrated in Figures 6 A and B. After turning laterally, (Aa-b) females make an initial forward and sideways step with the hindpaw ipsilateral to the direction of the dodge (Ab). This step is followed by a forward step by the hindpaw contralateral to the direction of the dodge (Ad) and a final forward step by the hindpaw ipsilateral to the direction of the dodge (Ae). In contrast, males use less lateral movement of the upper body (Ba) before taking a backward step with the hindpaw ipsilateral to the direction of the dodge (Bb). This is often followed by either, or both, a second backward step by the ipsilateral hindpaw (Bd) and a sideways step of the contralateral hindpaw into the approaching conspecific (Bd). The ipsilateral hindpaw then makes a final forward step (Be). To describe the pattern of hindlimb stepping, a simplified version of the Eshkol-Wachmann Movement Notation (EWMN) system was used (Eshkol & Wachmann, 1958). This system enables the observer to label numerically the direction of the hindpaw steps in relation to the position of the body at the time of the step. The numerical values were then compared to determine whether differences existed in the direction of the locomotor steps by the hindpaws.

To determine whether the stepping sequences made by the NOVX, POVX or AOVX animals were more male or female typical a note of the sequence that accompanied each dodge was made. Each step during the dodge was described as forward, backward, sideways, or at 45 degrees in between the three directions. This was achieved by adapting aspects of the EWMN system. Steps were described numerically in relation to the body at the time of the step. The direction of each hindpaw step was recorded in the horizontal plane with intervals of 45 degrees on the circumference. The intervals are read in a clockwise direction from 0, 1, 2, 3, 4, 5, 6, 7, and back to 0. The circle is placed so that 0 is always aligned with the snout and 4 with the pelvis. As the rat moves, the circle maintains the same orientation to the body (Fig. 6C). The direction of the hindpaw steps was then scored by notating the number that corresponded closest to the direction of the step. For example, a step backwards toward the pelvis is notated as 4. For numerical comparison, dodges to the right and left were all converted to the right. Therefore, a step to the left of the body (i.e., 6) was converted to the right side of the body (i.e., 2). Similarly, a step to 7 was converted to 1 and a step to 5 was converted to 3 (Eilam & Golani, 1988).

The Orientation of the Dodging Animal to the Robber at Dodge Completion: It has previously been shown that the orientation of the hindquarters of the dodging animal, in relation to the body of the robber at the end of a dodge, is also sexually dimorphic (Fig. 7) (Field et al., 1997b; Pellis et al., 1999). Female rats orient their hindquarters to the midbody of a robbing animal at the end of the dodge. In contrast, males orient their hindquarters to the head of the robbing animal. Thus, for each animal, the orientation of the dodging animal, to the head, midbody, or pelvis of the robber, is scored at the end of the dodge sequence.

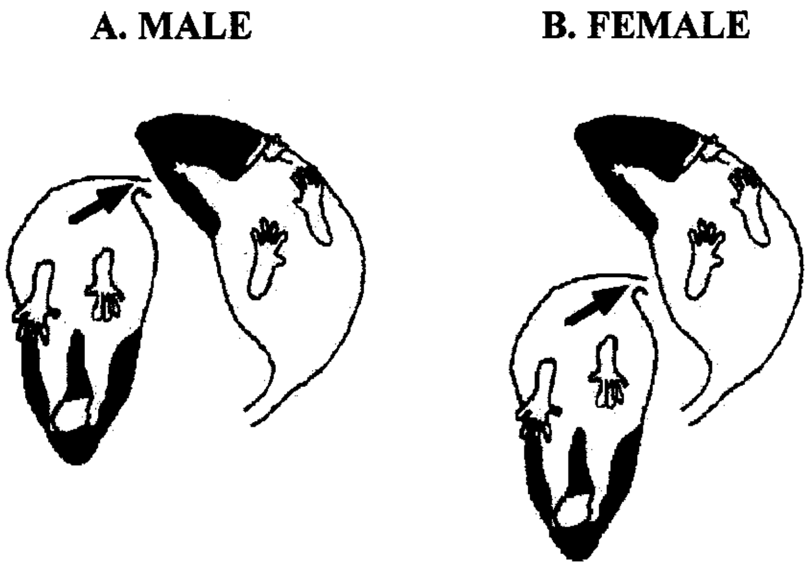
Analysis of the Robber's Behavior: Two measurements were taken of the robber's behavior during each dodge trial. This analysis was done to determine whether differences in the robber's behavior could account for the differences in the dodging patterns seen by the animals in each condition.

Figure 6



The number and direction of the steps taken by the hindpaws during a typical female (6A) and male (6B) dodge are shown. A simplified version of the Eshkol-Wachmann Movement Notation System is shown (6C). This system was used to analyze and compare the direction of the initial hindpaw steps (see Methods section for a description of the stepping patterns and the EWMN analysis; adapted from Field et al. 1997a).

Figure 7



The final position of the dodger with respect to the robber is shown here. The final, male-typical, position of the dodging rat is shown with its pelvis aligned to the head of the robber (7A). The final, female-typical, position of the dodging rat is shown with its pelvis aligned to the midbody of the robber (7B) (adapted from Field et al. 2004).

These were: (a) the angle between the longitudinal axis of the dodger's body and the robber's body, and (b) the distance on the video frame between the tips of the snouts of the dodging and robbing animals where the initiation of the dodge was visible (Pellis et al., 1999).

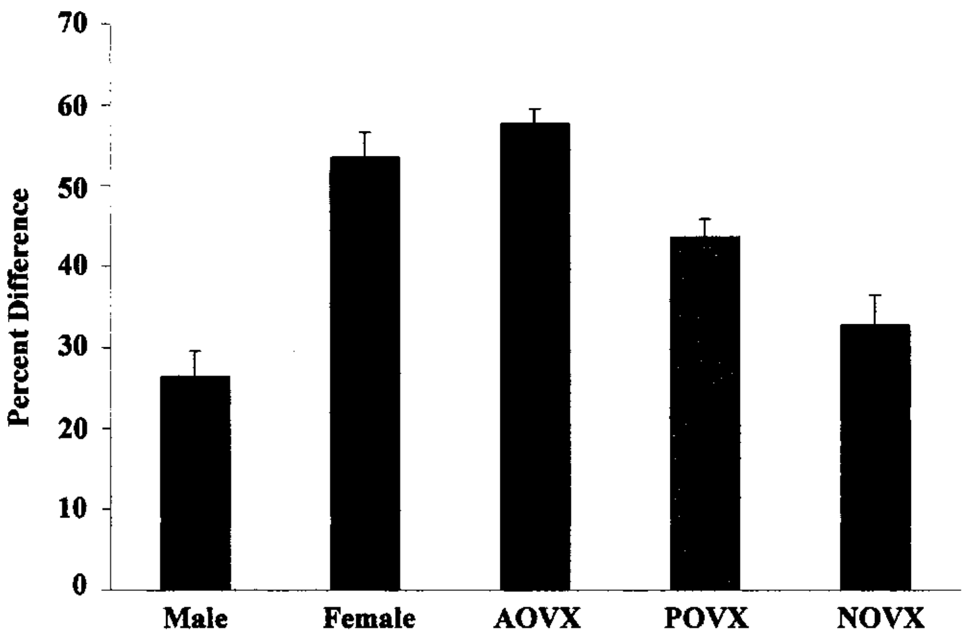
2.1.5 Calculation of Group Means

For each of the measures taken, group means and standard errors were calculated from the individual values obtained for each rat. The data were analyzed using analyses of variance followed by Fisher's partial least-squares difference post-hoc analyses for pairwise comparisons. For both kinds of tests, $p<0.05$ was the level for determining significant effects. For graphical purposes the mean and standard error of the mean were used.

2.2 Results

Distance Traveled by the Snout in Relation to the Pelvis: Dodging female rats have a large lateral displacement of the snout with only a small displacement of the pelvis (see Fig. 4A). In contrast, dodging male rats have a large displacement of the pelvis relative to the lateral displacement of the snout (see Fig. 4B). Comparison of the relative distance traveled by the pelvis as compared to the snout revealed significant group differences, $F(4,35)=20.35$, $p<0.0001$ (Fig. 8). Post hoc analysis showed that male Long-Evans rats were significantly different from the POVX, AOVX and female controls, but not the NOVX. Intact females were not significantly different from AOVX but were significantly different from POVX and NOVX females. The POVX females were significantly different from all groups.

Figure 8



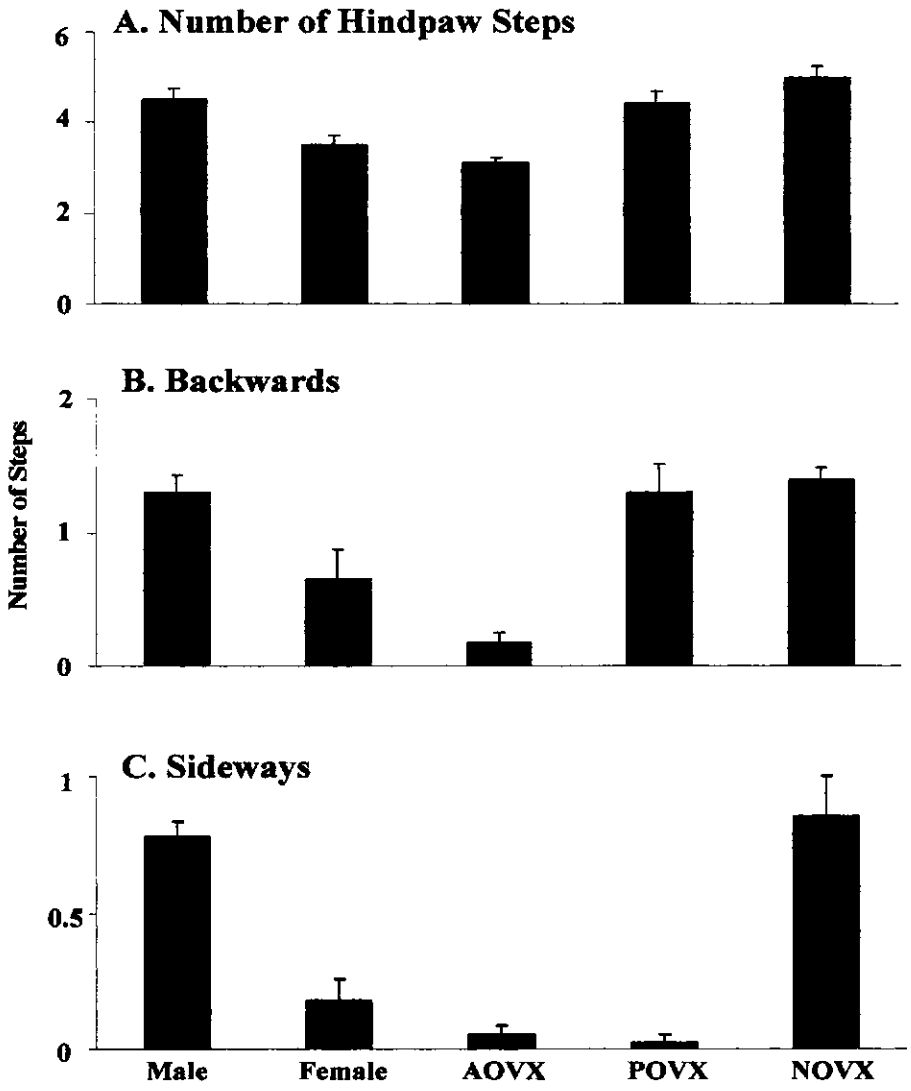
The difference, as a percentage, in the distance traveled by the snout relative to the pelvis is shown. The males and NOVX groups, while not significantly different from one another were significantly different from all other groups. The AOVX and intact females were not significantly different from each other but were different from the male and NOVX groups. The POVX group was significantly different from all other groups. **(Note: For this and all succeeding figures similar colors within the histogram bars represent groups that are not significantly different from one another. Different colors reflect significant differences between experimental groups.)**

Analysis of hindpaw stepping patterns: Hindpaw steps were compared both for frequency and placement. Comparisons of placement were made for the initial hindpaw steps that frequently occur during the dodge sequence. These were: (a) the number of backwards steps made by the hindpaw ipsilateral to the direction of the turn during the dodge (see Fig. 6Bb & Bd), and (b) the frequency of a sideways step, into the conspecific, made by the hindpaw contralateral to the direction of the turn (see Fig. 6Bd).

Number of Steps Taken by the Hindpaws: There were significant differences in the overall number of hindpaw steps used to complete a dodge F(4,35)=11.89, $p<0.0001$. Males, NOVX and POVX females used a significantly greater number of steps than intact females and AOVX females (Fig. 9A).

Direction of Hindpaw Steps: There were significant group differences in the patterns of stepping seen both for the number of backwards steps towards the pelvis by the paw ipsilateral to the direction of the turn F(4,35)=11.35, $p<0.0001$ (Fig. 9B), and for the likelihood of a step towards the robbing animal with the hindpaw contralateral to the direction of the dodge F(4,35)=24.02, $p<0.0001$ (Fig. 9C). Intact and AOVX females made significantly fewer steps backwards toward their pelvis than males, NOVX and POVX females. In contrast, only the males and NOVX groups were significantly more likely to make a step into the approaching conspecific with the hindpaw contralateral to the direction of the dodge.

Figure 9



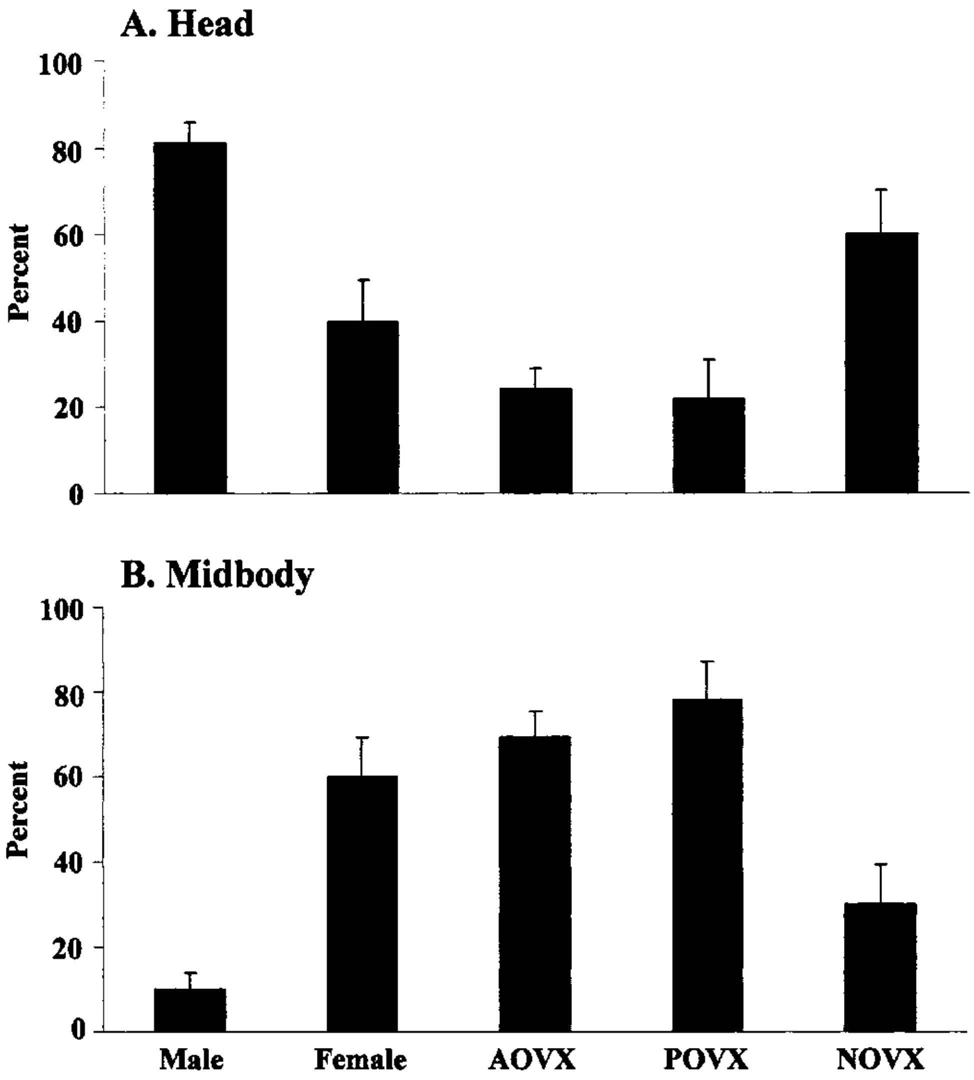
The difference in the overall number of hindpaw steps is depicted in Figure 9A. Males, NOVX and POVX animals all made significantly more hindpaw steps than intact and AOVX females. Males, NOVX and POVX animals were all significantly more likely to use an initial backward step of the hindpaw than intact females and AOVX animals (9B). In contrast, only the male and NOVX groups made significantly more steps into the robber with the contralateral hindpaw than the intact, POVX and AOVX females (9C).

Orientation of the Dodging Animal to the Conspecific at Dodge End: For all groups, the orientation of the pelvis to the body of the robber at the end of the dodge was notated. It has previously been shown that females typically align their pelvis to the midbody, (see Fig. 7B), whereas males typically orient their pelvis to the head of the approaching conspecific (see Fig. 7A) (Field et al. 1997b). Significant group differences were found in the likelihood of a dodging animal to orient their pelvis to the head of the conspecific $F(4,35)=10.18$, $p<0.001$ (Fig. 10A) or the midbody $F(4,35)=13.35$, $p<0.0001$ (Fig. 10B). Males and NOVX animals oriented their hindquarters to the head of the dodging animal significantly more than POVX, AOVX and control females. In contrast, the POVX, AOVX and female controls were significantly more likely to orient their pelvis towards the midbody of the conspecific, than NOVX and males, at the end of the dodge. There were no significant differences in the orientation of the dodger's hindquarters to the rump of the conspecific ($p>0.05$).

Angle of Approach of the Robber Relative to the Dodging Animal: There were no significant differences in the angle of approach of the conspecific to the dodging animal for any of the conditions ($p>0.05$).

Distance between the Snout of the Robber and Dodger at the Initiation of a Dodge: Significant group differences were found on this measure $F(4,35)=6.28$, $p<0.001$. Males and NOVX groups began to dodge at a significantly greater distance from the approaching conspecific than POVX, AOVX and control females.

Figure 10



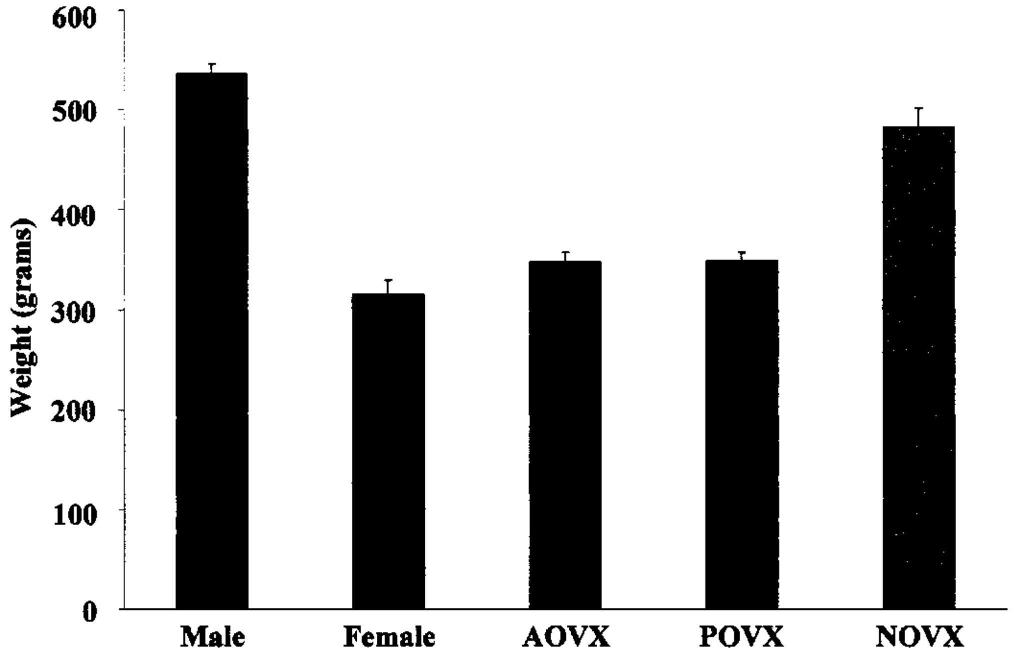
Males and NOVX animals were significantly more likely to align their pelvis with the head of the robber (10A). In contrast, control females, POVX and AOVX animals were more likely to orient their pelvis to the midbody of the robber (10B).

Body Weight: There were significant group differences in the average, free feeding, weight of each group F(4, 35)=56.057, $p<0.001$ (Fig. 11). Males were significantly heavier than all the female conditions. The NOVX group was also significantly heavier than all other female conditions. The POVX, AOVX and control females were not significantly different from one another.

2.3 Discussion

This experiment reveals that the absence of ovarian steroids either neonatally or during puberty, but not in adulthood, results in dodge patterns that are characterized by aspects of movement and stepping that are more typical of a male pattern of organization. Females ovariectomized as neonates were not significantly different from males on any measure. In contrast, females ovariectomized just prior to puberty exhibited dodge patterns that contained both female- and male-typical elements. This shows that the presence of ovarian steroids, either neonatally or during puberty, is necessary for the development of female-typical patterns of dodging. The removal of ovarian steroids in adulthood had no effect on the expression of the female-typical pattern. This suggests that the effects of ovariectomy prior to puberty are not due to activational effects of circulating ovarian steroids in adulthood, but rather, may be due to either the direct or the indirect organizational effects of ovarian steroids on the developing female phenotype.

Figure 11



The average body weight is depicted. The males were significantly heavier than all female groups. The NOVX animals, while significantly heavier than the POVX, AOVX and intact females, were significantly lighter than males.

Previous work has shown that following ovariectomy females will eat more and thus become significantly heavier than control females (Landau & Zucker, 1976). This finding was confirmed in the present study were it was found that the average weight of the NOVX group, whose dodge pattern was male-like, was significantly higher than the average weight of the POVX, AOVX, and control female groups. It is possible then that the differences in dodge patterns between the different female conditions are due, in part, to differences in the overall mass of the females. It is unlikely, however, that this can be the sole explanation. Differences in dodge patterns were also found between the POVX, AOVX and female controls, with the POVX group using a pattern of dodging that was composed of both male- and female-typical elements. These three groups, however, were not significantly different in their average weight. Further, although the NOVX females were significantly larger than the POVX, AOVX and females controls, they were still significantly smaller than the control males. Thus, whereas the overall mass of the dodging animal may influence the type of dodge pattern used, it is unlikely to be the sole explanation.

Finally, it has been shown previously that the organization of the motor and social components of dodging to protect a food item is dissociable (Field et al., 1997b; Pellis et al., 1999). In this experiment, in addition to showing that the dodge patterns used are influenced differentially by removal of ovarian steroids neonatally or pre-pubertally, it has been shown that the interaction of the dodging animal with the robber is also influenced by the absence of ovarian steroids. The orientation of the dodging animal to the conspecific remains female-typical with the removal of ovarian steroids, either just prior to puberty or in adulthood. In contrast, the removal of ovarian steroids neonatally leads to a more male-typical pattern of orientation. This suggests that the organization of a number of aspects of female behavior, during dodging to protect a food item, may be dependent on the presence of ovarian steroids during different critical periods.

Chapter 3 -Part A*

The Effects of Body Mass on the Expression of Sex Differences in Dodging to Protect a Food Item: An Analysis of Gravid Females

ABSTRACT

Previously it has been shown that males and females differ in how they organize their movements during turning to protect a food item from theft by a conspecific. While the role of gonadal steroids in the development of this sex difference has been studied the potential contribution of body mass differences has not. In this study gravid females that were significantly larger than nulliparous females but do not differ in mass from male controls were studied. Gravid females did not differ from nulliparous female in their kinematic composition of dodge patterns, they were however significantly different from intact males. This experiment provides evidence that sex differences in movement patterns during dodging to protect a food item are not due solely to differences in body mass and thus are likely mediated by the CNS.

*This chapter is modified and reprinted, with permission by the American Psychological Association, [© 2004], from:

Field, E. F., Whishaw, I. Q., Forgie, M. L., Pellis, S. M. (2004). Neonatal and pubertal, but not adult, ovarian steroids are necessary for the development of female-typical patterns of dodging to protect a food item. Behavioral Neuroscience, 118, 1293-1304.

3.0(A) Introduction

This experiment was conducted to determine whether the differences seen in the movement patterns exhibited by the female control and OVX groups of Chapter 2 could be explained by differences in body weight. It is possible that the male-typical pattern of dodging is more likely to be expressed in animals that are larger. Since both the males and neonatally ovariectomized (NOVX) groups were significantly larger than control, pre-pubertal and adult ovariectomized females the expression of the male-typical patterns of dodging in the male and NOVX groups may be related to differences in overall body mass.

To test this hypothesis, dodging to protect a food item was analyzed in males, nulliparous and gravid females (G19/20). If sex-typical patterns of dodging are determined by body mass one would predict that gravid females, that are similar in mass to control males, would be more male-like in their organization of movement and hindpaw stepping during dodging. Conversely, if body mass is not a relevant factor then gravid females should be more female-typical in their kinematic organization of dodging to protect a food item.

3.1(A) Methods

3.1.1(A) Subjects

Six adult male and twelve adult female, six nulliparous and six gravid (G19/20), Long-Evans rats were used. The animals were housed in same condition pairs, in Plexiglas cages, in accordance with the Canadian Council on Animal Care (1984) guidelines. They were maintained in the main colony room on a 12-hr light-dark cycle with food and water ad libitum.

3.1.2(A) Testing, Video Recording, and Behavioral Analysis

Trials were conducted using the same testing apparatus that was described in Chapter 2 (See Fig. 5). For both habituation and testing, however a new paradigm was used to maintain the large body mass of the gravid females. Two weeks of food deprivation are not necessary to elicit dodging behavior when a highly palatable food item is used. Furthermore, the magnitude of a dodge is dependent on the length of time it takes to eat the food item (Whishaw & Gorny, 1994). Therefore, whole almonds, which are highly palatable and hard, were used to induce dodging in animals that were not chronically food deprived.

All the animals were placed in the testing arena for four, one half hour sessions, one in the morning and one three hours later over a two day period. The first three sessions were to habituate the animals to the testing arena and the almonds. Following the first session all animals were observed to eat almonds and exhibit dodges. All the animals were food deprived for the three hours between the last habituation session and the fourth session to facilitate the occurrence of dodging. The fourth session was videotaped for subsequent analysis.

During the testing session almonds were placed in the testing arena individually and the animals were filmed until each animal had exhibited 5 dodges each. Video recordings were made of the dodging trials with a Sony Hi-8 Camcorder at a shutter speed of 1/250s. Additional light was provided by two 150-W spotlights. The Hi-8 video was subsequently transferred to a Sony VHS tape and a digital time code was added using a TRG-50 Horita Micro Window. All the behavioral analysis was done as described in Chapter 2.

3.1.3(A) Calculation of Group Means

For each of the measures taken, group means and standard errors were calculated from the individual values obtained for each rat. The data were analyzed using analyses of variance followed by Fisher's partial least-squares difference post-hoc analyses for pairwise comparisons. For both kinds of tests, $p<0.05$ was the level for determining significant effects. For graphical purposes the mean and standard error of the mean were used.

3.2(A) Results

Distance Traveled by the Snout in Relation to the Pelvis: Dodging female rats, gravid or not, had a large lateral displacement of the snout with only a small displacement of the pelvis. In contrast, male rats had a large displacement of the pelvis. Comparison of the relative distance traveled by the pelvis as compared to the snout revealed significant group differences, $F(2,15)=20.35$, $p<0.0001$ (Fig. 12). Post hoc analysis showed that male rats were significantly different from both the nulliparous and gravid females. Nulliparous and gravid females were not significantly different from one another.

Analysis of Hindpaw Stepping Patterns: Hindpaw steps were compared both in frequency and placement. Comparisons of placement were made for the initial hindpaw steps that frequently occur during the dodge sequence. These were the number of backwards steps made by the hindpaw ipsilateral to the direction of the turn during the dodge (see Fig. 6Bb & Bd) and the frequency of a sideways step, into the conspecific, made by the hindpaw contralateral to the direction of the turn (see Fig. 6Bd).

Number of Steps Taken by the Hindpaws: There were no significant group differences in the overall number of hindpaw steps used to complete a dodge F(2,15)=2.319, p=0.13.

Direction of Hindpaw Steps: There were significant group differences in the likelihood of stepping both for the number of backwards steps towards the pelvis by the paw ipsilateral to the direction of the turn F(2,15)=4.57, p<0.05 (Fig. 13A), and for the likelihood of a step towards the robbing animal with the hindpaw contralateral to the direction of the dodge F(2,15)=20.74, p<0.0001 (Fig. 13B). For the initial steps made by the hindpaw ipsilateral to the direction of the dodge, nulliparous and gravid females made significantly fewer steps backwards, toward their pelvis, than males. The males were significantly more likely to make a step into the approaching conspecific with the hindpaw contralateral to the direction of the dodge than either the nulliparous or the gravid females.

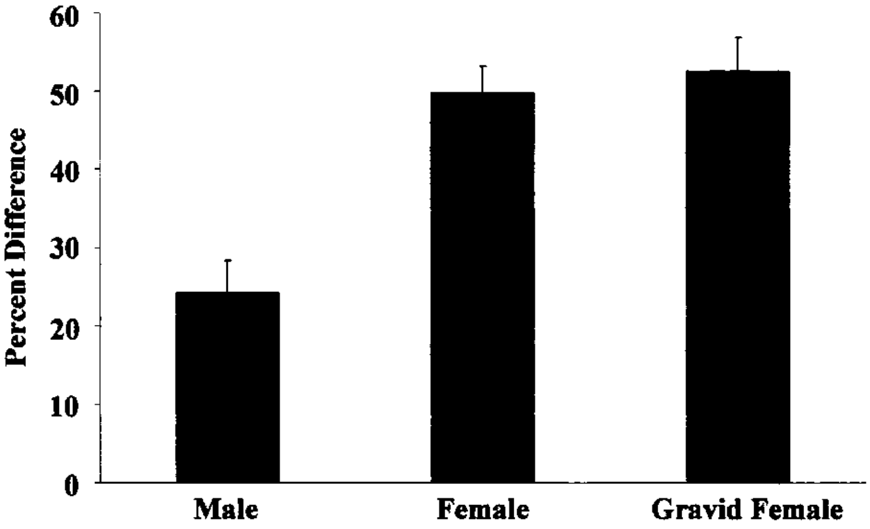
Orientation of the dodging animal to the conspecific at dodge end: For all groups, the orientation of the pelvis to the body of the robber at the end of the dodge was notated. It has previously been shown that females typically align their pelvis to the midbody (see Fig. 7A), whereas males typically orient their pelvis to the head of the approaching conspecific (see Fig. 7B) (Field et al. 1997b). Significant group differences were found in the likelihood of a dodging animal orienting their pelvis to the head of the conspecific F(2,15)=32.884, p<0.0001 or the midbody F(2,15)=26.373, p<0.0001. Males oriented their hindquarters to the head of the dodging animal significantly more than gravid and nulliparous females. In contrast, the nulliparous and gravid females were significantly more likely to orient their pelvis towards the midbody of the conspecific than were males (Fig. 14A and B). There were no significant differences in the orientation of the dodger's hindquarters to the rump of the conspecific ($p>0.05$).

Body Weight: There were significant group differences in body weight at the time of testing $F(2, 15)=54.892$, $p<0.001$ (Fig. 15). Males and gravid females were significantly heavier than nulliparous females but were not different from one another.

3.3(A) Discussion

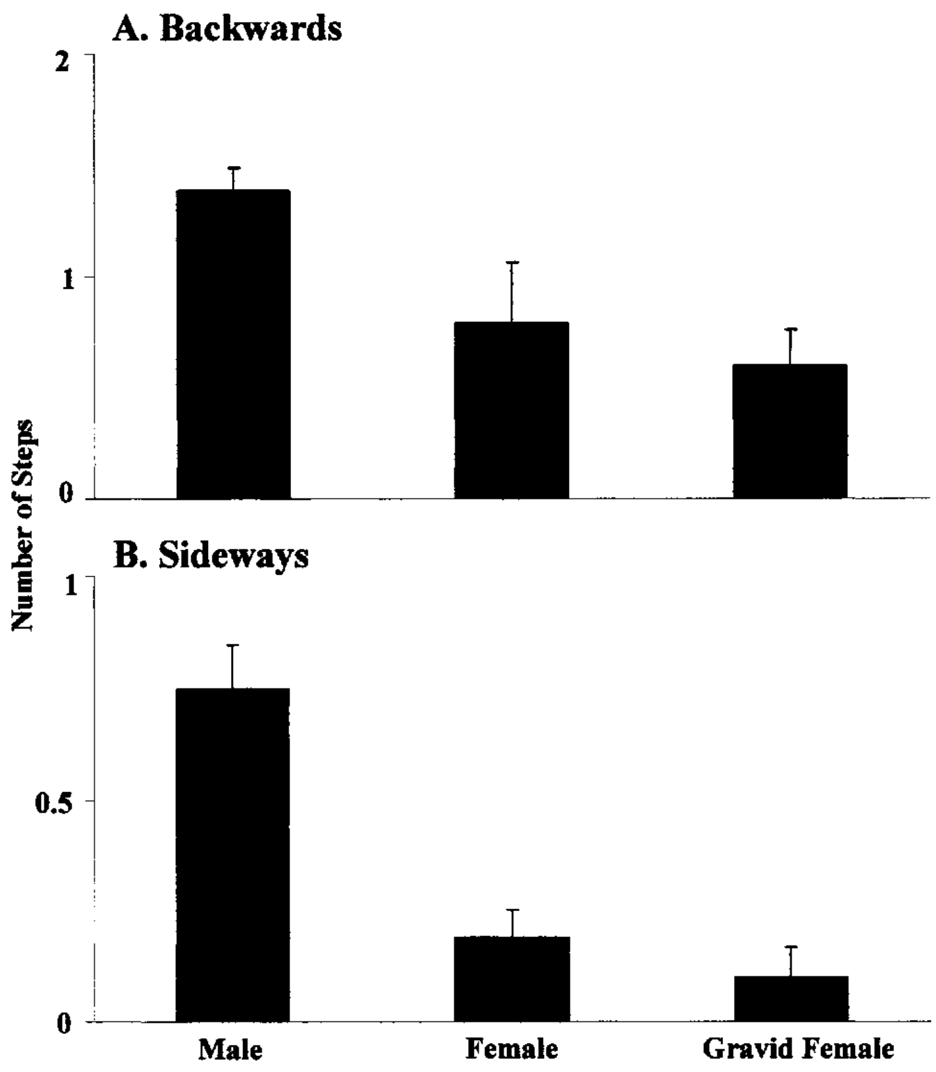
This study was done to confirm that body mass could not be the sole explanatory variable for the expression of sex-typical patterns of dodging. The kinematic organization of dodging in gravid females just prior to giving birth, at a time when they do not differ in body weight from adult males but are significantly larger than nulliparous females, was analyzed. The patterns of movement organization during dodging in gravid females were indistinguishable from the female-typical pattern used by the smaller nulliparous females. Thus, differences in body mass cannot explain the use, in males and females of sex-typical patterns of dodging. These data suggest that sex differences in movement organization may be determined by the CNS.

Figure 12



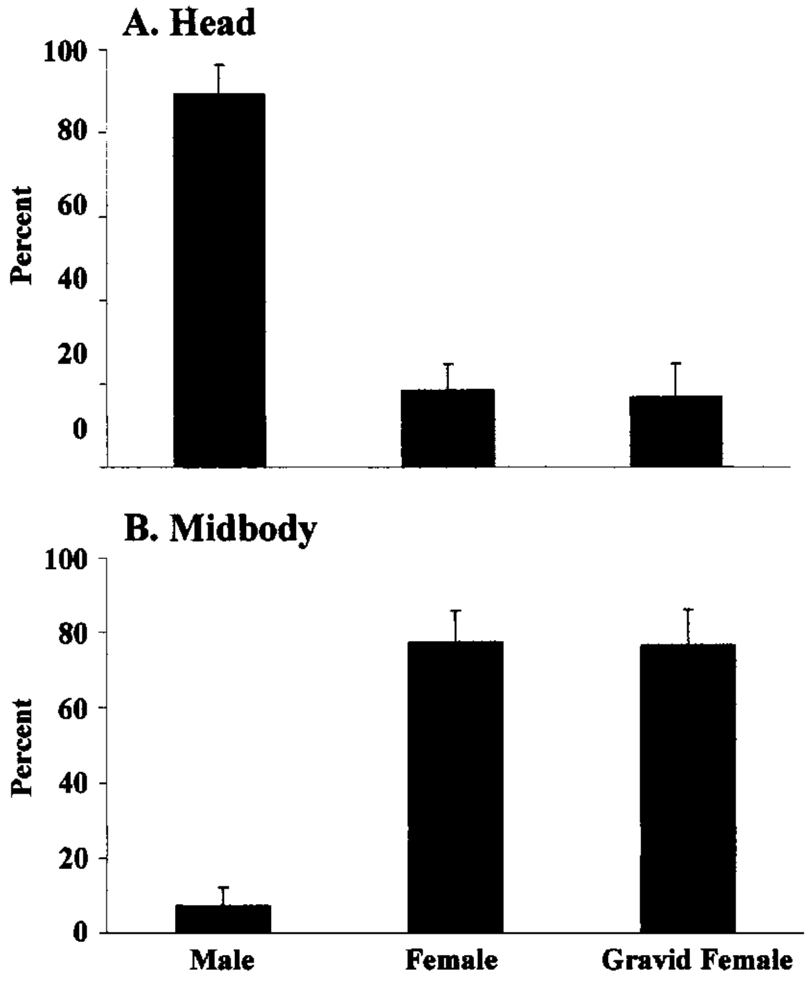
The difference, as a percentage, in the distance traveled by the snout relative to the pelvis is shown. The mean difference was significantly less for the males than the nulliparous and gravid females who did not differ from one another.

Figure 13



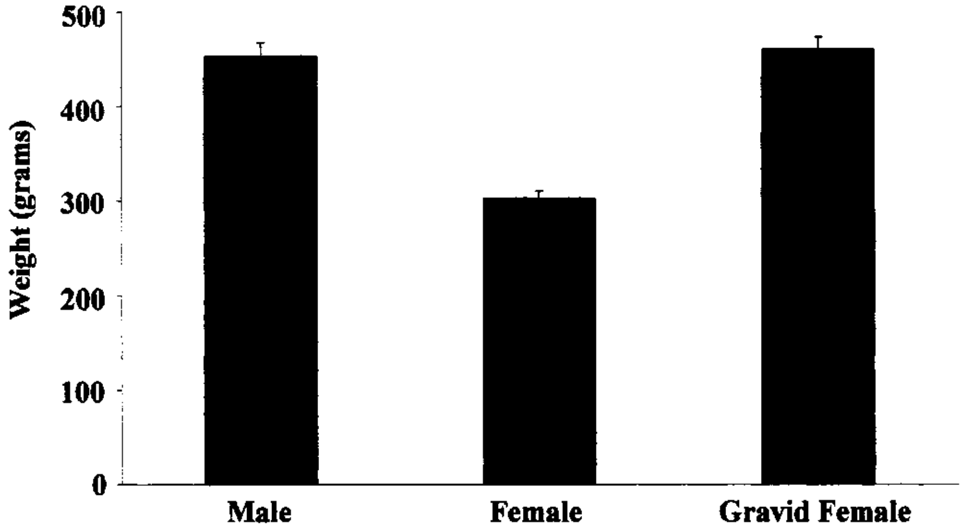
Males were significantly more likely to use backward steps (13A) of the ipsilateral hindpaw and made significantly more steps into the robber with the contralateral hindpaw (13B) than nulliparous and gravid females. The two female groups were not significantly different from one another.

Figure 14



Males were significantly more likely to align their pelvis with the head of the robber (14A). In contrast, nulliparous and gravid females were more likely to align their pelvis to the midbody of the robber (14B).

Figure 15



The average body weight of the nulliparous females was significantly less than that of males and gravid females. Males and gravid females were not significantly different from one another.

Chapter 3 - Part B*

The Role of Body Morphology in the Expression of Sex Differences in Dodging to Protect a Food Item: The *tfm* Model

ABSTRACT

Although sex differences in movement have been documented in rodents, the extent to which it relates to dimorphic neural control versus dimorphic body mass/structure is unclear. It has previously been shown that male and female rats are sexually dimorphic with regards to the lateral movements and hindpaw stepping they use to protect a food item. The question of whether this sexual dimorphism is due to sex differences in peripheral skeletomusculature or in the CNS was examined in *tfm* males and their wild-type male and female controls. The *tfm* male is a genetic male which develops internal testes that secrete testosterone, but is phenotypically female due to a failure of androgen receptor mediated masculinization of the periphery. Masculinization of the CNS of *tfm* males, however, is primarily accomplished by the actions of testosterone's aromatized metabolite, estradiol, acting via estrogen receptors. Thus the *tfm* male provides an assay by which the relative contributions of the skeletomusculature or CNS to sex differences in movement organization can be addressed. It was found that female wild-type animals were significantly different from both the *tfm* and wild-type males. There were no significant differences in dodge patterns used by *tfm* males and their wild-type male controls. This study provides evidence that the sex differences in dodging patterns are mediated primarily by CNS mechanisms and are not dependent on a male- or female-typical skeletomusculature.

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Field, E. F., Watson, N. V., Whishaw, I. Q., Pellis, S. M. (2005). A masculinized skeletomusculature is not necessary for male-typical patterns of food protective movement. Hormones and Behavior, 47, 49-55.

3.0(B) Introduction

As described in Chapter 2 and 3A, dodging to protect a food item, in Long-Evans rats, is sexually dimorphic. The dodge patterns used by male and female rats, while functionally similar, are comprised of different combinations of hindpaw stepping and movements of the body (Field et al. 1996a; 1997a; b). The sexual dimorphism in dodging can be modified by manipulating gonadal hormones neonatally (Field et al. 1997a, 2004). What is not clear, however, is whether the changes seen with neonatal hormone manipulations are due to changes within the CNS or are due to changes in the peripheral skeletomusculature. It is possible that the different patterns of dodging seen in male and female adult rats are due to sex differences in the periphery and not the CNS. While the data from Chapter 3A does not support this conclusion, with regards to the question of body mass, it does not address the question of whether sex differences in skeletomusculature are a contributing factor.

In the present study *tfm* male rats were used to evaluate how differences in peripheral skeletomusculature, in contrast to CNS mechanisms, contribute to sex differences in the organization of food-protective movements. The testicular feminized mutation has been documented in a number of species including horses (Crabbe et al., 1992; Kiefer, Burns, & Judge, 1976), cattle (Short, 1967), cats (Meyers-Wallen et al., 1989), dogs (Meyers-Wallen, 1993), mice (Lyons & Hawkes, 1970), non-human primates (Eli et al., 1980), and humans (Keenan, Meyer, Hadjian, Jones, & Midgeon, 1974; Pinsky, Kaufman, & Chudley, 1985; Warne, Gyorki, Risbridger, Khalid, & Funder, 1984). As described in the introduction to this thesis the *tfm* male rat (Stanley et al.

1973), is a consequence of a point mutation (*tfm*) in the androgen receptor gene (Yarbrough et al. 1990). Thus, *tfm* XY males are insensitive to the direct effect of androgens and express a feminine peripheral phenotype, including a blind end vagina, a nipple line (Stanley et al. 1973). They also have an unandrogenized skeletomusculature, as determined by femur length, diameter, and mass, and have an overall lower body mass than intact males (McGinnis et al. 2002; Vanderschueren et al. 1994).

Masculinization of the rodent CNS, in contrast to the periphery however, is accomplished primarily by the aromatization of testosterone to estrogen (Olsen, 1992). It has been demonstrated that *tfm* males can exhibit male sexual behavior as adults (Olsen, 1979b; Shapiro et al. 1980). In addition, it has been shown that castration during the neonatal period, prior to postnatal day ten (Olsen, 1979a), in contrast to castration after postnatal day ten (Olsen and Whalen, 1981), is necessary to induce lordosis with estrogen treatment in adulthood. Therefore, it is thought that the CNS of the *tfm* male is largely masculinized via the aromatization of testosterone to estrogen during the perinatal period.

The analysis of behavior in the *tfm* male provides an assay by which the relative contributions of a more female-typical skeletomusculature/body mass versus a more male-typical CNS to sex differences in movement organization could be dissociated. In this study the *tfm* males were compared to their wild-type female (WTF) and male (WTM) controls during dodging to protect a food item. The expression, by the *tfm* males, of a more male- or female-typical pattern of movement could thus be used to determine whether the hypothesis that a masculinized skeletomusculature is necessary for the expression of a male-typical pattern of dodging is correct.

3.1(B) Methods

3.1.1(B) Subjects

Ten WTM, 6 WTF and 10 *tfm* males, (bred on a Sprague-Dawley background), obtained from the breeding colony of Neil Watson at Simon Fraser University, were used. The WTM and WTF animals were obtained from the same stem colony as the *tfm* males. The absence of the *tfm* mutation on either of the X chromosomes of the WTF was confirmed using a PCR screening procedure (Fernandez, Collado, Garcia Doval, & Garcia-Falgueras, 2003) that is routinely used for genotyping this colony. At the conclusion of the experiment, the presence of internal testes was used to confirm the *tfm* mutation, in animals with an external female phenotype, through dissection of the reproductive tract. The average free feeding weights for the three conditions (mean, in grams +/- S.E.) were 228 +/- 3.9 for the WTF group, 404 +/- 9.2 for the WTM group and 290 +/- 7.1 for the *tfm* group.

The animals were housed, in accordance with the Canadian Council on Animal Care (1984) guidelines, in same condition pairs. They were maintained in the main colony room, at the University of Lethbridge, on a 12-hr light-dark cycle, when they were tested between 110 and 130 days of age. During testing subjects were maintained on a limited feeding schedule of approximately 20-30g/day (Purina Rodent Chow) to maintain their body weight at about 80-85% of their free feeding weight.

3.1.2(B) Testing and Video-recording

Trials were conducted between 0900 and 1200 hours, during the light part of the light dark cycle. Each pair was placed in a thin Plexiglas container, 40 cm in diameter and 45 cm high. The cylinder was placed on a table with a clear glass top. Under the table was a mirror inclined at 45 degrees from which the ventral side of the animals could be viewed and videotaped and a detailed analysis of the body movements and stepping patterns can then be performed (Pinel et al. 1992; Whishaw, 1988; Whishaw and Tomie, 1987; 1988) (See Fig. 5)

For both habituation and testing, a single food pellet, weighing approximately 2.5g, was placed in the testing apparatus with two-same condition cage mates. Habituation was complete when shortly after being placed in the cylinder, one of the rats began to eat and the other attempted to steal the food. Once one of the rats had completed at least 5 dodges of 135 degrees or more, the remaining food was removed and a new piece was given to the rat that had previously attempted to rob the food pellet. The trial continued until 5 dodges were obtained from both animals of each pair. Video recordings were made of the dodging trials with a Sony Hi-8 Camcorder at a shutter speed of 1/250s. Additional light was provided by two 150-W spotlights on the ceiling 125 cm above the glass table. The Hi-8 video was subsequently transferred to a Sony VHS tape and a digital time code was added using a TRG-50 Horita Micro Window.

3.1.3(B) Behavioral Analysis

An Analysis of the Relative Movement of the Snout to the Pelvis: Analysis of a dodge began on the video frame where the first movement of the snout was visible. Analysis continued until the rat had realigned its fore- and hindquarters and had resumed eating. For each animal the trajectories of the snout and pelvis were traced onto transparencies, and the distance of each trajectory was calculated, for the first five dodges of 135 degrees or greater, for each rat. The distance traveled by the base of the tail (pelvis; y) was divided by the distance traveled by the snout (x). This value was subtracted from 1 and the result was multiplied by 100 to obtain the percent difference in distance traveled by the snout relative to the pelvis. The larger the percent difference, the more distance traveled by the snout as compared to the distance traveled by the pelvis (see Field et al. 1996a for more details).

Analysis of the Hindpaw Stepping Patterns: It has previously been reported that male and female rats differ in their pattern of hindpaw steps used during a dodge (Field et al. 1996a). Females make an initial forward and sideways step with the hindpaw ipsilateral to the direction of the dodge. This step is followed by a forward step by the hindpaw contralateral to the direction of the dodge and a final step forward by the ipsilateral hindpaw. Males, in contrast, are more likely to make a backward step with the hindpaw ipsilateral to the direction of the dodge prior to the forward steps made by the ipsilateral and contralateral hindpaws (see Fig. 6A and B). A simplified version of the Eshkol-Wachmann Movement Notation (EWMN) system (Eshkol and Wachmann, 1958; Eilam and Golani, 1988) was used to describe the pattern of hind limb stepping and notate the likelihood of a step backwards towards the pelvis during the initial part of the dodge in the WTM, WTF and *tfm* animals (see Fig. 6C and Field et al.1997a; 2004 for more details).

The Orientation of the Dodging Animal to the Robber at Dodge Completion: It has been shown previously that the orientation of the hindquarters of the dodging animal, in relation to the body of the robber at the end of a dodge, is sexually dimorphic (see Fig. 7 and Field et al. 1997b; Pellis et al. 1999 for more details). Female rats orient their hindquarters to the midbody of a robbing animal at the end of the dodge. In contrast males orient their hindquarters to the head of the robbing animal. Thus for each animal the orientation of the dodging animal, to either the head, midbody, or pelvis of the robber, at the end of the dodge sequence was notated.

Analysis of the Robber's Behavior: Two measurements were taken of the robber's behavior during each dodge trial. This analysis was done to determine whether differences in the robber's behavior could account for the differences in the dodging patterns seen by the animals in each condition. These were: (a) the angle between the longitudinal axis of the dodger's body and the robber's body and (b) the distance between the tips of the snouts of the dodging and robbing animals on the video frame where the initiation of the dodge was visible (Pellis et al. 1999).

3.1.4(B) Calculation of Group Means

For each of the measures taken, group means and standard errors were calculated from the individual values obtained for each rat. The data were analyzed using analyses of variance followed by Fisher's partial least-squares difference post-hoc analyses for pairwise comparisons. For both kinds of tests, $p<0.05$ was the level for determining significant effects. For graphical purposes the mean and standard error of the mean are shown.

3.2(B) Results

Distance Traveled by the Snout in Relation to the Pelvis: Dodging WTF animals have a large lateral displacement of the snout with only a small displacement of the pelvis (Fig. 16A). In contrast, dodging WTM and *tfm* rats have a large displacement of the pelvis (Fig. 16B). Comparison of the relative distance traveled by the pelvis as compared to the snout revealed significant group differences, $F(2,23)=30.45$, $p<0.0001$.

Post hoc analysis showed that WTF rats were significantly different from WTM and *tfm* groups (Fig. 17). No difference was found between the WTM and *tfm* groups on this measure.

Number of Steps Taken by the Hindpaws: There were no significant differences in the overall number of hindpaw steps used to complete a dodge $F(2,23)=.889$, $p>0.05$.

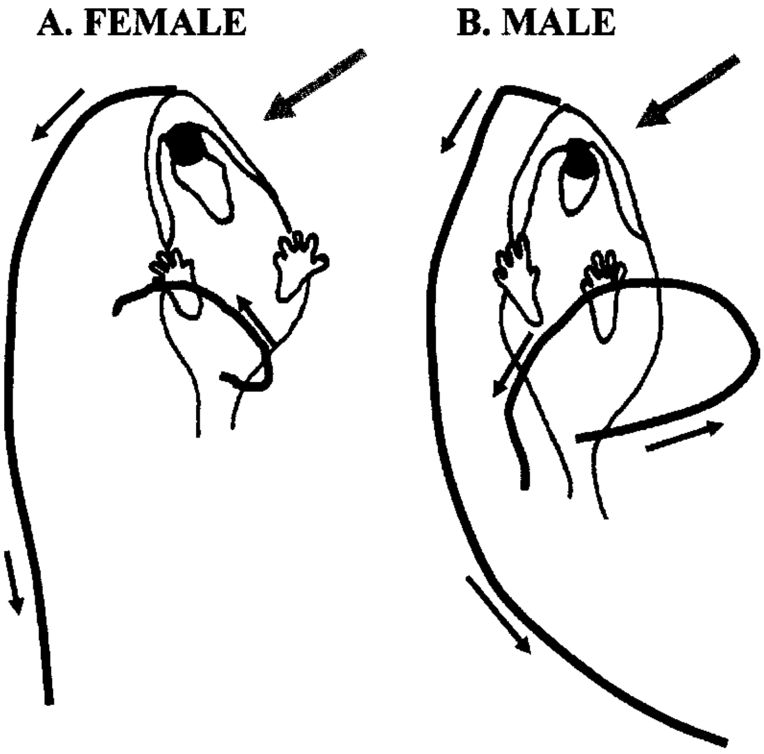
Direction of Hindpaw Steps: There were significant group differences in the likelihood of a backwards step, towards the pelvis, by the paw ipsilateral to the direction of the dodge $F(2,23)=15.73$, $p<0.0001$ (Fig. 18). WTM and *tfm* animals were more likely to make a backward step towards the pelvis than WTF. There was no significant difference between the WTM and *tfm* animals on this measure.

Orientation of the Dodging Animal to the Conspecific at Dodge End: For all groups, the orientation of the pelvis to the body of the robber at the end of the dodge was noted. Significant group differences were found in the likelihood of a dodging animal to orient their pelvis to the head of the conspecific $F(2,23)=18.481$, $p<0.0001$ or the midbody $F(2,23)=16.140$, $p<0.0001$. The WTM and *tfm* groups oriented their hindquarters to the head of the dodging animal significantly more than the WTF animals (Fig. 19A). In contrast, the WTF animals were significantly more likely to orient their pelvis towards the midbody of the conspecific, than the WTM and *tfm* groups, at the end of the dodge (Fig. 19B). There were no significant differences in the orientation of the dodger's hindquarters to the rump of the conspecific.

Angle of Approach of the Robber relative to the Dodging Animal: There were no significant differences in the angle of approach of the conspecific to the dodging animal for any of the conditions.

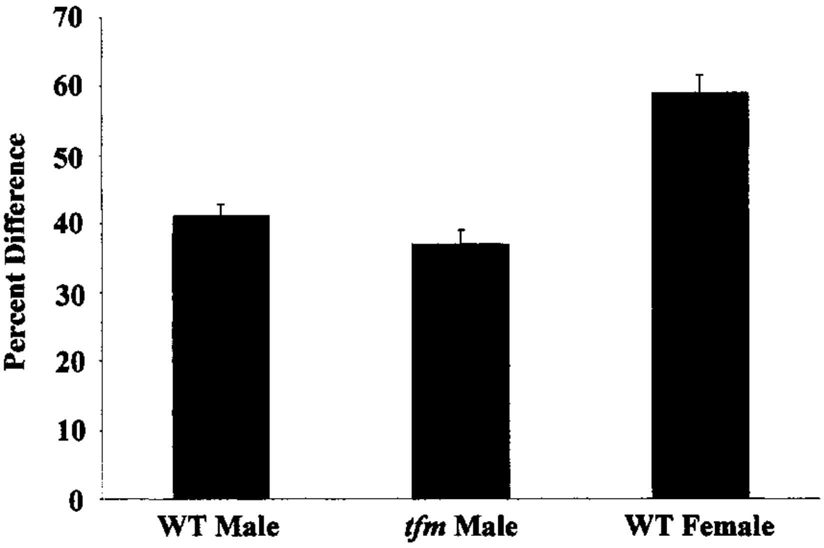
Distance between the Snout of the Robber and Dodger at the Initiation of the Dodge: Significant group differences were found on this measure F(2,23)=4.045, $p<0.05$). WTM animals began to dodge at a greater distance from the robber and thus were significantly different from the WTF and *tfm* groups.

Figure 16



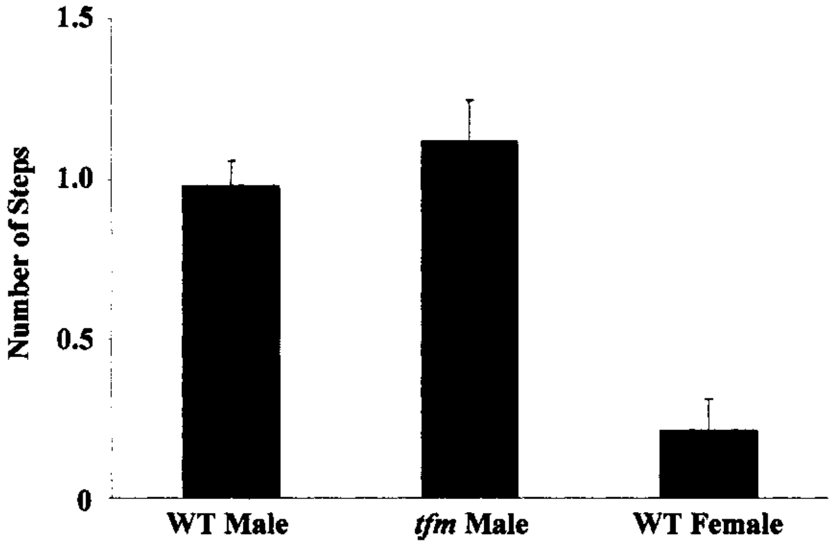
The trajectories for the snout and pelvis of a typical wild-type female (A) and male (B) dodge are shown. The black arrows indicate the direction of movement. Note that unlike the female, the male makes a large outward swing of the pelvis prior to realigning the pelvis with the snout at the completion of the dodge.

Figure 17



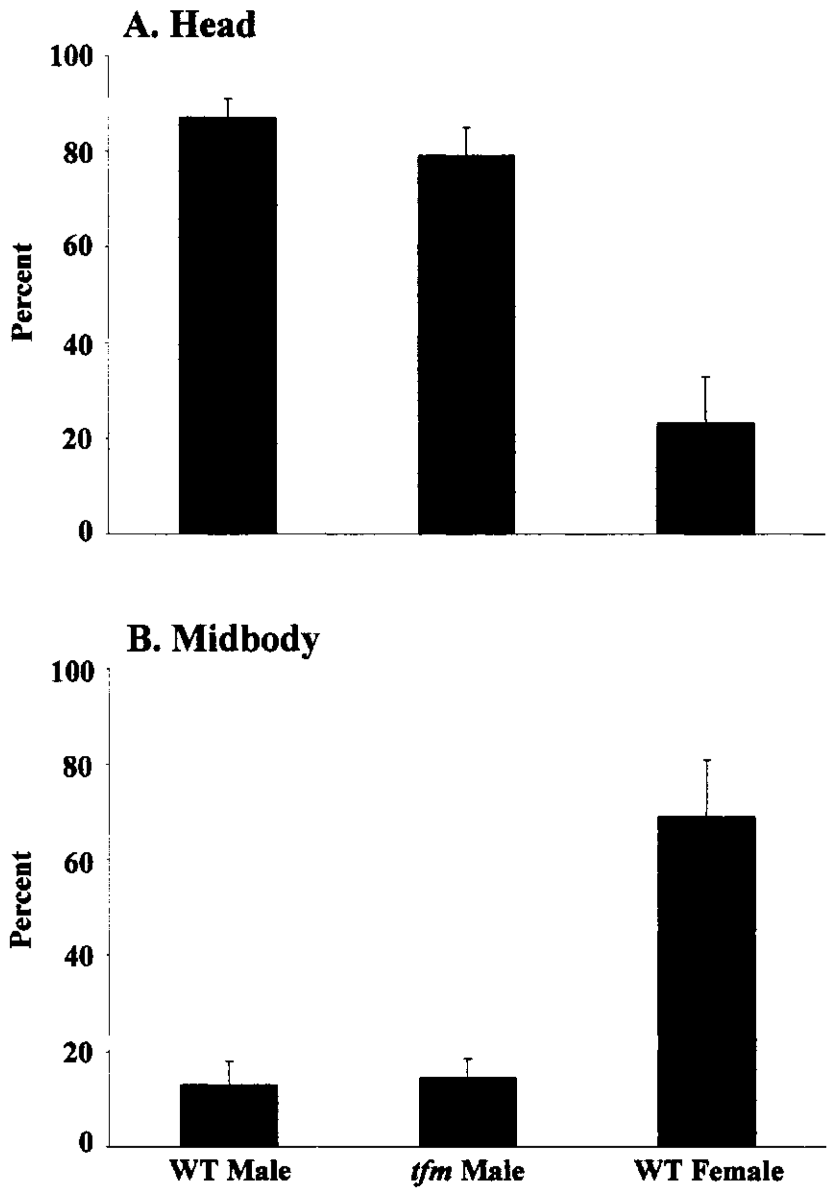
The percent difference in the distance traveled by the snout relative to the pelvis is depicted for, WTM, *tfm* and WTF animals. WT and *tfm* males were significantly different from WT females.

Figure 18



The frequency of steps back, towards the pelvis at the initiation of a dodge, is shown for WTM, *tfm* and WTF animals. WT and *tfm* males were significantly different from WT females.

Figure 19



The likelihood of the dodging animal to orient their pelvis to either the head (A) or the midbody (B) of the robber is depicted for WTM, *tfm* and WTF animals. WT and *tfm* males were significantly different from WT females.

3.3(B) Discussion

The present study was conducted to address the question of whether the composition of a non-reproductive behavior, dodging to protect a food item, is dependent on either a sex-typical peripheral phenotype or CNS by using the *tfm* model. The pattern of dodging used by the *tfm* males and WT males, while not different from one another, was significantly different from the WT females. At the initiation of the dodge both the *tfm* and WT males groups exhibited more movement of the pelvis relative to the snout and were more likely to step back towards the pelvis with the hindpaw ipsilateral to the direction of the dodge at its initiation. WTF females, in contrast, rarely stepped back towards the pelvis during the initiation of a dodge and the overall distance traveled by the pelvis relative to the snout was less than that for the WTM and *tfm* animals. The results of this study show that a masculinized skeletomusculature is not necessary for the expression of male-typical patterns of movement and that a functional androgen receptor and thus the direct influence of androgens on skeletomusculature development are not required for the development of male-typical patterns of dodging. In addition, these results suggest that the sex differences in dodge patterns are likely dependent on the masculinization of CNS mechanisms by the action of testosterone's aromatized metabolite, estradiol, acting via estrogen receptors.

It has been shown previously that the orientation of the dodging animal to the robber at the end of the dodge is also sexually dimorphic (Field et al. 1997b). Both the *tfm* and WTM orient their pelvis to the head of the robber at the completion of the dodge in contrast to the WTF animals who oriented their pelvis to the midbody of the robbing animal. This provides further evidence that the *tfm* males are masculinized in their patterns of movement and social interaction during a dodging encounter.

Currently the *tfm* model is used primarily to study the interplay of genes, gonadal hormones and the development of neuromuscular anatomy (Freeman, Watson, & Breedlove, 1996; Monks, Vanston, & Watson, 1999; Monks & Watson, 2001; Watson, Freeman, & Breedlove, 2001). In general, however, there has been little analysis of their non-reproductive behavior (McGinnis et al., 2002) with the exception of play behavior, (Field, Watson, Whishaw, & Pellis, in press; Meaney, Stewart, Poulin, & McEwen, 1983) and more recently spatial behavior in the Morris water maze (Jones & Watson, 2005). The *tfm* model, in rats and other species, (Couse & Korach, 1998; McGinnis et al., 2002), provides a unique opportunity to explore further the effects of genes and gonadal hormones on neural development, versus the peripheral skeletomusculature, and the subsequent expression of sex differences in the kinematic organization of non-reproductive behaviors.

Chapter 4*

Sex Differences in Righting from Supine to Prone: A Masculinized Skeletomusculature is not required.

ABSTRACT

It has previously been documented that sex differences exist in the composition of lateral movements. An unresolved question is whether sex differences are present in other movements, such as rotation around the longitudinal axis and whether this difference is dependent on a feminine or masculine skeletomusculature. Female rats, when placed supine on a solid surface, first rotate their forequarters and then their hindquarters; both in the same direction. Male rats, in contrast, exhibit rotation of the hindquarters counter to the direction of forequarter rotation. *tfm* males, who have a feminized skeletomusculature and masculinized CNS, are similar to wild-type male controls. This study provides evidence that sex differences in movement integration are not restricted to the lateral plane, are not due to sex differences in skeletomusculature, and thus are likely mediated by the CNS.

* This Chapter is adapted and modified, with permission by the American Psychological Association, [© 2005], from:

Field, E. F., Watson, N. V., Martens, D. J., Pellis, S. M. (2005). Sex differences in righting from supine to prone in rats (*Rattus norvegicus*): A masculinized skeletomusculature is not required. Journal of Comparative Psychology, 119, 238-245.

4.0 Introduction

It has been documented in the preceding chapters that adult male (MLE) and female (FLE) Long-Evans rats differ in how they organize the lateral movements they use to protect a food item from an approaching conspecific (Field, Watson, Whishaw, & Pellis, 2005; Field, Whishaw, Forgie, & Pellis, 2004). In general, females initiate lateral movements with their forequarters, while their hindquarters are recruited only for realignment with the forequarters; that is, the forequarters lead and the hindquarters follow. In contrast, males often move their hindquarters simultaneously with their forequarters. Furthermore, their hindquarter movements are often opposite or counter to the direction of the forequarters. It thus appears that males 'dissociate' the movements of their fore- and hindquarters, in that they move them in independent directions of one another.

A question that arises, however, is whether these sex differences in the integration of fore- and hindquarter movements are also present in behaviors that are not exclusive to the lateral plane. For example, righting, or the ability of an animal to rotate from supine to prone, requires that an animal integrate rotatory movements, around the longitudinal axis, of the upper body or forequarters with the lower body or hindquarters. Righting has been studied developmentally (Pellis et al., 1992; Pellis et al., 1991), and with regards to how visual (Pellis, Whishaw, & Pellis, 1991), tactile and vestibular systems (Pellis, Pellis, & Teitelbaum, 1991; Pellis et al., 1996a; Pellis et al., 1991) control the coordination of this behavior. All of this work, however, while demonstrating that righting reflexes can be impaired, has not addressed whether males and females are different in their kinematic organization of righting movements. In this chapter, the question of whether there are sex differences in the integration of rotation of the fore- and hindquarters around the longitudinal axis by comparing patterns of contact righting, the rotation of an animal around the longitudinal axis from supine to prone when placed on a solid surface, in intact male (MLE) and female (FLE) Long-Evans rats was examined.

A second question that arises in relation to sex differences in movement organization is whether these differences are merely due to sex differences in rats' skeletomusculature and overall body mass. In the previous chapter evidence was provided that sex differences in the organization of lateral dodging are not likely due to differences in skeletomusculature. In the present study FLE and MLE rats were also compared to *tfm* male rats and their wild-type male (WTM) and female (WTF) controls; animals that were introduced in the last chapter. This was done to evaluate how differences in peripheral skeletomusculature, in contrast to CNS mechanisms, contribute to sex differences in the organization of contact righting. Thus, the *tfm* animal provides a model with which to address the question of whether sex differences in the integration of fore- and hindquarter movements during rotation around the longitudinal axis are primarily due to sex differences in skeletomusculature or are likely mediated by differences at the level of the CNS.

4.1 Methods

4.1.1 Subjects

Eight FLE and eight MLE rats, from the University of Lethbridge animal colony, and eight WTM, six female WTF and eight *tfm* males, (bred on a Sprague-Dawley background) obtained from the breeding colony of Neil Watson at Simon Fraser

University, were used. The WTM and WTF animals were obtained from the same stem colony as the *tfm* males. The absence of the *tfm* mutation on either of the X chromosomes of these females was confirmed using an RT-PCR screening procedure (Fernandez et al., 2003) that is routinely used for genotyping this colony. At the conclusion of the experiment, the presence of testes was used to confirm the *tfm* mutation through dissection of the reproductive tract. The animals were housed in same condition pairs, in clear Plexiglas tubs, in accordance with the Canadian Council on Animal Care (1984) guidelines. They were maintained in the main colony room on a 12-hr light-dark cycle until adulthood, and were tested between 3 and 4 months of age. All animals were provided with food (Purina Rodent Chow) and water *ad libitum.*

4.1.2 Testing and Video Recording

Trials were conducted between 0900 and 1200 hours. Each animal was placed on a table with a transparent Plexiglas top. Under the table was a mirror inclined at 45 degrees from which the ventral side of the animals could be viewed and videotaped (Pinel et al., 1992).

Video recordings were made of the contact righting trials with a Sony Hi-8 Camcorder at a shutter speed of 1/250s. Additional light was provided by two 150-W spotlights on the ceiling 125 cm above the glass table. The Hi-8 video was subsequently transferred to a Sony VHS tape and a digital time code was added using a TRG-50 Horita Micro Window.

4.1.3 Testing Procedures

All animals were videotaped for at least five trials. Contact righting was tested by placing the rat supine on the testing surface and gently restraining both the fore- and hindquarters. To prevent the initiation of rotatory movements, prior to release of the fore- and hindquarters by the experimenter, each rat was swung gently backwards and forwards several times while holding it around the chest just under the forelimbs. This action functioned to make the animals temporarily immobile prior to their placement on the testing surface and subsequent release (Pellis, Teitelbaum, & Meyer, 1990).

4.1.4 Movement Analysis

The first five videotaped sequences, obtained for each rat, were analyzed using a simplified version of the Eshkol-Wachman Movement Notation (EWMN) system (Eshkol & Wachmann, 1958). This provided a detailed analysis of the spatiotemporal relationship of the movements of the fore- and hindquarters of each rat. In brief, EWMN is designed to record relations and changes in relations between body parts. The body is treated as a system of articulated axes (i.e., body and limb segments). A limb is any part of the body that either lies between two joints or has a joint and a free extremity. These are imagined as straight lines (axes), of constant length, which move with one end fixed to the center of a sphere. Given that the longitudinal axis of the body involves many anatomical units (vertebra), for current purposes, the body was divided into three segments: the head and the neck, the upper torso, and the lower body. The upper and lower body was divided at the lumbar area.

For the analysis of sex differences in contact righting the rotatory, lateral and dorsoventral movements of the head, neck, upper torso and pelvis were notated for a FLE and a MLE rat. A comparison of the notated sequences revealed the typical pattern of rotation for each animal. To confirm further that the patterns of rotation that were notated were common for each group, five examples of contact righting for each rat were inspected frame-by-frame and two features of the typical patterns were quantified. The features scored and their analyses are described in the next section.

4.1.5 Calculation of Group Means

For each of the measures that were quantified, group means and standard errors were calculated from the individual values obtained for each rat. The data were analyzed using analyses of variance followed by Fisher's partial least-squares difference post-hoc analyses for pairwise comparisons. For both kinds of tests, $p<0.05$ was the level for determining significant effects. For graphical purposes the mean and standard error of the mean were used.

4.2 Results

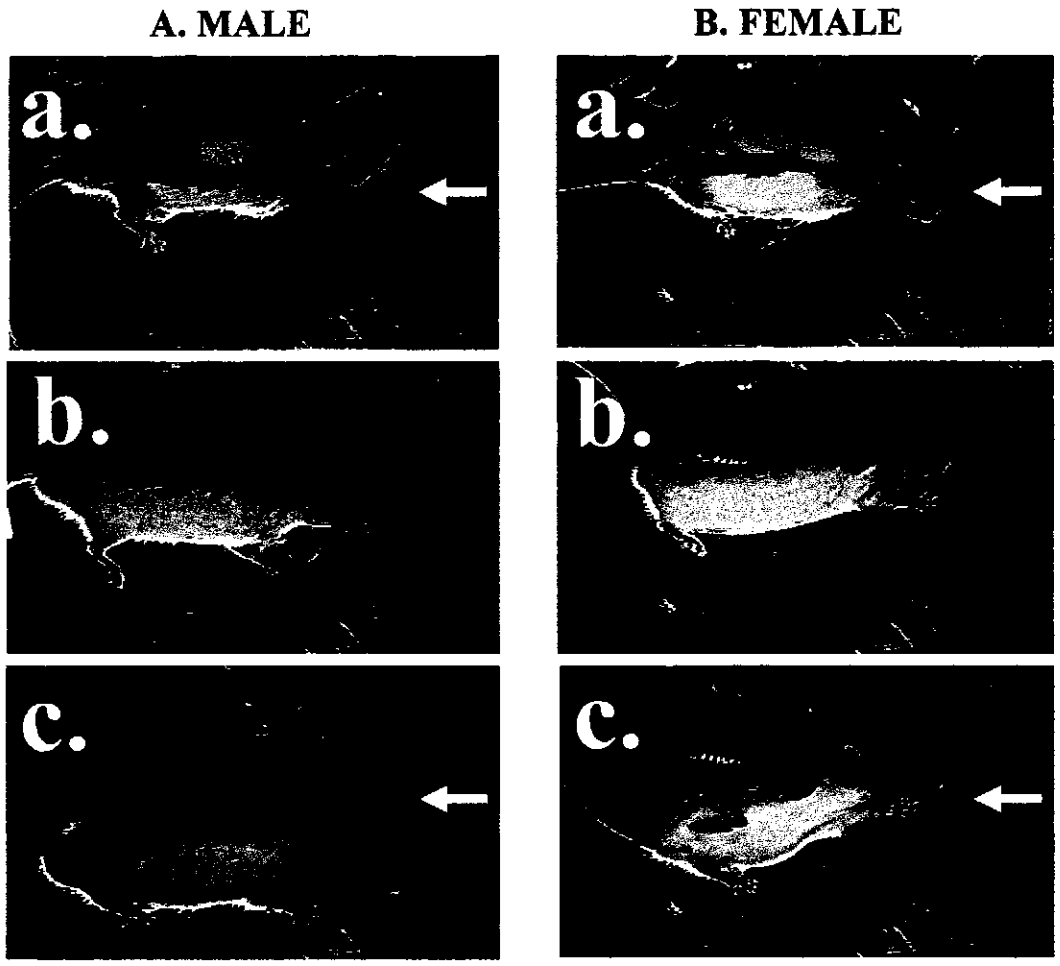
Qualitative Description of Righting Sequences: It was found that in all animals, rotation to prone began with a rotation of the shoulders, and then continued with a passive carrying of the neck and head towards prone, until one or both forepaws were placed on the ground (See Fig. 20A and B, panels a and b). In contrast, the recruitment of the hindquarters was different between the female and male groups. In the FLE their hindquarters rotated in the same direction as their forequarters once their forepaws had established firm contact with the ground. In addition, in order for them to maintain the alignment of their longitudinal axis between the upper and lower body, a slight dorsiflexion was evident as their hindquarters rotated to prone (Fig. 20Bb). As a consequence of the fixation of the forepaws and snout in one location and the stabilized alignment of the hindquarters with the forequarters, the rotation of the hindquarters to prone led to a large angular change in the longitudinal axis of the FLE relative to their starting point when supine. (Fig. 20Ba versus. Bc).

In contrast to the FLE, in the MLE there was a small rotation by the hindquarters in the opposite direction to that of the forequarters within a few frames of the initiation of forequarter rotation. Because of this initial counter rotation of the hindquarters, the males' forepaws typically did not achieve contact with the ground in as firm a manner as did the female animals. Consequently, their forequarters, including the head, were displaced away from the point of origin (Fig. 20Aa versus Ab). It was only after the initial counter rotation of their hindquarters, in the opposite direction to that of their forequarters, that the males rotated their hindquarters in the same direction of the forequarters so as to complete the righting sequence and achieve a prone position. In addition, while the female animals exhibited a slight dorsiflexion of the body in order to maintain the alignment of their fore- and hindquarters, the males exhibited a slight ventroflexion of the body as it was rotated to prone. Thus, from the initial supine position to the final prone position, there was little angular displacement of the longitudinal axis of the body. (Fig. 20Aa versus Ac).

An analysis of the lateral view of rotation from supine to prone showed that the tail, in the MLE, also moves during rotation. The tail is lifted away from the surface and is recruited as part of the counter rotation of the pelvis (Fig. 21Aa-d). In contrast, the tail of the FLE remained approximately at the same horizontal angle to the ground throughout the righting sequence and was rotated along with the hindquarters in the same direction as the forequarters (Fig. 21Ba-d).

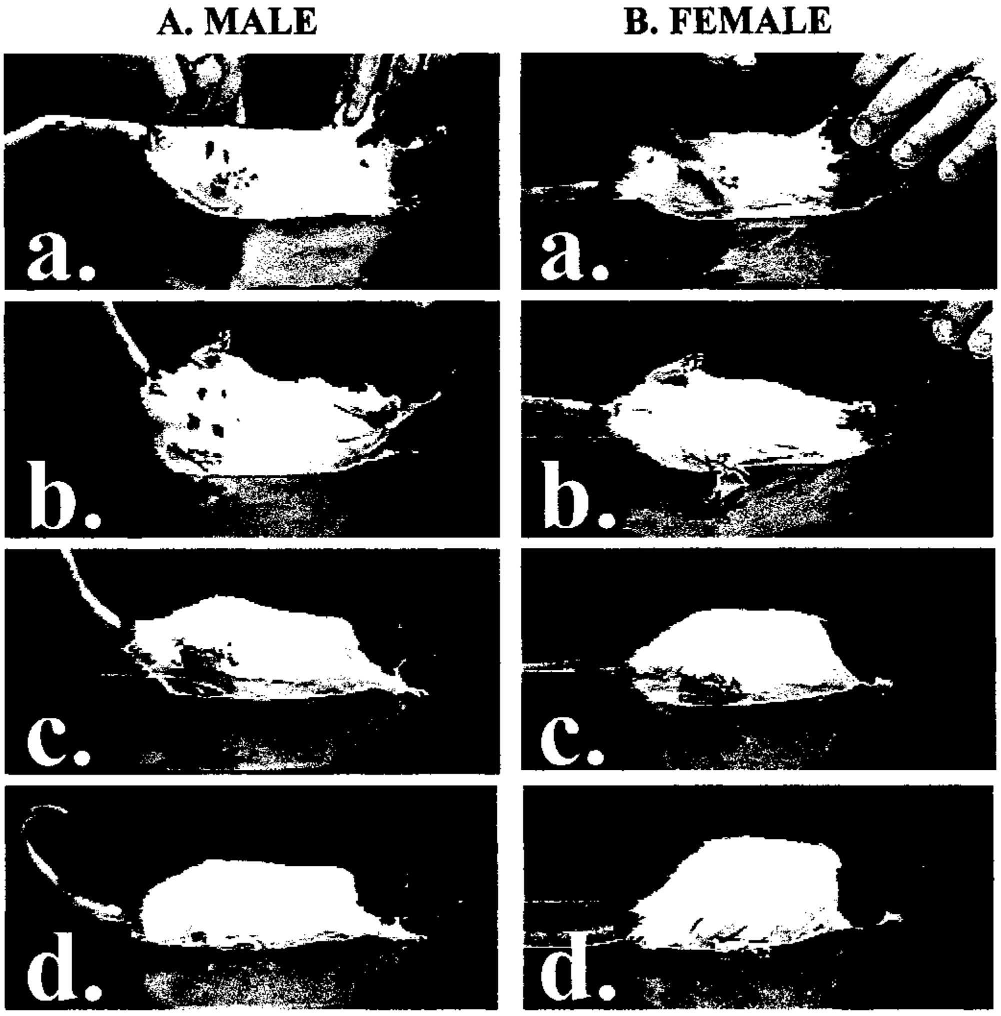
Comparison of the overall sequence of righting by the FLE, MLE, *tfm*, WTM and WTF rats showed that the female animals stabilized their snouts relative to their initial position, but in doing so, exhibited a large angular displacement of their longitudinal axis. In contrast, the longitudinal axis of the male animals remained relatively parallel with regard to their initial supine position and their final prone position. Thus, while the males did not undergo a large angular displacement, they did have a large displacement of the whole body away from the initial supine position. (These differences are illustrated in Figure 20, where the white arrow indicates the angle of the body prior to the commencement of righting. Comparing the rats' final positions to this arrow illustrates the greater angular displacement by the female).

Figure 20



The pattern of contact righting, from the ventral perspective, is shown for a male (A) and a female (B) Long-Evans rat. Note that the female undergoes a large angular displacement along the longitudinal axis, whereas the male has a large whole body displacement. The white arrow represents the initial supine position for each animal.

Figure 21



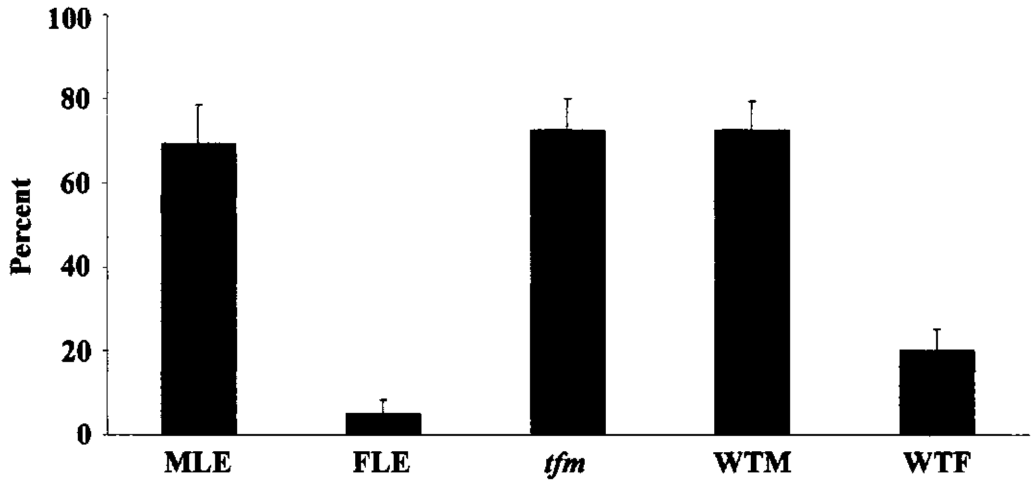
Rotation around the longitudinal axis, from the lateral perspective, during contact righting for a male (A) and female (B) Long-Evans rat. Note the lifting of the tail by the male but not the female. See text for details.

Quantification of Features from the Qualitative Description: In order to validate, quantitatively, these sex differentiated patterns of righting, two features were measured. The first measure scored was whether the righting sequence contained the counter rotation of the pelvis that had been seen in the males. The likelihood of rotation was scored as either present (1) or not (0), with a total possible score of 5 for any animal. The obtained score was then divided by 5 to obtain a score that denoted the probability of counter rotation by the hindquarters for each animal. The second feature measured was that of the angular displacement of the longitudinal axis of the body between the initial supine position and the final prone position. If the descriptive analysis was correct, it was predicted that the males would score much higher than the females on the likelihood of hindquarter rotation but have a significantly smaller angular displacement of the longitudinal axis.

Likelihood of Hindquarter Rotation: There was a significant group difference in the likelihood of hindquarter rotation in the direction opposite the rotation of the forequarters $F(4,33)=23.405$, $p<0.0001$. Post hoc analysis revealed that the MLE, WTM and *tfm* groups were significantly more likely to exhibit active hindquarter rotation in the direction opposite to the forequarters than were the FLE and WTF groups (Fig. 22).

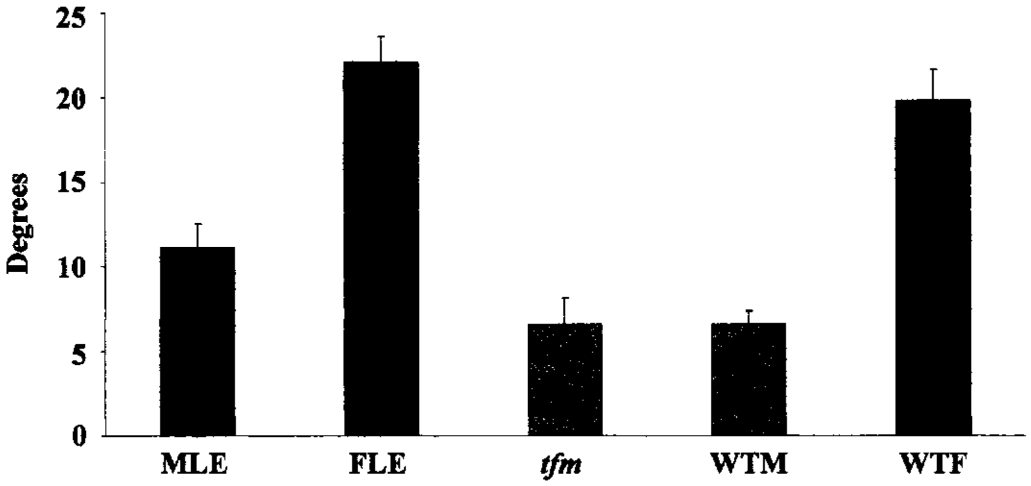
Angle of Displacement of the Longitudinal Axis: There were also significant group differences in the angular displacement of the longitudinal axis during righting $F(4,33)=25.301$, $p<0.0001$. Post hoc analysis revealed that the FLE and WTF animals were significantly different from all other groups. In addition, the MLE were significantly different from the WTM and *tfm* animals. The WTM and *tfm* animals were not different from one another (Fig. 23).

Figure 22



The likelihood of rotation of the hindquarters in the direction opposite to the forequarters is depicted. The FLE and WTF animals were significantly less likely to rotate their hindquarters in the direction opposite the forequarters than were the MLE, WTM and *tfm* animals.

Figure 23



The angular displacement of the longitudinal axis from the initial supine position to the prone position is depicted. The FLE and WTF animals had a significantly higher angular displacement of the longitudinal axis than did all the male groups. In addition, the MLE animals were significantly different from the WTM and *tfm* males. The WT and *tfm* males were not significantly different from one another.

4.3 Discussion

The results of this study reveal that sex differences are present in rotatory movements around the longitudinal axis. Furthermore, *tfm* males, with their feminized periphery, are indistinguishable from the WTM and MLE males in their patterns of rotation from supine to prone. Therefore, the sex differences that are present in the organization of movement and postural adjustments during contact righting are not likely due to differences in peripheral skeletomusculature, but rather, are due to differences in the CNS mechanisms that control these behaviors.

In addition to the sex differences shown here, with regard to the integration of the fore- and hindquarters during rotation from supine to prone, it has previously been shown that sex differences in the integration of fore- and hindquarter movements are present in other behavioral paradigms such as dodging to protect a food item (Field et al. 1996a; 1997a; 1997b; 2003; Pellis et al. 1999), spontaneous turning (Field and Pellis, 1998) and changes in postural stability during haloperidol-induced catalepsy (Field, Whishaw & Pellis, 2000). These findings, in conjunction with those of this study, suggest that there are fundamental differences in how males and females integrate their fore- and hindquarter movements that are not task specific and are due to sex differences within the CNS.

CHAPTER 5*

Sexually Dimorphic Postural Adjustments are used in a Skilled Reaching Task in the Rat

ABSTRACT

Sex differences occur not only in the sexual repertoire of animals but are also evident in other aspects of movement. The present study was conducted to determine whether sexually dimorphic motor behavior extends to the skilled movements used in reaching for food in the rat. It has previously been shown that males and females are sexually dimorphic in their organization of lateral and rotatory movements. Since postural adjustments are incorporated into skilled movements, both body posture and limb movements were examined during a skilled reaching task. Male and female Long-Evans rats were filmed, from both the front and ventral views, and their stepping patterns, postural adjustments, and forelimb movements, during a successful reach were analyzed. While males and females did not differ in forelimb movements, they were significantly different in how they approached and oriented their body to the food item during a successful reach.

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Field, E.F., Whishaw, I. Q. (2005). Sexually dimorphic postural adjustments are used in a skilled task in the rat. Behavioural Brain Research, 163, 237-245.

5.0 Introduction

Adult male and female rats differ in how they organize the lateral movements they use to protect a food item from an approaching conspecific (Field et al., 1996a; Field et al., 1997a; Field et al., 1997b). Male and female rats also use different tactics to rotate around the longitudinal axis from supine to prone (Field, Martens, Watson, & Pellis, 2005). Thus, in general, females integrate the movements of their hindquarters and forequarters differently than do males.

The question examined in this study was whether sex differences in the integration of fore- and hindquarter movements are also present primarily in the anterior or forward plane of movement as an animal performs a skilled reach. Reaching is a skill that is widespread among mammalian taxa, including the rat (Iwaniuk & Whishaw, 2000). While the limb movements that occur during a reach may be similar between species, in each case for a successful reach to occur a number of movements and postural adjustments must be made to orient an animal to the target object of the reach. Once the target has been found there are further shifts of posture that must occur as an animal lifts its paw or hand toward the object and grasps it (Metz & Whishaw, 2000; Metz & Whishaw, 2002; Whishaw, 1996; Whishaw, 2003; Whishaw & Gorny, 1994; Whishaw & Pellis, 1990; Whishaw, Pellis, & Gorny, 1992).

The question of whether males and females use different combinations of orienting, stepping and postural shifts, and forelimb movements to attain a food item has not been addressed. Potentially, sex differences in skilled movements or the postural adjustments used during skilled movements would be relevant to the evolutionary origins of skilled movements, to understanding the relation between whole body movements and skilled movements, and to the study of potential sex differences in recovery and compensation after brain injury (Di Carlo et al., 2003; Groswasser, Cohen, & Keren, 1998; Hurn, Vannucci, & Hagberg, 2005).

5.1 Methods

5.1.1 Subjects

Subjects were 7 male and 8 female six month old Long-Evans rats that were born and raised in the University of Lethbridge vivarium. They were housed, in same sex pairs, in clear Plexiglas cages from weaning (P23). Prior to testing all animals were provided with food and water ad libitum. The colony was maintained on a 12/12 h light/dark cycle (lights on 8:00-20:00h). The experiments were approved by the University of Lethbridge Animal Care Committee and conducted according to the principles outlined by the Canadian Council on Animal Care (1984).

5.1.2 Reaching Boxes

Single pellet boxes (25cm x 35cm x 30 cm high) were made of clear Plexiglas. Five centimeters from the side of each wall was a 1cm wide slot that extended from the floor to the height of 15cm. On the outside of the wall in front of the slot, mounted 3cm above the floor, was a 2cm wide by 4cm long shelf. Food pellets (90mg Rodent Chow food pellets, Bioserve Inc., PO Box 250 Frenchtown, NJ) were placed in one of two small indentations on the surface of the shelf. The indentations were 2cm away from the inside wall of the box and were aligned with the edges of the slot through which the rat reached (Fig. 24). See (Whishaw & Metz, 2002) for details.

5.1.3 Food Deprivation and Training

One week before training began the animals were placed on a food restricted schedule. The rats were reduced to a body weight of approximately 90 percent of their free feeding weight. This weight was maintained throughout the testing period.

Training was conducted over two weeks during which each animal was habituated to the apparatus and learned to reach for food pellets. For each rat, food was placed in the indentation contralateral to the limb with which the rat reached. Training was administered in such a way that when the rat made a successful reach, a short pause preceded presentation of the next food pellet, during which a second food pellet was dropped into the back of the box. The second pellet ensured that the rat left the food aperture after each reach and had to re-approach and reposition itself at the food slot for each successive reach for a food pellet. During the training period each rat was presented with twenty food pellets daily. Training was complete when all rats attempted to reach for twenty successive pellets.

5.1.4 Video Recording

Video records were made with a Sony Video 8 portable camera at a shutter speed of 250th of a second. Each animal was filmed from the front and ventral views simultaneously. For all animals the camera position and zoom was maintained between trials to maintain the scale of the movements analyzed. Frame-by-frame analysis, at 30 frames per second, was provided by a JVC (HR-VP628U) video recorder with frame by frame capability. All the rats were videotaped after two weeks of training and these video records were used for the behavioral analysis.

5.1.5 Scoring Reaching Success

Overall reaching performance was compared for males and females by counting the hits and misses for each rat during ten trials. If a rat made a reaching movement in which a paw was inserted through the slot of the testing apparatus, the movement was scored as a "reach". If the rat obtained a piece of food and consumed it, the reach was scored as a "hit". In addition, hits were further subdivided into two categories. Rats when attempting to attain a food pellet reach through the slot, pronate their paw over the food pellet and subsequently close their digits around the pellet. It is possible, however, for an animal to "miss" the pellet upon closure of the paw. Often in these circumstances the animal will maintain its paw position on the reaching tray, open its digits, re-orient the paw, and re-attempt to grasp the pellet.

Hits were analyzed as the following: (a) Overall Hits. These were hits where the animal successfully attained the food item, either initially or upon successive opening and closing of the digits to attain the food item. (b) Initial Hits. These were hits where the animal attained the food upon the initial advance of the arm and closure of the digits over the food item. For each category of hits a percent score was obtained using the following formulae - Hit percent = (number of hits/number of reaches) x 100.

The time taken to attain a food item during "initial hit" successful reaches was also calculated. This time period started with the animal engaging the food item by placing its snout into the reaching slot and ended upon closure of the digits over the food pellet.

5.1.6 Movement Analysis of the Reach

Movements were analyzed using a rating scale derived from and Eshkol-Wachmann Movement Notation (EWMN) (Eshkol & Wachmann, 1958) analysis of reaching (Whishaw & Pellis, 1990). In brief, EWMN is designed to express relations and changes of relation between parts of the body. The body is treated as a system of articulated axes (i.e. body and limb segments). A limb is any part of the body that either lies between two joints or has a joint and a free extremity. These are imagined as straight lines (axes) of a constant length, which move with one end fixed to the center of a sphere. An important feature of EWMN is that the same movements can be notated in several polar coordinate systems. The coordinates of each system are determined with reference to the environment, to the midline axis of the animal's body, and/or to the next proximal or distal limb or body segment. By transforming the description of the same behavior from one coordinate system to the next, invariances in that behavior may emerge in some coordinate systems but not in others. Thus, the behavior may be invariant in relation to some or all of the following: the animal's longitudinal axis, gravity, or bodywise in relation to the next proximal or distal segment. On the basis of the descriptions obtained from EWMN, rating scales of reaching movements were derived.

Ten reaches for each rat were rated for qualitative features of the movement (Whishaw & Metz, 2002). For all animals ten components of each reach were rated as present (1) or absent (0).

(1) ***Digits to the Midline.*** Using mainly the upper arm, the reaching limb is lifted from the floor so that the tips of the digits are aligned with the midline of the body.

(2) ***Digits Flexed.*** As the limb is lifted, the digits are flexed and the paw is supinated and the wrist is partially flexed.

(3) ***Elbow in.*** Using an upper arm movement, the elbow is adducted to the midline while the tips of the digits retain their alignment with the midline of the body.

(4) ***Advance.*** The limb is advanced directly through the slot toward the food target.

(5) ***Digits Extend.*** During the advance, the digits extend so that the digit tips are pointing towards the target.

(6) ***Arpeggio.*** When the paw is over the target, the paw pronates from digit 5, (the outer digit) through to digit 2, and at the same time the digits open.

(7) ***Grasp.*** The digits close and flex over the food, with the paw remaining in place, and the wrist is slightly extended to lift the food.

(8) ***Supination I.*** As the paw is withdrawn, the paw supinates by almost 90 degrees.

(9) ***Supination II.*** Once the paw is withdrawn from the slot to the mouth the paw further supinates by about 45 degrees to place the food in the mouth.

(10) ***Release.*** The mouth contacts the paw and the paw opens to release the food.

5.1.7 Kinematic Analysis of Body Movements during the Reach

In addition to the analysis of reaching success and the movements of the reaching limb and paw, a kinematic analysis of five successful reaches where the food pellet was obtained during an initial hit, was conducted for each rat. All reaches were analyzed, by using tracings of the movements on acetate sheets, and aspects of the EWMN system, described previously, from both the front and ventral view. For all measures, the starting point of the reach was the placement of the snout into the reaching slot. The conclusion of the reach occurred when the animal closed its digits over the food pellet. The following measures were scored:

Front View

1) ***Rotation of the Head during the Reach:*** To calculate rotation of the head, a line was drawn on a transparency, between the tips of each ear at the initiation and conclusion of a reach. The angular change between the initial position of the ears and the final position was calculated and used as a measure of head rotation during the reach. (Fig. 24Aa as compared to Fig. 24Ce)
2) ***Vertical Displacement of the Snout:*** To calculate the vertical displacement of the head the distance traveled by the tip of the snout during the reach was calculated by subtracting the initial position of the snout from the final position (Fig. 24Cd).
3) ***Horizontal Displacement of the Reaching Paw:*** The initial placement of the reaching paw relative to the center of the reaching slot was compared (Fig. 24Ab).
4) ***The Vertical Displacement of the Reaching Paw*:** The maximum height of the reaching paw, relative to the shelf where the pellet was placed was calculated. (Fig. 24Bc)

Ventral View

1) ***The Angle of the Longitudinal Axis to the Reaching Slot:*** For each animal a straight line was drawn from the tip of the snout to the base of the tail (anus) at

both the initiation and conclusion of a reach. (Fig. 25Ba) The angular displacement from a line perpendicular to the reaching slot was calculated. In addition the change between the initial and final angles was calculated

2) ***The Angle of the Forequarters to the Hindquarters:*** In order to determine whether differences in the angle of the longitudinal axis were due to differences in the angle of the forequarters to the hindquarters the body was divided into two segments and the angle between the two segments was calculated. The forequarter segment consisted of the head and upper torso and the hindquarter region consisted of the midbody and the pelvis. (Fig. 25Aa the heavy dashed lines represent the fore and hindquarters respectively, the lightly dashed, curved line represents the angle that was calculated).
3) ***Number of Steps.*** The numbers of steps taken by the fore and hindpaws from the initiation of the reach until its conclusion were counted. (Fig. 25Ab arrows indicate paws that are about to step)
4) ***The Likelihood of being in Midstride when the Food is Engaged by the Snout:*** For all animals, at the video frame where the snout was placed into the slot, it was noted whether they were in a stationary position with no steps occurring or whether they were making steps and thus adjusting body position simultaneously to engaging the food with the snout.
5) ***The Occurrence of Uncoupled Steps:*** In order to determine whether the steps made, between the engagement of the food with the snout, and the subsequent reach were locomotor or postural adjustment steps the occurrence of an

'uncoupled' step was noted. An uncoupled step was defined as a step that was not preceded or followed by a forward locomotor step of a contralateral limb.

6) ***The Direction of the Hindlimb Steps relative to the Position of the Body***: To determine whether the steps made by males and females were different in their direction of movement a simplified version of the EWMN system, described above, was used. This allows the direction of the steps to be notated in relation to the body of the animal. For the current analysis there were three directions scored, these were: (a) steps forward toward the snout, (b) steps sideways into the midline of the body, or (c) steps sideways away from the midline of the body. (See Chapters 2 for more details of the analysis of step direction).

7) ***The Angle of the Three Supporting Paws to the Reaching Slot:*** The angle of the paws relative to the slot at the conclusion of a reach was calculated. For each of the three paws that maintained substrate contact during the reach a line was drawn from the tip of the second digit through the heel. (Fig. 25Bb)

8) ***The Shift of the Body over the Limbs***: To determine whether males and females differed in the amount of lateral displacement of the body, during the reach, the outline of the body was traced at the initiation of the reach and compared to the position of the body at the conclusion of the reach. At the point of maximum displacement (Fig. 28Ab arrow) the distance between the initial and final positions was calculated.

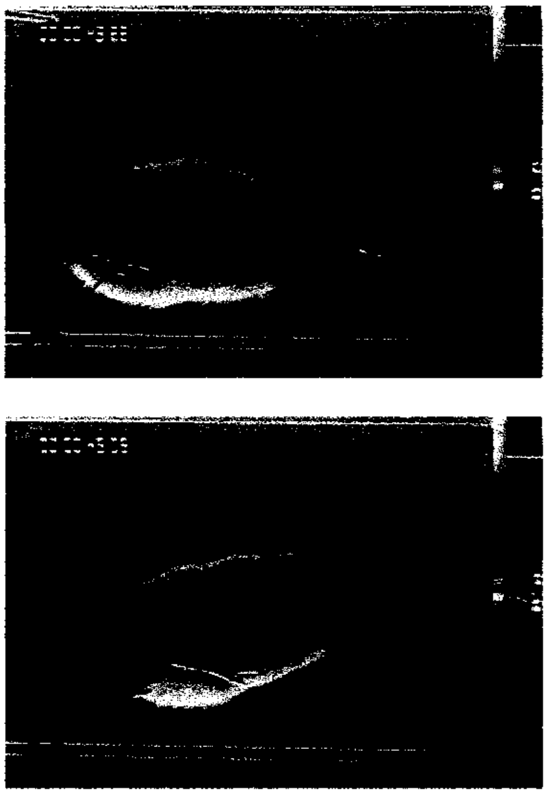
5.1.8 Calculation of Group Means

For each of the measures taken, group means and standard errors were calculated from the individual values obtained for each rat. For the analysis of the ten-point reaching scale a one way repeated measures ANOVA was used. For all other data analysis was done using an unpaired student t-test. $P<0.05$ was used for determining significant effects. For graphical purposes the mean and standard error of the mean were used.

Figure 24

In this figure the aspects of the reach that were quantified from the front view are depicted. Figure 24A represents the initial position of the animal when engaging the food pellet with its snout. In Figure 24B the animal is advancing the reaching paw forward to grasp the pellet. Figure 24C represents the conclusion of the reach when the animal successfully grasps the food pellet. The following measures were scored: (a) the horizontal displacement of the reaching paw (Ab), (b) the vertical displacement of the reaching paw relative to the shelf (Bc), (c) the vertical displacement of the snout (Cd), and (d) the angular rotation of the head using the initial and final position of the ears (Aa and Ce). See text for further details.

Figure 25



The reach from the ventral view is depicted. Figure 25A represents the initiation of a reach sequence when the animal engages the food pellet by placing its snout in the reaching slot. Figure 25B represents the conclusion of the reach when the animal attains the food pellet. The measures that were scored from the ventral view included: (a) the angle of the longitudinal axis at the video frame where the reach was initiated and the pellet was grasped (Ba), (b) the angle of the forequarters to the hindquarters (Aa), (c) the number of steps taken during the reach (Ab) the arrows are pointing to paws that are about to step, and (d) the angle of the supporting paws relative the front wall of the reaching box (Bb). See text for further details.

5.2 Results

5.2.1 Reaching Success and Movement Analysis of the Reach

There was no significant difference between the males and females on overall reaching success ($p>0.05$). Males and females were also not different in their likelihood of scoring a hit either initially or with repeated attempts at grasping the food item ($p>0.05$). The amount of time male and female animals took to successfully obtain a food pellet on an initial hit, from the point when the animals placed their snout in the reaching slot until their digits closed over the food item, was not different. An analysis of reaching movements using the ten point scale described previously also revealed no significant differences, between males and females, in the arm or forepaw movements used to obtain a food item.

5.2.2 Kinematic Analysis

The Front View

Males and females were not significantly different on any measure analyzed from the front view ($p>0.05$).

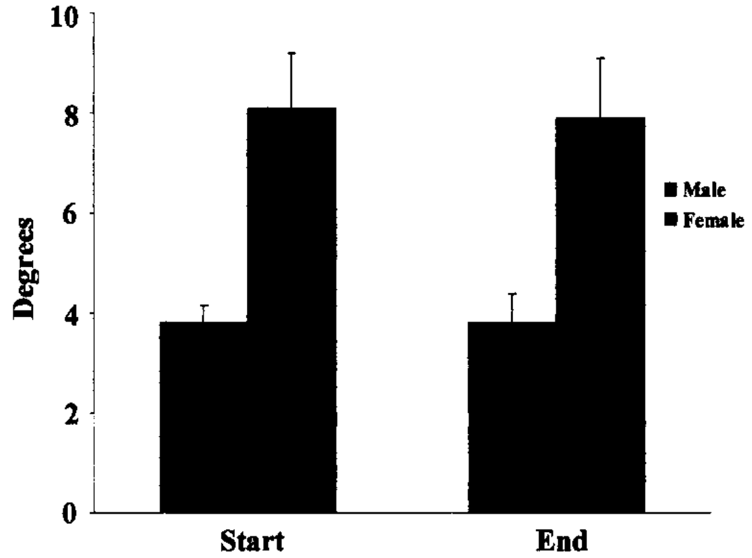
The Ventral View

Angular Change in the Longitudinal Axis: Males and females were significantly different in the angle of their longitudinal axis to the front wall of the testing enclosure at both the initial orientation of the snout to the food pellet $t = 3.212$, $p<0.05$ and at the point when the animal closed its digits around the food pellet $t = 2.963$, $p<0.05$ (Fig. 26). There was no significant difference between males and females in the change of the angle of the longitudinal axis during the reach. It is possible however, that the differences in the angle of the longitudinal axis to the reaching slot are, in part, determined by differences in the angle of the fore- and hind-quarters to one another. The angle of the forequarters to the hindquarters, at both the start and the end of the reach, were not significantly different between males and females ($p<0.05$).

Analysis of Stepping: Females made significantly more steps while orienting and reaching for a food pellet than males $t = 3.392$, $p<0.05$ (Fig. 27A). Females were also more likely to orient their snout to the food item during a step; males in contrast, were stationary $t = 11.222$, $p<0.05$ (Fig. 27B). In addition, the steps made by females were more likely to be postural adjustment steps that were not coupled to a forward locomotor step $t = 3.188$, $p<0.05$ (Fig. 27C). While males and females were different in the number and type of steps they used they were not different in the direction of the steps, relative to the body, ($p>0.05$) or in the angle of the three supporting paws relative to the front wall of the reaching box ($p>0.05$).

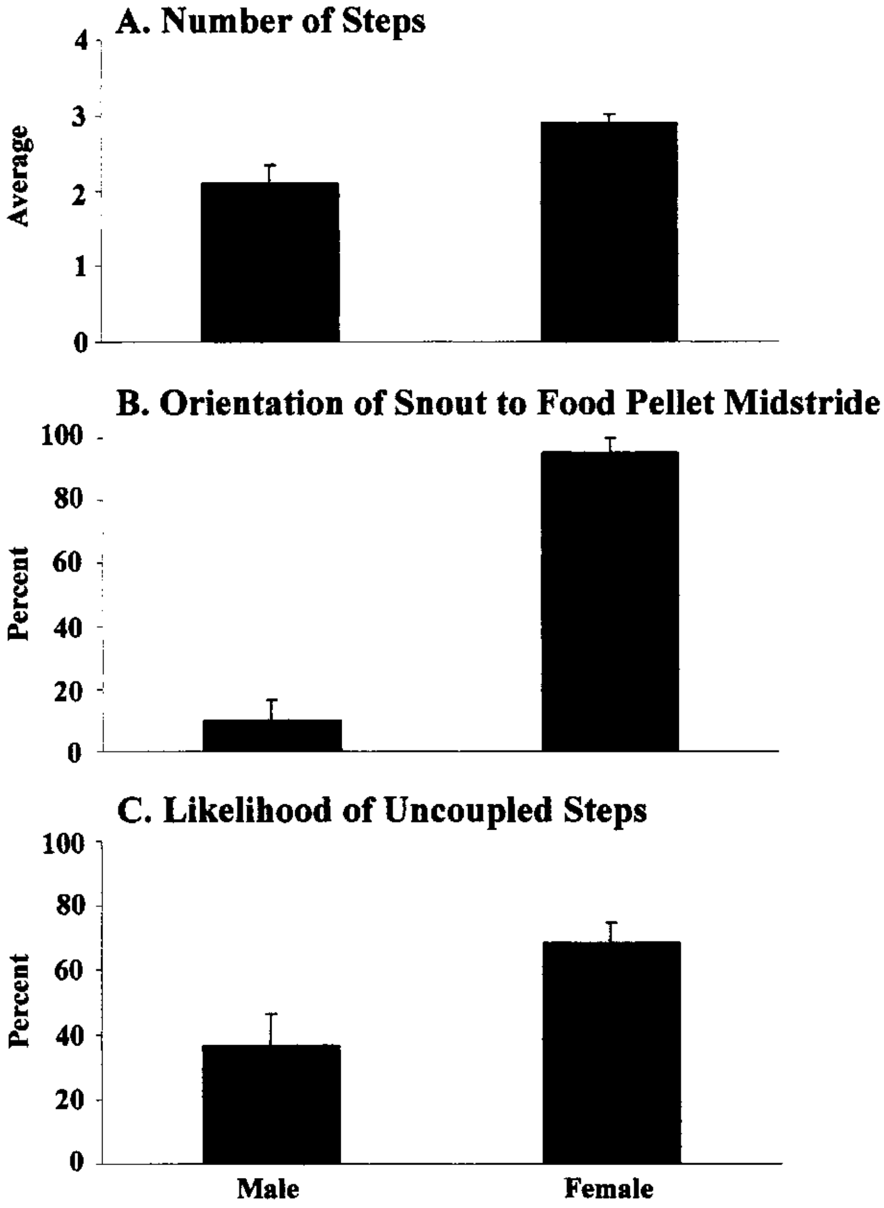
Lateral Displacement: While there was no significant difference in the change of the angle of the longitudinal axis to the food pellet during a reach tracings of the outline of the body show that females were more likely to shift their midbody laterally, away from the reaching paw, while maintaining their head and pelvis stationary (Fig. 28Aa-b for a female and 28Ba-b for a male example). Quantification of this difference by measuring the maximum displacement of the midbody during the reach revealed a significant difference between males and females $t = 4.613$, $p<0.05$ (Fig. 28C).

Figure 26



The degree of displacement of the longitudinal axis from perpendicular to the reaching slot is depicted. Females had a significantly greater angular displacement than males at both the initiation of a reach where the animal places its snout into the reaching slot and at the conclusion of the reach when the food pellet is grasped.

Figure 27

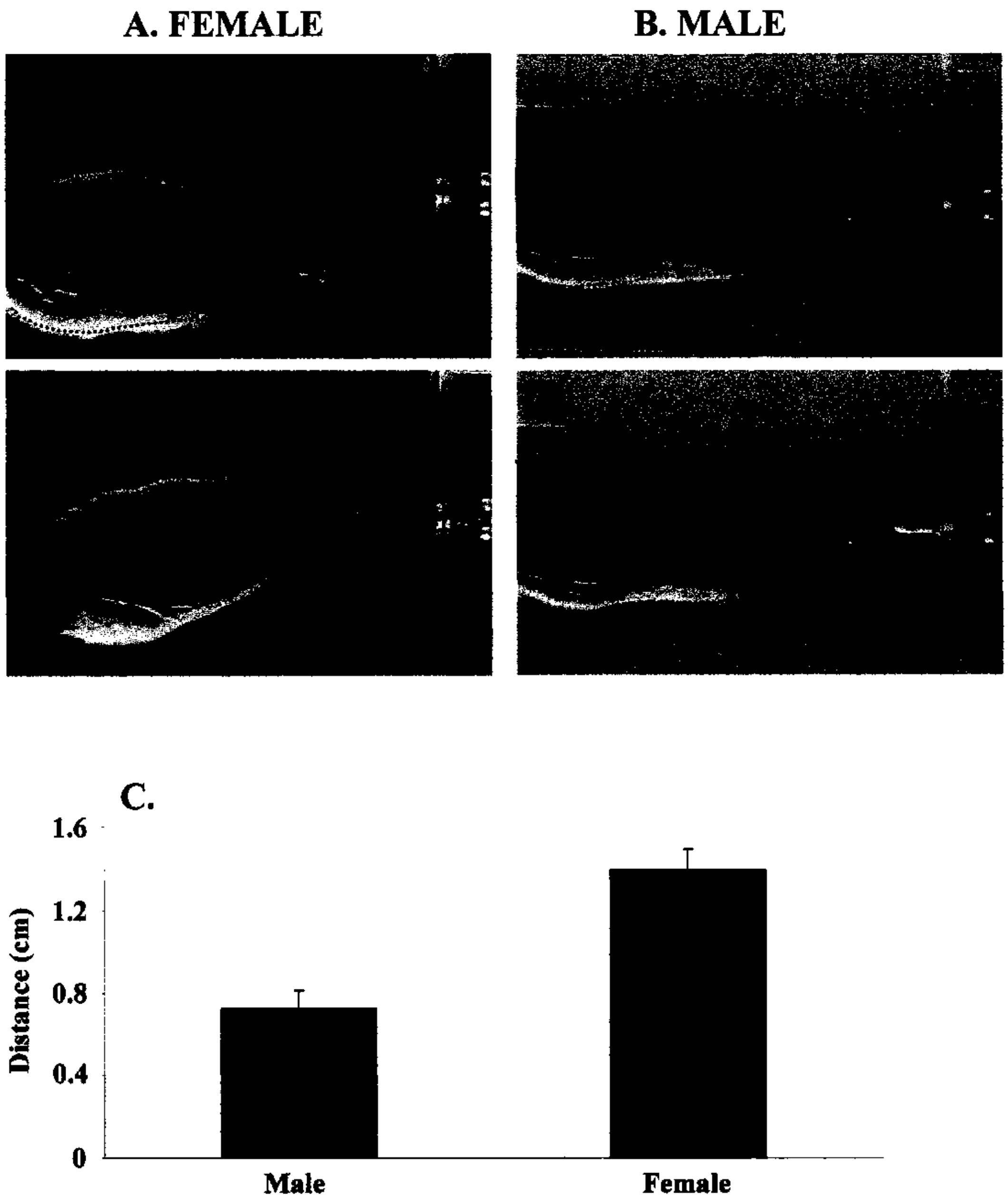


Females made significantly more steps during the initiation of a reach than males (A) and were more likely to orient to the food pellet during a step (B). Females were also more likely to make a postural adjustment step, with either the fore- or hindpaw that was not coupled to a forward locomotor step (C).

Figure 28 - Figure Caption

In this figure the position of a female and male at both the initiation of a reach, when the animal engages the food pellet with its snout (Figure 28 Aa and Ba) and at the conclusion of the reach when the pellet is grasped (Figure 28 Ab and Bb) are depicted. The angle of the longitudinal axis of the body is designated by the large dashed line. Females have a greater angular displacement from perpendicular to the reaching slot than do males at both the beginning and end of a reach. Females are also more likely to exhibit a greater lateral shift of the midbody during a reach than males. This can be seen by comparing the initial body position (the small dashed lines Ab and Bb) to the final body position. The arrow (Ab) is pointing to the area of greater midbody displacement in the female. The quantification of the maximum displacement of the midbody for a female and male is depicted (Fig. 28C). Females exhibit a greater lateral displacement of the midbody than males during a reach.

Figure 28



5.3 Discussion

Sex differences in posture and movement organization are important not only for understanding differences in sex-related behavior but also for understanding the neural control of movement more generally. In this study the question of whether skilled reaching, and the postural adjustments that accompany it, are different in male and female rats was asked. No sex differences in forelimb movements were found. Females did however use a different combination of stepping and postural adjustments to complete a successful reach. Females were more likely to be in midstride as they oriented their snout to the food pellet, to make more postural adjustments steps, to show greater displacement of the longitudinal axis from perpendicular to the food pellet, and to exhibit a greater lateral shift of their midbody as they reached, than did males.

The laboratory rat has been used extensively to study the neural control of skilled movements (Miklyaeva, Castañeda, & Whishaw, 1994; Whishaw, Gorny, & Sarna, 1998; Whishaw & Metz, 2002; Whishaw, O'Connor, & Dunnett, 1986; Whishaw, Pellis, Gorny, Kolb, & Tetzlaff, 1993) however a direct comparison of male and female movements had not been made. Sex differences in the integration of fore- and hindquarter movements are present in other whole body movements such as dodging to protect a food item (Field et al., 1996a; Field et al., 1997a; Field et al., 1997b), spontaneous turning (Field & Pellis, 1998), changes in postural stability during haloperidol-induced catalepsy (Field et al., 2000) and rotation from supine to prone (Field et al., 2005). This study provides added support that sex differences in the organization of movements and posture by the laboratory rat may be an integral part of how males and females execute behavioral tasks.

Chapter 6*

Sexually Dimorphic Postural Adjustments during Vertical Behavior are altered in a Unilateral 6-OHDA Rat Model of Parkinson's disease.

ABSTRACT

The study of sex differences in the onset, progression and symptoms of Parkinson's disease, in humans, has led to mixed results. In this study a unilateral 6-hydroxydopamine animal model of Parkinson's disease was used, to address whether there are sex differences in the composition of the movements used during vertical exploration within a confined cylinder. Tyrosine hydroxylase staining and apomorphine induced rotation were used to confirm lesion magnitude. There were no sex differences or lesion effects in the frequency of occurrence of a vertical bout or the average time spent vertical. Both male and female 6-OHDA treated animals exhibited equal impairments in the use of the forelimb contralateral to the lesion during vertical exploration. In contrast, 6-OHDA males, as compared to 6-OHDA treated females, showed a significant reduction in the use of the hindlimbs. An analysis of hindlimb step direction revealed that while sham treated females were more likely to step forward, sham treated males were more likely to step backwards during a vertical bout. This sex difference was no longer present in the 6-OHDA treated animals. Finally, 6-OHDA treated males were significantly more likely to place their dorsal surface in contact with the wall of the cylinder to maintain an upright posture during a vertical bout than animals in any other condition. These results demonstrate that the use of a confined cylinder task is appropriate for the kinematic analysis of sex differences in vertical behavior and shows

that males and females use different strategies to maintain postural support after CNS injury.

*This chapter is in preparation for submission to Behavioural Brain Research

6.0 Introduction

In the current study the question of whether sex differences in the integration of fore- and hindquarter movements are present in vertical behaviors was addressed (Gharbawie, Whishaw, & Whishaw, 2004; Johnston, Schallert, & Becker, 1999). Given previous findings (Field et al., 2005; Field, Metz, Pellis, & Whishaw, 2004; Field et al., 2005; Field & Whishaw, 2005a; Field et al., 2004) it was hypothesized that any differences found in the postural adjustments used during vertical behavior would exist in the integration of fore- and hindquarter movements and in the stepping patterns of the hindpaws. This would be consistent with the sex differences that have been described in the kinematic organization of other non-reproductive behaviors and would support the hypothesis that there are sex-typical patterns of movement organization and postural adjustments that are generalizable across a wide variety of rat behavior.

It is also possible that sex differences in the kinematic organization of vertical exploration in the rat can be modified or are no longer present after CNS injury. In contrast, sex differences in movement organization may only become apparent after CNS injury. This would suggest that males and females use different compensatory mechanisms to maintain postural integrity and organize their movements after neural damage. In order to address these issues the vertical behavior of male and female rats that received a unilateral 6-hydroxydopamine (6-OHDA) nigrostriatal bundle lesion, a rodent model of Parkinson's disease were compared (Schallert et al., 2000; Tillerson et al., 2002; Tillerson et al., 2001; Whishaw et al., 1997; Whishaw et al., 2003; Whishaw et al., 2002; Woodlee & Schallert, 2004) to sham-treated males and females. This model also allows for comparison of the movements by the side of the body affected by the lesion to the unaffected side (Metz, Tse, Ballerman, Smith, & Fouad, 2005; Miklyaeva & Whishaw, 1996; Miklyaeva et al., 1997; Whishaw et al., 1997; Whishaw et al., 2003). Thus, it is possible to determine whether the integration of movements between the impaired and non-impaired side are differentially affected in males and females.

6.1 Methods

6.1.1 Subjects

Twelve male and twelve female six month old Long-Evans rats, that were born and raised in the University of Lethbridge vivarium, were used in this study. They were housed, in same sex pairs, in clear Plexiglas cages from weaning. All animals were provided with food and water ad libitum. The colony was maintained on a 12/12 h light/dark cycle (lights on 8:00-20:00h). The experiments were approved by the University of Lethbridge Animal Care Committee and conducted according to the principles outlined by the Canadian Council on Animal Care (1984).

6.1.2 Surgery

Six male and six female rats received unilateral lesions of the left nigrostriatal bundle. Thirty minutes prior to surgery, the rats received a 25mg/kg i.p. injection of desmethylimipramine (Sigma Chemicals, St. Louis, MO, USA). The rats were anesthetized with isoflurane (4% for initiation, 1.5% for maintenance). Unilateral neurotoxic lesions of the left nigrostriatal bundle were performed with injections of 6-hydroxydopamine (6-OHDA) hydrobromide (2 µl of 4mg/ml in 0.9% saline with 0.02% ascorbic acid) (Whishaw, O'Connor, & Dunnett, 1986) at the following coordinates: 4.0 mm posterior to bregma, 1.5 mm lateral to the midline, and 8.5 mm ventral to the skull surface with the skull flat between lambda and bregma (Paxinos & Watson, 1998). Infusion took place over 5 minutes, with five additional minutes of diffusion. The remaining six males and females served as sham operated controls. The surgical procedure was identical for the sham treated animals, however only saline and ascorbic acid was injected into the nigrostriatal bundle.

6.1.3 Lesion Quantification

Apomorphine Rotation Testing: Three weeks after surgery an apomorphine rotation test was used to determine the severity of the lesion (Metz, Piecharka, Kleim, & Whishaw, 2004; Ungerstedt, 1971). All animals were injected subcutaneously with apomorphine-hydrochloride (0.05 mg/kg (Sigma Chemicals, St. Louis, MO, USA) dissolved in a 0.9% saline solution with 0.02% ascorbic acid). Immediately after injection the animals were placed individually into a 30 cm diameter round rotometer bowl. A cuff was wrapped around the trunk of the rat and connected to a lead and swivel that was connected to a computer. A custom-made computer program recorded the number of turns in the direction ipsiversive and contraversive to the lesion during a forty minute period.

***Tyrosine Hydroxylase Staining*:** In order to confirm the severity of the lesion as indicated by the apomorphine rotation scores the brain tissue from all the lesioned animals was removed, sliced, and stained for tyrosine hydroxylase (TH), a marker of catecholaminergic neurons. After the behavioral testing was complete the animals were deeply anesthetized and perfused through the heart with a 0.9% NaCl solution followed by picric acid (Lana's fix). The brains were removed and postfixed for a minimum of 2 days. The brains were then cut using a vibratome (50µm sections). For the TH immunocytochemistry the sections were washed in phosphate buffer and incubated overnight, at room temperature, with anti-TH monoclonal antiserum. (1:10 000, Sigma). The sections were then processed using the ABC method (Vector, Vectastain, Burlingame, CA) with anti-mouse antiserum IgG and horse serum and reacted with 3,3' - diamino-bezidine tetrachloride (0.06%), hydrogen peroxide (0.3%) and nickel solution (Metz et al., 2004; Metz & Whishaw, 2002)

For quantification of substantia nigra TH cell loss in the lesioned hemisphere three separate sections were chosen that showed the highest TH staining in the substantia nigra and ventral tegmental area on the non-lesioned side. The analyzed sections were between 4.8 and 5.8 mm posterior to bregma. A TH-positive cell was defined as a cell, whose cell body, was visibly stained on the section being analyzed. The cell counts for the three sections for each animal were averaged to obtain a score for the intact and lesioned hemisphere for each animal that was used for statistical analysis.

6.1.4 Behavioral Testing

Testing Apparatus: The testing apparatus was a clear Plexiglas cylinder (20 cm in diameter, 30 cm high). The cylinder was placed on a table with a clear Plexiglas top. Under the table was a mirror inclined at 45 degrees from which the ventral side of the animals could be viewed and videotaped and a detailed analysis of the body movements and stepping patterns could be performed (Gharbawie et al., 2004; Pinel, Hilton Jones, & Whishaw, 1992). The cylinder was sufficiently heavy to prevent the animals moving the cylinder on the table during vertical bouts.

Video Recording: Video recordings were made of each animal's vertical behavior with a Sony Hi-8 Camcorder at 30 frames per second at a shutter speed of

1/250s. Illumination was provided by two freestanding 150-W spotlights placed at a fourty-five degree angle to the glass table. The Hi-8 video was subsequently transferred to a Sony VHS tape and a digital time code was added using a TRG-50 Horita Micro Window to allow for frame-by-frame analysis of the videotaped trials.

Testing Procedure: Trials were conducted between 0900 and 1200 hours, during the light part of the light-dark cycle. During the three weeks between surgery and testing all animals were handled weekly for 10 minutes. Three weeks after surgery, a point in time when any recovery from the lesion is considered to be largely complete (Whishaw et al., 2003) each animal, naïve to the testing environment, was filmed in the testing apparatus for one ten minute trial. From the video records their vertical activity was analyzed. The following section describes the behavioral measures that were scored for each animal.

6.1.5 Behavioral Analysis

***Number and Time of Vertical Bouts*:** The number of vertical bouts during each ten minute trial was counted for each animal. A vertical bout was initiated when both forepaws were released from the floor surface and vertical movement of the longitudinal axis was observed. Termination of a vertical bout occurred when one forepaw regained contact with the floor. The duration, in seconds, of the first ten vertical bouts was also calculated. The average time spent vertical for each of the ten bouts per animal was averaged. This average was subsequently used for statistical comparisons.

6.1.6 Kinematic Analysis

In addition to calculating the total number of vertical bouts, and the average time spent vertical for the first ten bouts for each animal, a detailed analysis of the fore- and hindpaw steps and postural adjustments made by each animal during the first ten vertical bouts was conducted. The kinematic measures scored were as follows:

1) ***Forepaw Release and Subsequent Contact with the Floor upon Initiation and Termination of a Vertical Bout:*** A vertical bout was initiated when both forepaws were released from the floor surface and vertical movement of the longitudinal axis was observed. At the time of initiation of a vertical bout the forepaw that released first from the substrate was recorded. Termination of a vertical bout occurred when one forepaw regained contact with the floor. The forepaw that made the first contact with the substrate upon completion of a vertical bout was also recorded.
2) ***The use of the Forepaws to Explore the Cylinder Surface:*** The likelihood of contact with the vertical surface of the cylinder, for each vertical bout, was calculated for the forepaw ipsi- and contralateral to the lesion for each animal. In addition, the likelihood of forepaw stepping during a vertical bout, as a measure of vertical exploration (Gharbawie et al., 2004), was conducted for each forepaw.
3) ***Frequency of Hindpaw Steps:*** For each animal the average number of vertical bouts, where hindpaw steps occurred, was calculated. For the hindpaw steps that were exhibited by each animal the likelihood of whether the step was made by the hindpaw ipsi- or contralateral to the lesion was also calculated. For this analysis two lesioned males and one lesioned female were excluded due to a lack of hindpaw steps.
4) ***The Direction of Hindpaw Steps:*** In addition to calculating the frequency of hindpaw steps a simplified version of the Eshkol-Wachmann Movement Notation (EWMN) system (Eshkol & Wachmann, 1958) was used to describe the pattern of hindpaw stepping. The EWMN system enables the observer to label numerically the direction

of the hindpaw steps in relation to the position of the body at the time of the step. The numerical values obtained were then compared to determine whether differences existed in the direction of the locomotor/postural adjustment steps made by the hindpaws. Each step during the vertical bout was described as forward, backward, sideways or, to a point 45°, between the three. This was achieved by adapting aspects of the EWMN system.

Steps were described numerically in relation to the body at the time of the step. The direction of each hindpaw step was recorded in the horizontal plane with intervals of 45° on the circumference. The intervals are read in a clockwise direction from 0, 1, 2, 3, 4, 5, 6, 7 and back to 0. The circle is placed so that 0 is always aligned with the snout and 4 with the pelvis. As the rat moves, in either the horizontal or vertical direction, the circle and the associated numbers, maintain the same orientation to the body (See Fig. 6). The direction of the hindpaw steps was then scored by notating the number that corresponded to the direction of the step; for example, a step backwards toward the pelvis is notated as 4. For final quantitative and statistical comparison steps were categorized as forward (between 7 and 1) sideways (between 1 and 3 or 5 and 7) or backwards (between 3 and 5) (Eilam & Golani, 1988).

5) ***Hindquarter Bracing:*** It is possible that an animal may use the cylinder wall as an aid to postural support during a vertical bout. This can be achieved by leaning against the cylinder surface with a body part other than the forepaws. For all animals the likelihood of using the cylinder wall, as a brace for the body to maintain postural support while vertical, was scored.

6.1.7 Calculation of Group Means

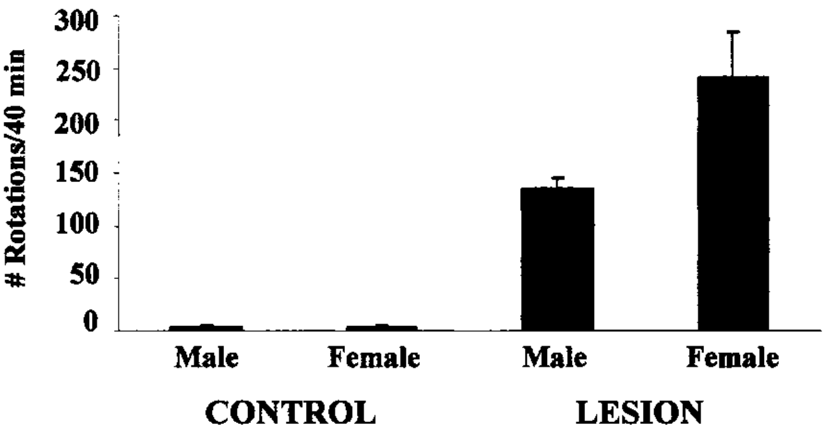
Results were analyzed using a two way ANOVA, with two between-groups factors. Significance was set at p = or < 0.05. Post hoc analysis was done using a Tukey's HSD test where appropriate. For graphical purposes the mean and standard error of the mean are shown.

6.2 Results

Apomorphine Rotation: The analysis of the likelihood of contraversive rotation revealed a significant main effect of sex F(1,20) = 5.44, p<.05, lesion F(1,20) = 64.86, p<.0001 and a sex by lesion interaction F(2,20) = 5.38, p<0.05. Post hoc analysis revealed that both males and females with unilateral nigrostriatal bundle lesions had significantly higher contraversive rotation scores than sham operated controls. In addition, 6-OHDA treated females showed significantly higher contraversive rotation scores than did 6-OHDA treated males (Fig. 29).

Tyrosine Hydroxylase Immunoreactivity: Analysis of tyrosine hydroxylase staining of the mesencephalic dopamine system in the 6-OHDA treated animals revealed a significant main effect of sex F(1,20) = 6.45, p<0.05, and lesion F(1,20) = 494.69, p<0.0001, however, there was no significant sex by lesion interaction. Representative sections for a lesioned male and female are shown in Figure 30A and B. Post hoc analysis revealed that both male and female 6-OHDA treated animals had a greater loss of TH immunoreactive neurons on the 6-OHDA treated side than the intact hemisphere. (Fig. 30C)

Figure 29

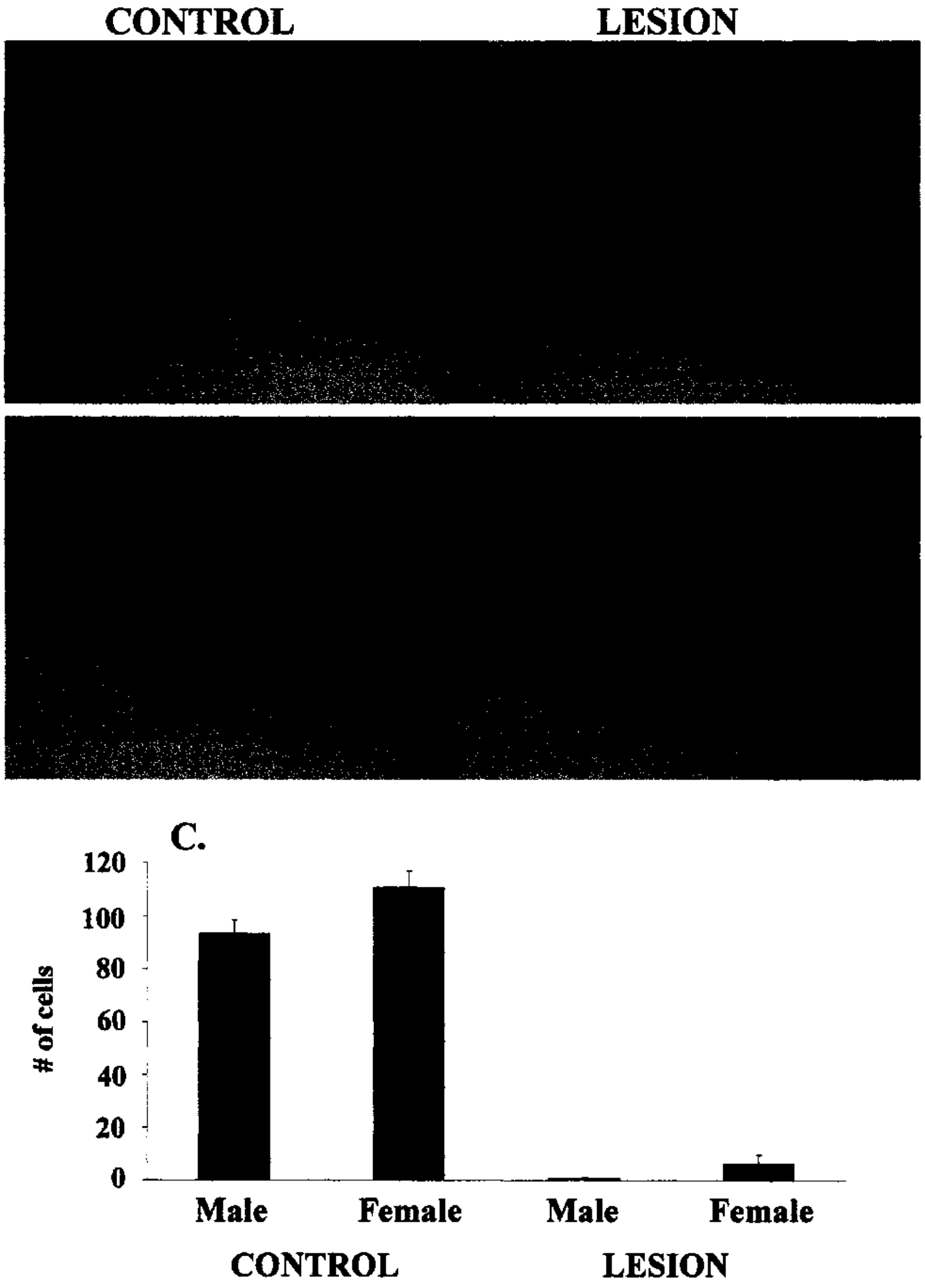


The number of contraversive turns in response to a 0.05mg/kg dose of apomorphine during a 40 minute trial is depicted. Both the 6-OHDA treated males and females had significantly higher rotation scores than did the sham operated animals. The female 6-OHDA treated animals also showed significantly higher levels of rotation than did the male 6-OHDA treated animals.

Figure 30 - Figure Caption

A representative photomicrograph of a coronal section through the mesencephalon of a male (A) and female (B) rat that had received a 6-hydroxydopamine lesion of the left nigrostriatal bundle is shown. The lack of tyrosine hydroxylase staining on the left versus the right reveals an almost complete loss of dopaminergic cells and fibers in both the ventral tegmental area and the substantia nigra. Magnification: 250x. The number of TH immunopositive cells that were counted in the intact versus 6-OHDA treated hemisphere is shown in Figure 30C.

Figure 30


CONTROL
LESION
C.
120
100
80
60
40
20
0
# of cells
Male
Female
Male
Female
CONTROL
LESION

Number of Vertical Bouts: The number of vertical bouts during the ten minute test was calculated for each animal. There were no significant main effects for sex or lesion.

Time Spent in the Vertical Position: The average time spent vertical was calculated for the first ten vertical bouts exhibited by each animal. There were no significant main effects for sex or lesion. There was, however, a significant sex by lesion interaction $F(2,20) = 5.02$, $p<0.05$. Post hoc analysis revealed that 6-OHDA treated males spent less average time in the vertical position than sham treated males.

Preferred Forepaw use during Initiation and Termination of a Vertical Bout: There were no significant group differences in the likelihood of whether the ipsi- or contralateral forepaw was released first, from the substrate, upon the initiation of a vertical bout. In addition, no group differences were found in the likelihood of whether the ipsi- or contralateral forepaw contacted the ground first at the end of the vertical bout.

Forepaw Contact and Exploration of the Cylinder Surface: A significant main effect of lesion was found for the likelihood of contact, with the cylinder surface, with the forepaw contralateral to the lesion $F(1,20) =281.7$, $p<.0001$. Post hoc analysis revealed that both female and male 6-OHDA treated animals were significantly less likely than female or male sham treated animals to contact the cylinder wall with the contralateral forepaw. A comparison of the likelihood of forepaw contact, with the cylinder surface, with the forepaw ipsilateral to the lesion revealed a significant main effect for lesion $F(1,20) = 8.09$, $p<.0001$ and a sex by lesion interaction $F(2,20) = 4.09$, $p=0.05$. Post hoc analysis revealed that 6-OHDA treated males were significantly less likely to contact the cylinder surface with the paw ipsilateral to the lesion than sham treated male controls. (Fig. 31A & B).

Analysis of the frequency of forepaw stepping on the vertical surface, as a measure of exploration, was conducted for the forepaw ipsilateral to the lesion. There was a main effect of sex F(1,20) = 5.4, p<.05, and lesion F(1,20) = 6.3, p<.03, but no sex by lesion interaction. Post-hoc analysis revealed that 6-OHDA treated males were significantly less likely to exhibit stepping on the cylinder wall with the forepaw ipsilateral to the lesion than sham treated females. 6-OHDA treated males were not significantly different from 6-OHDA treated females or sham operated males. No other differences between the individual groups were found.

Hindpaw Stepping Frequency: An analysis of the likelihood of vertical exploratory bouts that incorporated hindpaw stepping revealed significant main effects for sex F(1,20) = 6.18, p<.05, and lesion F(1,20) = 11.7, p<.01. Post hoc analysis revealed that 6-OHDA treated males were less likely to incorporate hindpaw stepping with vertical exploration than any other group (Fig. 32). Whether the hindpaw steps that do occur are made primarily with the ipsi- or contralateral hindpaw was also analyzed. There was a significant main effect of lesion for the likelihood of steps by the contralateral hindpaw F(1,17) = 19.16, p<0.001. Post hoc analysis revealed that both male and female 6-OHDA treated animals were significantly less likely to use the contralateral hindpaw to step as compared to the sham treated controls. A significant main effect of lesion was also found for the likelihood of stepping with the hindpaw ipsilateral to the lesion F(1,17) = 19.15, p<.001. 6-OHDA treated males and females made significantly fewer steps with the ipsilateral hindpaw than sham treated females.

Direction of Hindpaw Steps: In addition to determining the overall likelihood of the occurrence of hindpaw steps the direction of the hindpaw steps used, during vertical exploration, was also analyzed. As described above, a modified version of EWMN was used to notate the direction of hindpaw steps while the animals maintained a vertical orientation. Due to the lack of hindpaw steps by the 6-OHDA treated animals, with the hindpaw contralateral to the lesion, and the lack of significant differences in the likelihood of step direction for either the ipsi- or contralateral hindpaw in the intact animals the data for the two hindpaws was collapsed. The likelihood of hindpaw direction for each hindpaw step, as a percentage of the total number of hindpaw steps for each animal, was calculated.

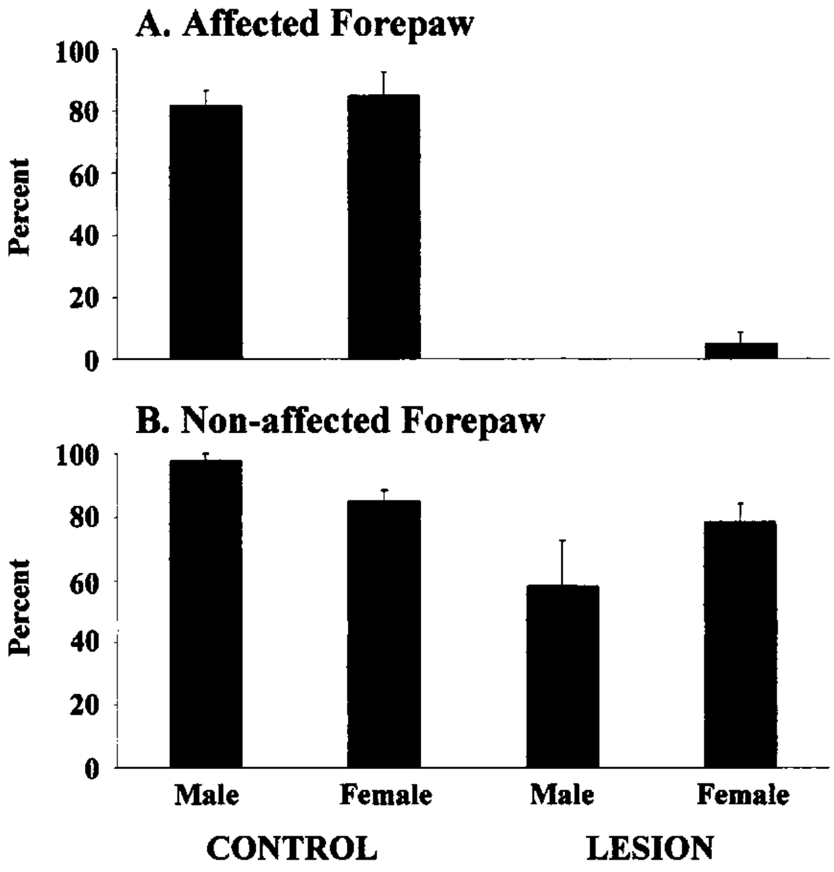
Steps Forward: A significant main effect for sex $F(1,17) = 31.4$, $p<0.0001$, and lesion $F(1,17) = 9.58$, $p<0.01$, was found. Post hoc analysis revealed that sham treated females were more likely to step forward with their hindpaws during a vertical exploratory bout than any other group (Fig. 33A). 6-OHDA treated females were also significantly more likely to use forward steps during vertical bouts than were 6-OHDA treated males. The two male groups were not different from one another.

Steps Sideways: There was a significant main effect of lesion $F(1,17) = 33.57$, $p<0.0001$, and a significant sex by lesion interaction $F(2,17) = 5.65$ $p<0.05$, for the likelihood of sideways steps during vertical exploratory bouts. Post hoc analysis revealed that male 6-OHDA treated animals were significantly more likely than male and female sham treated animals to use sideways steps during a vertical bout. 6-OHDA treated females were also significantly different from sham treated males. Sham treated males and females were not significantly different from one another (Fig. 33B).

Steps Backward: A significant main effect for sex F(1,17) = 20.77, $p<0.001$, lesion F(1,17) = 14.9, $p<0.01$, and a sex by lesion interaction F(2,17) = 16.7, $p<0.001$, was found in the likelihood of steps backward during vertical exploratory bouts. Sham operated males were significantly more likely to step backwards during an exploratory bout than were any other group. There were no significant differences between the other three groups on this measure (Fig. 33C).

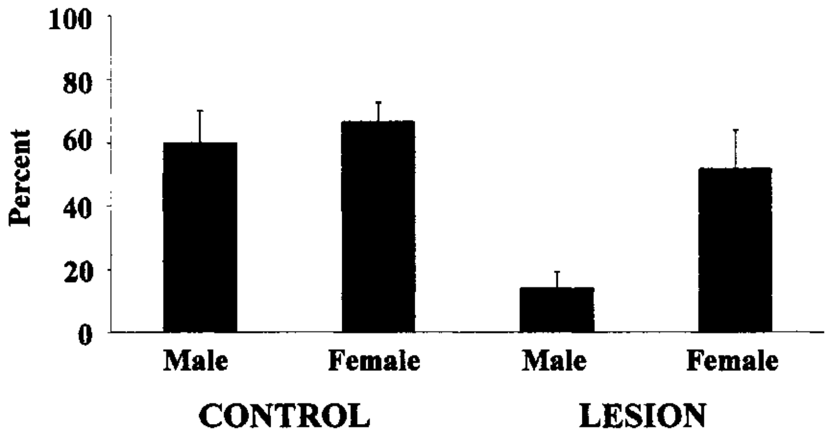
Hindquarter Bracing: A significant main effect for sex F(1,17) = 13.39, $p<0.01$, lesion F(1,17) = 4.69, $p<0.05$, and a sex by lesion interaction F(2,17) = 5.52, $p<0.05$, was found for the likelihood of hindquarter bracing. 6-OHDA treated males were significantly more likely to perform this behavior than any other group (Fig. 34). No significant differences were found between the 6-OHDA treated females and sham operated males and females.

Figure 31



The likelihood of forepaw contact with the cylinder surface is depicted for the forepaw either contralateral to (A) or ipsilateral (B) to the lesion. For the non-affected (ipsilateral) forepaw 6-OHDA treated males were significantly different from sham treated males but not from the females of either condition.

Figure 32



The average number of vertical bouts where hindpaw steps occurred is depicted. 6-OHDA treated males were significantly less likely to incorporate hindpaw steps with a vertical bout than any other group.

Figure 33 - Figure Caption

A modified EWMN analysis (see Fig. 6) of the direction of the steps made by the hindpaws revealed a sex difference in the likelihood of step direction for the sham treated animals. Sham treated females were significantly more likely to step forward toward the anterior of the body than sham treated males during a vertical bout. Sham treated males, in contrast, were more likely to step backwards during a vertical bout (A vs C). While 6-OHDA treated females maintained a propensity to step forward during a vertical bout 6-OHDA treated males no longer exhibited the male-typical strategy of stepping backwards (A vs C). In contrast to sham treated animals both 6-OHDA treated males and females show an increased likelihood to step sideways during a vertical bout (B).

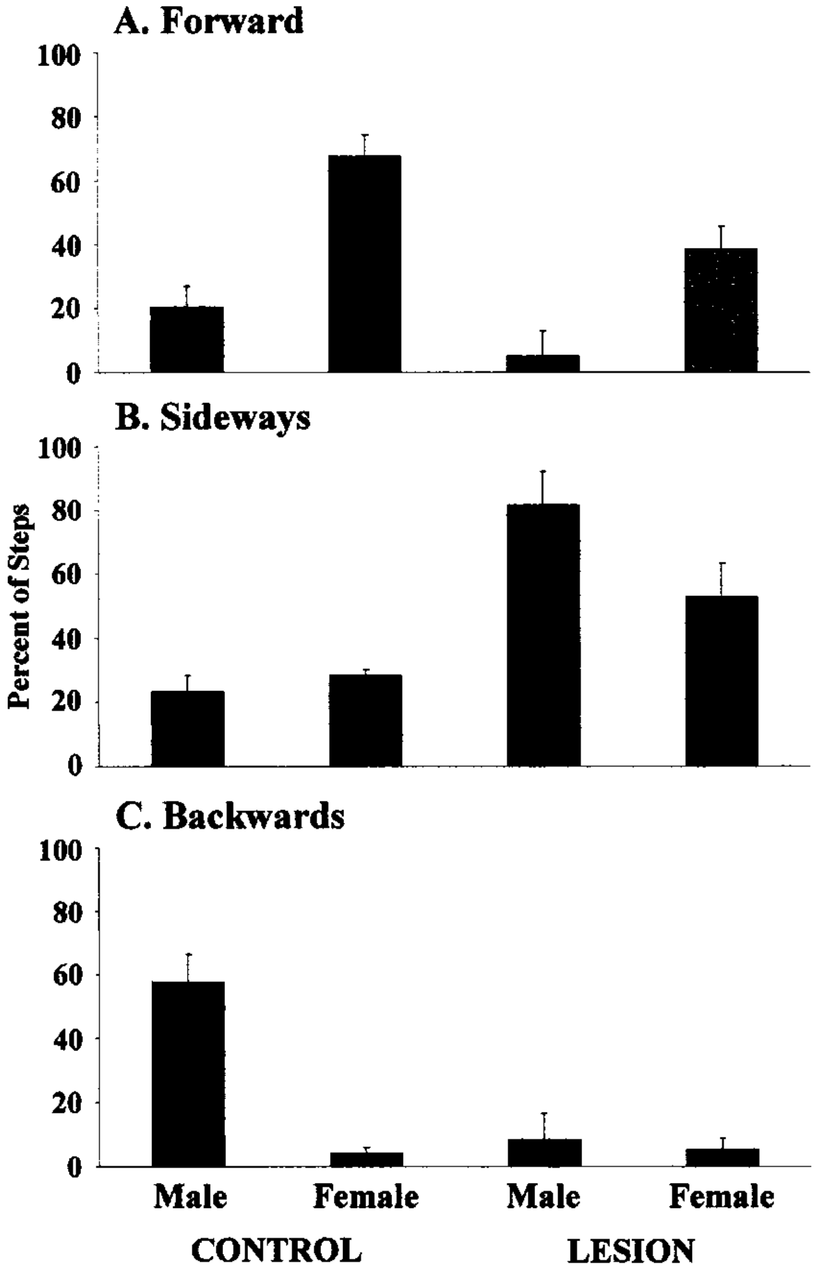
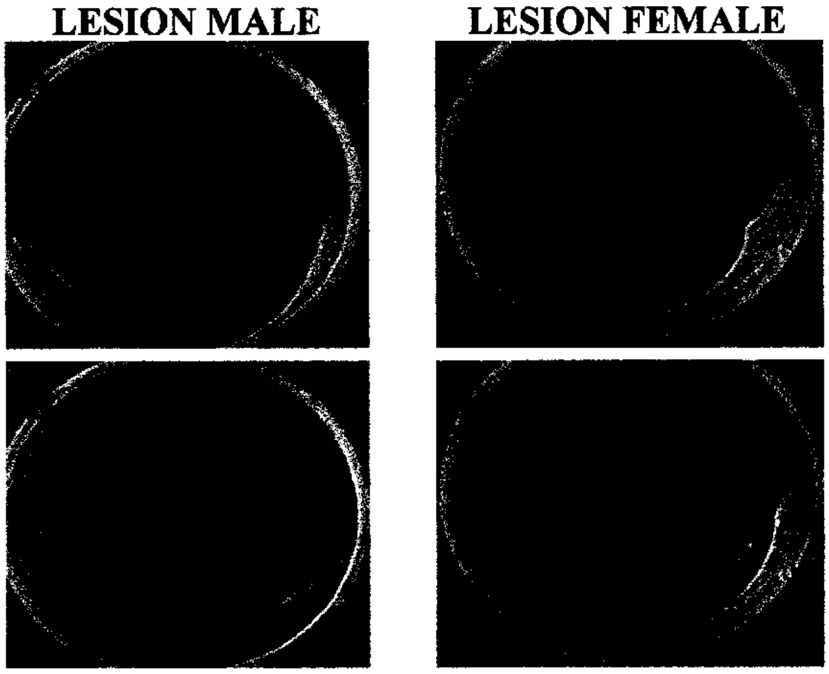
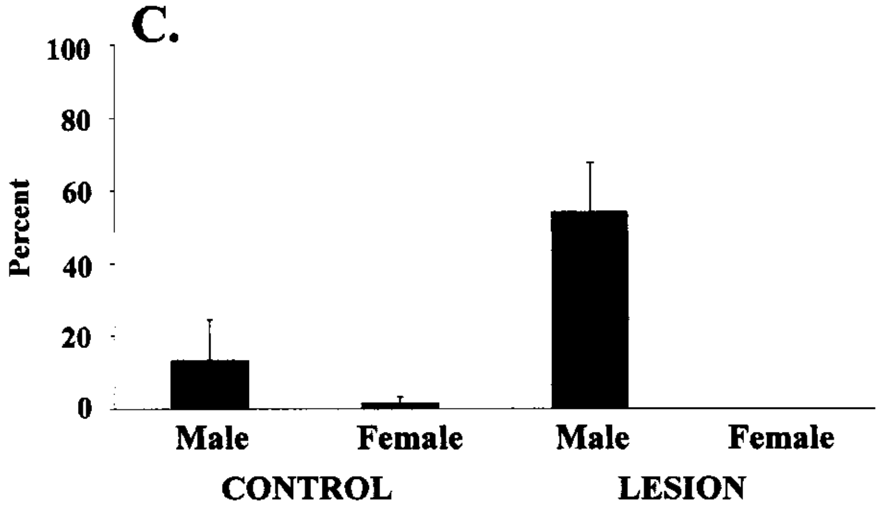

A. Forward
B. Sideways
C. Backwards
Percent of Steps
100
80
60
40
20
0
Male
Female
Male
Female
CONTROL
LESION

Figure 34 - Figure Caption

In this figure an example of a male 6-OHDA treated animal using the wall for support of the dorsal surface is shown. (Lesion Male A vs B) In panel A the 6-OHDA treated male or female is shown with their forepaws in contact with the substrate. Panel B shows a typical posture for a 6-OHDA treated male or female during a vertical bout. 6-OHDA treated males used the wall for support significantly more often during a vertical bout than either 6-OHDA treated females or sham treated animals of both sexes (C). The arrows indicate hindpaws that are about to step sideways. (The size of the cylinder relative to body mass cannot explain this difference since the sham treated males, who are larger than 6-OHDA treated males, rarely displayed this behavior Fig. 34C).


LESION MALE
LESION FEMALE


C.
100
80
60
40
20
0
Percent
Male
Female
Male
Female
CONTROL
LESION

6.3 Discussion

The results of this study reveal that there are kinematic differences in the way that males and females organize their movements during vertical exploratory behavior both before and after CNS injury. Males, with a unilateral 6-OHDA lesion of the nigrostriatal bundle, made fewer hindpaw steps and were more likely to maintain their vertical posture by using the cylinder wall for support of the body, than 6-OHDA treated females or sham operated males and females. These results suggest that males and females need to be considered separately when looking at the kinematic organization of motor behaviors and when assessing how CNS injury alters the organization and integration of movement and postural control.

Endpoint measures of success, such as counting the overall number of vertical bouts or calculating the time elapsed during a vertical bout did not reveal any differences between the 6-OHDA and sham treated males and females. Detailed kinematic analysis did reveal, however, that the stepping patterns of the hindpaws during vertical exploratory movements in sham treated males and females were sexually dimorphic. Females were significantly more likely to step forward, towards the wall, during a vertical bout than were males. In contrast, males were more likely to step backwards away from the wall - usually during the descent portion of the vertical bout. These sex differences were no longer apparent after the lesion. There were, however, changes in the kinematic organization of vertical behavior, after the unilateral 6-OHDA lesions of the nigrostriatal bundle, that were sex-typical. 6-OHDA treated males were more likely to: (a) reduce the number of hindpaw steps used during vertical exploration, (b) alter the direction of the hindpaw steps they did make, (c) use the wall for postural support of the hindquarters, a difference that cannot be due to differences in body mass since it was not seen in intact males, and (d) exhibit a trend towards a decrease in the use of the non-affected forepaw to contact and explore the wall of the cylinder. These results show that sex-differences in the kinematic organization of vertical behavior can be found both before and after CNS injury. These differences would not have been revealed if only endpoint measures of behavior, such as the frequency of vertical bouts or time elapsed during a vertical bout, were used.

The amount of dopamine depletion following 6-OHDA injection into the nigrostriatal bundle is often assessed by the number of contraversive apomorphine rotations that are made within a given time frame (Metz, Piecharka, Kleim, & Whishaw, 2004; Ungerstedt, 1971). The number of contraversive rotations exhibited by 6-OHDA treated males and females suggested that both groups had large lesions. 6-OHDA treated females, however, exhibited significantly higher numbers of contraversive rotation than did 6-OHDA treated males. The sex difference in contraversive rotation was not corroborated by the histological analysis of mesencephalic TH loss; which indicated equivalent cell loss in males and females. It has recently been demonstrated that while contraversive rotation with apomorphine is indicative of a large lesion it does not correlate well with behavioral impairments (Metz & Whishaw, 2002). Reported contraversive apomorphine rotation scores, using the same dose of apomorphine that was used in the current study, vary from three rotations per minute in a study that used males (Metz et al., 2004), to fourteen rotations per minute in a study that used females (Metz et al., 2005). Further investigation into the relationship between sex differences in, the behavioral impairments, contraversive apomorphine rotation scores, and possible dopamine receptor super-sensitivity in response to a nigrostriatal lesion will need to be conducted.

The significantly higher apomorphine rotation scores in 6-OHDA treated females, coupled with the lower severity of behavioral impairments suggest that changes in striatal function may be different in males and females after a 6-OHDA lesion of the nigrostriatal bundle. Sex differences in striatal function have been reported (Becker, 1999; Robinson, Becker, & Presty, 1982; Savageau & Beatty, 1981). Recently, a number of authors have suggested that estrogens may play a neuroprotective role in animal models of nigrostriatal damage (Dluzen, 2000; Dluzen & McDermott, 2000; Gillies, Murray, Dexter, & McArthur, 2004). It has also been suggested that estrogens in human females may lower the likelihood of developing Parkinson's disease in humans (Currie, Harrison, Trugman, Bennett, & Wooten, 2004; Dluzen, 2000; Dluzen & McDermott, 2000; Gillies, Murray, Dexter, & McArthur, 2004; Kompoliti, 2003). The results of the present study suggest that further investigation into the role of estrogen and its relationship to sex-typical anatomical and behavioral changes after a nigrostriatal lesion is warranted.

DISCUSSION

7.0 General Discussion

In the final chapter of this thesis, the data presented for each behavioral task that support or refute the hypotheses, stated in the Introduction to this thesis, will be reviewed. These hypotheses were: (a) there are general sex differences in the organization of movement in rats, (b) exposure to gonadal hormones neonatally can affect the development of male- and female-typical patterns of movement, (c) sex differences in movement organization are due to differences in body morphology, and (d) alterations of CNS dopaminergic function, in adulthood, can differentially affect the expression of patterns of movement organization in male and female rats. Additional evidence supporting the role of the CNS in orchestrating the expression of sex-typical patterns of movement both from a developmental and adult perspective will be discussed.

Prior to addressing the theoretical issues of why sex differences in movement organization may have evolved, and the possible evolutionary history of these differences, preliminary data from a comparative analysis of sex differences in spontaneous turning in five species: *Mus musculus, Octodon degus, Dasyurus hallacatus, Gryllus bimaculatus,* and *Gromphadorhina portentosa* will be presented. The comparison of the organization of movement in placental mammals, marsupials and insects, provides empirical data that can be used to speculate about the evolutionary origins of sex-typical patterns of movement organization. In light of the findings presented in this thesis, the following will be discussed: (a) possible genetic contributions to sex differences in movement organization, (b) how these differences may have ultimately evolved, and (c) the relevance of these findings to the study of proximate mechanisms in humans. The discussion will be concluded by presenting the theory that sex differences in movement organization are phylogenetically old and may underlie the subsequent evolution of a sexually differentiated nervous system.

7.1 Summary of Research Findings

In this thesis, evidence has been provided that in rats, sex differences in the organization of movement are present in a diverse group of behaviors, independent of the success of the chosen behavior. At present, sex differences in movement organization have been documented in locomotion (Pellis et al., 1997), spontaneous turning (Field & Pellis, 1998), during dodging to protect a food item (Field et al., 2005; Field et al., 2004; Field et al., 1996a; Field et al., 1997a; Field et al., 1997b; Pellis et al., 1999), contact righting, or the rotation from supine to prone on a solid surface (Field et al., 2005), during the postural adjustments and movements that are made during skilled reaching (Field & Whishaw, 2005a), vertical exploratory behavior (Field et al., 2004), and during postural adjustments in response to challenges of postural stability (Field et al., 2000).

Sex differences in movement and in the organization of posture, in behaviors such as dodging, are modifiable by gonadal hormonal exposure neonatally (Field et al., 2004; Field et al., 1997a) and are not due to differences in body morphology (Field et al., 2005; Field et al., 2004; Pellis et al., 1999). After CNS injury, sex-typical alterations in the organization of movement can also be found (Field et al., 2004; Field, Sherren, Pellis, & Whishaw, 2005d; Field et al., 2004; Pellis, Field, Sherren, Yamazaki, & Whishaw, 2005). These data show that there are differences between males and females in how they integrate their posture and movement, in the lateral, forward, vertical and rotatory, planes of movement. Furthermore, these data suggest that these sex differences are determined primarily by CNS mechanisms.

7.2 Summary of the Sex Differences in Movement Organization in Each Task

Dodging to Protect a Food Item: Male and female Long-Evans rats, during a dodge turn in the lateral plane, approximately 180 degrees, away from an approaching conspecific or robber. Females and males, while equally successful in maintaining possession of a food item (Whishaw, 1988; Whishaw & Tomie, 1987) use different movement tactics to turn away from an approaching robber. Females typically turn away from a robber by pivoting around a point located near the pelvis. Males pivot away from the robber by turning around a pivot point located near the midbody. This leads to a larger excursion of the snout relative to the pelvis in females than in males. The difference in the movement of the snout relative to the pelvis is accompanied by differences in the stepping patterns of the hindpaws. Females generally initiate a dodge by stepping forward and out to the side of the body with the hindpaw that is ipsilateral to the turn. This is followed by hindpaw steps forward in the direction of the snout. Males, in contrast, initiate a dodge by primarily stepping backwards, with the ipsilateral hindpaw, towards the pelvis. This step is generally followed by a sideways step with the hindpaw contralateral to the turn. The dodge is completed with steps forward, by the hindpaws, in the direction of the snout (Field et al., 2005; Field et al., 2004; Field et al., 1996a; Field et al., 1997a; Field et al., 1997b; Pellis et al., 1999).

The sex difference in dodging is present in both Long-Evans rats and the wild-type male and female rats that were used for comparison to the *tfm* males. This suggests that sex differences in dodging are not a function of the strain of rat tested. An interesting note is that while the *tfm* and wild-type males are significantly different from wild-type females, they are also different from Long-Evans males in the ratio of their snout to pelvis movement (Fig. 35; see Chapter 2 for details of this analysis)[6]. This difference is due to a lack of occurrence of the sideways step with the contralateral hindpaw, in the direction of the robber, that is exhibited by Long-Evans males (Field, Whishaw, Watson, & Pellis, 2005b) (Fig. 36)[7]. These data suggest that the differences between males and females in the movement kinematics of the turn, used during dodging to protect a food item, are not strain specific. However, there may be elements of the dodge pattern that vary with the strain of rat tested. Further research will be needed to determine how sex-typical patterns of dodging differ with rat strain and how this interacts with exposure to gonadal steroids.

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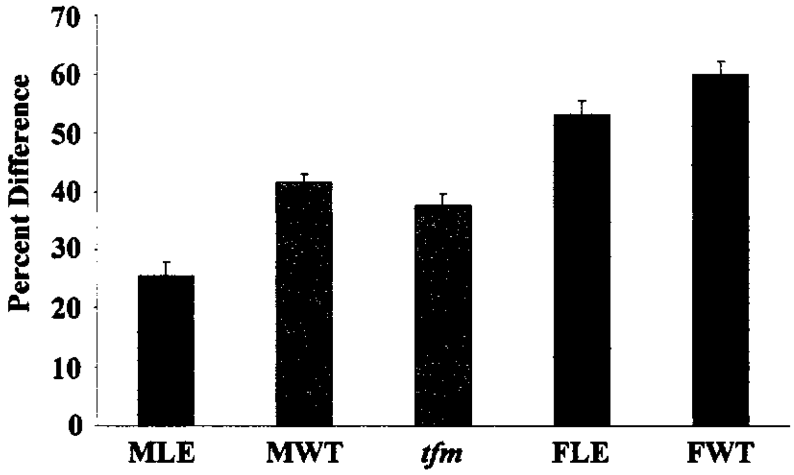
[6] 10 Female and 10 male Long-Evans, 10 *tfm*,6 female and 10 male wild-type controls were used. ANOVA for snout to pelvis ratio F(4, 41) = 32.49, p<.0001. Post hoc analysis was done using a Fisher PLSD.

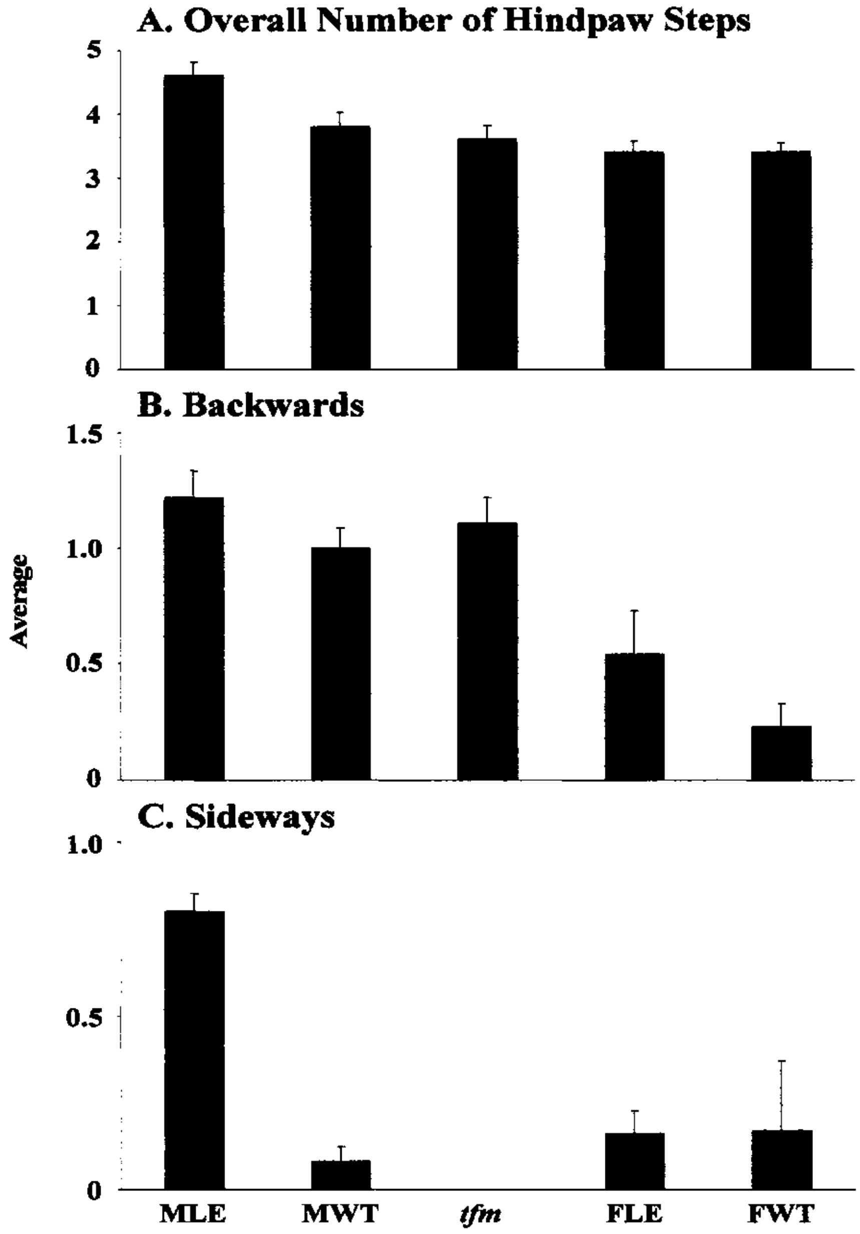
[7] ANOVAs for the overall number of steps F(4,41) = 6.022, p<.001., backwards steps F(4,41) = 8.226, p<.0001., and sideways steps F(4,41) = 60.24, p<.0001. Post hoc analysis was done using a Fisher PLSD test to compare individual group means.

Figure 35



The ratio of snout to pelvis movement is depicted for Long-Evans male and female rats (MLE and FLE), *tfm* males and their wild type male and female controls (MWT and FWT). The MLE is significantly different from all other groups. The *tfm* and MWT animals, while not different from one another, were significantly different from both female groups. The female groups, while not different from one another, are different from all male groups on this measure.

Figure 36



tfm males and their wild-type male controls do not exhibit the same frequency of hindpaw stepping during dodging as Long-Evans males (A). While they do exhibit backward steps with the hindpaws that are typical of Long-Evans males (B), they do not incorporate the lateral step, with the hindpaw contralateral to the direction of the turn, towards the robber (C).

Contact Righting: Contact righting is a behavior that involves the coordination of the fore- and hindquarters to rotate around the longitudinal axis, from supine to prone, on a solid surface. For both male and female Long-Evans rats, rotation to prone began with a rotation of the shoulders, and then continued with a passive carrying of the neck and head towards prone. In contrast, the recruitment of the hindquarters was different between the female and male groups.

In the female animals, once their forepaws had established firm contact with the ground, their hindquarters rotated in the same direction passed their forequarters. This led to a large angular change in the longitudinal axis of the female animals relative to their starting point when supine. Males, in contrast, exhibited a small rotation by the hindquarters, in the opposite direction to that of the forequarters, within a frame or two of the initiation of forequarter rotation. It was only after the initial counter rotation of their hindquarters, in the opposite direction to that of their forequarters, that the males rotated their hindquarters in the same direction as the forequarters to complete the righting sequence and achieve a prone position. Thus, from the initial supine position to the final prone position, there was little angular displacement of the longitudinal axis of the body. Comparing the performance of male and female Long-Evans rats to the wild-type males and females used for comparison to the *tfm*-affected males again suggests that these sex differences in movement organization are not due to a strain effect (Field et al., 2005).

Skilled Reaching: Skilled reaching is a task that allows for the analysis of sex differences in movement and postural adjustments that occur primarily in the forward plane of movement as the animal approaches, and reaches through the reaching slot, for a food item. Sex differences in the way that female and male rats transported their limb to the target or in the way they grasped or withdrew their limb once the food item was attained were not found. Nevertheless, there were sex differences in the postural adjustments made to grasp a food pellet successfully. Females exhibited a greater displacement of their longitudinal axis from perpendicular to the food pellet, showed a greater lateral shift of their mid-body as they reached, were more likely to be in mid-stride as they oriented their snout to the food pellet, and made more postural adjustment steps as they aligned themselves to the food pellet (Field & Whishaw, 2005a).

These data suggest that the sexually dimorphic components of postural adjustments and whole body movements are dissociable from a skilled forelimb movement. This may reflect a dissociation of the neural control of skilled movements from the neural control of the postural adjustments that underlie them. One male animal that was tested, but not included in the study, was found to be hydrocephalic when perfused and upon gross inspection it was established that he had very little cortex (Field & Whishaw, 2005b) (Fig. 37). Behaviorally, this male was indistinguishable from other males in the tactics used to rotate from supine to prone or during dodging to protect a food item. He also did not differ from other males in overall appearance or body weight. When attempting to reach, however, the hydrocephalic male would approach the reaching tray using male-typical stepping and postural adjustments, but would not reach with its forepaw. Any attempts made to acquire the food pellet were done by sticking its tongue through the reaching slot (Field & Whishaw, 2005b). This male provides an example of the dissociation of the ability to perform a skilled movement, such as reaching, from the ability to express male-typical patterns of postural adjustments and movement. Thus, the neural control of skilled movements, such as reaching, may be dissociable from the neural control of sex-typical postural adjustments and movement organization. Again, further research will be necessary to determine the veracity of this hypothesis.

Figure 37

The brain of a hydrocephalic male rat, cut down the midline, is depicted. The anterior of the brain is at the top of the photograph. Note the large cavities under the thin layer of cortex especially in the right hemisphere.

Vertical Exploratory Behavior: Sex differences were also found in the postural adjustments that occur during vertical exploratory behavior. While the overall number of vertical bouts or the time elapsed during a vertical bout did not reveal sex differences in the behavior of males and females, the stepping patterns of the hindpaws were sexually dimorphic. Females were significantly more likely to step forward towards the wall with their hindpaws during a vertical bout than were males. In contrast, males were more likely to step backwards away from the wall, usually during the descent portion of the vertical bout. These findings are corroborated by previous data showing that males and females distribute their weight differently on the hindpaws during a vertical exploratory bout. Females tend to shift their weight onto the anterior portion of the hindpaws while males primarily use the posterior portion of the hindpaw for weight bearing[8] (Field, Pellis, & Whishaw, 1998). Like the behaviors previously described males and females thus integrate changes in postural support with hindpaw use differently during vertical exploratory behavior (Field et al., 2004).

The Common Element: The findings presented in this thesis reveal that there are fundamental differences in how males and females integrate their fore- and hindquarter movements, in both social and solitary behaviors, that are independent of the success of the behavior being studied. These differences are present in behaviors that occur in lateral, forward, vertical and rotatory movement, and thus occur in all planes of movement (Eilam & Golani, 1988; Golani et al., 1981). In general, females lead with the

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[8] For a description of the methods used in this analysis refer to Miklyaeva, E. I., & Whishaw, I. Q. (1996). HemiParkinson analogue rats display active support in good limbs versus passive support in bad limbs on a skilled reaching task of variable height. Behavioral Neuroscience, 110, 117-125.

forequarters and follow with the hindquarters. Males, in contrast, appear to have a greater dissociation of the direction of the movement of the fore- and hindquarters, with the movement of the hindquarters often occurring counter to the direction of the movement of the forequarters. Females and males were equally successful in protecting a food item from theft during dodging, rotating from supine to prone, and in attaining a food item during a skilled reaching task. During vertical exploratory behavior they were as likely to initiate a vertical posture and maintain that posture for equal lengths of time. It is the manner in which they performed these actions that differed between the sexes. Thus, the results of this thesis show that the use of endpoint measures is not adequate for an investigation of how or why males and females differ in their performance of a given task - a detailed kinematic analysis is necessary.

While in this thesis the primary focus has been on sex differences in the organization of movement previous research has shown that sex differences in postural adjustments can also occur independent of movement (Field et al., 2000). Animals made cataleptic with haloperidol (a dopamine antagonist) will actively maintain a static, stable posture, but will no longer initiate voluntary movements (Teitelbaum, 1982). When their postural stability is challenged, such rats will make a variety of postural adjustments to protect their stability. For example, when pushed sideways, rats will shift their body weight in the direction of the displacing force (De Ryck, Schallert & Teitelbaum, 1980; Pellis, de la Cruz, Pellis & Teitelbaum, 1986). Similarly, when on an inclined plane, haloperidol-treated rats will shift their weight away from the direction of gravity, so that when facing downward, they will shift their body's backward (Morrissey et al., 1989). Untreated rats will turn to face upwards rather than brace against the downward pull of gravity. A kinematic analysis of the postural adjustments used by haloperidol-treated males and females to maintain their position on the inclined plane revealed that females mostly use their forelimbs to brace against slipping downwards, whereas males primarily used their hindlimbs (Field, Whishaw & Pellis, 2000). The results presented above, describing how males and females shift their weight differently during a skilled reaching task, provides further support for differences between the sexes in the use of postural support mechanisms (Field & Whishaw, 2005a).

Sex differences in the organization of movement are thus due to sex differences in both the maintenance of posture, and in the selection of sex-typical patterns of movement. Sex differences in movement organization may underlie the performance of any non-sexual behavior that includes a movement component, even when the success, or functional endpoint, of the behavior is the same for males and females. Thus sex differences in the organization of movement needs to be considered as a contributing factor in all facets of sex-typical patterns of behavior, such as sex differences in the performance of cognitive tasks, (McCarthy & Konkle, 2005) or in drug-induced behaviors (Becker, 1999; Field, Whishaw & Pellis, 1996b).

7.3 The Role of Gonadal Steroids

Once a sex difference in the organization of a behavior is documented the possible roles that gonadal steroids may have, with regards to the development and expression of this difference, are often investigated (Baum, 2003; Beach, 1975; Beach, 1981; Becker et al., 2005). Traditionally, the model for sexual differentiation of mammals has viewed the female as the default condition (Jost, 1983). Testes are necessary to produce the external genitalia typical of genetic males, ovaries, however, are not needed to produce the external genitalia typical of females (Jost, 1960). Thus, in the absence of testicular hormones, the genetic male develops the female phenotype (Voutilainen, 1992). This classical model of sexual differentiation has been extended to the CNS:

> The intrinsic pattern of CNS development is assumed to be organized along lines that are appropriate for the homogametic sex. In the heterogametic sex, differentiation away from this pattern occurs as a result of hormones produced by the gonads. Thus, in mammals the intrinsic pattern is female, with differentiation toward masculine patterns of... behavior occurring in the male as a result of exposure to testicular hormones during development. (MacLusky & Naftolin, 1981, p. 1294)

The time of exposure to gonadal steroids may also be critical for the expression of sex-typical patterns of behavior. There are periods of time during development when exposure to gonadal steroids may organize the neural circuits that underlie the expression of sex-typical patterns of non-sexual behavior in adulthood. In adulthood, these same neural circuits, may subsequently rely on exposure to gonadal steroids for activation before the sex-typical behavior pattern in question is expressed (Phoenix et al., 1959). Thus, the performance of sex-typical patterns of behavior may depend on both the organizational and activational effects of gonadal steroids (Baum, 2003). It is possible that the sex differences in behavior that have been described in this thesis require the exposure of male and female rats to gonadal steroids either around birth (organizational effects), in adulthood (activational effects), or at both time points. In order to address this line of questioning the role of testicular and ovarian hormones has been studied using the paradigm of dodging to protect a food item.

Testicular Hormones: Previous research has shown that testicular hormones are necessary for the development of a male-typical pattern of dodging to protect a food item. Males castrated the day after birth use a female-typical pattern of dodging as adults. In contrast, females injected with 200 µl of testosterone propionate, on the day of birth and the subsequent day, are more male-typical in their kinematic organization of a dodge as adults. Castration of males just prior to puberty or in adulthood had no effect on the sex-typical expression of dodging. This suggests that sex-typical patterns of dodging are dependent on the organizational effects of testicular hormones but do not require their presence for the sex-typical expression of this behavior in adulthood (Field et al., 1997a). A question that arises from this work is whether this is due to the direct effect of androgens on the developing brain or whether it is due to the masculinization of the CNS via the aromatization of androgens to estrogens (Breedlove & Hampson, 2002; McCarthy, 1994)

The experiments that are necessary to answer this question definitively have not been done. The work presented in this thesis, describing the behavior of the *tfm* males, suggests that it may be the conversion of androgens to estrogens that is necessary for the masculinization of the neural circuits that underlie the expression of the male-typical patterns of movement. The *tfm* males do have testes and produce androgens, but since they have a non-functional androgen receptor they cannot be masculinized by the direct effects of androgens on the body or developing CNS (Shapiro et al., 1980; Stanley et al., 1973). The masculinization of their behavior is therefore dependent on the effects of estrogens aromatized from androgens (Olsen, 1992; Purvis et al., 1977). The kinematic analysis of their patterns of dodging to protect a food item and contact righting revealed that the behavior of the *tfm* males is male-typical. These results provide support for the hypothesis that it is the masculinizing effects of estrogens on the CNS that are necessary for the expression of male-typical patterns of movement.

Ovarian Hormones: As previously mentioned female-typical development has often been considered the default condition; that is gonadal steroids are not necessary for the development of female-typical form and behavior (Jost, 1983; MacLusky & Naftolin, 1981). There is growing evidence however, that female-typical patterns of behavior are not simply the default condition, but are actively determined by both genetic (De Vries et al., 2002) and hormonal processes (Collaer & Hines, 1995). In rats, the ovaries become active around postnatal day 7 (Sokka & Huhtaniemi, 1995; Weniger, Zeis, & Chouraqui, 1993) and begin to exhibit regular ovulatory surges of ovarian steroids associated with sexual receptivity after vaginal opening at puberty (Brown et al., 1994). A number of behavioral studies have shown that the action of ovarian hormones are necessary for the female-typical development of sexual (Gerall, Dunlap, & Hendricks, 1973; Hendricks & Duffy, 1974) and non-sexual (Field et al., 2000; Forgie & Stewart, 1994; Pellis, 2002; Stewart & Cygan, 1980) behaviors.

The presence of ovarian steroids, directly after birth or at puberty, but not in adulthood, is necessary for the development of the female-typical behavioral pattern of dodging. Females ovariectomized just after birth were indistinguishable from intact males. Females ovariectomized prior to puberty exhibited patterns of dodging that qualitatively appeared female-like but incorporated backward steps of the hindpaw that are typical of males. This suggests that for females there may be an organizational effect of ovarian steroids during puberty on the organization of motoric behaviors. Ovariectomy in adulthood had no effect. These data provide evidence that the female phenotype of movement organization is not a default, but is actively feminized and demasculinized (Fitch, Cowell, & Denenberg, 1998; Fitch & Denenberg, 1998; Pellis, 2002) by ovarian steroids. Thus, the organization of the female pattern of dodging is dependent on the presence of neonatal and pubertal gonadal steroids (Field et al., 1997a).

Further research will need to be conducted to determine the relative contributions of ovarian and testicular steroids, and their associated receptor subtypes to the development of sex-typical patterns of movement and its neural control. For example, the effects of estrogen can be exerted via two intracellular receptor subtypes; ER-alpha & ER-beta (McEwen, 2001). Estrogenic effects on behavior can be specific to receptor subtype; the running wheel activity of female mice is dependent on the presence of ER-alpha, not ER-beta (Ogawa, Chan, Gustafsson, Korach, & Pfaff, 2003). It has also been shown that female hormones, most specifically estrogen, can have influences on the biochemical and behavioral output of the basal ganglia dopaminergic system (Becker, 1990a; Becker, 1990b; Becker, 1999); a system that is involved in the coordination and selection of motor patterns (Hikosaka, 1998; Mink, 1996). Therefore, it is possible that the effects of estrogens on the development and the function of the basal ganglia dopaminergic system may be, in part, responsible for the development of sex-typical patterns of movement organization in females.

7.4 The Role of Body Morphology

One possible confound or explanatory variable, for the sex differences in movement patterns that have been discussed in this thesis is the presence of sex differences in overall body mass and underlying skeletomusculature. Male Long-Evans rats, as adults, are larger than females and this is due in part to the influence of gonadal steroids during early development (Wade, 1976). Gonadectomy just after birth influences final mass of the adult body. Males castrated at birth are smaller (Field et al., 1997a), and females ovariectomized at birth are larger, than their intact male and female counterparts (Field et al., 2004). Thus, it is possible that sex differences in the organization of movement and posture may arise from peripheral differences in overall body mass.

Similarly, since there are sex differences in the morphology of the pelvis for a number of species, including humans (Coleman, 1969), rats (Bernstein & Crelin, 1967), mice (Shimizu & Awata, 1984) and rabbits (Lowrance, 1968), as well as in muscle and organ weights (Stewart & German, 1999), and skeletal mass (DeMoss & Wright, 1998), it is possible that sex differences in movement are a byproduct of sex differences in body form. Two experiments were conducted in order to determine whether sex differences in body mass or skeletomusculature morphology were the sole explanatory variable for sex differences in movement organization. The dodging to protect a food item and contact righting paradigms were used for analysis.

Pregnancy: To address the question of whether differences in body mass could explain the differences between males and females in their choice of sex-typical tactics of movement, the dodging behavior of gravid females was examined. Gravid females at G19/20 are indistinguishable from normal males with regards to overall mass (Field et al., 2004). If overall body mass contributes to the expression of sex-typical patterns of movement then gravid females should be more similar to intact males than females. The results from this experiment revealed that gravid females were similar to intact females, not to intact males. Thus, differences in body mass cannot be the sole reason for the expression of sex-typical patterns of dodging in Long-Evans male and female rats (Field et al., 2004).

***The tfm Model*:** Male and female rats also differ in their underlying skeletomusculature (Bernstein & Crelin, 1967; DeMoss & Wright, 1998; Stewart & German, 1999). The *tfm* model was used to examine the behavior of a genetic male with a peripheral female phenotype and skeletomusculature (Vanderschueren et al., 1994; Vanderschueren et al., 1993), but a masculinized CNS (Olsen, 1979b; Olsen, 1992; Purvis et al., 1977). This was done to determine whether sex differences in movement organization are determined by sex differences in an animal's skeletomusculature or in the CNS. The analysis of the behavior of *tfm* males, during dodging to protect a food item and contact righting, revealed that even though they were smaller and more feminized in their body morphology and skeletomusculature, they were indistinguishable from control wild-type males in their performance of male-typical patterns of dodging and contact righting.

The results of these two experiments, in combination with a previous experiment showing that sex differences in dodging are present prior to puberty (Pellis et al., 1999), and thus prior to the sexual differentiation of pelvic morphology (Bernstein & Crelin, 1967), provides evidence that sex differences in movement organization are not due to differences in overall body mass or to differences in skeletomusculature. Thus, sex differences in movement organization must be due, in large part, to sex differences in how the CNS orchestrates the organization of sex-typical patterns of movement.

7.5 The Role of the Nervous System

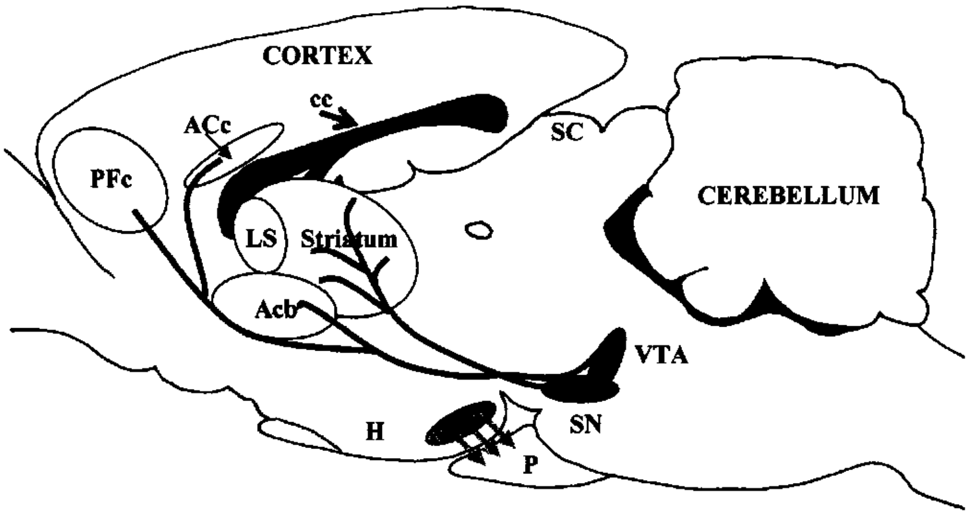
One of the major roles of the central nervous system is to generate instructions for the production of a behavior in response to the environment within which an organism exists. That similar non-reproductive behaviors, such as those previously described, differ in form suggests that there are fundamental differences in how males and females have evolved to interact with their environment. These differences are likely reflected in the sexually dimorphic CNS.

Sex differences in CNS anatomy and function have been described for numerous CNS systems and nuclei, in the rat (Connell, Karikari, & Hohmann, 2004; De Vries, 2004; McCarthy & Konkle, 2005; McEwen, Alves, Bulloch, & Weiland, 1998; Segovia et al., 1999), that are involved in the control and expression of behavior. Sex differences in the structure and function of the components of the nervous system that are involved in movement control in the rat, have also been described for: (a) neuromuscular junction kinetics (Villarroel, 2001), (b) spinal cord anatomy and function (Johansen, Jordan, & Breedlove, 2004; Rand & Breedlove, 1987; Rand & Breedlove, 1995; Watson et al., 2001), (c) the cerebellum (Lavaque, Mayen, Azcoitia, Tene-Sempere, & Garcia-Segura, 2006; Nguon, Baxter, & Sajdel-Sulkowska, 2005; Nguon, Ladd, Baxter, & Sajdel-Sulkowska, 2005), (d) pontine nuclei anatomy (Khankasikam, Khunwittaya, Srisai, Bjaalie, & Kotchabhakdi, 2005), (e) basal ganglia anatomy and function (Becker, 1999; Creutz & Kritzer, 2002; Di Paolo, 1994; Miller, 1983), and (f) motor cortex (Kritzer, 2004).

While many CNS systems and structures are likely relevant to the expression of sex differences in movement organization, the remainder of this discussion will be constrained to the possible role of dopaminergic systems, and their development, in the control of sex differences in the kinematic organization of sex-typical patterns of behavior. The dopaminergic system of the CNS is composed of three primary nuclei; the substantia nigra, ventral tegmental area and arcuate nucleus. The substantia nigra projects primarily to the striatum and is referred to as the nigrostriatal dopaminergic system. Dopaminergic projections from the ventral tegmental area project to the nucleus accumbens, cingulate and prefrontal cortex to form the mesolimbocortical dopaminergic system. The dopaminergic neurons of the arcuate nucleus of the hypothalmus release dopamine into the anterior pituitary which affects prolactin release. This system is called the tuberoinfundibular dopaminergic system (Nestler, Hyman, & Malenka, 2001) (See Fig. 38). (Note: for the rest of this discussion the tuberoinfundibular system will not be considered)

Figure 38

The Three Major Dopaminergic Pathways



This figure depicts a sagittal section of the rat brain. Three nuclei, the substantia nigra (SN, in red), ventral tegmental area (VTA, in blue), and arcuate nucleus (in green) contain the majority of dopaminergic neurons within the rodent brain. The SN dopaminergic neurons send projections primarily to the striatum (solid red line). The VTA neurons primarily send dopaminergic projections (solid blue lines) to the nucleus accumbens (Acb) anterior cingulate cortex (ACc) and prefrontal cortex (PFc). The arcuate nucleus of the hypothalamus releases dopamine into the anterior pituitary (solid green arrows). Other abbreviations: hypothalamus (H), pituitary (P), lateral septum (LS), superior colliculus (SC) and corpus callosum (cc).

Sex differences in the anatomy and function of dopaminergic neurons have been described during early development (Andersen, Rutstein, Benzo, Hostetter, & Teicher, 1997; Andersen, Thompson, Kreznel, & Teicher, 2002; Beyer, Eusterschulte, Pilgrim, & Reisert, 1992; Beyer, Pilgrim, & Reisert, 1991; Pilgrim, Beyer, & Reisert, 1999), after treatment with drugs, such as amphetamine, apomorphine and cocaine (Becker, 1999; Becker, Molenda, & Hummer, 2001; Becker, Robinson, & Lorenz, 1982; Robinson, Becker, & Ramirez, 1980; Savageau & Beatty, 1981) and in the substantia nigra before (Ravizza, Galanopoulou, Velíšková, & Moshé, 2002; Ravizza, Velíšková, & Moshé, 2003) and after damage (Cass, Peters, & Smith, 2005; Murray et al., 2003; Tamás, Lubics, Szalontay, Lengvári, & Reglődi, 2005). In the following two sections, I will present data that support the hypothesis that sex differences in movement organization may be related, in part, to sex differences in the development and adult function of the nigrostriatal and mesolimbocortical dopaminergic systems.

Lesions of the Dopaminergic Systems in Adulthood: As presented in Chapter 6, the organization of sex-typical patterns of movement, in adult male and female Long-Evans rats, can be altered by lesions of the dopaminergic systems in adulthood. Males and females given unilateral 6-OHDA lesions of the ascending dopaminergic systems were differentially impaired when rearing to explore a vertical surface. 6-OHDA treated males were less likely to exhibit hindpaw steps during vertical exploration and were more likely to use the cylinder wall for support of their dorsal surface (See Figs. 32 and 34). They also no longer incorporated backward steps by the hindpaws towards the pelvis when rearing. 6-OHDA treated females, in contrast, did not show a decrease in the use of the hindpaws and did not use the vertical surface for postural support of their dorsal surface. While they did exhibit a decrease in the likelihood of sex-typical forward stepping with the hindpaws, it was not as dramatic as the decrease in the backwards hindpaw stepping that was seen in the males. These data provide evidence that lesions of the ascending dopaminergic systems, in adulthood, can affect the kinematic organization of movement differently in males and females.

The basal ganglia is considered to be important not only for the integration of movement but also for the selection of a pattern of movement (Hikosaka, 1998; Mink, 1996). Thus, it is possible that the choice of a sex-typical pattern of movement can also be affected by lesions of the ascending dopaminergic systems which alter basal ganglia function. A comparison of the pattern of contact righting, in adult rats with a unilateral 6-OHDA lesion, towards either the affected or non-affected side of the body, can be used to determine whether the choice of a sex-typical pattern of movement can be impaired by CNS injury[9].

Contact righting has been used previously as a behavioral model to address questions regarding how the nervous system coordinates sensorimotor information and how this behavior is modified following CNS injury (Martens, Whishaw, Miklyaeva, & Pellis, 1996; Pellis, Pellis, Chen, Barzci, & Teitelbaum, 1989; Pellis, Pellis, Morrissey, & Teitelbaum, 1989; Pellis, Pellis, & Teitelbaum, 1987). Lesions to the nigrostriatal system

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[9] Dodging to protect a food item was not chosen for this analysis. It has been shown previously that when a robber approaches the impaired side of the body, in an animal with a unilateral 6-OHDA lesion of the nigrostriatal bundle, the animal has difficulty in initiating a dodge. This often results in the theft of the food by the robber. Thus a kinematic comparison of the movements made during dodging for the two sides of the body is not feasible.

Whishaw, I. Q., & Tomie, J. (1988). Food wrenching and dodging: A neuroethological test of cortical and dopaminergic contributions to sensorimotor behavior in the rat. Ibid., 102, 110-123.

have been shown to impair the coordination of body segments during righting (Martens et al., 1996; Masuda & Yamaguchi, 2000). All of this work, however, while demonstrating that righting reflexes can be impaired by CNS injury, has not addressed whether males and females are affected differently by CNS manipulations.

Given the sex-typical differences in how fore- and hindquarter movements are organized during contact righting, it seems likely that CNS manipulations that affect the coordination of the righting reflex may have different effects in males and females. To test whether males and females used male- or female-typical patterns of contact righting, after a unilateral lesion of the ascending dopaminergic systems, both sham and 6-OHDA treated males and females were tested in a manner similar to that described in Chapter Four[10]. The likelihood of hindquarter rotation and the angle of displacement of the longitudinal axis were recorded for five rotations to either the contralateral, or side of the body affected by the lesion, and to the ipsilateral or non-affected side. The results for each side were compared for sham and 6-OHDA treated males and females.

The results of this study revealed that the choice of male- or female-typical patterns of righting can be influenced by a unilateral lesion of the ascending dopaminergic systems. Male and female sham treated animals showed a sex-typical pattern of contact righting irrespective of the side to which they rotated. In contrast, 6-OHDA treated males and females showed a bias towards the use of a male- or female-typical pattern of rotation that was dependent on the side of the body they rotated towards, irrespective of the sex of the animal. Both 6-OHDA treated males and females had a displacement of the longitudinal axis that was similar to sham treated males and

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[10] Twenty-four, twelve male and twelve female, Long Evans rats were used for this study. Six animals of each sex received a unilateral 6-OHDA lesion of the left nigrostriatal bundle. The remaining animals received a sham lesion. For details of the surgical procedure see Chapter 6.

used a male-typical strategy of hindquarter rotation when rotating towards the affected side of the body (Figs. 39B and 40B)[11]. In contrast, both lesion treated males and females had an angular displacement of the longitudinal axis and a lack of hindquarter rotation that was more female-typical when rotating towards the non-affected side of the body (Figs. 39A and 40A)[12]. These differences in the selection of sex-typical patterns of hindquarter movement were independent of forequarter movement. There were no differences between the 6-OHDA treated males and females in the presence of forequarter rotation or in the likelihood and timing of forepaw placement.

These data provide evidence, for the first time, that the choice of a sex-typical movement pattern is dependent on the function of intact ascending dopaminergic systems. How the choice of a sex-typical motor program is governed by these systems will need further investigation. This study also provides definitive evidence that sex differences in movement organization are not due to differences in body morphology, but are due to sex differences in the selection of movement patterns by the CNS since males, and females, are now exhibiting both sex-typical strategies,

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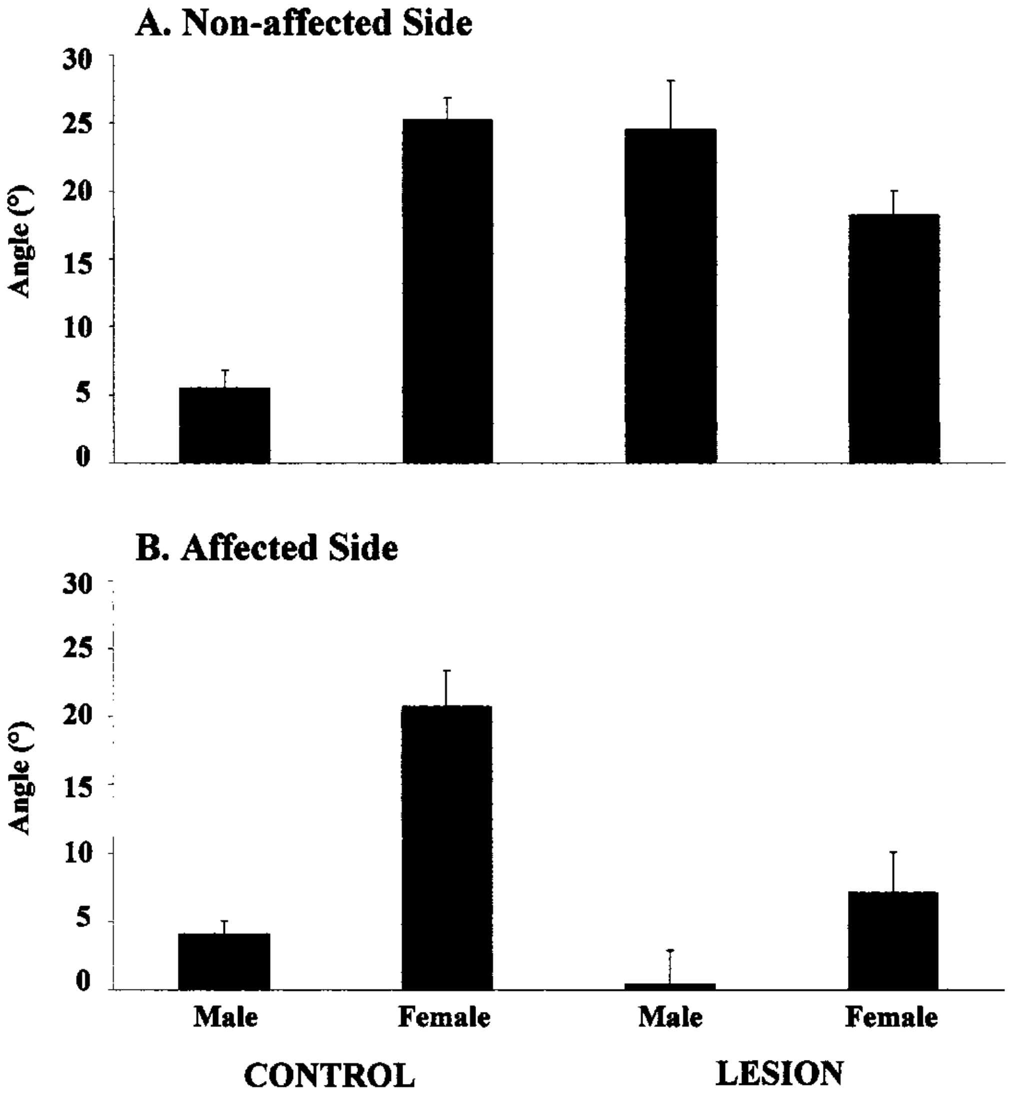
[11] When rotating towards the affected side of the body, contact with the substrate is primarily with the affected side. The opposite is true for rotation towards the non-affected side.

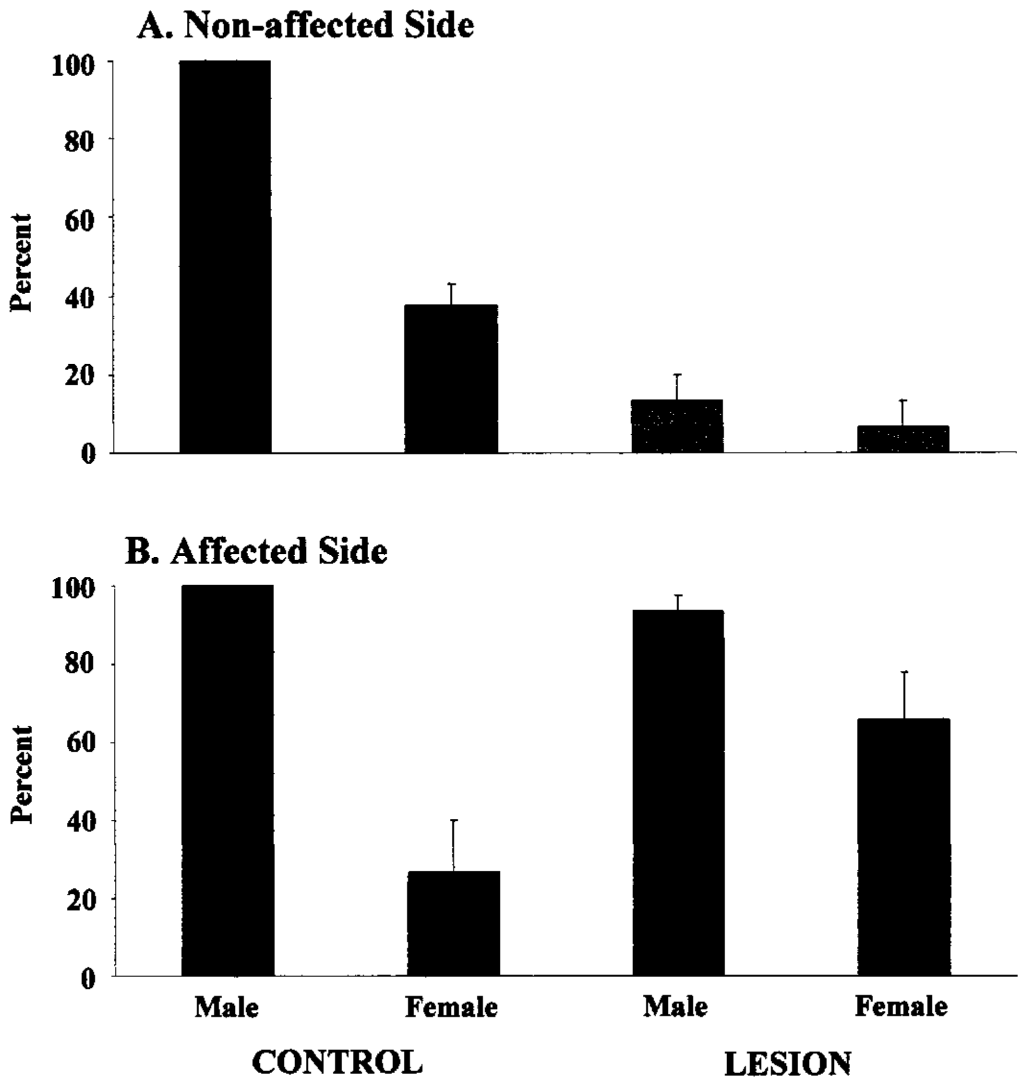
[12] Results were analyzed using a two-way ANOVA with two between-groups factors. Tukey HSD post hoc tests were used to compare individual group means. There were significant main effects for sex $F(1,20) = 9.03$, $p<0.01$ and lesion $F(1,20) = 33.88$, $p<0.0001$, for the displacement of the longitudinal axis, during contact righting, to the non-affected side. A significant sex by lesion interaction $F(2,20) = 7.1$, $p<0.05$, was also found. Post hoc analysis revealed that sham treated males were significantly different from all other groups. For the displacement of the longitudinal axis when rotating to the affected side of the body significant main effects for sex $F(1,20) = 23.75$, $p<0.0001$, lesion $F(1,20) = 13.1$, $p<0.01$, and a sex by lesion interaction $F(2,20) = 4.22$, $p=.05$ were found. Post hoc analysis revealed that sham treated females were significantly different from all other groups. For the likelihood of hindquarter rotation to the non-affected side significant main effects were found for sex $F(1,20) = 40.33$, $p<0.0001$, and lesion $F(1,20) = 117.6$, $p<0.0001$. A significant sex by lesion interaction was also found $F(2,20) = 26.3$, $p<0.0001$. Post hoc analysis revealed that 6-OHDA treated males and females, while not significantly different from one another, were different from sham treated males and females. Sham treated males and females were also significantly different from one another. For the likelihood of hindquarter rotation to the affected side a significant main effect was found for sex $F(1,20) = 29.65$, $p<0.0001$. A significant sex by lesion interaction was also found $F(2,20) = 6.13$, $p<0.05$. Post hoc analysis revealed that 6-OHDA treated males and females and sham treated males were all significantly different from sham treated females.

Figure 39



The angle of displacement of the longitudinal axis during contact righting in males and females that have been either sham treated or given a unilateral 6-OHDA lesion of the ascending dopaminergic systems is depicted. Both male and female 6-OHDA animals are female-like when rotating towards the side of the body not affected by the lesion (A) and are more male-like when rotating towards the affected side of the body (B).

Figure 40



This figure depicts the likelihood of contralateral hindquarter rotation during contact righting in males and females that have been either sham treated or given a unilateral 6-OHDA lesion of the ascending dopaminergic systems. Both male and female 6-OHDA animals were more female-like when rotating towards the side of the body not affected by the lesion (A) in contrast to being more male-like when rotating towards the affected side of the body (B).

Lesions of the Dopaminergic System in Early Development: Evidence has been provided that sex differences in movement organization are generalizable to many non-reproductive behaviors and that manipulation of the ascending dopaminergic systems, in adulthood, can alter the sex-typical expression of a movement pattern. The question of whether these differences are present early in development and whether manipulation of the dopaminergic system developmentally can alter, in a sex-typical manner, the expression of sex-typical patterns of movement organization in adulthood has not been addressed. The analyses of behavior in adult rats that have received neonatal lesions of the ascending dopaminergic systems[13] reveals that altering the development of this system can affect, in a sex-typical manner, the coordination of motoric behavior in adulthood.

It has been shown that there are impairments in movement integration during skilled reaching in adult Long-Evans rats that received a bilateral intraventricular 6-hydroxydopamine lesion of the ascending dopaminergic systems early in development (Whishaw et al., 1994). An analysis of sex differences in the organization of movements has not been conducted using this model of CNS injury. To address the hypothesis that males and females would show different impairments in motor coordination after neonatal lesions of ascending dopaminergic systems, 6-OHDA treated males and females, and their sham treated controls, were tested during dodging to protect a food item, contact righting and a food handling task.

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[13] For this study, thirty-two Long-Evans rats were used. Seven males and nine females received bilateral intraventricular lesions of the ascending dopaminergic systems on postnatal day 5. Each animal was injected with 20mg/kg desmethylimipramine (Sigma Chemicals, St. Louis, MO, USA) I.P. 45 minutes prior to surgery. The surgery consisted of two injections, one into each lateral ventricle, of 1.5 μl containing 100μg of free base 6-OHDA in PBS with 0.2% ascorbic acid. The remaining seven males and nine females served as sham operated controls.

The analysis of sex-typical patterns of dodging behavior revealed that there were no impairments in the kinematic organization of dodging in 6-OHDA treated males and females as compared to controls (Fig. 41A & B)[14]. 6-OHDA treated males and females also used, in general, sex-typical patterns of contact righting from supine to prone as determined by the presence or absence of counter rotation by the hindquarters (Fig. 42A). A deficit was found, however, in the 6-OHDA treated males with regards to the proper timing and placement of the forepaw during rotation (See Fig. 42C). These males would often place the forepaw, ipsilateral to the direction of the turn, onto the substrate earlier than control males. This corresponded to an overall greater angle of displacement of the longitudinal axis of the 6-OHDA treated males as compared to their male controls (Fig. 42B)[15]. 6-OHDA treated males also had greater difficulties in coordinating the forepaws with oral movements and upright posture, than animals in any other group, during a food handling task (See Figs. 43 and 44)[16].

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[14] For a description of the methods used to analyze the kinematic organization of a dodge, refer to Chapters 2 and 3. Results were analyzed using a two-way ANOVA with two between-groups factors. Tukey HSD post hoc tests were used to compare individual group means. There was a main effect of sex for the snout to pelvis ratio $F(1,28) = 61.53$, $p<0.0001$, and frequency of hindpaw steps $F(1,28) = 24.15$, $p<0.0001$. Post hoc analysis revealed that males and females were significantly different from one another irrespective of treatment condition.

[15] For a description of the methods use to analyze the kinematic organization of contact righting, refer to Chapter 4. Results were analyzed using a two-way ANOVA with two between-groups factors. Tukey HSD post hoc tests were used to compare individual group means. For the likelihood of hindquarter rotation there was a main effect of sex $F(1,28) = 120.39$, $p<0.0001$, and lesion $F(1,28) = 6.09$, $P<0.05$. Post hoc analysis revealed that males and females were significantly different from one another irrespective of treatment condition. For the displacement of the longitudinal axis there was a main effect of sex $F(1,28) = 48.68$, $p<.0001$ and lesion $F(1,28) = 13.36$, $p<0.01$. Post hoc analysis revealed that sham treated males were significantly different from all other groups. 6-OHDA treated males were significantly different from 6-OHDA treated females; however, neither 6-OHDA treated group was different from sham treated females. There was a main effect of sex $F(1,28) = 82.45$, $p<0.0001$, and lesion $F(1,28) = 5.58$, $p<0.05$, for the time of forepaw placement, of the paw closest to the substrate, as a percent of the total time taken to rotate. There was also a significant sex by lesion interaction $F(2,28) = 5.3$, $p<0.05$. Post hoc analysis revealed that sham treated males were significantly different from all other groups. 6-OHDA males were also significantly different from all other groups. The two female groups were not significantly different from one another.

[16] For the analysis of food handling ability each animal was filmed eating two almonds. Results were analyzed using a two-way ANOVA with two between-groups factors. Tukey HSD post hoc tests were used

These results provide evidence that 6-OHDA treated males were more impaired in their organization of movements by neonatal lesions of the ascending dopaminergic systems than 6-OHDA treated females. In general, the 6-OHDA treated males had greater difficulties completing behaviors that required the integration of their forelimb movements with other aspects of movement. These data support the hypothesis presented earlier, during the discussion of skilled reaching, that the control of the fore- and hindquarters by the CNS are dissociable. In addition, this study suggests that the integration of the movements of the fore- and hindquarters, and their organization by the CNS, may follow different trajectories during the development of males and females. Thus, it should be possible to find sex differences in the kinematic organization of movement and posture early in development.

Studies have reported that male and female rats, within the first few days of life, exhibit a difference in how they maintain their posture. Males are more likely to have a tail position bias in the opposite direction to their head position than females (Afonso, Santana, & Rodriguez, 1993; Ross, Glick, & Meibach, 1981) (Fig. 45). The analysis of patterns of contact righting and spontaneous turning in rats within the first few days of birth also suggests that males and females differ in how they integrate their movements (Field unpublished observations). Male neonates, like adults, appear to have a greater

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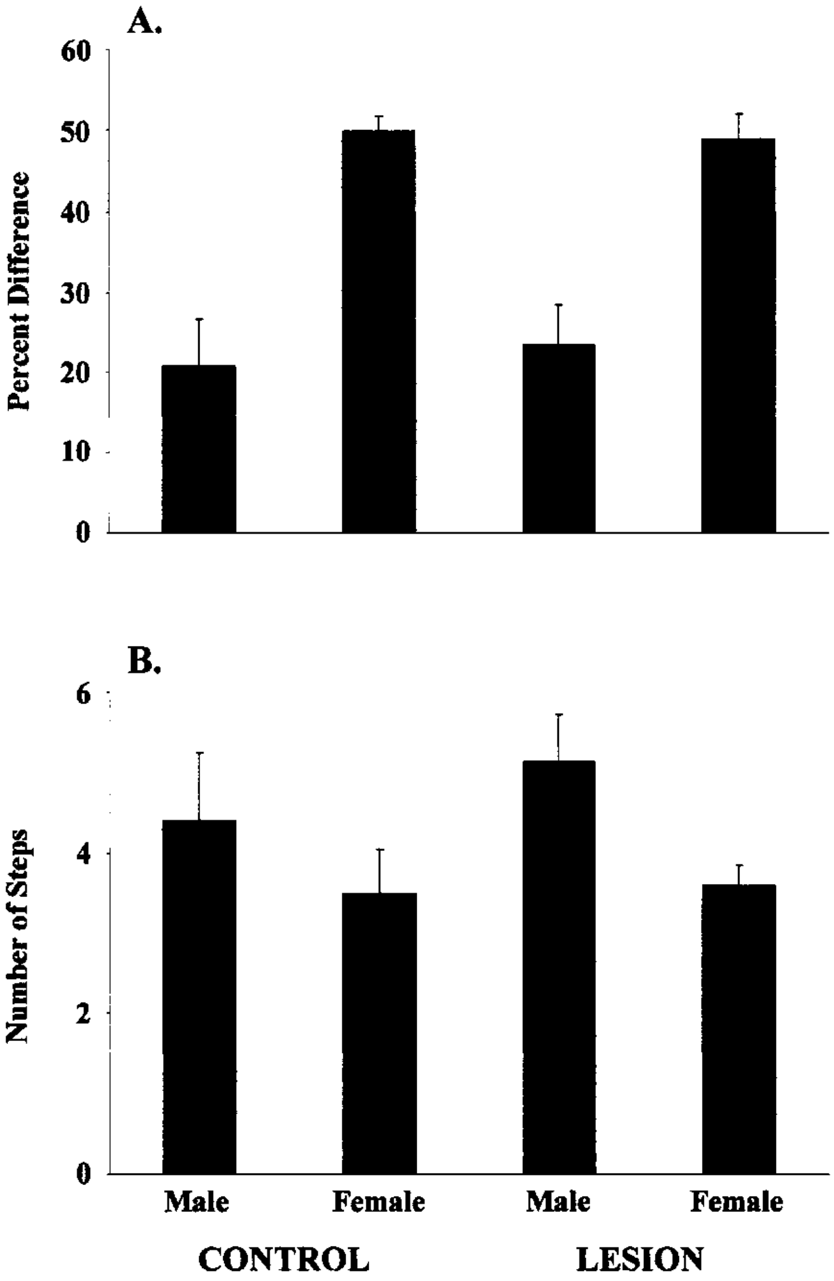
to compare individual group means. The numbers of attempts to remove the food item from the mouth for consumption, and the number of rotations of the food item during consumption, were counted as a measure of motoric impairment. There was a significant main effect for sex F(1,28) = 32.64, $p<0.0001$, lesion F(1,28) = 47.98, $p<0.0001$ and a significant sex by lesion interaction F(2,28) = 31.56, $p<0.0001$ for the number of attempts made to remove the food item from the mouth. Post hoc analysis revealed that 6-OHDA treated males were significantly different from all other groups. There were no other group differences. There was also a significant main effect for sex F(1,28) = 12.22, $p<0.01$, lesion F(1,28) = 21.15, $p<0.0001$ and a significant sex by lesion interaction F(2,28) = 10.32, $p<0.01$ for the number of rotations made during food consumption. Post hoc analysis revealed that 6-OHDA treated males were significantly different from all other groups. There were no other group differences.

dissociation of the direction of the movement of the fore- and hindquarters with the direction of the movement of the hindquarters often opposite to that of the forequarters. These findings provide support for the hypothesis that sex differences in CNS development and the control of movement are present within days of birth. A great deal of further study, however, is needed to verify and expand these findings.

Figure 42



The snout to pelvis ratio (A) and the overall number of hindpaw steps used (B) during dodging to protect a food item in rats that received either a sham or bilateral intraventricular 6-OHDA lesion on postnatal day five are depicted. Note that, respective of sex, there are no differences between the lesion and control groups for either measure.

Figure 42 - Figure Caption

The likelihood of hindquarter rotation (A), the angle of displacement of the longitudinal axis (B), and the temporal occurrence of the placement of the ipsilateral forepaw as a percentage of the total time taken to rotate from supine to prone (C) during contact righting in rats that received either a sham or bilateral intraventricular 6-OHDA lesion on postnatal day five are depicted. While both males and females that received 6-OHDA lesions exhibited a sex-typical likelihood of hindquarter rotation (A), the 6-OHDA treated males were more impaired in the timing of the placement of the forepaw ipsilateral to the substrate (C). This resulted in a greater displacement of the longitudinal axis for these animals as compared to their same sex controls (B).

Figure 42

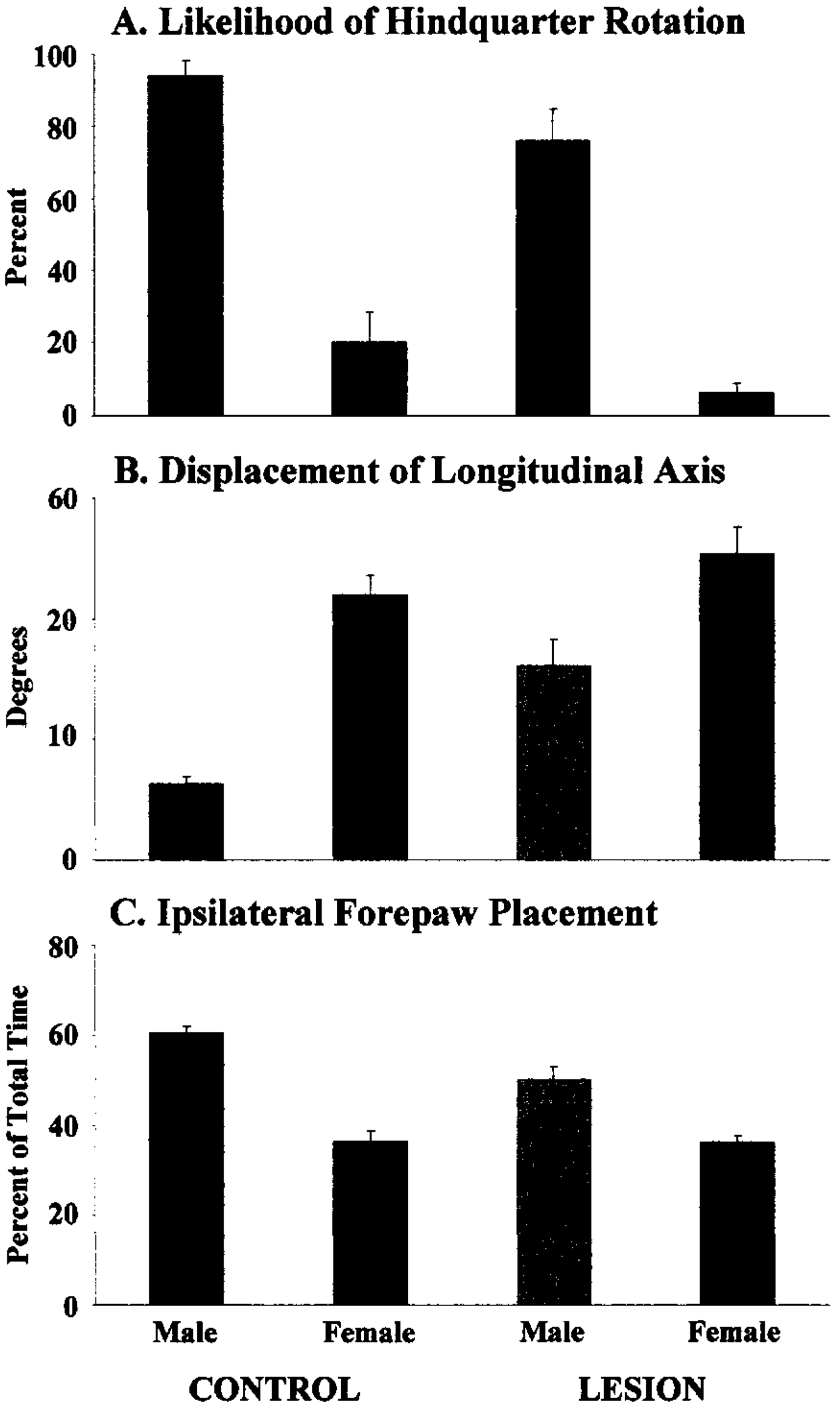
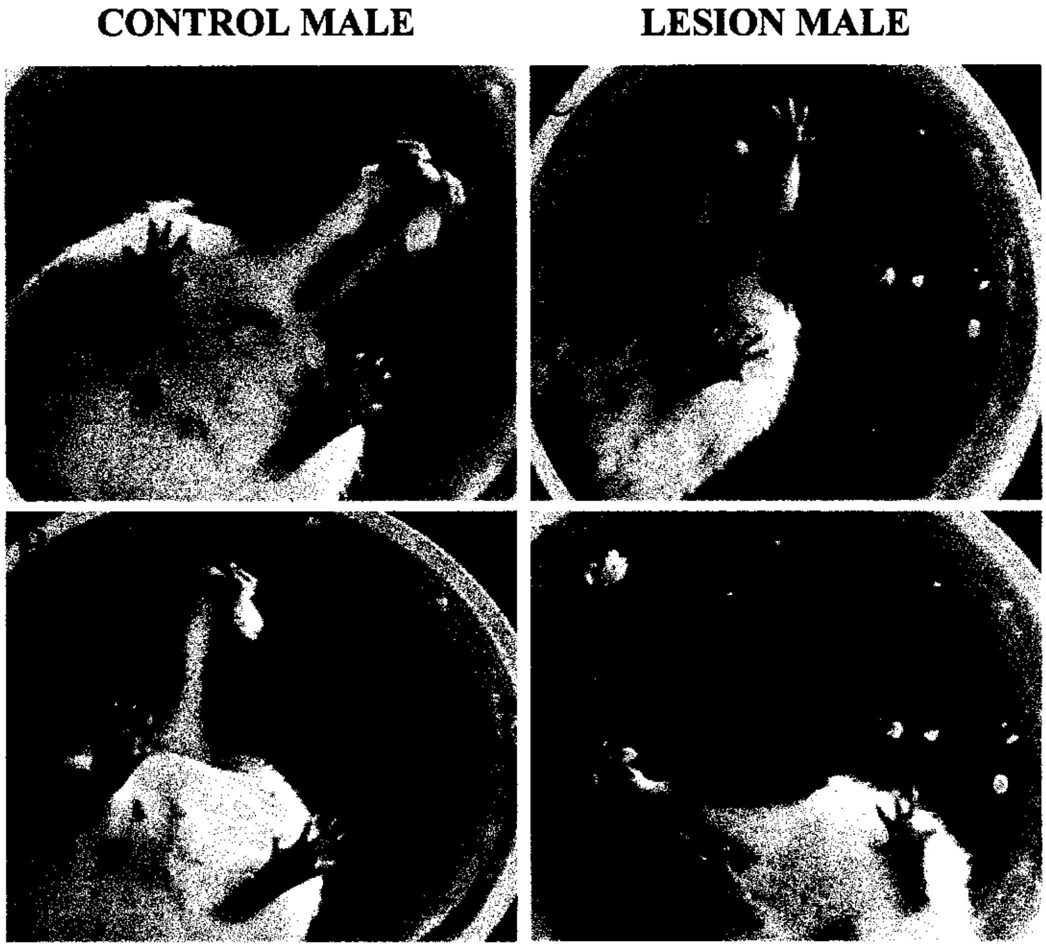

A. Likelihood of Hindquarter Rotation
Percent
100
80
60
40
20
0
B. Displacement of Longitudinal Axis
Degrees
60
20
10
0
C. Ipsilateral Forepaw Placement
Percent of Total Time
80
60
40
20
0
Male
Female
Male
Female
CONTROL
LESION

Figure 43

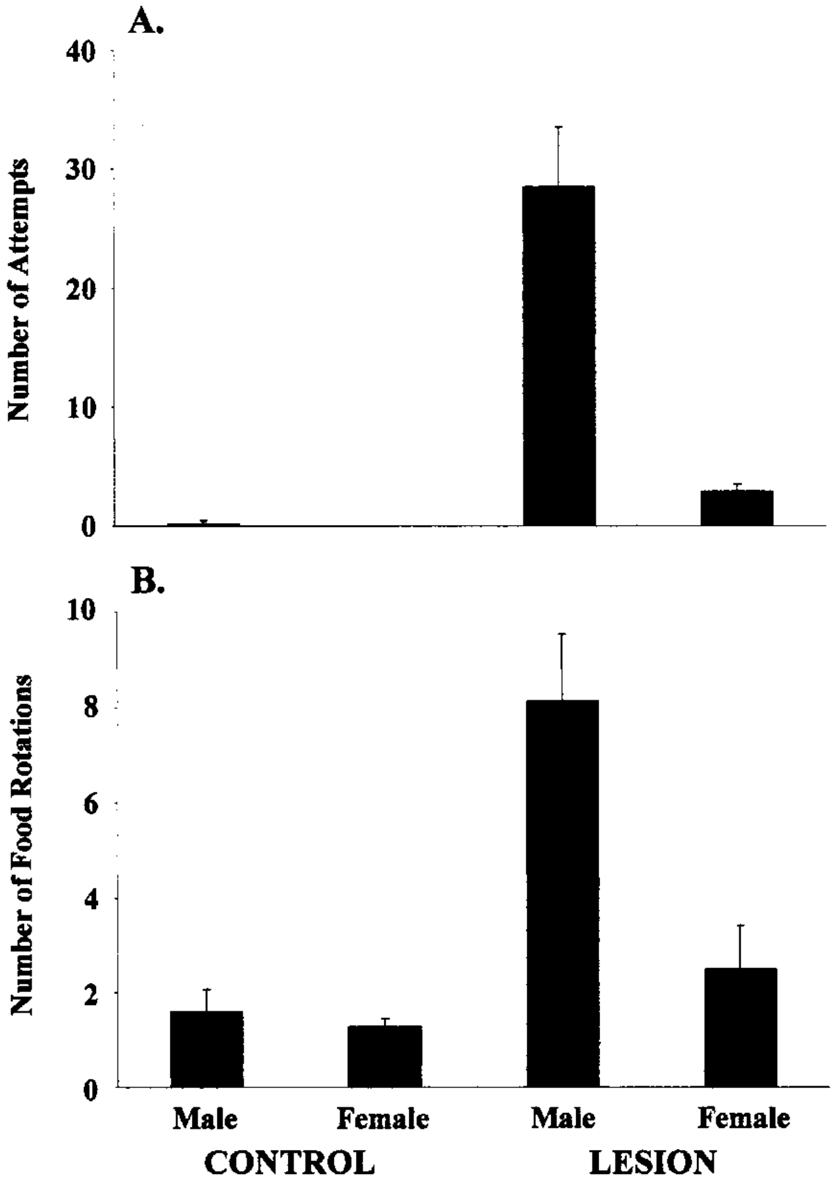


CONTROL MALE

LESION MALE

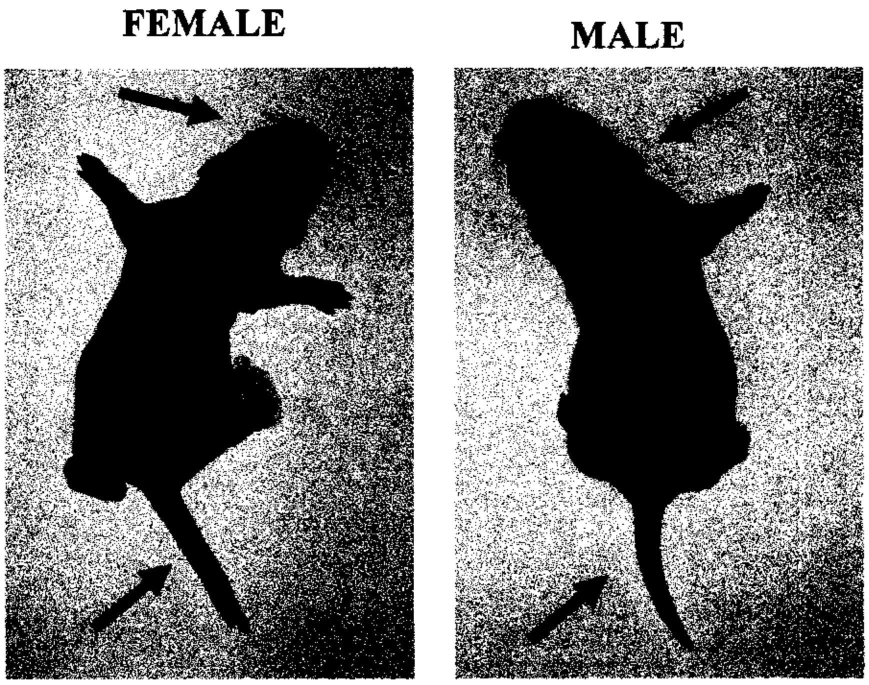
A male that received a sham lesion and a male that received a neonatal intraventricular 6-OHDA lesion on postnatal day five are depicted while consuming an almond. Note the use of the forelimb for postural support (C), the position of the food in the mouth, which is about to fall (D), and the number of food pieces on the floor of the testing arena (D) for the lesion treated male animal as compared to the sham treated control (A & B).

Figure 44



The number of attempts to remove the food item from the mouth (A) and the number of rotations of the food (B), during a food handling task that involved the consumption of almonds, in rats that received either a sham or bilateral intraventricular 6-OHDA lesion on postnatal day five are depicted. 6-OHDA treated males were significantly more impaired than the 6-OHDA treated females or sham treated males and females.

Figure 45



Two, two day old male and female rats are depicted. In the female the hindpaw ipsilateral to the direction of the turn has just stepped forward in the direction of the forequarters. In the male, the step that occurred subsequent to the photo displayed was with the hindpaw contralateral to the direction of the turn away from the side of the body, thus moving the hindquarters in the direction opposite to that of the forequarters. Note the difference in the direction of the tail in contrast to the head (indicated by arrows). Adapted from (Afonso et al., 1993; Pictures by E. Field)

The data described in the previous sections provide evidence that the development of motor coordination during development may be different in males and females. Furthermore, perturbations of the CNS dopaminergic systems during development and in adulthood, can affect the adult expression of sex-typical patterns of movement organization differently. While the concept that developmental perturbations of the dopaminergic system can affect the development of males and females differently is not new (Berger-Sweeney & Hohmann, 1997; Castagné, Cuénod, & Do, 2004) these are the first studies that describe sex differences in the kinematic organization of sex-typical patterns of movement in response to lesions of the ascending dopaminergic systems (Field et al., 2005d). While further study of sex differences in the kinematic organization of movements in rats and the CNS systems that subserve them, is necessary, there is one experimental question, raised at the beginning of this discussion, that has not been addressed. Are there sexually differentiated patterns of organization of movement in species other than the rat?

7.6 A Comparative Analysis

As described in the introduction to this thesis, it is often assumed that sex differences in motor or kinematic performance are due to sex differences in body mass or skeletomusculature. In the previous sections of this thesis, evidence has been provided showing that this is not the case. Sex differences in the kinematic organization of behavior, in rats, are due to differences in the function of the CNS. It is possible, however, that the differences that have been described are specific to rats and not generalizable to other species. In this section, evidence will be provided that sex differences in the organization of movement are not restricted to rodents but can be found in species with very different body morphologies and life histories. The existence of sex differences in a diverse selection of species provides a basis for speculation about how and why these sex differences may have evolved.

In order to compare movement organization across diverse species and determine whether sex differences in the organization of motoric organization are present, spontaneous turning was chosen for analysis. Spontaneous turning can be defined as a transitional act that occurs between bouts of forward locomotion (Eilam, 1994). Spontaneous turning has been studied in numerous organisms ranging from single cell paramecium (Machemer, 2001), to insects (Bell & Schal, 1980; Dürr & Ebeling, 2005; Fish & Nicastro, 2003; Frantsevich & Cruse, 2005), fish (Gerstner, 1999), crayfish (Copp & Jamon, 2001), and cetaceans (Fish, 2002). Thus, it can be argued that spontaneous turning is a behavior that is likely expressed by all motile animals. While the kinematics of turning behavior are being increasingly studied possible sex differences in this behavior are not (although for a recent exception documenting sex differences in the turning kinematics of sea lions, see Fish, Hurley, & Costa, 2003).

The data gathered from a comparative study of possible sex differences in spontaneous turning can be used to: (a) further confirm that sex differences in the organization of movement are not dependent on sex differences in the form or body morphology of the species in question, (b) determine when these sex differences may have evolved, and (c) further our understanding of why males and females have evolved different patterns of movement organization.

In order to address these questions and ascertain whether consistent sex differences exist in the organization of spontaneous turning, five species were chosen for analysis. Two rodents, the laboratory mouse (C57/Black6) (*Mus musculus*) and degus (*Octodon degus*), one marsupial, the marsupial cat (*Dasyurus hallacatus)* and two insects, the African field cricket (*Gryllus bimaculatus*) and the giant Madagascan hissing cockroach (*Gromphadorhina portentosa*). Spontaneous turns were operationally defined as a cessation of forward movement and a change in position of the head and upper body by 90 degrees or greater (Eilam, 1994). Spontaneous turns that were immediately preceded by grooming or rearing or began without all limbs having contact with the substrate, were not included. For each example (five per animal), the trajectory of movement for the anterior and posterior end of the body were traced onto transparencies and the distance traveled for each point was determined. Ratios of the relative distance traveled for the anterior versus posterior ends of the body were calculated in the same manner as the snout to pelvis ratios that were calculated for dodging to protect a food item in rats (See Chapters 2 and 3 for details). For all five species analyzed, it was found that males moved the posterior end of their bodies through a significantly greater distance, relative to the anterior end of the body, than females during spontaneous turns (Fig. 46)[17]. In addition, it appears that the males, of all five species, are more likely to step back towards the posterior of the body, or sideways away from the body, with a limb on the contralateral side of the body relative to the direction of the turn, than are females (Field, unpublished observations).

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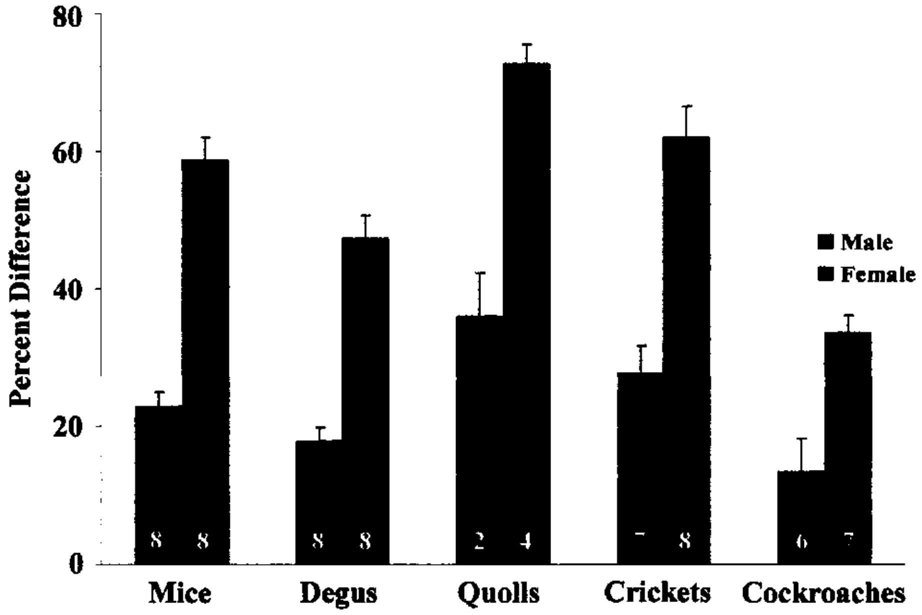
[17] The t values for the male to female comparison conducted for each species are as follows: (a) *Mus musculus*, $t = 8.70$, $p<0.0001$, (b) *Octodon degus* $t = 7.65$, $p<0.0001$, (c) *Dasyurus hallacatus* $t = 6.13$, $p=0.004$, (d) *Gryllus bimaculatus* $t = 5.52$, $p<0.0001$, and (e) *Gromphadorhina portentosa* $t = 3.83$, $p=0.003$.

Figure 45 - Figure Caption

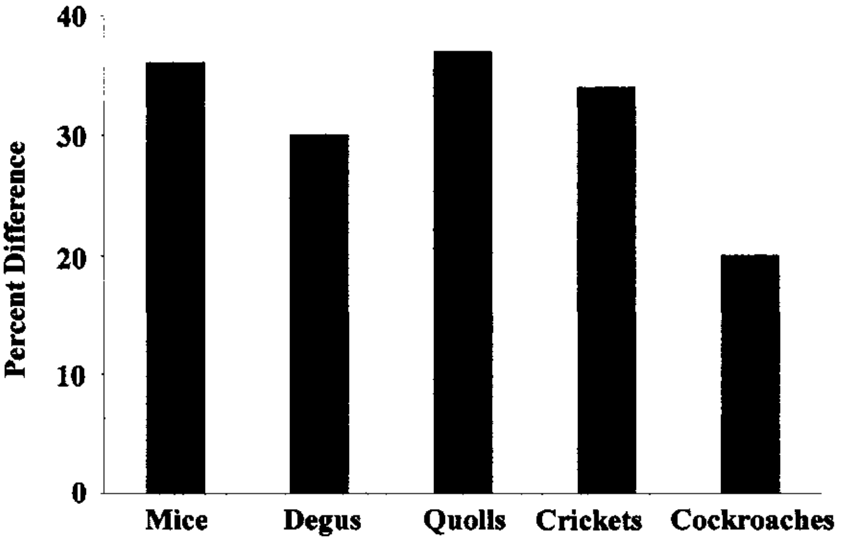
The ratio of distance traveled by the anterior versus the posterior end of the body during spontaneous turns is depicted for male and female laboratory mice (C57/Black6) (*Mus musculus*), degus (*Octodon degus*), marsupial cats (*Dasyurus hallacatus),* African field crickets (*Gryllus bimaculatus*) and giant Madagascan hissing cockroaches (*Gromphadorhina portentosa*) (A). The numbers, in white on each column, denote the number of animals for each group. For all species the overall percent difference between the group averages is depicted in B.

Figure 46

A. Distance Traveled Anterior vs. Posterior



B. Male vs. Female



The existence of sex differences in the organization of spontaneous turning in these species suggests that the evolution of sex differences in the organization of movement may precede the divergence of the Bilateria into protostomes and deuterostomes. The Arthropod phylum, to which crickets and cockroaches belong, is a member of the protostomes whereas the remaining species are members of the Chordata phylum, one of the deuterostomes phyla. This would date the evolution of these sex differences to before the Cambrian explosion (circa 530 mya); when most current body plans are thought to have evolved and the protostomes and deuterostomes diverged from a common ancestor.

A conserved sex difference in the organization of movements across species with widely different body plans and developmental histories suggest that these differences are due to conserved mechanisms within the nervous system. Thus, sex differences in the neural control of movement may be one of the earliest sex differences to have evolved. It is also possible that these sex differences have evolved multiple times in different lineages. Data from additional species from wide ranging lineages are needed to test whether homoplasy (multiple origins) or homology (conservation of an ancestral condition) best explains the above findings. Nevertheless, the data presented above represents a mammalian lineage with species that have diverged at least 100 mya (placentals versus marsupials) (Clemens, 1977), and an insect lineage with a split of 100 mya (crickets versus cockroaches) (Grimaldi & Engel, 2005). The divergence of the arthropod-chordate phyla has been dated to prior to the Cambrian explosion (Dawkins, 2004). The divergence of these species over millions of years strongly suggests that the conservation of sex differences in movement organization is homologous rather than homoplastic.

A difference in the kinematic organization of non-reproductive behaviors suggests that the environmental milieu faced by males and females differed early in the evolution of Animalia. Darwin proposed that sex differences in behavior must have evolved due to differences in what is the optimal phenotype for a male and a female to ensure reproductive success (Rhen, 2000). A trait that has similar effects with regards to the success of reproduction would not become sexually dimorphic because it would not yield a reproductive advantage for either sex. It is only when a trait differentially affects the reproductive success of a male or a female that the selected trait is spread within a particular sex (Gaulin, 1995).

Sex differences in the organization of movement patterns may have evolved in conjunction with the evolution of sex differences in a number of other behaviors such as maternal care, courtship or sexual behaviors, or aggression. It is also possible that sex differences in movement organization are vestigial; that is, they played an important role 'historically'. It has been argued that through much of animal evolution basic developmental processes have been conserved (Arthur, 1997). Thus, the maintenance of such sex differences, in how the nervous system organizes movement, may have been conserved from an earlier adaptation by animals rather than having any current adaptive value. Even though these sex differences may not have a current adaptive function, the conserved neural circuitry that sub-serves these behavioral differences may be involved in the control of multiple functions that are necessary for the development and subsequent survival and reproduction of the animal (Bass & Baker, 1997; Gerhart &

Kirschner, 1997). Nonetheless, it is still possible that sexually-differentiated movement strategies are being maintained currently due to a common problem that is different between the sexes.

I argued previously that differences in maternal/paternal care could explain the presence of sex differences in non-sexual behaviors in rats (Field, 1996). The demands of gestation and lactation require certain combinations of posture and movement that make it 'easier' for a female to cope with the demands of gestation, parturition and lactation. However, neither the African field crickets nor the giant Madagascan hissing cockroaches bear the physical constraint of having to gestate internally and subsequently care for their offspring. Thus, the current comparative results suggest that the demands of gestation and maternal care cannot be the adaptive explanation.

While all of these species do exhibit courtship and sexual behavior, its expression is not the same. For example, during sexual behavior in rodents, males mount the female after establishing a snout to nape contact. Once the nape is contacted, the male's forepaws grasp the female's upper back behind the shoulders. He then shifts his hindquarters in line with the female's pelvis and attempts to mount. If the male does not orient properly to the female, she may swerve away laterally but will often maintain the orientation of her rump in the direction of the male (Whishaw & Kolb, 1985). In this context, the movements of males and females appear similar to the sex differences described for dodging and spontaneous turning. Females pivot around a point more posterior than males and thus maintain a relatively fixed position of the posterior end of the body as compared to males.

Sexual behavior in African field crickets and giant Madagascan hissing cockroaches again do not support the adaptive explanation that sex differences in non-reproductive behaviors are related to the sex differences in the movements used during sexual behavior. In the African field cricket the female mounts the male and retrieves a spermatophore from this position (Huber, Moore, & Loher, 1989). In the giant Madagascan hissing cockroach copulation and transfer of the spermatophore is completed by posterior to posterior contact (Guthrie & Tindall, 1968). The actual mechanics of reproductive behavior in these two species are very different from mammals and thus an adaptive explanation that invokes current reproductive behavior is weak.

With regards to aggression all five species that were studied do engage in aggression and it is possible that there are sex-typical patterns of aggression that consistently favor sex differences in movement. While detailed comparisons will be needed to evaluate this possibility fully, what is currently known about aggression in rodents and crickets would suggest that current aggressive tactics are not the adaptive explanation. When fighting, male rodents do use lateral maneuvers that require mid-body pivoting that are consistent with the turning patterns that have been described for dodging to protect a food item (Pellis, 1997). The analysis of aggressive movements in crickets revealed that the movements they exhibit occur primarily in the anterior-posterior plane (Alexander, 1961). Thus, similar to the discussion of sexual behavior above, each species has developed its own unique style of aggressive combat. That being the case, it seems unlikely that the sex-typical organization of movements has been conserved across widely divergent species for use solely in aggressive encounters.

While at this point a definitive conclusion regarding an adaptive explanation for sex differences in movement organization is not possible, it does seem that a number of the most likely factors such as maternal care, sex and aggression cannot be the sole candidates. Whatever the historical, or current selective factors are that have contributed to the development of these differences, the fact that sex differences are present in a diverse selection of species suggests that there may be some important genetic factors that underlie the similarities across species.

7.7 Possible Genetic Contributions

In rodents, the current evidence suggests that gonadal steroids during early development likely underlie the differentiation of male- and female-typical patterns of movement. At present, the possibility that hormones determine the sexual differentiation of movement in the species previously mentioned cannot be excluded. However, given that sex differences in movement organization are present in a diverse selection of species with different developmental histories, it is possible that genetic differences may contribute to the differentiation of the underlying nervous system circuitry that give rise to the behavioral differences.

It has been demonstrated in mice, rats, cattle and humans that male blastocysts and embryos, prior to hormonal events, develop faster than female ones (Kochhar, Peippo, & King, 2001; Mittwoch, 1993; Mittwoch, 2000; Mittwoch, 2001; Scott & Holson, 1977). Contributions of sex chromosome genes to development have also been reported in wood lemmings (Fredga, Setterfield, & Mittwoch, 2000) and marsupials (Arnold, 1996; Renfree, 1994). In mice, differences in gene expression can precede the hormonal events that are related to sexual differentiation (Dewing, Shi, Horvath, & Vilain, 2003). It has also been shown that cultures of mesencephalic neurons from female rat embryos, prior to gonadal steroid exposure, contain more TH positive neurons, and have these neurons have a greater outgrowth of tyrosine hydroxylase-immunoreactive processes than cultures from male embryos (Beyer et al., 1992; Beyer, Pilgrim, & Reisert, 1991; Reisert, Engele & Pilgrim, 1989). Male neurons in dissociated diencephalic cultures, however, were found to be thirty percent larger than female neurons (Kolbinger, Trepel, Beyer, Pilgrim & Reisert, 1991). These data suggest that sex differences in dopaminergic neurons, and putatively systems, are present prior to exposure of the embryo to gonadal steroids and are thus genetically determined.

Recently, a model has been created in mice that addresses whether there is a genetic contribution to the development of a sexually differentiated CNS. In this model the testis-determining gene *Sry*, which normally resides on the Y chromosome, and is necessary for male genital development, has been moved to an autosome (Arnold, 2003; Arnold & Burgoyne, 2004). Thus, one can have a male that is genetically XY but without the *Sry* gene. This genetic male will develop ovaries and be exposed to ovarian steroids during development in contrast to an XY male with the *Sry* gene that develops testes and is exposed to testicular hormones. Corollary variants are available for females. This allows for the comparison of two animals both of which have a female phenotype (i.e. ovaries and estrogen) but that have either an XX or XY sex chromosome compliment. The use of this model has revealed that sex differences at the neural level can be linked to the presence of either a XX or XY chromosome complement, independent of whether the animal has ovaries or testis (Carruth et al., 2002; De Vries et al., 2002). Cultures of mesencephalic TH positive neurons from these mice show that XY embryos, regardless of the presence of *Sry,* contain greater numbers of TH positive neurons (Carruth et al., 2002). Therefore, as Reisert & Pilgrim (1991) have suggested, differences between males and females may be genetically determined and gonadal steroids may simply further exaggerate these differences.

The findings mentioned above suggest that further study of the possible contributions of the sex chromosomes to sex differences in the organization of movement may be worthwhile. Work has been done showing that patterns of spontaneous turning can be altered by genetic mutations of X-linked genes (Fuji, Tanabe, Fukuda, & Ooshima, 2003). This model, in addition to the one described above, could provide a rich source of material to study the effects of altered gene expression on the development of sex differences in movement organization and how this interacts with gonadal hormone exposure. The comparative data from the previous section also suggests that any genetic differences that may contribute to sex differences in movement organization should be present in all species that are sexually dimorphic in their behavior; thus possible genetic contributions beyond the sex chromosomes may need to be considered.

Work during the last few decades has shown that there is a large degree of conservation of the *Hox* genes that provide the initial blueprint for the organization of the body (Du & Taylor, 2004; Hughes & Kaufman, 2002; Manning, Callow, & Bundred, 2003) and the nervous system (Akin & Nazarali, 2005; Briscoe & Wilkinson, 2004; Hoshiyama et al., 1998). Further study of the genetic contribution of genes such as *Engrailed-1, Engrailed-2,* (Simon, Thuret, & Alberi, 2004) *Hb9* (Arber et al., 1999), *Pax-6* (Osumi et al., 1997), *Phox2b* (Pattyn, Hirsch, Goridis, & Brunet, 2000), genes that provide part of the blueprint for mid- and hindbrain development, and *Pitx3* (Asbreuk, Vogelaar, Hellemons, Smidt, & Burbach, 2002; Smidt et al., 2004; Smidt, Smits, & Burbach, 2004; Smits, Mathon, Burbach, Ramakers, & Smidt, 2005) which is important for the development of dopaminergic neurons, may provide clues as to how the nervous system is differentially organized during development in males and females.

Further research will be needed to determine whether genes, other than those that are important for sex determination, can influence the development of the nervous system and its control of movement in a sex-typical manner. At present, it appears that questions of this sort are not often asked with regards to nervous system development in mammals. The idea is not implausible. In *D. melanogaster* the sex of the cells at the anterior-posterior (A/P) borders of the genital discs can influence the subsequent development of the genital primordia as male or female irrespective of the sex of the cells that constitute the genital primordia (Christiansen, Keisman, Ahmad, & Baker, 2002; Keisman, Christiansen & Baker, 2001). In *C. elegans,* the genes that regulate the development of male reproductive structures are also involved in regulating the development of the neurons that coordinate the behavioral interaction, and thus movement, of the male with the hermaphrodite during sex (Yi, Ross, & Zarkower, 2000; Zarkower, 2001). The role of regulatory genes in the development of the body and sexual differentiation of the nervous system and behavior may be a fertile ground for further research.

7.8 The Ultimate Issue - How did Sex Differences in Movement Organization Evolve?

The comparative data described in this thesis suggest that sex differences in motoric organization are phylogenetically old and may have originated with the evolution of bilaterality and sexually dimorphic organisms (Haag & Doty, 2005). I would speculate that sex differences in the organization of movement may be found in numerous other species beyond those discussed in Section 7.6. Furthermore, I would speculate that 'sex differences' or variation between individuals in how they organize their motoric behavior may have been a factor in the original evolution of sexually dimorphic behavior.

One way to test this would be to determine whether differences in movement organization are related to the likelihood of reproductive success as a male or a female. The organism chosen would need to be an organism that diverged early from the original common bilaterian ancestor, such as a simultaneous hermaphrodite that does not consistently engage in reciprocal sex. Experiments could then be conducted to determine whether differences in the performance of a non-sexual behavior can predict the likelihood of playing the role of a female who receives sperm and carries the metabolic cost of reproduction, or a male who contributes sperm to the conspecific and does not carry the metabolic costs of reproduction.

One of the earliest phyla to diverge from the original bilaterian ancestors were the Platyhelminths (i. e., flatworms). Most of the free swimming species are simultaneous hermaphrodites. It has been documented in the *Cotylea* sp. that pairs of animals rear up towards one another and fence with their penes. The first one to stab the body of the other injects sperm. Thus, while they are simultaneous hermaphrodites, they compete to be 'male' (Michiels & Newman, 1998). Interestingly, it has been stated that another species of free swimming turbellarian flatworms (*Stenostomum oesophagium*), which are also spontaneous hermaphrodites and normally cross-fertilize, manage to inseminate others but avoid being inseminated themselves (Forsyth, 2001). In the simultaneously hermaphroditic flatworm, *Schmidtea polychroa,* it has been shown, using genetic analysis, that reciprocal fertilizations were found in only 41 out of 110 mating combinations, demonstrating that fertilization is not equitable between members of mating pairs (Pongratz & Michiels, 2003).

These data suggest that the worms that are 'male-like' and manage to avoid being the recipients of sperm, may have a different organization of motoric behavior than the ones that do receive sperm. A correlation between non-sexual patterns of movement, or behavior, and the likelihood of inseminating a conspecific, would suggest that 'sexually' differentiated patterns of movement organization may have preceded the emergence of fully sexually dimorphic life histories. In turn, these differences may have become crucially linked to the female or male-typical phenotype with successive generations and the subsequent loss of hermaphroditism. Further research will be needed to determine the merit of this hypothesis.

7.9Proximate Issues - Implications for Human Studies

Irrespective of whether variations in movement ultimately led to the sexual differentiation of movement organization across diverse phyla, the presence of sex differences in diverse species has implications for the study of humans. Sex differences have been documented in human locomotion (Cutting, Proffitt, & Kozlowski, 1978; Kozlowski & Cutting, 1977; Li, Wang, Crompton, & Gunther, 2001; Pollick, Lestou, Ryu, & Cho, 2002; Schache, Blanch, Wrigley, & Bennell, 2003). Sex differences have also been documented in humans, as well as other primates, in the performance of sensorimotor/motor tasks such as carrying, (Jenni & Jenni, 1976) throwing, (Cleveland, Rocca, Wendt, & Westergaard, 2003; Watson & Kimura, 1989; Westergaard, Liv, Haynie, & Suomi, 2000) and intercepting a moving target (Peters, 1997). Recently, a growing amount of literature has revealed that men and women organize their movements differently in a large number of behaviors that are dynamic; that is the movements are occurring in multiple planes of movement simultaneously (Fleisig, Nicholls, Elliott, & Escamilla, 2003; Gourgoulis et al., 2002; Lindbeck & Kjellberg, 2001; Salci, Kentel, Heycan, Akin, & Korkusuz, 2004; Schade, Arampatzis, Bruggemann, & Komi, 2004).

Sex differences in movement organization are also present during development. Sex differences in sensorimotor behaviors have been reported both pre- and postnatally (Almli, Ball, & Wheeler, 2001; Davies & Rose, 2000; DiPietro et al., 2001; Piek, Gasson, Barrett, & Case, 2002), and with advancing age (Cao, Ashton-Miller, Schultz, & Alexander, 1997; Frandin, Sonn, Svantesson, & Grimby, 1995; Pavol, Owings, Foley, & Grabiner, 1999; Sayers, Guralnik, Thombs, & Fielding, 2005; Schultz, Ashton-Miller, & Alexander, 1997; Wojcik, Thelen, Schultz, Ashton-Miller, & Alexander, 1999). While the effects of developmental experience (Hall & Kimura, 1995) and the integration of visual information with sex differences in movement organization have been addressed (Tottenham & Saucier, 2004), possible sex differences in movement organization and posture and how they might contribute to sex differences in sensorimotor tasks, have not. Thus, it is possible that sex differences in movement during a goal directed task, such as throwing accuracy, are dependent, in part, on sex differences in postural support and movement organization.

Kinematic analysis, in humans, has primarily focused on how differences in body morphology determine differences in movement. It has recently been argued that this cannot explain the sex differences found in the organization of dynamic movements (Zeller, McCrory, Kibler, & Uhl, 2003). There are differences in the throwing accuracy of homosexual and heterosexual males who have similar body morphology (Hall & Kimura, 1995; Sanders & Wright, 1997) and the selection of movement patterns can be influenced by an individual's perception of their gender relative to their physical sex (Barlow, Mills, Agras, & Steinman, 1980; Rekers & Morey, 1989). Girls with congenital adrenal hyperplasia (CAH), who are prenatally androgenized (Collaer & Hines, 1995), also exhibit alterations of their movement. Girls with the simple-virilizing variant of CAH, who have a more strongly masculinized body morphology, are more feminized in their movements, whereas girls with the salt-wasting variant, who are more female-like in body morphology, are more masculine in their movements (Dittman, 1992). These observations support our findings in rats, and suggest that at least some of the sex differences in movement arise from sex differences in neural organization, rather than as a byproduct of differences in body morphology.

Consistent with what has been documented for rats, preliminary data shows that when humans turn after walking forward, they do so using a sequence of movements that is different for males and females (Field, unpublished observations). Seven female and seven male undergraduates were videotaped, walking toward the camera. They then turned, at a marked location on the floor, 180 degrees away from their initial direction of locomotion, and walked away from the camera. The pattern of shifting body weight and the timing of the rotation of the pelvis relative to the change of direction of the feet differed between the sexes. Males began to turn their pelvis in the direction of the turn, and thus away from the camera, prior to a change in the direction of movement of the feet. Thus, for males, the rotation of the pelvis during a change in direction of the movement of the body was initiated by a rotation of the pelvis. This was followed by a change in the direction of the movement of the feet. In contrast, in females, the rotation of the pelvis during the turn occurred simultaneously with a change in the direction of movement of the feet. Thus, for females, the change in direction was initiated by the stepping of the feet (Fig. 47)[18]. In males, this pattern of movement was accompanied by a shift of their body weight to the leg furthest away from the direction of the turn, whereas the females shifted their body weight to the leg closest to the direction of turning.

The present thesis, and previously described work, suggests that a specific examination of differences in movement organization and postural changes before and after CNS damage should be examined at a kinematic level in both sexes - especially for movement that occurs in multiple planes of movement simultaneously. The finding that male and female rats use different patterns of stepping and postural support during vertical exploratory behavior and that different patterns of contact righting are chosen after a lesion suggest that there may also be sex differences in humans in the compensatory movements that occur following CNS injury - even if the likelihood of the occurrence of a behavior is not compromised.

There is some evidence that impairments and recovery of function can differ, as a function of sex (Di Carlo et al., 2003; Groswasser et al., 1998; Hurn et al., 2005; Roof &

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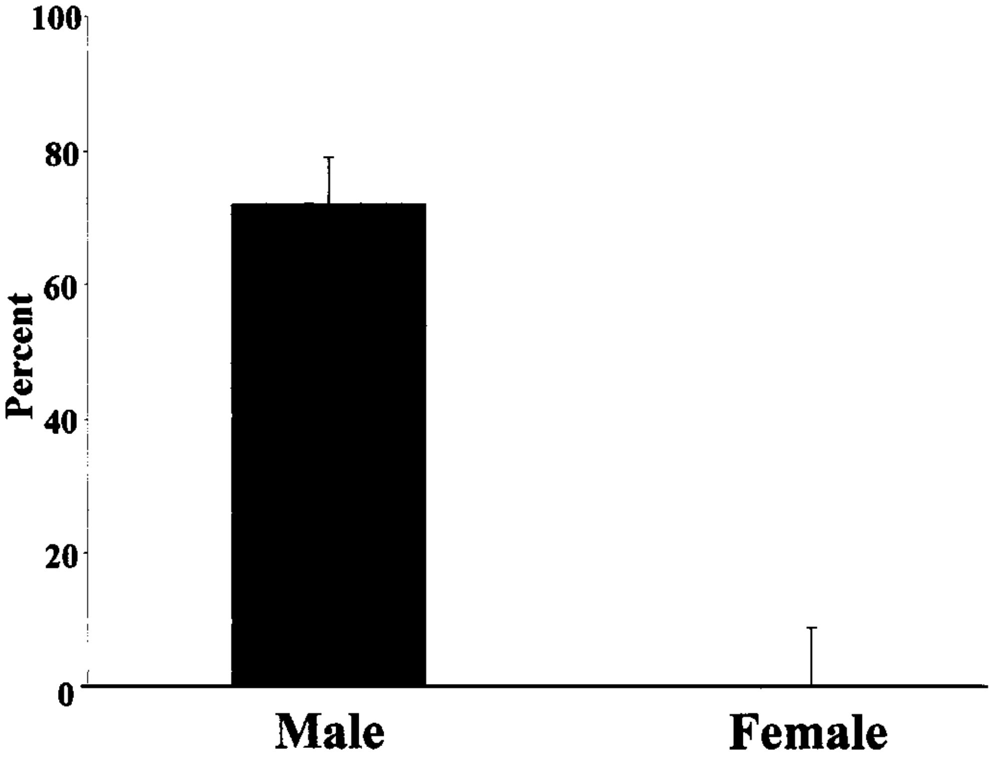
[18] The t value for the comparison of human males and females with regards to the likelihood of beginning a turn with pelvic movement, $t= 5.143$, $p<0.001$.

Hall, 2000; Scivoletto, Morganti, & Molinari, 2004; Sipski, Jackson, Gómez-Marín, Estores, & Stein, 2004), after CNS injury. There is also some suggestion in the literature that males and females suffering from PD, may differ in their symptomology (Dunnewold et al., 1998; Lyons et al., 1998; van Hilten et al., 1993). At present, the presence, or not, of sex differences in the kinematic organization of behavior in parkinsonian patients, or individuals with CNS injury, have not been well studied. In addition, the kinematic analysis of possible sex differences in the organization of movement in individuals who have neurodevelopmental disorders that involve the dysfunction of the CNS, such as, obsessive compulsive disorder, schizophrenia, attention deficit hyperactivity disorder, or Tourette's syndrome, and autism have not been studied. This is surprising, since sex differences in the prevalence and symptomology of these disorders is well known and these disorders have been linked to dysfunction of dopaminergic systems. Furthermore, movement abnormalities are commonly mentioned and are often apparent prior to cognitive and social impairments (Melillo & Leisman, 2004).

When motor behaviors are studied in neurodevelopmental disorders, or after CNS injury, the tasks used are often analyzed from the perspective of whether a behavior has occurred. When detailed kinematic analysis is done, it often focuses on one aspect of a movement without considering the movement of the rest of the body. Furthermore, the movements that are often studied do not directly address how shifts in attention, that direct conscious attention away from the movements that are occurring, affect the expression of a movement. For example, during vertical exploratory movements in the rat, the animal must maintain postural integrity in the vertical plane and incorporate this with forward, backward and/or lateral movements of the hindquarters. These movements have to occur while the animal is actively exploring and attention is focused on the surrounding environment. Thus, it is possible that sex differences in performance and underlying neural activation may become apparent when attention is dissociated from the task being completed (Gorbet & Sergio, 2005). Future research into possible sex differences in humans in the kinematic organization of behavior with altered CNS function may need to take into account this relationship

Figure 47



In this figure the likelihood, in humans, of pelvis rotation occurring prior to a change in direction of the feet, when initiating a 180 degree turn, is depicted. See text for details.

7.10 Final Conclusions and a Theoretical Perspective

The advent of video technology has allowed for the analysis of fine detailed differences in the kinematic organization of movement by males and females. While much of what has been studied in the past, and even currently, with regards to sex differences in behavior, has focused on endpoint measures, it is clear that a great deal of valuable information is lost with this approach. In addition, the view that the form of the animal dictates the organization of behavior, and thus the underlying neural circuitry (Balaban, 1994), has constrained theoretical and empirical investigation into the question of whether patterns of behavior may be conserved independent of differences in body form. In this thesis, evidence has been provided showing that sex differences in movement organization are orchestrated by the nervous system and can be conserved, according to the sex of the animal, even though the form of the animal may vary. An understanding of sex-typical movement organization can provide the foundation for further inquiry into how sex differences in the neural control of movement have been conserved and how these differences may have influenced the development of, and become integrated with, sex differences in other cognitive and social systems.

To advance a theoretical perspective can be a risky proposition. The theoretical perspective of Charles Edward Brown-Sequard, regarding the role of gonadal steroids and rejuvenation of the body,[19] led to his being remembered, not for his lifelong contributions to science, but rather, for one badly supported theory. It has been argued, by Ludwig v. Graff over a hundred years ago, that 'a sound species description is more valuable to science than the most brilliant idea' (Reiger, 1998). Many brilliant ideas, for example, Mendelian inheritance and the Darwin-Wallace theory of evolution, are

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[19] See thesis Introduction for details.

grounded in data, or sound species descriptions, that took years to gather and subsequently organize before the theories of genetic inheritance and evolution were put forth. While I have not had the luxury of decades to gather data to support a theoretical position, the data that I have presented in this thesis does provide a basis for theoretical speculation. I would caution that while speculation can be a fertile ground for scientific advancement, it cannot replace empirical data, nor must it be used to validate the existence of its own position. That said, I would like to advance the following theoretical position.

'Sex differences' in the organization of movements are phylogenetically very old. These differences may have been present prior to the evolution of Bilateria, and may precede the emergence of fully sexually dimorphic life histories. Motoric activity has been suggested to be the driving evolutionary force behind the subsequent development of the nervous system (Llinás, 2001). Thus, sex differences in the evolution of the neural control of movement may have provided the initial template for the subsequent evolution of sex differences in many facets of behavior; as animals developed increasingly complex body plans and nervous systems. In other words, the existence of sexually dimorphic neural systems for the control of movement may provide a neural 'blueprint' for the development of sex differences in other non-motoric behaviors such as cognitive and social behaviors. These sexually differentiated motor, cognitive, and social systems may be differentially sensitive to the effects of environmental perturbations on their sex-typical patterns of development. This difference may explain, in part, the sex differences in the likelihood of males and females to develop neurodevelopmental disorders such as

ADHD, autism, Tourette's syndrome and schizophrenia - all of which have alterations of movement organization in common (Melillo & Leisman, 2004).

It will take many years of research to elucidate the constituent parts of a sexually dimorphic nervous system that contribute to sex differences in movement organization, cognitive and social function. The evolution of sex differences in all these areas will also require further investigation from a comparative perspective. In the proximate world, we can use these differences and the theoretical position outlined above to attempt to understand how these differences may contribute to sex differences, in both normal and altered nervous system function, across the lifespan in humans.

"With respect to biological science as a whole, and particularly to traditional endocrinology, it is abundantly clear that explication of functional relationships involving behavior, the endocrine system, [the nervous system, genetics,] and the environment will greatly increase our understanding of ...evolutionary survival and change and an individual's adaptation to the demands of daily existence."

Behavioral endocrinology: An emerging discipline. F. A. Beach

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