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SEX DIFFERENCES IN THE DEVELOPMENT OF SPATIAL BEHAVIOR IN MONTANE VOLES: EXPERIENTIAL AND HORMONAL INFLUENCES

by

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A dissertation submitted to the Faculty of the
Graduate School at the
University of North Carolina at Greensboro
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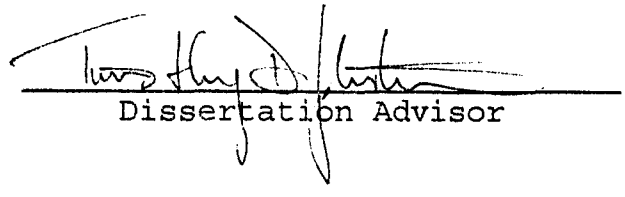

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In animals with polygamous mating systems, in which males' territories overlap the territories of two or more females, males perform spatial tasks with fewer errors than do females. While it has been suggested that these differences persist due to natural selection, this does not explain how they develop.

In this study, I investigated the development of sex differences in spatial abilities of montane voles (Microtus montanus), a polygamous species, as influenced by environmental and hormonal factors. Litters were culled to three same-sex pups and raised in clear plastic cages, either small (21x20x23cm = Restricted) or large with objects for exploration (21x38x48cm = Expanded). The pups were weaned at 21 days of age and remained in their natal environment.

In each litter for each rearing condition, on the day of birth, one pup received testosterone propionate and one cholesterol. These animals were marked by a toe clip to allow for identification. They also were gonadectomized (i.e., GNX-T and GNX-C). The third pup received a sham operation (CONT). Surgeries for males were on the day of birth; for females at thirty days of age. At 45 days of age, when montane voles typically become sexually active,

the GNX-T animals were injected with testosterone enanthenate. The GNX-C animals received cholesterol injections at the same time.

Behavioral testing commenced when the animals were 50 days old. Spatial behavior was measured by performance on an eight-arm radial maze, as recorded by number of arms visited per trial, test duration, number of baits taken per trial, and number of revisits per trial.

Results show that hormonal milieu and rearing conditions are important for the development of effective spatial behavior. There were significant interactions for gender by rearing and rearing by hormonal treatment. In the Expanded condition, males scored higher than females, whereas Restricted females were comparable to the Expanded males. Also, animals with testosterone did better than those animals without androgens. Expanded groups without androgens showed the worst spatial performance. This indicates that without some form of androgens, too much stimulation in the form of exploratory opportunities can impair spatial effectiveness in these animals.

APPROVAL PAGE

This dissertation has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

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CHAPTER I

INTRODUCTION

Sex differences in the ability to negotiate the environment have been observed in many animals, including rodents (e.g., Gaulin & Fitzgerald, 1989) and humans (e.g., Galea & Kimura, 1993). Often these differences are considered, implicitly or otherwise, to be genetic in origin. For example, O'Keefe and Nadel (1978) argue that spatial behavior is ". . . part of the innate machinery of the organism (p.52)", with the neural components in place in the neonate, or arising as a result of maturation. Indeed, it has been hypothesized that there is a gene for spatial behavior located on the X-chromosome, with a recessive allele conferring superior ability (McGee, 1979). However, because males affected by androgen-insensitivity syndrome or testicular feminizing syndrome were similar to control females in spatial scores (Masica, Money & Ehrhardt, 1969), Bock and Kolokowski concluded that, while there was a gene for spatial competency and that it was sex-linked, it possibly was influenced by hormonal states, specifically by testosterone. Therefore, while allowing for genetic expression to be testosterone mediated, any variation not explained by the X-allele or hormonal abnormalities must be due to ". . . polygenic variation and measurement error

(p.1)."

When a particular characteristic is labelled as genetic in origin, the label tends to become explanatory (e.g., review in Johnston, 1987). This has been a tendency in the investigation of spatial behavior, particularly from an ecological perspective. There is resistance to accepting that experiential factors may influence the development of behavior that, presumably, has been subject to natural selection (Gaulin & Wartell, 1990). However, as early as 1948, Tolman suggested that specific spatial abilities develop as a result of experience with the environment. Tolman's suggestion has been supported by more recent experiments, like that of Juraska, Henderson & Muller (1984), who found that rats reared in a complex environment outperformed those reared in a more impoverished environment when tested on a radial maze task. Other studies have revealed more specific and less obvious early experiential effects. For example, Cramer, Pfister and Haig (1988) found that simply limiting the opportunity for neonatal rats to shift nipples while suckling adversely affected adult performance on spatial tasks.

Gender differences in the effects of experience on both behavior and neuroanatomy suggest that steroidal hormones may also play a role in the development of spatial behavior (Juraska et al., 1985). In humans, the prenatal hormonal milieu has been shown to be correlated with later adult

performance on spatial tasks (Reinisch & Sanders, 1992). In rodents, manipulation of neonatal steroidal hormones affected maze navigation at adulthood, suggesting an organizational effect (Williams, Barnett, & Meck, 1990), presumably in the hippocampus, which numerous studies have shown to be involved in spatial behavior (e.g., Cramer, 1988; Fordyce, Bhat, Buraban & Whener, 1994; Juraska, 1991; Juraska, Fitch, Henderson & Rivers, 1985; Sherry, Jacobs & Gaulin, 1992; Silva, Stevens, Tonegawa & Wang, 1990). In support of this conclusion, Roof and Havens (1992) were able to induce male-phenotypic hippocampi in female rats by injecting testosterone at birth.

In summary, early experiences have been shown to alter subsequent performance on spatial tasks in adult animals. Both pre- and postnatal hormonal factors, specifically androgens, have been shown to have effects on spatial behavior, both in humans and in nonhumans. The research reported here was designed to determine the interactive influence of these factors on the development of spatial behavior and therefore to demonstrate that various developmental influences contribute the proficiency of spatial behavior.

Theoretical Background

The rationale for this research is based on evidence for sex differences in spatial behavior. Suggested mediating factors include early experience and hormonal milieu. An

overview of relevant research provides the background for this rationale.

Gender Differences: Differential performance by males and females in navigation is a well-documented and robust phenomenon (e.g. Ellis, 1986). In general, males tend to show superior ability in spatial behavior when compared to females. In humans, these differences are not present prior to puberty (Jacklin, Wilcox & Maccoby, 1988), but become pronounced in adolescence and adulthood (Newcombe & Dubas, 1992; Oosthuizen, 1991). This is consistent with data from non-humans, in which sex differences in spatial ability are most extreme during breeding seasons (Galea, Kavaliers, Ossenkopp, Innes & Hargreaves, 1994) or during fertile periods of the reproductive cycle (Galea, Kavaliers, Ossenkopp & Hanson, 1995). Overall, these differences are most apparent in animals with polygamous mating systems, in which males either disperse or show larger territories than females. For example, Gaulin & Fitzgerald (1986 & 1989) have investigated several species of voles. Montane voles (Microtus montanus) and meadow voles (Microtus pennsylvanicus) are polygynous. Males have large territories that may overlap several smaller female home ranges. Correspondingly, males of these species solve spatial problems with fewer errors than do conspecific females. In contrast, prairie voles (Microtus ochrogaster) and pine voles (Microtus pinetorum) are monogamous. Males

and females share home ranges. In these species, there are no observed sex differences in navigational ability as measured by maze performance.

There are also sex differences in both spatial behavior and hippocampal size in kangaroo rats (Dipodomys sp.). These animals are polygynous and both brain and behavioral sex differences are consistent with those found in other polygynous species. However, two species of polygynous kangaroo rats differ in their foraging styles, which is also reflected in their measured spatial abilities and brain anatomy. Bannertails (D. spectabilis) do not cache seeds but Merriams' kangaroo rats (D. merriami) do (Schroder, 1979). The bannertails have larger hippocampi than do Merriams', with corresponding sex differences in the behavior and brains of both species. Sherry, Jacobs and Gaulin (1992) conclude that these behavioral and anatomical differences are determined ". . .partially by the cognitive demands of foraging and food storage and . . . partially by the cognitive demands imposed by the mating systems (p. 302)". An important question raised by these results concerns how the "cognitive demands of foraging. . . and . . . mating" give rise to these differences. Do these differences result only by selection on the population or also by effects on the developing organism as it copes with the opportunities provided by its rearing circumstances?

It has been suggested (McGee, 1979) that the persistent

sex differences in spatial ability observed in rodents, as well as humans (Smith & Scholly, 1994), are due to an evolutionary history of polygamy. The human male advantage in navigational ability is thought to be reflected in prehistoric sex roles, observed today in hunter/gatherer societies (Galea & Kimura, 1992; Silverman & Eals, 1992). Males range farther afield when hunting large game than do females when collecting plant items (Gaulin & Fitzgerald, 1989). However, it should also be noted that male children in these societies tend to be less restricted culturally in their movements than are females (McGee, 1979), which may enhance to the development of navigational skills. Early locomotor behavior has been shown to influence the development of spatial behavior in human children. For example, Bai & Bertenthal (1987) found that increased opportunity to crawl resulted in improved spatial ability in human infants, as demonstrated by use of landmarks for orientation. Also, as noted by Stumpf and Kleime (1989), there has been a trend towards a convergence of scores on spatial tasks for females and males in industrial societies that may reflect increasing similarities in parental treatment of children as well as educational experiences that provide more opportunities for girls to explore their environment and have more exposure to technical tools. So, there is abundant evidence to suggest that early experiences influence adult spatial behavior and that differences in

exploratory opportunities during rearing may contribute to sex differences in adult spatial behavior.

Potential Mediating Factors: It is well known that manipulation of the rearing environment results in changes in the brain that are correlated with changes in behavior. Greenough (1975) has shown that adult rat brains change as a result of experience; however, the most dramatic changes occur as a result of manipulations early in life. In a classic study, Krech, Rosenzweig and Bennett (1962) placed juvenile rats, after weaning, in environments that varied in complexity. After 30 days of exposure to the different environments, the rats were tested on learning tasks for 18 days, and then sacrificed for analysis of brain anatomy. The researchers found that there were differences in cortical weights of the rats that correlated positively with environmental complexity and were also reflected in previous performance on reversal discrimination tasks. The rats raised in the most complex environment had the largest cortical weight ratios and also had the fewest errors in the problem solving tasks. These results have been replicated a number of times in studies involving adult or post-weaning animals (Greenough, Juraska & Volkmar, 1979; Rosenzweig, Bennett & Diamond, 1972).

Other studies using neonatal subjects have suggested that very early experiences may influence adult spatial behavior specifically. For example, Braithwaite and

Guilford (1995) reared pigeons either in a loft that allowed the birds to see the landscape or in a loft that was covered with frosted Perspex that provided comparable light levels but no view. The birds reared in the former condition were better navigators than those reared in the latter condition, and also were more likely to use visual landmarks while homing.

Further evidence for the importance of early visual input on developing spatial competency was found by Tees, Burhmann and Hanley (1990). They reared rats either on a 12 hour light/12 hour dark schedule (LR) or in continuous darkness (DR). Those rats reared in the light performed better, with shorter latencies in a Morris Water maze than did those reared without the benefit of visual experience. This work was replicated with comparisons to animals reared in a complex environment, who outperformed the LR rats, suggesting that the richer the visual experience, the better adult navigational skills will be. Hyatt (1990) investigated a similar phenomenon by rearing rats either in clear cages that provided a view of the environment in all three dimensions or in opaque, covered cages. She found that those with the ability to view distant room cues and objects were better able to solve allocentric learning tasks than those without the benefit of wide visual experience. The benefit appeared to be unique to allocentric tasks since there was no difference in performance on egocentric

learning tasks; therefore, the early visual experience influenced how the animal oriented in its environment, with those animals with a richer visual field relating to landmarks as well as place cues for orientation.

Other investigators have demonstrated non-obvious relationships between early experiences and subsequent spatial behavior. For example, if hamster pups are restrained by a barrier from leaving their nest, or if they are maintained on a liquid diet that affects the rate of retrieval by the mothers, they perform very poorly in spatial tasks at maturity (Tomlinson, 1989; 1991). Cramer, Pfister & Haig (1988) discovered that the lack of opportunity to shift nipple sites during suckling by neonatal rats impaired later maze performance, independent of other types of learning, such as association or perceptual tasks.

The obvious conclusion to be drawn from these latter studies is that early experiences influence later spatial behaviors. Because of the evidence for increased spatial competency resulting from complex rearing environments, it would appear that navigational development requires an "organizational framework" that is dependent on a variety of early experiences (Tees et al, 1990).

Despite these findings, some have argued that early experience contributes minimally, if at all, to the development of spatial behavior, particularly the superior

performance of males versus females (e.g., Gaulin & Wartell, 1990; Gladue, Beatty, Larson, & Staton, 1990; Oosthuizen, 1991). Taking a non-developmental additive view, these writers suggest that genetic factors are more important than experience in the development of navigation. However, because adult spatial abilities are not perfectly correlated with chromosomal sex, some researchers (e.g., Bock & Kolakowski, 1973; Galea, Kavaliers, Ossenkopp, Innes & Hargreaves, 1994; Silverman & Eals, 1992) acknowledge that the expression of the behavior, while genetically mediated, also may be influenced by hormones . Early hormonal exposure allows the brain to organize differentially, which will be reflected later in variation in adult navigational competencies. Specifically, the hypothesized organizing factor for sex differentiation in mammalian brain morphology is testosterone and related metabolites (Williams & Meck, 1990). The most frequently observed sensitive period for exposure is during the early postnatal period. For example, in rodents, the sensitive period occurs during the first week of life (Baum, 1987) for the development of receptor sites for steroids in the telencephalon of the rat brain. These are transitory; they exist in the first two weeks of life but are not present in the adult (Loy, Gerlach, & McEwen, 1988; Williams, Barnett, & Meck, 1990).

There are several hypothesized mechanisms for steroidal-based brain organization. Tobet, Chickering,

Hanna, Crandall and Schwarting (1994) propose that steroid hormones direct cell positioning and migration which results in permanent differences in brain structure that ultimately underlie functional sex differences. Kuhnemann, Brown, Hockberg and MacLusky (1994) argue that testosterone secretion by males during early development permanently alters the capacity of the brain to respond to circulating estrogens. In rats, this change in estrogen responsiveness is associated with a decrease in estrogen receptor levels which occurs as early as 24 hours after birth. Juraska (1991) suggests that early hormonal milieu affects the plasticity of the cells in the dentate gyrus which will influence the animal's response to environmental cues as well as the extent to which the animals benefit from differential rearing conditions.

Interestingly, estrogen can also play an organizational role in the masculinization of the brain and the subsequent development of adult behavior (Leger, 1992). In many male mammals, testosterone is converted to estradiol in the brain, which results in masculinizing effects. This does not happen to females because alphafetoproteins bind circulating estrogens and keep them from entering the brain. They do not bind with testosterone. If there is an excess of estrogens, then the binding capacity of the alphafetoproteins is swamped and the brain may become masculinized. Artificial sources of estrogens (e.g., DES)

are not bound by the alphafetoproteins, so they may result in masculinizing effects, which may explain DES effects in humans (Reinisch & Sanders, 1992). As indicated previously, this is a short-lived phenomenon, present only during the perinatal sensitive period (see Loy et al, 1988).

Correspondingly, there has been experimental evidence in support of hormonal influences on brain organization independent of chromosomal sex of the subjects. For example, Roof and Havens (1992) were able to masculinize the hippocampi of female rats by administering testosterone neonatally. Cells in the dentate gyrus of the hippocampus were larger and more asymmetrical laterally in intact males and in females with neonatal testosterone treatments than in control females. Correspondingly, the treated females were equal to control males and superior to control females on navigation tasks.

Williams, Barnett and Meck (1990) manipulated neonatal hormones in rats by gonadectomizing male subjects at birth and treating newborn females with testosterone. At adulthood, these animals were compared to intact male and female littermates on performance on a seventeen-arm radial maze. Again, females masculinized through neonatal treatment with androgens showed maze performance equal to intact males and superior to females. In summary, these results also support an argument for organizational effects of steroidal hormones. Therefore, adult spatial behavior

may be more closely correlated with neonatal hormonal state than with chromosomal sex, which suggests an "extra-genetic" component to the development of navigational abilities.

While neonatal steroids may be necessary for the organization of brain structures that are correlated with the superior spatial abilities typical of males, they are not sufficient for the manifestation of the male phenotypic behavior. There also appears to be an activational role of gonadal hormones after the age of sexual maturity. Sex differences in navigational abilities as measured by maze performance are most dramatic when animals are in breeding condition as compared to non-reproductively active animals (Steven J. Gaulin, personal communication, September, 1993). These sex differences disappear in non-reproductive animals when steroid hormones are at their lowest levels (Galea, Kavaliers, Ossenkopp & Hampson, 1995; Galea, Kavaliers, Ossenkopp, Innes & Hargreaves, 1994). In adult mammals, the sex hormones have different effects, depending on gender. Frye (1995) has shown that in rats, estrous females or ovariectomized females with estradiol replacement exhibit poor performance in a water-maze task compared to diestrous females and intact males. This effect was most pronounced during acquisition, when the task was novel.

Differential performance on the water maze task that was correlated with particular hormonal states have been seen in meadow voles (Galea, Kavaliers, Ossenkopp & Hampson,

1995). This species is polygamous and in previous research has exhibited reliable sex differences in navigation (Gaulin & Fitzgerald, 1986; 1989). Diestrous females did better than estrous females. Analysis of hormonal assays allowed for more specific conclusions regarding hormonal influences. Females with high estrogen levels took significantly longer to swim to the submerged platform than either intact males or females with low estrogen levels. The results of this research further supports the contention that while testosterone may help increase adult spatial abilities through early organization of critical brain areas (i.e., the hippocampus), presence of estrogens may actually suppress spatial performance. Even in male rats, injections of estrogen will result in poor maze performance (Thiessen, 1976).

Acknowledgement of the influence of adult hormonal state on spatial performance is particularly important in interpreting those few reports in which no sex differences were observed in species which otherwise have shown sexual dimorphism in navigation. Sawrey, Keith & Backes (1994) found male and female prairie, montane and meadow voles to be equally efficient in a Morris water-maze task. However, as has been previously indicated, without reference to reproductive condition and associated hormonal states, it is difficult to predict adult spatial performance accurately.

In humans, too, there appears to be an activational effect of sex-related hormones. In six-year old boys and girls, who are presumably pre-pubertal, there was no difference in measured cognitive abilities, including spatial tasks, as reported by Jacklin, Wilcox and Maccoby (1988), but post-pubertal performance has been shown to reflect hormonal states (Hampson, 1990; Hampson & Kimura, 1988). Specifically, females who are at the mid-luteal stage of their menstrual cycle, with high circulating levels of estrogen show impaired spatial performance while their performance improves during menses, when low circulating levels of estrogen are present. Females on birth control pills perform worse than any other group (Genetta-Wadley & Swirsky-Sacchetti, 1990).

It is evident that gender specificity in spatial abilities are better predicted by knowledge of hormonal condition than by genetic sex. Correspondingly, there has been interest in identifying the hormonal contributions to differential spatial performance of males and females. Literature on both human and non-human behavior acknowledges sex differences, with regard to either hormonal or experiential factors (e.g., Hassler, 1991 & 1992; McKeever, 1986; Pearson & Ferguson, 1989). Indeed, hormonal influence may be mediated by experiential factors. For example, it is necessary for meadow vole pups to be exposed to the appropriate photoperiod for induction of hormonal release to

occur as adults, as well as for organization of neonatal brain tissue that correlates with sex differences in adult navigational ability (Kelly, 1993). In the absence of long day (i.e., 14 hour light/10 hour dark) exposure, responsiveness of the central nervous system to the masculinizing effects of perinatal testosterone decreases.

In conclusion, it is apparent that the development of sex differences in spatial behavior is a complex phenomenon. Experiential and hormonal milieu contribute to the organization and maintenance of navigational abilities. The research reported here is designed to examine the interactions among these potential influences.

In this study, I investigated the development of spatial behavior in montane voles (Microtus montanus), a polygynous species (Jannett, 1982) that shows sex differences in spatial ability (Jacobs, Gaulin, Sherry, & Hoffmann, 1990). Influences of early experience were determined by varying the opportunities for exploration and hormonal influences were manipulated through neonatal gonadectomies and testosterone injections. Dependent measures involved performance in an eight-arm radial-arm maze.

The eight-arm radial maze was selected for measuring spatial performance because it has been used previously to show sex differences in spatial behavior in rodents (e.g., Cramer, Pfister & Haig, 1988; Juraska, Henderson & Muller,

1984). It was also chosen because it is a reasonable approximation of the sort of challenges that a montane vole might encounter in its natural habitat. Voles spend a large amount of time foraging which could feasibly require orienting to stable landmarks (Jannett, 1982), a skill that is necessary for successfully solving the eight-arm radial maze. The Morris Water maze was rejected because voles do not typically spend much time swimming.

It was predicted that females receiving testosterone and reared in an environment rich with exploratory opportunities (i.e., "Expanded") would have behavioral scores similar to intact males reared in the same condition. Conversely, males without postnatal testosterone, reared in an environment with few exploratory opportunities (i.e., "Restricted") would have behavioral measures similar to the control females reared in the same condition. Values found for the females receiving testosterone but reared in the "Restricted" condition and males without testosterone reared in the "Expanded" environment were predicted to be intermediate.

Unlike previous research in which hormonal or experiential influences have been studied in separate experiments, this study combined experiential and hormonal manipulations in a single experimental design. Furthermore, this study examined the effects of exploratory experiences from birth, whereas other studies of developmental

influences on subsequent behavior have manipulated animals' post-weaning environments (e.g., Greenough, 1975 and Rosenzweig & Bennett, 1969). As suggested by the research of Cramer and her colleagues (1988), pre-weaning experience clearly contributes to the development of spatial proficiency.

CHAPTER II

GENERAL EXPERIMENTAL METHODS

Subjects

Pairs of montane voles (*Microtus montanus*) were bred until twenty viable litters of the appropriate number and sex composition had been reared. Litters were culled to three pups, each consisting of either males or females and were raised in one of two rearing conditions. The voles were selected from a captive population in the laboratory colony housed at the Animal Care Facility at the University of North Carolina, Greensboro, with known lineages recorded to avoid inbreeding. They were reared in the same facility, maintained on Rabbit Chow and water ad libitum under a 14/10 hour light/dark schedule. They were provided with wood shavings for bedding and nesting material. These conditions have been approved by the Institutional Animal Care and Use Committee.

Design and Procedure

Single-sex litters (three animals per litter) were reared with their parents from birth in one of two conditions, designated *Expanded* and *Restricted*, that offered different opportunities for early exploratory behavior. In the Expanded condition, animals were housed in large (38 x 48 cm) clear plastic cages and provided with wooden objects

of varied shapes and sizes with which the pups could interact (after Rosenzweig & Bennett, 1969; see Figure 1). These "toys" were made of pine, the same material commonly used for the bedding. In the Restricted condition, the voles were housed in small (20 x 23 cm) clear plastic cages with no additional objects for handling or exploration (see Figure 2). To manipulate hormonal status, one animal in each litter was injected on the day of birth with testosterone propionate while the other two served as controls, as described in more detail below. The pups were weaned at 21 days of age by removing their parents. Testing on the spatial orientation task described below began at 50 days and continued daily for 14 days. The animals were then sacrificed by sodium pentobarbital injection and transcardial perfusion and their brains removed for a neuroanatomical study. Those results will not be reported here due to time constraints.

Sex of litter was matched to each condition, and alternated sequentially when possible. For example, one litter of males was reared in the Expanded condition, and a corresponding litter of males was reared in the Restricted condition concurrently.

The study used ten litters of each sex per rearing condition, each litter consisting of three animals, for a total of twenty litters or 60 animals. This number allowed for five replications of the experiment per condition,

repeated with males and females. Three litters per condition has been suggested previously as adequate for studies of this type (e.g., Cramer et al, 1988), but five per condition were included in this investigation to ensure adequate replication for statistical analysis.

Manipulation of early hormonal milieu involved hormonal injections performed on the day of birth. Injections consisted of a single dose of either 0.5 mg testosterone propionate dissolved in 0.05 ml peanut oil (after Smale, Nelson & Zucker, 1985) or a control injection of 0.5 mg cholesterol in a vehicle of 0.05 ml peanut oil. Sex was determined with the use of a dissecting microscope to ensure that litters consisted of same sex pups.

In each male litter for each rearing condition, the two animals to receive injections were gonadectomized. The surgery was performed on the day of birth, using cryanesthesia (see Figure 3). One of the gonadectomized animals in each litter received a subcutaneous injection of testosterone (GNX-MT); cholesterol was injected into the other (GNX-MC). The third same sex pup (CONT-M) received a sham operation. This involved the same procedure as the GNX animals, with incisions in the skin and muscle wall, except that the gonads were left intact. Incisions were closed with Nu-Skin (R), a surgical glue. Animals were marked by a toe clip after surgery. This allowed for individual identification of the animal for later injections and at

termination of testing. After surgery, all animals were returned to their parents.

The litters of female pups received the same hormonal treatment on the day of birth as did the males, using cryanesthesia for the injection and toe-clipping procedure; however, due to the high mortality rate of ovariectomized neonatal females in pilot work, gonadectomy was postponed until after weaning, at 30 days of age. This was a feasible modification since ovaries are essentially non-functional in the neonatal and juvenile rodent (Ellis, 1986; Williams & Meck, 1990). Because they begin producing hormones at puberty, which occurs at approximately 45-50 days of age in Montane voles (Jannett, 1982), ovariectomies on day 30 ensured that the gonads were removed prior to secretion of hormones that might influence behavior but after the animal was sufficiently mature to survive the surgery. In this procedure, the females were anesthetized with an intraperitoneal injection of sodium pentobarbital (i.e., dosage of 0.05ml/40gm of animal weight). The ovaries were removed from the two animals identified by clipped toes as the testosterone propionate and cholesterol recipients. These became the GNX-FT and GNX-FC animals. The third received a sham operation (CONT-F). The incisions in the muscle wall and skin were closed with surgical suture and the animals returned to their home cages to recover.

Other investigators have found that the most reliable sex differences in maze performance by voles occurs during the breeding season, or when the animals are reproductively active (Steven J. Gaulin, personal communication, September 1993), which suggests that there may be an activational as well as organization role for testosterone in spatial orientation. In order to maximize the likelihood of obtaining clear sex differences in this study, a single subcutaneous dose of 0.50 mg of testosterone enanthenate in 0.05 ml of peanut oil (after Zielinski & Vandenbergh, 1993) was injected into the GNX-T animals at 45 days of age, when montane voles typically become sexually active (Jannett, 1982). The GNX-C animals received cholesterol injections (0.50mg in 0.05 ml of peanut oil) at the same time. While it is unlikely that this manipulation exactly mimicked the natural onset of puberty, these potential differences could be assessed by comparisons with the CONT groups. Behavioral testing commenced on day 50 to allow for metabolization of the hormones.

A summary of the research design is as follows:

	Expanded Condition	Restricted Condition
Males	GNX-MT	GNX-MT
	GNX-MC	GNX-MC
	CONT-M	CONT-M
Females	GNX-FT	GNX-FT
	GNX-FC	GNX-FC
	CONT-F	CONT-F

Five litters for each of two conditions per sex, with three animals per litter = 60 subjects.

Spatial orientation was measured using a wooden eight-arm radial maze. The dimensions of each arm were 10.5cm wide, 8cm high, and 12cm long. The central arena was octagonal, each side measuring 15 cm. Clear Plexiglas covered all arms and the central arena (see Figure 4) so that the animal could look up and out. This also allowed a view of the salient features of the room which could function as orientation cues (e.g., cabinet location, pictures on the wall).

Each arm of the mzae was baited with a small piece of apple, which my pilot studies established as a desirable food item for voles.

Testing began at 50 days of age. At the start of each trial, a single animal was placed in the center of the maze.

The animal was free to choose any alley (see Figure 5). A choice was scored when the animal's shoulders had entered the arm (Olton & Samuelson, 1976). Repetition of choices was possible since all arms were continuously available; however, revisits would not garner the vole further rewards, because the arms were not rebaited. The animal was allowed to explore the maze until all eight rewards had been depleted or ten minutes had elapsed, after which it was removed and returned to its home cage. After each litter's trial, the maze was swabbed with a 50/50 solution of rubbing alcohol and tap water to control for odor cues for subsequent subjects. Behavioral tests were conducted in a room in the same building as the Animal Care facility, under fluorescent lighting.

Dependent measures included number of arms visited per day, time elapsed until all eight baits were taken, number of baits obtained per trial and number of revisits to arms from which baits had been removed. An additional dependent measure was utilized to assess performance. Observation during pilot work had shown that low scores of arm visits and revisits, or errors, did not necessarily mean spatial competency. Some animals had few visits because of inactivity (e.g., grooming in the corner of one arm). Few numbers of revisits sometimes reflected lack of efficiency in finding baits. An error was scored if the vole returned to an arm from which the bait had already been taken. If

the animal did not find the bait, but returned to the arm, or was inactive, it would also achieve a low error rate. In order to gain a more accurate picture of the overall ability of the animals, the ratio of visits per minute was calculated. This provided a means of assessing efficiency, since it related how much time the animal spent in the maze to the number of arms traversed.

While a number of dependent variables were used, they were not independent assessments of behavior. All measure spatial ability and so are expected to produce similar results. Because spatial behavior is a complex phenomenon, multiple measures provide a better idea of overall performance.

An optimal spatial performance in respect to these dependent measures would be a low mean number of visits to arms, a short average time spent in the maze until task completion, a high mean number of baits taken, with a low mean number of revisits to arms from which the bait had been taken, and a high mean visits per minute ratio.

After 14 test days had elapsed, behavioral tests were concluded for that litter. This was justified because other research has shown that most animals have mastered the task by then, and that if they haven't, performance does not substantially improve by further exposure to the maze (e.g., Cramer, 1988). This is consistent with pilot data obtained with voles from this colony.

Three-Way ANOVAs for repeated measures, with interaction terms of gender, hormonal treatment, and rearing condition were used (Cramer, 1988) for statistical analyses with an alpha level set at ≤ 0.05. For post-hoc pair-wise comparisons, Tukey tests on collapsed interactions were computed, with an alpha level set at ≤ 0.01 (as suggested by Grace Kissling, UNC-G Department of Mathmatics, personal communication, July 1995). The statistical analyses were based on the last 12 days of testing, which allowed days 1 and 2 for the animals to become familiar with the maze as well as the introduction of apples as an acceptable food. Data from the pilot study mentioned previously indicated that by day 3 the majority of the voles were beginning to take baits and complete the task under the ten-minute limit. Allowing uncounted trials for the animals to gain exposure to the maze is consistent with other studies evaluating maze performance (e.g., Cramer, Pfister & Haig, 1988).

Predictions

The following results were predicted, with "good performance" defined as the least amount of time spent in the alley, with the fewest number of alleys visited, the lowest error rate, and the highest visits per minute ratio: 1) Expanded GNX-FT will show overall performance comparable to the Expanded CONT-M and Expanded GNX-MT; 2) Restricted GNX-MC will have the poorest scores, similar to Restricted CONT-F and Restricted GNX-FC; 3) Restricted GNX-FT will be

approximately equal to Expanded GNX-MC and show superior performance to Restricted CONT-F; 4) All GNX-MT will perform similarly to CONT-M reared under similar conditions; 5) GNX-FC should be similar to CONT-F in the same environmental regimen.

CHAPTER III

RESULTS

The guiding proposition for this research, that developmental influences do not act in isolation was strongly supported, since there were no significant main effects for any of the dependent measures. Although the three-way interaction of sex by rearing by hormonal treatment was not significant, the Three-Way ANOVA for repeated measures revealed a significant interaction of rearing by sex for four of the five dependent measures and a significant interaction of rearing by hormonal treatment for three dependent measures. Thus, animals reared in the Expanded condition differed from each other in ways that were unlike the differences among animals reared in the Restricted conditions, depending upon their sex and hormonal treatment. For all measures, there was significance for trials over time, meaning that performance consistently changed as the testing days progressed. The changes in performance showed either a linear or quadratic trend, with improvement across trials.

Gender by Rearing Interactions

Visits: While there was a significant gender by rearing interaction for number of visits per trial ($F = 6.51$, $p = .0137$), there were no significant differences among means

for the conservative alpha level of .01 (see Tables 1 and 3). Although it was predicted that the fewest alley visits (i.e., best performance) would be made by the animals in the Expanded condition, examination of the means showed that the Expanded females and males were very similar in numbers of visits to the Restricted females. Surprisingly, the lower scores tended to be made by the Restricted males (see Table 2 and Figure 6).

Time: There was a significant interaction for time spent in the maze ($F = 7.04$, $p = .0106$), as well as significant differences among the group means (see Tables 4-5) when rearing and gender are taken into account. Females in the Expanded condition spent more time in the alleys than any of the other groups, which all had similar times (see Table 6 and Figure 7). It was expected that the Expanded females would spend more time navigating the alleys than their male counterparts, and conceivable that the Restricted males would take less time to locate the baits, given the potential advantage of prenatal androgens, but it was not expected that the Restricted females would perform more like males, regardless of rearing condition, and have shorter latencies than the females who had the benefit of more opportunities to engage in exploratory behavior.

Number of Baits Taken:

There was no significant gender by rearing interaction for the number of apple pieces retrieved during the testing

period for ($F = 1.47$, $p = .2309$; see Tables 7-8 and Figure 8). This reflects a ceiling effect when all animals are considered together; as the number of trials increased, animals tended to become proficient, finding all the baits. However, the overall perspective is clarified by examining the interaction of rearing and hormone (see below).

Revisits: Males differed significantly from females with regard to the conditions in which they were raised for number of revisits, or errors made ($F = 4.14$, $p = .0472$; see Tables 9-10). As predicted, the Restricted females had the most errors and the Expanded males had the fewest; however, the Restricted females did not differ significantly from either the Restricted males or Expanded females. While the Expanded males were very different from the Restricted females, they tended to make similar scores to the intermediate groups of Restricted males and Expanded females (see Table 11 and Figure 9).

Visits per Minute: There was a significant interaction for the compound variable of visits per minute, designed to give a more accurate account of those animals who had low numbers of alley visits because they spent time in alternate activities (e.g., grooming in one arm) for extended periods of time ($F= 7.41$; $p=.0088$; see Tables 12-13). The Expanded females, who spent the most time in the maze per trial, also had the lowest ratio for visits per minute, indicating inefficiency relative to the other groups, who achieved

similar scores (see Table 14 and Figure 10).

Summary: From an examination of the results of the four dependent measures that revealed significant interaction effects for rearing and sex, it is clear that accurate prediction of spatial phenotype cannot be made upon the basis of either rearing or sex alone. Females reared in the Expanded condition tended to do poorly on navigational tasks relative to the other groups, but males were not necessarily superior to females in general. Depending upon the measure, Restricted females were comparable to Restricted as well as Expanded males.

Rearing by Hormonal Treatment Interactions

Visits: There was no significant rearing by hormonal treatment interaction for number of visits ($F = 0.35$, $p = .5591$; see Table 15 and Figure 11).

Time: There was a significant interaction for latencies to obtain all the apples ($F = 3.48$, $p = .0382$; see Tables 16-17). Expanded GNX-Cs spent significantly more time in the maze compared to either Restricted Controls or Restricted testosterone-treated animals. Also, the Expanded GNX-Cs were significantly slower than the voles reared comparably in the Expanded condition but who had received testosterone injections (see Table 18 and Figure 12). These results are consistent with those found for the rearing by sex interaction; Expanded females had longer latencies relative to the other groups, suggesting that the lack of hormones,

particularly testosterone together with an early rearing history that is rich with exploratory opportunities may actually impair adult navigation, at least in terms of time.

Number of Baits Taken: The animals varied according to hormonal milieu and by exposure to varying opportunities to explore in terms of how many apples were obtained ($F = 3.18$, $p = .0498$; see Table 7). The Expanded GNX-Cs acquired the fewest number of baits relative to the Expanded and Restricted testosterone-treated animals and all the Restricted voles (see Tables 19-20 and Figure 13). This is particularly revealing, given that these animals spent the most time in the maze, but apparently not in profitable ways.

Revisits: There was not a significant interaction effect of rearing by hormone for number of errors made (see Tables 9 and 21 and Figure 14).

Visits per minute: There was a significant interaction of rearing and hormone for the ratio score of visits per minute, which clarifies the relationship between number of arms entered and time per trial ($F = 3.41$, $p = 0.0409$; see Tables 22-23 and Figure 15). Pairwise comparisons revealed significant differences among the extreme groups (see Table 24). The Restricted CONT animals had the highest ratios which were significantly different from the Expanded CONT and GNX-C animals exhibiting the lowest ratios respectively. This is consistent with the time measures, which showed that

the Restricted animals with endogenous sources of hormones spent the least amount of time in the maze. The Restricted males also had the fewest number of arm visits in the Rearing by Gender analyses. That the Expanded GNX-C animals had the lowest efficiency rating is not surprising, given that they spent the most time in the maze and found the fewest baits.

Summary: Lack of male hormones seems to be the most critical factor in producing an inefficient spatial phenotype, given that in all of the three dependent measures for which a significant rearing by hormonal treatment interaction was found, GNX-C animals had the poorest performance. Another result of particular interest is the low ranking of the animals reared in the Expanded condition. As indicated above, without the benefit of male hormones rearing in an Expanded environment may actually impair navigational abilities.

Specific Predictions

Discussion of the specific hormonal effects by sex and rearing initially hypothesized was not possible because the three-way interaction effects were not significant for any dependent measure.

CHAPTER IV

DISCUSSION

Because there were significant interaction effects for gender by rearing and rearing by hormonal treatment, the overall prediction that knowledge of hormonal milieu and rearing conditions with regard to gender, rather than just knowledge of chromosomal sex, is necessary for accurate prediction of spatial ability was upheld.

For rearing by gender effects, the males tended to score overall higher than the females in the Expanded condition. These females had the worst scores on half the dependent measures (i.e., worst times, and lowest number of visits per minute ratios). Their counterparts in the Restricted condition were comparable to the Expanded males on time and visits per minute, but had the highest error rate. So, depending on the measure, the females were distinguished by rearing.

Also, Restricted females tended to be more like their male counterparts and the Expanded males on two of the measures. It is possible that without androgens, it may be better to be reared in an environment without too many exploratory opportunities. This indicates that without some form of androgens, too much stimulation can impair spatial effectiveness in these animals. It may be that encoding

complex spatial information in a meaningful way (i.e, for use in navigation) may be dependent on post-natal testosterone.

A more complete story is obtained through examination of the rearing by hormonal treatment effects. When testosterone was present, the voles tended to rank at the top on all dependent measures for which effects were found. Expanded groups without androgens were at the bottom. The animals that had some source of androgens, whether exogenous or endogenous, had similar scores, regardless of whether they were reared in either Expanded or Restricted environments. The Expanded GNX-Ts, Restricted GNX-Ts and Restricted CONTs had the best performances on the three dependent measures that showed significance (i.e., fewest mean number of visits per trial, highest average number of baits taken per trial and highest average visit per minute ratio). This indicates the importance of post-natal testosterone in improving spatial ability, regardless of genetic sex. It might be that the restricted rearing also induced stress, leading to increased adrenal hormone production, with resulting masculinization of the females, mimicking the effects of testosterone. However, without knowledge of circulating titres of adrenal hormone secretion, it is difficult to draw conclusions about potential effects of stress.

Another interesting aspect of these analyses concerns the similarity of the Restricted CONTs to the Expanded and Restricted testosterone treated animals. There was variability in the ranking of the Restricted CONT animals. This is logical since these animals included males with endogenous sources of hormones. These CONT males may have had variable levels of androgens, depending upon their reproductive condition or timing of puberty. While montane voles usually achieve puberty around 40 days of age, there is undoubtably some variation, even though they were maintained on a light schedule that mimics the photoperiod supporting an active reproductive state in nature. Also, within this group are intact females, presumably with circulating estrogens, whose scores may have helped lower the average of those males who did have normal levels of post-pubertal androgens. That they were able to compete successfully with the testosterone animals in the Expanded condition is further evidence of the developmental complexity of navigational skills. There is more than one developmental route to the outcome of high spatial ability, therefore supporting the embryological principle of developmental equifinality for behavior (Brunswik, 1952; Gottlieb, in press).

The second worst performance was by the Expanded CONTs (i.e., high mean number of visits per trial and low average visit per minute ratio). Half of these animals were intact

females with estrogens that were expected to suppress spatial competency, and as was supported with the rearing by gender analysis. The other half were intact males that should have had the benefit of some level of androgens. It could be that the females' performance was sufficiently poor that it lowered the mean to such a degree that the competency of the males was not apparent. For reasons discussed above (e.g., variation in onset of puberty and differential levels of endogenous hormones), the males may have indeed behaved more like their female counterparts than their testosterone-enhanced brothers.

Conclusion

It is apparent from these findings that the development of sex differences in spatial behavior is a complex phenomenon. Experience and hormonal milieu contribute to the organization and maintenance of navigational abilities, but in some surprising ways. Enriched rearing confers an advantage, but only if androgens are present. If they are not, then increased amounts of sensory stimulation appears to be detrimental, resulting in poor navigational performance. Observation of the animals in these groups (i.e., Expanded GNX-Cs) suggests that they developed deficient spatial abilities since they did not seem to traverse the maze with any "scheme." They entered a large number of arms in a hit-or-miss fashion. They did not adopt an adjacent alley strategy or appear to benefit from peering

to orient to landmarks in order to find the arms with baits remaining. Indeed, they seemed not to look for the baits when visiting arms. They also were not able to adopt a win-shift strategy, returning again and again to alleys from which the apple piece had been removed. This was in marked contrast to the animals with sources of androgens that moved through the maze methodically, peering after every bait was removed and frequently gazing up at the peripheral edge of the maze from the center during a trial. These animals made few revisits to arms from which the bait had been removed. It was often observed that they would pause by a doorway that had been entered previously, hesitate, and peer upward, then move on to an unvisited arm.

As these findings indicate, while knowledge of chromosomal sex makes it possible to predict spatial ability with partial accuracy, it is not the "whole story." Which males or females will do better apparently is dependent upon hormonal state as well as rearing condition. These influences may be contributing to the differences in the behaviors in subtle ways, such as changes in reactivity, attention, fearfulness, activity level or ability to process complex information efficiently. This supports the idea that if we are to gain a complete understanding of the development of a particular phenotype, we must not rely on reductionist explanations that attribute phenotypes to single causes, but acknowledge the complexities of the

phenomenon and identify as many interacting influences as possible (Gottlieb, 1991).

Directions of Future Research

While the results of this research support the prediction that hormonal milieu as well as early exploratory opportunities contribute to adult navigational abilities, there remain several avenues of investigation to pursue to clarify the nature of their interactions.

1. Is the effect of testosterone organizational, activational, or both?

While the findings of many studies support the notion that masculine phenotypes, including spatial ability, result from perinatal exposure to testosterone (e.g., Loy, Gerlach & McEwen, 1988; Williams & Meck, 1990), others have argued that while testosterone may be necessary for the development of masculine behaviors in males, it is ineffective in producing masculinization in females (e.g., Baum, 1979; Peterson, 1985). The studies that indicated variability in spatial ability with seasonal fluctuations in hormonal state suggest that hormones may exert an activational effect (e.g., Frye, 1995; Galea, Kavaliers, Ossenkopp & Hampson, 1995; Galea, Kavaliers, Ossenkopp, Innes & Hargreaves, 1994).

In light of this confusion, it would be beneficial to determine the period during which the steroid is most influential, at least in this species. If its role is

organizational, then the critical period for its introduction would be perinatally. If its role is activational, then it would be essential for testosterone to be present during adulthood, at the time of testing. This could be investigated by manipulating hormonal condition through neonatal gonadectomies and then administering testosterone replacement at birth or at 45 days of age, when puberty normally occurs. These findings would help clarify the time period when androgens confer an advantage, and also when over-stimulation from the environment may interact with a lack of hormones to impair spatial performance.

2. *Whether the role of testosterone is activational, organizational, or both, is the effect dose dependent?*

In the original experiment, the amount of testosterone administered was .05 mg/.05 ml of peanut oil. Results from the GNX-T males suggest that these are high levels of testosterone which resulted in improved performance. In order to determine the level of androgen sufficient to produce the differences in behavior, doses of testosterone could be varied (e.g., .01 mg; .02 mg, etc). This would give an indication of whether or not the highest titres of androgens are necessary for enhancing spatial ability or if there is an optimal level that may be intermediate.

3. *What are the circulating hormonal levels of GNX-T, GNX- C and CONT animals?*

Because little is known about circulating hormonal levels in montane voles, it would be useful to perform hormonal assays on animals from experiments 1 through 2. These findings would provide a baseline for others researching the effects of exogenous hormones on montane vole behavior.

This variation could also be useful in clarifying the role of high circulating androgens for each sex in producing differences in spatial competency. For humans, there are conflicting results found in the literature concerning the effective levels of androgens in the production of superior spatial performance. Some studies have found an interaction of androgen levels and gender as influencing spatial ability. Shute, Pellagrino, Hubert and Reynolds (1983) found that human females with high levels of androgens tended to do well on spatial tasks, whereas males with high androgen levels did poorly. However, in another study, McKeever, Rich, Deyo and Conner (1987) found no relationship between androgen levels, regardless of sex, on measures of spatial ability. By examining the correlations of spatial efficiency with endogenous hormones, and then comparing the findings with the results from the proposed experiments in #2 above, the generalizability of the original experimental design to performance of wild populations could be evaluated. It would also provide a basis for comparison for examining hormonal titres and their influence on sex

differences in spatial learning for other species, such as humans.

4. *What is the role, if any, of female hormones, such as estrogen and progesterone?*

Baum (1987) has stated that it is the estrogenic metabolites of circulating androgens that are responsible in the perinatal defeminization of male rodents. Introduction of synthetic estrogens prenatally has different developmental effects on human male and female fetuses (Reinisch & Sanders, 1992). Also, as has been mentioned previously, estrogen has been suspected as an inhibiting agent for spatial ability in both rodents and humans (Hampson & Kimura, 1988; Genetta-Wadley & Swirsky-Sacchetti, 1990).

Given these findings, and the interaction of rearing condition and sex in the research reported here, with females in the Restricted condition uniformly outperforming their peers in the Expanded condition, the next logical step would be to investigate the influences of estrogen. In this replication, the hormonal treatment would involve estrogen rather than testosterone. This would be particularly interesting given the previous finding that males without postnatal androgens, but reared in the Expanded environment, are not able to benefit from the prenatal androgen advantage that their littermates had. This may help determine whether female hormones alter the developmental process associated

with spatial behavior or suppress adult expression of previously developed navigational skills.

Since Baum (1979) has proposed that in some mammalian species, progesterone is essential for development of female phenotypes, it would be of interest to investigate the potential influence of progesterone on the development of sex differences in spatial phenotypes as well.

6. *Do early hormonal and rearing conditions affect spatial performance only, or do they effect other abilities that involve learning and memory?*

There is ample evidence to suggest that spatial learning tasks involve different behavioral strategies and different neural substrates from non-spatial tasks (e.g., Sutherland & Rudy, 1989; Worden, 1992). Support for these distinctions comes from an investigation by Kamil and Balda (1990), who have demonstrated differences in spatial abilities in seed-caching species of birds versus non-seed caching birds, but find comparable performance in other behaviors, such as operant learning tasks. This trend in differential performance on spatial tasks, but not on association tasks has been seen in rats (Juraska, Henderson & Muller, 1984) and humans (Reinisch & Sanders, 1992). Cramer, Pfister and Haig (1988) confirmed that early developmental experiences (i.e., opportunities to nipple-shift) influenced spatial tasks, such as performance in the eight-arm radial maze, but did not affect efficiency on

operant tasks requiring differential response rates, light/dark discrimination tasks, or operant tasks based on visual discrimination.

Studies using radial-arm mazes seem to present good evidence for the separation of learning tasks (Olton & Samuelson, 1976). While a position response, or association-based strategy may explain an animal's route through the maze, it does not seem to explain why rotation of the maze, or moving the distal visual array disrupts performance. It is necessary for animals to use landmarks and respond in terms of their relationships.

Sutherland & Rudy (1989) suggested that performance on the radial-arm maze can be separated into two "versions." To assess spatial performance, the arms of the maze are made indistinguishable from each other, and may be differentiated only by orienting to distal landmarks. To measure learning based on associations, each arm would provide visual cues, as it would be made distinctive in some way.

If behavioral differences exist for each type of task, it is logical to assume that there are different learning process underlying each, with different neural correlates. Sutherland and Rudy (1989) make the distinction between a "Simple Association System," (SAS) which is based on the elementary pairing of stimuli, such as light or position and food, and a "Configural Association System" (CAS). The CAS functions to join the representations of the simple

associations and to synthesize new representations. It is also hypothesized to store associations between the configural representation and elementary representations, creating the basis for the more complex task of orienting to landmarks rather than position. These researchers also propose that these systems function in different areas of the brain. An intact hippocampus is necessary for CAS to operate normally, but is not required for the SAS.

Literature on pathological conditions involving the hippocampus and resulting behavioral deficits support this division (e.g., Kolb & Whishaw, 1990). Lesions in the hippocampus have resulted in poor spatial performances but not deficits in association learning in rats (Jarrard, 1978; Roberts, Dember & Brodwick, 1962) and humans (Milner, 1965). More specifically, Silva, Stevens, Tonegawa and Wang (1992) engineered mice that lacked a particular enzyme necessary for the normal activity of hippocampal cells correlated with normal spatial behavior. When the mutant mice who had these enzyme-deficient hippocampal cells were tested behaviorally (Silva, Paylor, Wehner & Tonegawa, 1992), they were much slower in mastering the task of locating the hidden platform in a Morris water maze than the "wild type" mice. When tested with the association component of the task (i.e., instead of a hidden platform, a flag marked its location), the mutant mice equaled the wild types in trials to acquisition as well as latency for location.

Given the support for the notion that there are two different systems involved in the two types of learning, it would be of interest to determine the relative influence of the interaction of sex, rearing and hormonal milieu on various types of learning. In this way, it may be possible to assess whether differences in behaviors such as reactivity, motivation, fearfulness, and activity levels exhibited among the animals for each condition contributed only to the differences in spatial performance, or influence performance on association type tasks also. In this variation, the original study would be replicated, with substitution of dependent measures that would assess types of learning that are not strictly spatial in nature (e.g., operant learning tasks, win/shift strategies, visual discrimination tasks).

Previous investigators have attempted to quantify the relative contribution of factors (e.g., genes and environment) to the appearance of a particular characteristic (e.g., Plomin, 1986). As stated previously, this results in misguided notions of "'how much' and not how (Gottlieb, in press; p. 25)" each factor contributes to the development of a trait. That spatial performance in the montane vole is influenced by gender, hormonal condition and early exploratory opportunities, offers support for a systems view of development. Taken together, the results of the current work, and proposed future research, should

provide insight into the development of a ubiquitous behavior that has traditionally been explained broadly in terms of differences in chromosomal sex. These studies offer explanations that address the complexity of the production of a phenotype and the importance of examining multiple mediating influences rather than reliance on explanations based on single-factor, linear causality (Gottlieb, 1991).

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Appendix A
Table A1

Analysis of visits per trial by rearing, gender and hormonal treatment.

Rearing	Gender	Hormone	N	Mean	Std Dev	SE
Expanded	Female	GNX-T	5	17.350	10.376	1.339
Expanded	Male	GNX-T	5	22.000	16.983	2.192
Expanded	Female	GNX-C	5	20.650	10.987	1.418
Expanded	Male	GNX-C	5	20.667	13.026	1.681
Expanded	Female	CONT	5	21.900	8.875	1.145
Expanded	Male	CONT	5	16.633	8.810	1.137
Restricted	Female	GNX-T	6	17.569	10.712	1.262
Restricted	Male	GNX-T	5	15.183	6.135	.792
Restricted	Female	GNX-C	6	22.333	15.905	1.874
Restricted	Male	GNX-C	5	17.883	7.764	1.002
Restricted	Female	CONT	6	23.375	16.447	1.938
Restricted	Male	CONT	5	20.783	11.473	1.480

Table A2

Analysis of amount of time for task completion per trial by rearing, gender and hormonal treatment.

Rearing	Gender	Hormone	N	Mean	Std Dev	SE
Expanded	Female	GNX-T	5	3.975	3.070	.396
Expanded	Male	GNX-T	5	4.458	3.692	.477
Expanded	Female	GNX-C	5	6.467	3.671	.474
Expanded	Male	GNX-C	5	5.117	3.599	.464
Expanded	Female	CONT	5	7.583	2.720	.351
Expanded	Male	CONT	5	3.433	2.689	.347
Restricted	Female	GNX-T	6	3.701	3.103	.366
Restricted	Male	GNX-T	5	3.533	2.174	.281
Restricted	Female	GNX-C	6	4.465	3.201	.377
Restricted	Male	GNX-C	5	4.700	3.137	.405
Restricted	Female	CONT	6	4.069	3.181	.376
Restricted	Male	CONT	5	3.333	2.184	.282

Table A3

Analysis of number of baits taken per trial by rearing, gender and hormonal treatment.

Rearing	Gender	Hormone	N	Mean	Std Dev	SE
Expanded	Female	GNX-T	5	7.700	.889	.115
Expanded	Male	GNX-T	5	6.700	2.431	.314
Expanded	Female	GNX-C	5	6.117	2.598	.335
Expanded	Male	GNX-C	5	6.283	2.929	.378
Expanded	Female	CONT	5	6.017	3.006	.388
Expanded	Male	CONT	5	7.867	.566	.073
Restricted	Female	GNX-T	6	7.694	1.096	.129
Restricted	Male	GNX-T	5	7.783	1.090	.141
Restricted	Female	GNX-C	6	7.625	1.168	.138
Restricted	Male	GNX-C	5	7.267	1.921	.248
Restricted	Female	CONT	6	7.278	2.064	.243
Restricted	Male	CONT	5	7.967	.181	.023

Table A4

Analysis of number of revisits per trial by rearing, gender and hormonal treatment.

Rearing	Gender	Hormone	N	Mean	Std Dev	SE
Expanded	Female	GNX-T	5	5.467	5.531	.714
Expanded	Male	GNX-T	5	4.567	6.001	.774
Expanded	Female	GNX-C	5	5.467	5.706	.736
Expanded	Male	GNX-C	5	5.000	6.079	.784
Expanded	Female	CONT	5	7.517	6.663	.860
Expanded	Male	CONT	5	4.883	5.059	.653
Restricted	Female	GNX-T	6	6.278	6.874	.810
Restricted	Male	GNX-T	5	5.133	4.485	.579
Restricted	Female	GNX-C	6	8.569	9.272	1.093
Restricted	Male	GNX-C	5	5.200	3.874	.500
Restricted	Female	CONT	6	7.347	8.816	1.039
Restricted	Male	CONT	5	8.883	8.487	1.095

Table A5

Analysis of number of visits per minute per trial by rearing, gender and hormonal treatment.

Rearing	Gender	Hormone	N	Mean	Std Dev	SE
Expanded	Female	GNX-T	5	5.545	2.651	.342
Expanded	Male	GNX-T	5	6.130	2.832	.366
Expanded	Female	GNX-C	5	4.492	2.946	.380
Expanded	Male	GNX-C	5	5.293	2.516	.325
Expanded	Female	CONT	5	3.234	1.505	.194
Expanded	Male	CONT	5	6.320	3.030	.391
Restricted	Female	GNX-T	6	6.351	2.848	.336
Restricted	Male	GNX-T	5	5.074	2.058	.266
Restricted	Female	GNX-C	6	6.154	3.330	.392
Restricted	Male	GNX-C	5	5.026	2.306	.298
Restricted	Female	CONT	6	6.630	2.298	.271
Restricted	Male	CONT	5	6.862	2.293	.296

Table 1

General Linear Models Procedure with Repeated Measues ANOVA for the second degree polynomial contrast for number of visits.

Source	DF	Type III SS	Mean Square	F value	Pr > F
Mean	1	819.878	819.878	6.69	0.0126
Rearing	1	48.425	48.425	0.40	0.5324
Hormone	2	9.190	4.595	0.04	0.9632
Rearing X Hormone	2	326.879	163.440	1.33	0.2725
Gender	1	270.739	270.739	2.21	0.1433
Rearing X Gender	1	798.112	798.112	6.51	0.0137
Hormone X Gender	2	81.595	40.797	0.33	0.7184
Rearing X Hormone Gender	2	371.525	185.763	1.52	0.2294
Error	51	6249.913	122.547		

Table 2
Analysis of visits per trial by rearing and gender.

Rearing	Gender	N	Mean	Std Dev	SE
Expanded	Female	180	19.967	10.244	.763
Expanded	Male	180	19.767	13.484	1.000
Restricted	Female	216	21.093	14.737	1.003
Restricted	Male	180	17.950	8.995	.670

Table 3

Tukey's Studentized Range (HSD) Test for comparison of means of visits for the interaction of rearing and gender.

Alpha = .01; MSE = 145.946
Critical Value of Studentized Range = 4.418
Minimum Significant Difference = 3.8943

Means with the same letter are not significantly different.

General Linear Models Procedure

Tukey Grouping	Mean	N	Interact
A	21.093	216	Restricted Females
A			
A	19.967	180	Expanded Females
A			
A	19.767	180	Expanded Males
A			
A	17.950	180	Restricted Males

Table 4

General Linear Models Procedure with Repeated Measues ANOVA for the second degree polynomial contrast for time spent in the maze per trial.

Source	DF	Type III SS	Mean Square	F value	Pr > F
Mean	1	7.360	7.360	0.69	0.4114
Rearing	1	2.367	2.367	0.22	0.6406
Hormone	2	8.860	4.430	0.41	0.6640
Rearing X Hormone	2	13.973	6.987	0.65	0.5257
Gender	1	5.280	5.280	0.49	0.4862
Rearing X Gender	1	75.550	75.550	7.04	0.0106
Hormone X Gender	2	0.785	0.393	0.04	0.9641
Rearing X Hormone Gender	2	24.315	12.158	1.13	0.3300
Error	51	547.220	10.730		

Table 5

Analysis of time per trial by rearing and gender.

Rearing	Gender	N	Mean	Std Dev	SE
Expanded	Female	180	6.008	3.504	.261
Expanded	Male	180	4.336	3.410	.297
Restricted	Female	216	4.079	3.163	.215
Restricted	Male	180	3.856	2.596	.226

Table 6

Tukey's Studentized Range (HSD) Test for comparison of means of the times for the interaction of rearing and gender.

Alpha = .01; MSE = 9.48152
Critical Value of Studentized Range = 4.418
Minimum Significant Difference = 0.9926

Means with the same letter are not significantly different.

General Linear Models Procedure

Tukey Grouping	Mean	N	Interact
A	6.008	180	Expanded Females
B	4.336	180	Expanded Males
B B	4.079	216	Restricted Females
B B	3.856	180	Restricted Males

Table 7

General Linear Models Procedure with Repeated Measues ANOVA for the second degree polynomial contrast for number of baits taken per trial.

Source	DF	Type III SS	Mean Square	F value	Pr > F
Mean	1	12.911	12.911	3.37	0.0722
Rearing	1	1.791	1.791	0.47	0.4972
Hormone	2	0.425	0.213	0.06	0.9460
Rearing X Hormone	2	24.377	12.188	3.18	0.0498
Gender	1	6.422	6.422	1.68	0.2012
Rearing X Gender	1	5.631	5.631	1.47	0.2309
Hormone X Gender	2	5.667	2.833	0.74	0.4972
Rearing X Hormone Gender	2	12.927	6.463	1.69	0.1951
Error	51	195.325	3.830		

Table 8

Analysis of number of apples retrieved per trial by rearing and gender.

Rearing	Gender	N	Mean	Std Dev	SE
Expanded	Female	180	6.611	2.461	.183
Expanded	Male	180	6.950	2.309	.172
Restricted	Female	216	7.532	1.512	.103
Restricted	Male	180	7.672	1.307	.097

Table 9

General Linear Models Procedure with Repeated Measues ANOVA for the second degree polynomial contrast for number of revisits per trial.

Source	DF	Type III SS	Mean Square	F value	Pr > F
Mean	1	35.437	35.437	0.79	0.3791
Rearing	1	17.688	17.688	0.39	0.5335
Hormone	2	57.118	28.559	0.63	0.5343
Rearing X Hormone	2	198.379	99.190	2.20	0.1208
Gender	1	63.284	63.284	1.41	0.2412
Rearing X Gender	1	186.206	186.206	4.14	0.0472
Hormone X Gender	2	113.068	56.534	1.26	0.2935
Rearing X Hormone X Gender	2	4.166	2.083	0.05	0.9548
Error	51	2295.593	45.012		

Table 10

Analysis of number revisits per trial by rearing and gender.

Rearing	Gender	N	Mean	Std Dev	SE
Expanded	Female	180	6.150	6.032	.450
Expanded	Male	180	4.817	5.703	.425
Restricted	Female	216	7.398	8.399	.572
Restricted	Male	180	6.406	6.198	.462

Table 11

Tukey's Studentized Range (HSD) Test for comparison of means of the number of revisits per trial for the interaction of rearing and gender.

Alpha = .01; MSE = 44.97164
Critical Value of Studentized Range = 4.418
Minimum Significant Difference = 2.1617

Means with the same letter are not significantly different.

General Linear Models Procedure

Tukey Grouping	Mean	N	Interact
A	7.398	216	Restricted Females
A			
B A	6.4056	180	Restricted Males
B			
B	6.150	216	Expanded Females
B			
B	4.817	180	Expanded Males

Table 12

General Linear Models Procedure with Repeated Measues ANOVA for the third degree polynomial contrast for the ratio score of visits per minute per trial.

Source	DF	Type III SS	Mean Square	F value	Pr > F
Mean	1	0.000	0.000	0.00	1.0000
Rearing	1	4.850	4.850	1.71	0.1966
Hormone	2	5.270	2.635	0.93	0.4012
Rearing X Hormone	2	7.627	3.814	1.35	0.2694
Gender	1	12.218	12.218	4.31	0.0429
Rearing X Gender	1	21.005	21.005	7.41	0.0088
Hormone X Gender	2	1.816	0.908	0.32	0.7273
Rearing X Hormone X Gender	2	3.473	1.737	0.61	0.546
Error	51	144.508	2.833		

Table 13

Analysis of visits per minute per trial by rearing and gender.

Rearing	Gender	N	Mean	Std Dev	SE
Expanded	Female	180	4.424	2.612	.195
Expanded	Male	180	5.914	2.821	.210
Restricted	Female	216	6.378	2.850	.194
Restricted	Male	180	5.654	2.370	.177

Table 14

Tukey's Studentized Range (HSD) Test for comparison of means of the number of visits per minute per trial for the interaction of rearing and gender.

Alpha = .01; MSE = 6.80141
Critical Value of Studentized Range = 4.418
Minimum Significant Difference = 0.8407

Means with the same letter are not significantly different.

General Linear Models Procedure

Tukey Grouping	Mean	N	Interact
A	6.378	216	Restricted Females
A			
A	5.914	180	Expanded Males
A			
A	5.654	216	Restricted Males
B	4.424	180	Expanded Females

Table 15

Analysis of number of visits by rearing and hormonal treatment.

Rearing	Hormone	N	Mean	Std Dev	SE
Expanded	GNX-T	120	19.675	14.206	1.296
Expanded	GNX-C	120	20.658	11.999	1.095
Expanded	CONT	120	19.267	9.194	.839
Restricted	GNX-T	132	16.485	8.976	.781
Restricted	GNX-C	132	20.311	13.007	1.132
Restricted	CONT	132	22.197	14.407	1.254

Table 16

General Linear Models Procedure with Repeated Measues ANOVA for the first degree polynomial contrast for the ratio score of time per trial.

Source	DF	Type III SS	Mean Square	F value	Pr > F
Mean	1	277.637	277.637	25.77	0.0001
Rearing	1	1.873	1.873	0.17	0.6785
Hormone	2	40.495	20.248	1.88	0.1631
Rearing X Hormone	2	75.020	37.510	3.48	0.0382
Gender	1	14.025	14.025	1.30	0.2592
Rearing X Gender	1	27.373	27.373	2.54	0.1171
Hormone X Gender	2	13.510	6.755	0.63	0.5382
Rearing X Hormone X Gender	2	39.562	19.781	1.84	0.1698
Error	51	549.438	10.773		

Table 17

Analysis of times per trial by rearing and hormonal treatment.

Rearing	Hormone	N	Mean	Std Dev	SE
Expanded	GNX-T	120	4.217	3.390	.310
Expanded	GNX-C	120	5.792	3.683	.336
Expanded	CONT	120	5.508	3.405	.312
Restricted	GNX-T	132	3.625	2.712	.236
Restricted	GNX-C	132	4.572	3.162	.275
Restricted	CONT	132	3.734	2.787	.243

Table 18

Tukey's Studentized Range (HSD) Test for comparison of means of the time spent in the maze per minute per trial for the interaction of rearing and hormonal treatment.

Alpha = .01; MSE = 9.482
Critical Value of Studentized Range = 4.775
Minimum Significant Difference = 1.311

Means with the same letter are not significantly different.

General Linear Models Procedure

Tukey Grouping			Mean	N	Interact
	A		5.792	120	Expanded GNX-C
	A				
B	A		5.508	120	Expanded CONT
B	A				
B	A	C	4.572	132	Restricted GNX-C
B		C			
B		C	4.217	120	Expanded GNX-T
		C			
		C	3.735	132	Restricted CONT
		C			
		C	3.625	132	Restricted GNX-T

Table 19

Analysis of number of apples retrieved per trial by rearing and hormonal treatment.

Rearing	Hormone	N	Mean	Std Dev	SE
Expanded	GNX-T	120	7.200	1.890	.173
Expanded	GNX-C	120	6.200	2.758	.566
Expanded	CONT	120	6.942	2.345	.234
Restricted	GNX-T	132	7.735	1.090	.095
Restricted	GNX-C	132	7.462	1.560	.136
Restricted	CONT	132	7.591	1.562	.136

Table 20

Tukey's Studentized Range (HSD) Test for comparison of means of the numbers of apples retrieved per trial for the interaction of rearing and hormonal treatment.

Alpha = .01; MSE = 3.5295
Critical Value of Studentized Range = 4.775
Minimum Significant Difference = 0.8001

Means with the same letter are not significantly different.

General Linear Models Procedure

Tukey Grouping	Mean	N	Interact
A	7.735	132	Restricted GNX-T
A			
A	7.591	132	Restricted CONT
A			
A	7.462	132	Restricted GNX-C
A			
A	7.200	120	Expanded GNX-T
A			
B A	6.942	120	Expanded CONT
B			
B	6.200	120	Expanded GNX-C

Table 21

Analysis of number of revisits per trial by rearing and hormonal treatment.

Rearing	Hormone	N	Mean	Std Dev	SE
Expanded	GNX-T	120	5.017	5.764	.526
Expanded	GNX-C	120	5.233	5.875	.537
Expanded	CONT	120	6.200	6.037	.551
Restricted	GNX-T	132	5.758	5.916	.515
Restricted	GNX-C	132	7.038	7.496	.652
Restricted	CONT	132	8.045	8.669	.755

Table 22

General Linear Models Procedure with Repeated Measues ANOVA for the first degree polynomial contrast for the ratio score of visits per minute per trial.

Source	DF	Type III SS	Mean Square	F value	Pr > F
Mean	1	74.771	74.771	14.80	0.0003
Rearing	1	0.001	0.001	0.00	0.9869
Hormone	2	9.732	4.867	0.96	0.3885
Rearing X Hormone	2	34.426	17.213	3.41	0.0409
Gender	1	18.628	18.628	3.69	0.0604
Rearing X Gender	1	3.630	3.630	0.72	0.4006
Hormone X Gender	2	2.754	1.377	0.27	0.7625
Rearing X Hormone X Gender	2	14.164	7.082	1.40	0.2555
Error	51	257.680	5.053		

Table 23

Analysis of visits per minute per trial by rearing and hormonal treatment.

Rearing	Hormone	N	Mean	Std Dev	SE
Expanded	GNX-T	120	5.838	2.747	.251
Expanded	GNX-C	120	4.893	2.757	.252
Expanded	CONT	120	4.777	2.842	.258
Restricted	GNX-T	132	5.770	2.590	.226
Restricted	GNX-C	132	5.641	2.953	.257
Restricted	CONT	132	6.735	2.290	.020

Table 24

Tukey's Studentized Range (HSD) Test for comparison of means of the numbers of visits per minute per trial for the interaction of rearing and hormonal treatment.

Alpha = .01; MSE = 6.80141
Critical Value of Studentized Range = 4.775
Minimum Significant Difference = 1.1107

Means with the same letter are not significantly different.

General Linear Models Procedure

Tukey Grouping		Mean	N	Interact
	A	6.736	132	Restricted CONT
	A			
B	A	5.837	120	Expanded GNX-T
B	A			
B	A	5.770	132	Restricted GNX-T
B	A			
B	A	5.641	132	Restricted GNX-C
B				
B		4.893	120	Expanded GNX-C
B				
B		4.777	120	Expanded CONT

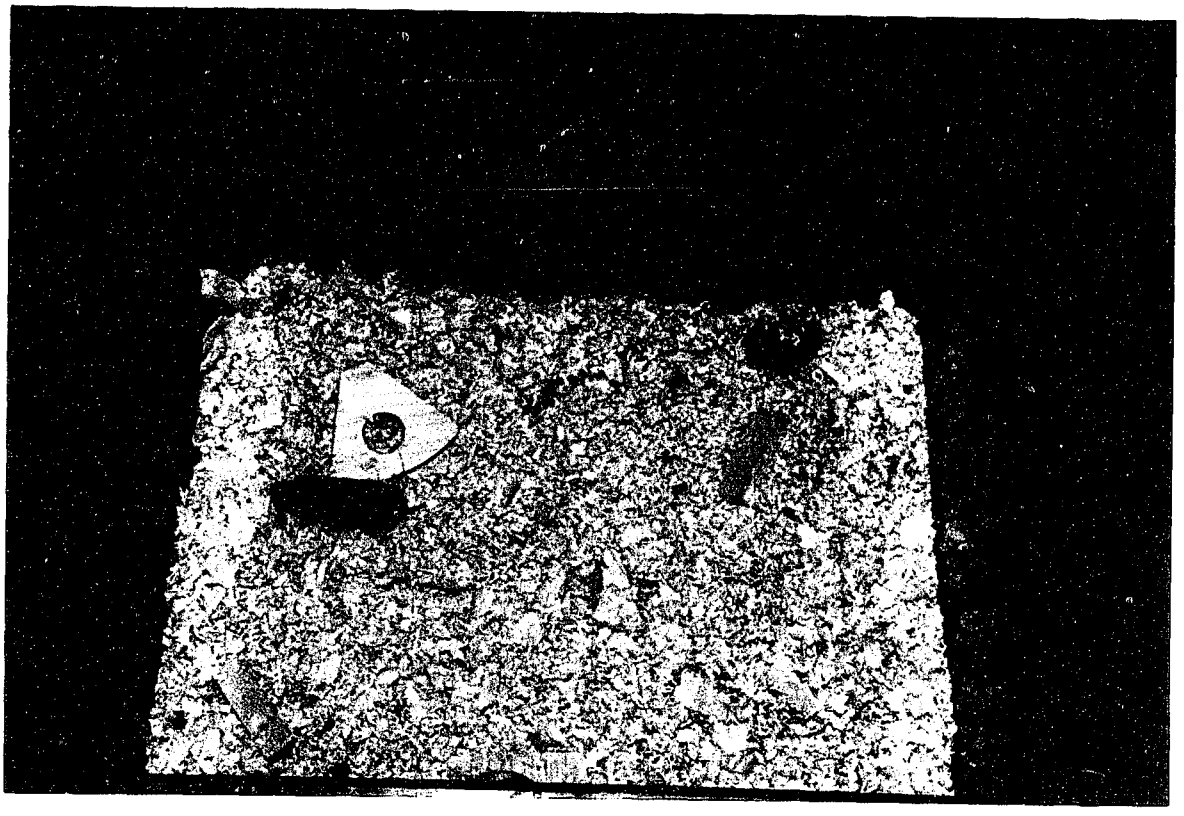

Figure 1. The Expanded condition provided exploratory opportunities, with a relatively large area in which to wander and wooden objects for interaction.

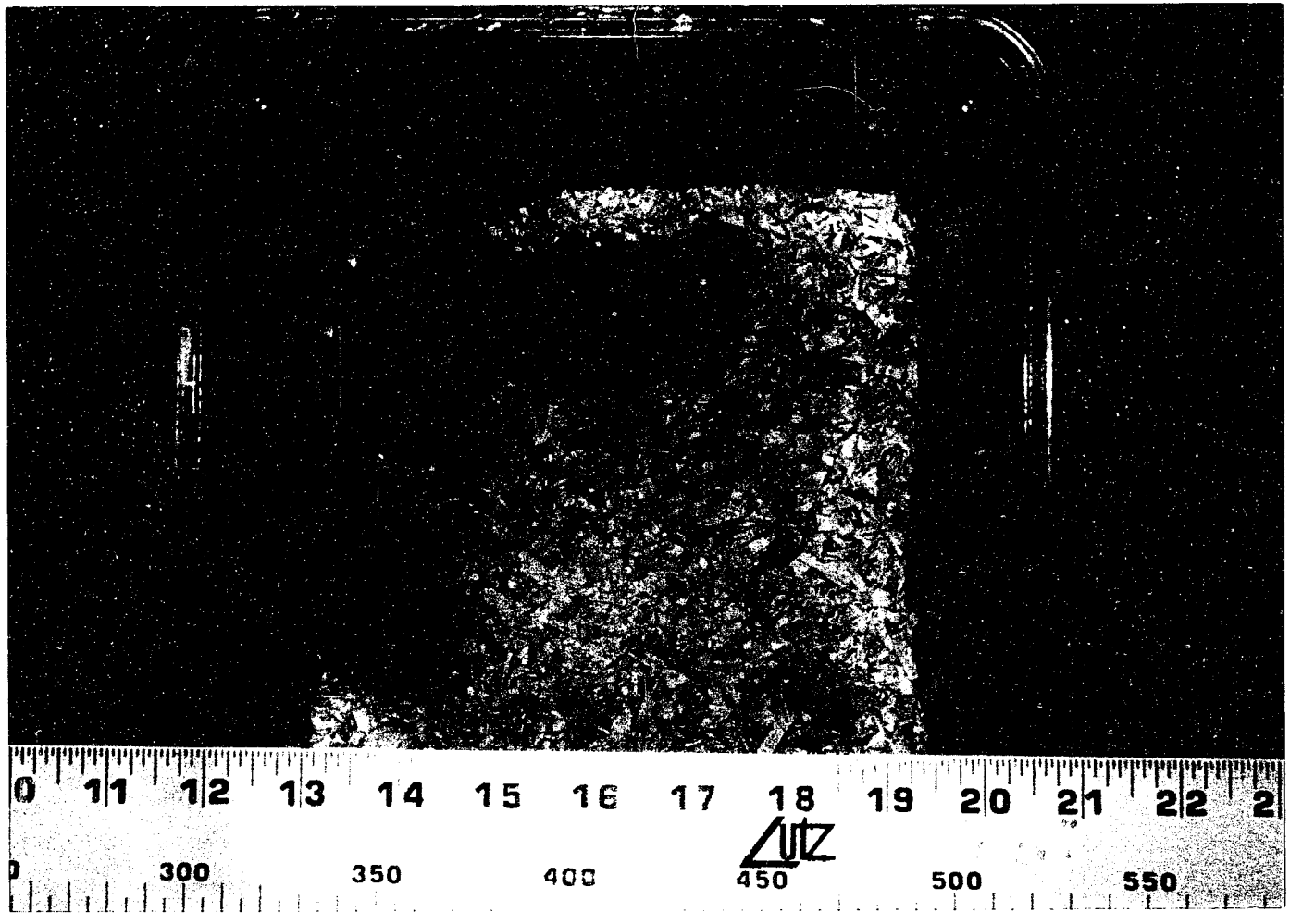

Figure 2. The Restricted condition represented a more impoverished environment, with limited space for movement and no objects for interaction.

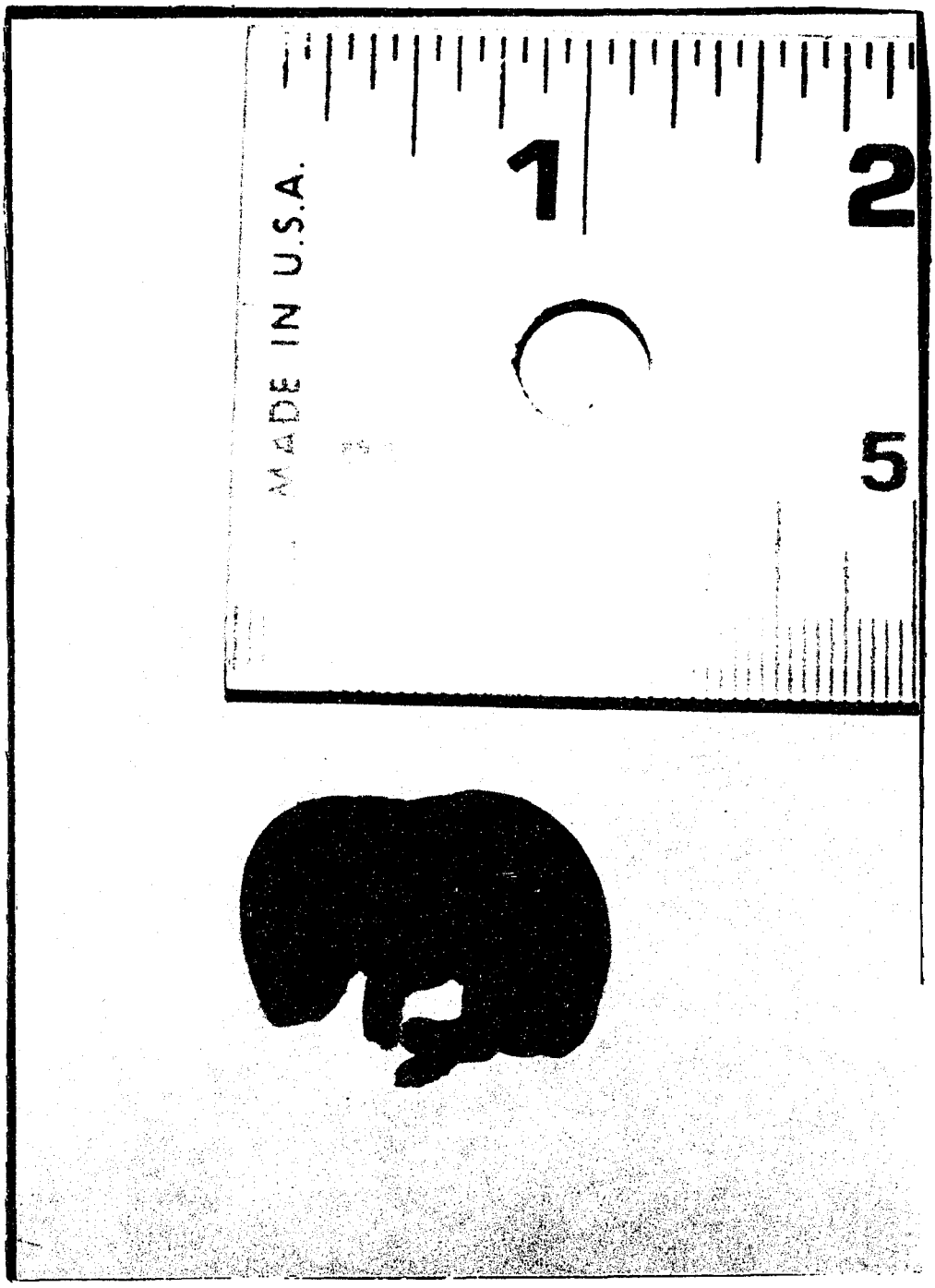

Figure 3. The pups were gondadectomized on the day of birth.

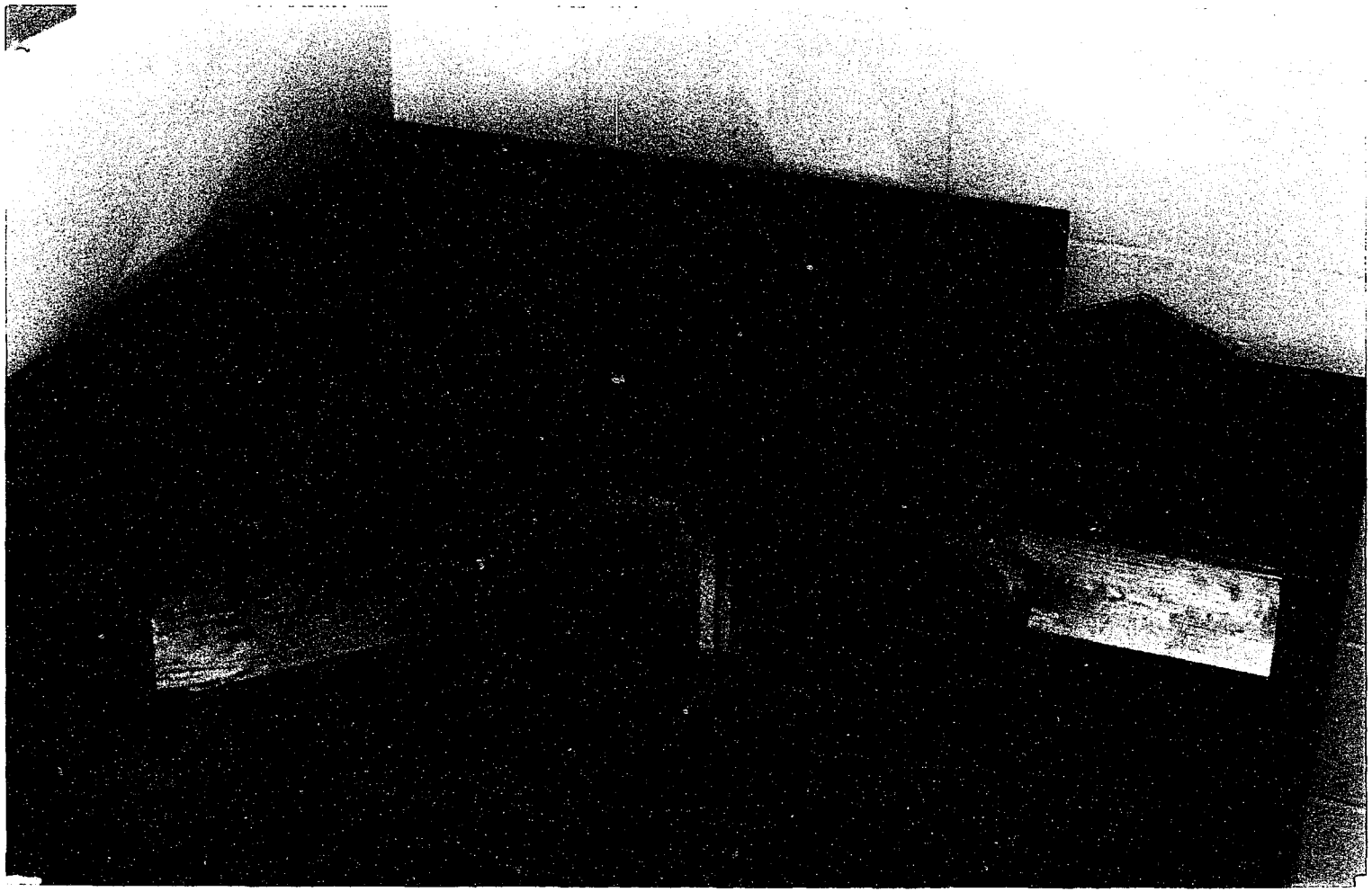

Figure 4. The eight-arm radial maze has been used in a number of studies to test for sex-differences in navigational ability. It is essential that the maze allow the animal to look up and out, because distal cues are used for navigation.

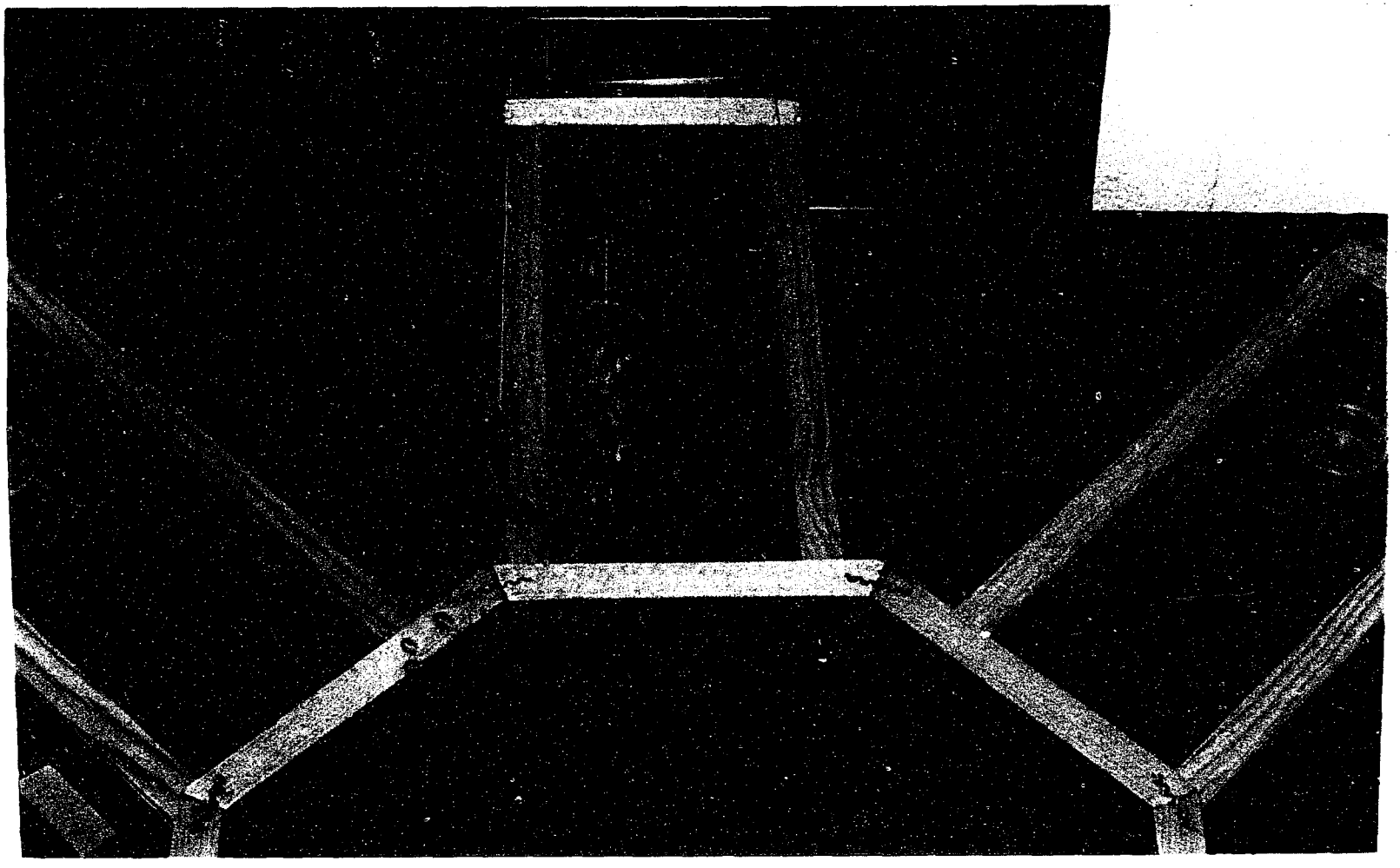

Figure 5. A montane vole navigates an arm of the maze, looking for an apple bait that is placed in the cup. Because the cups are opaque, this prevents the subject from detecting the bait from the doorway.

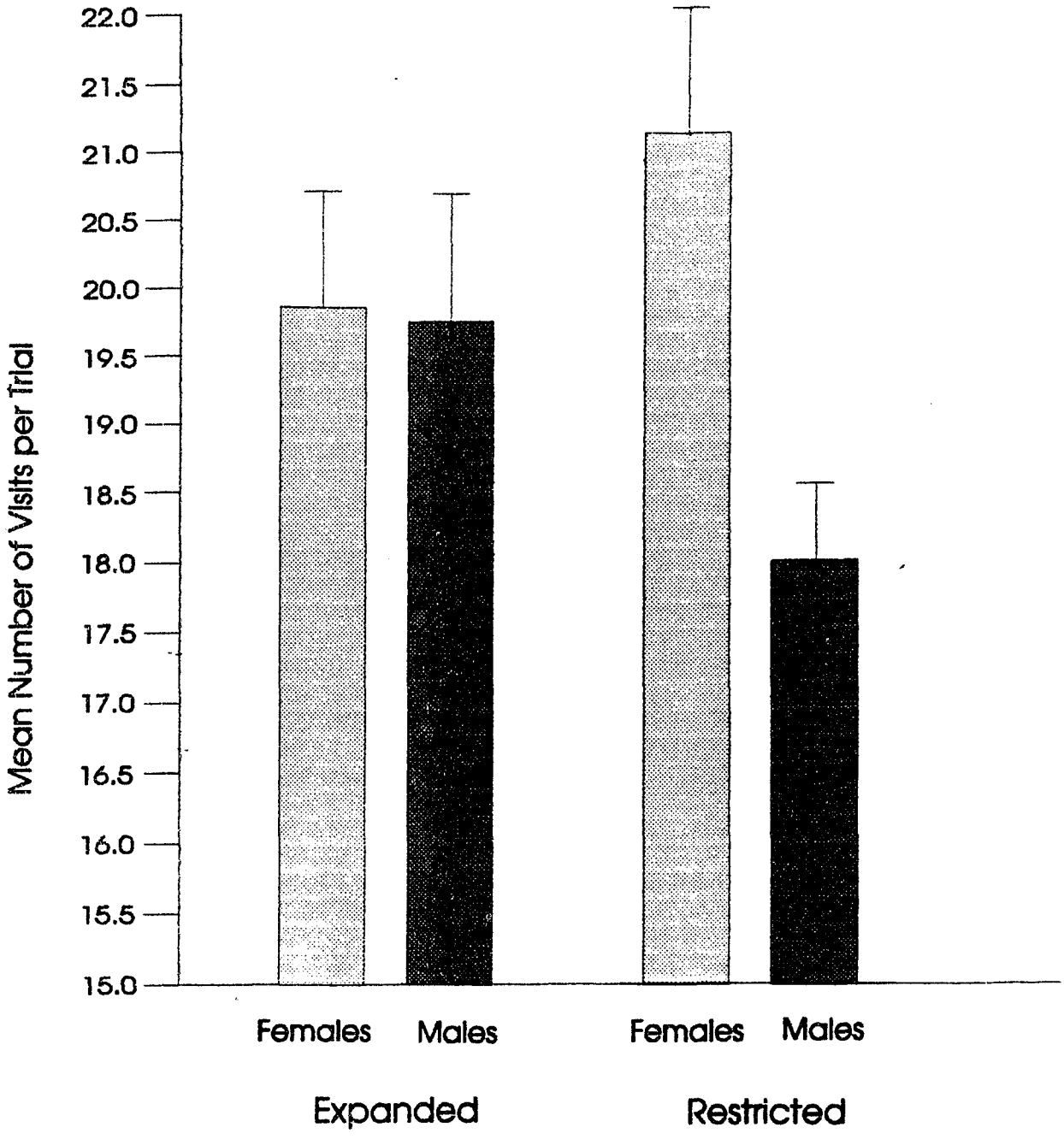

Figure 6. Average number of visits per trial for interaction of rearing by gender. Error bars equal Standard Error of the Mean.

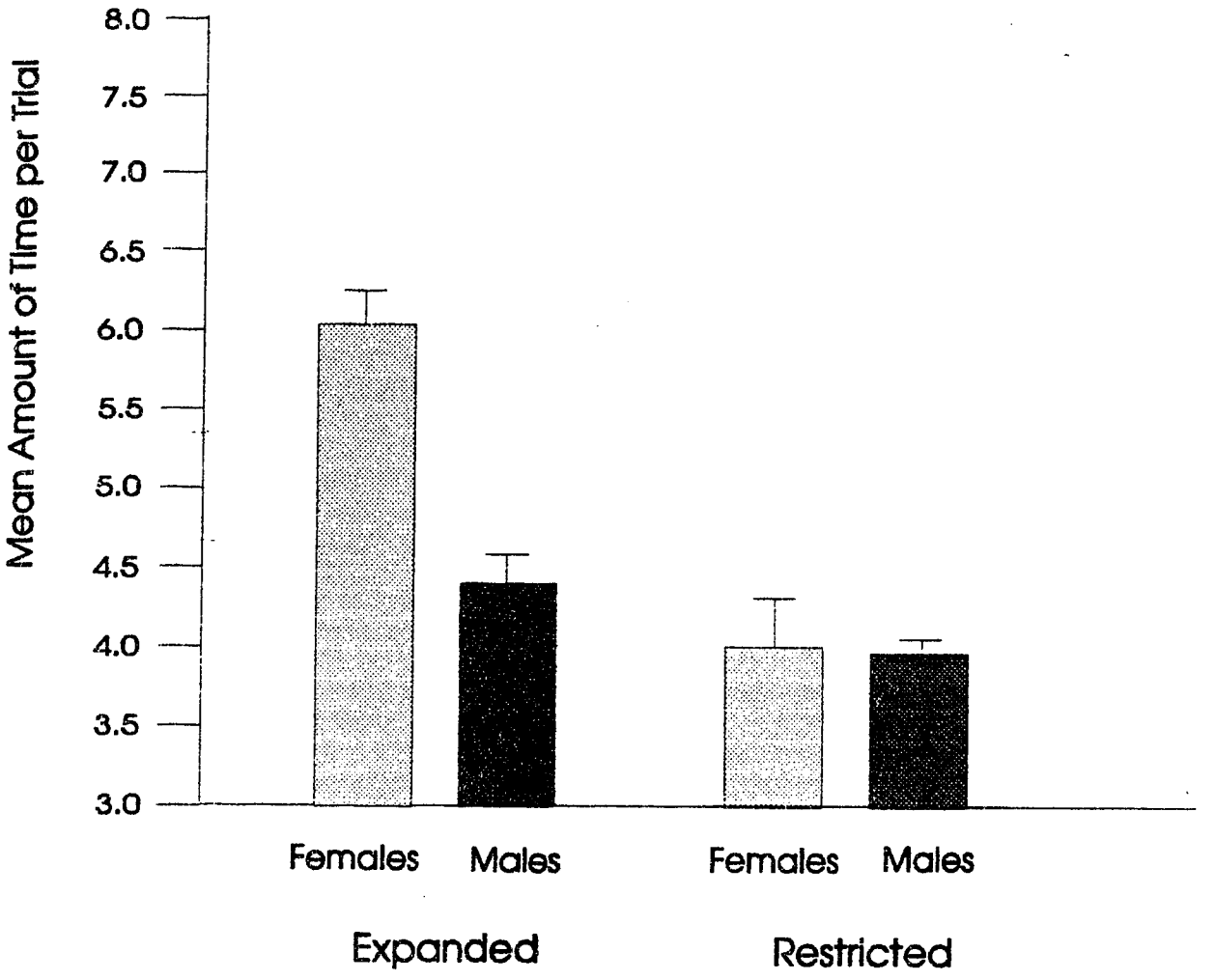

Figure 7. Average amount of time spent in the maze per trial for interaction of rearing by gender. Error bars equal Standard Error of the Mean.

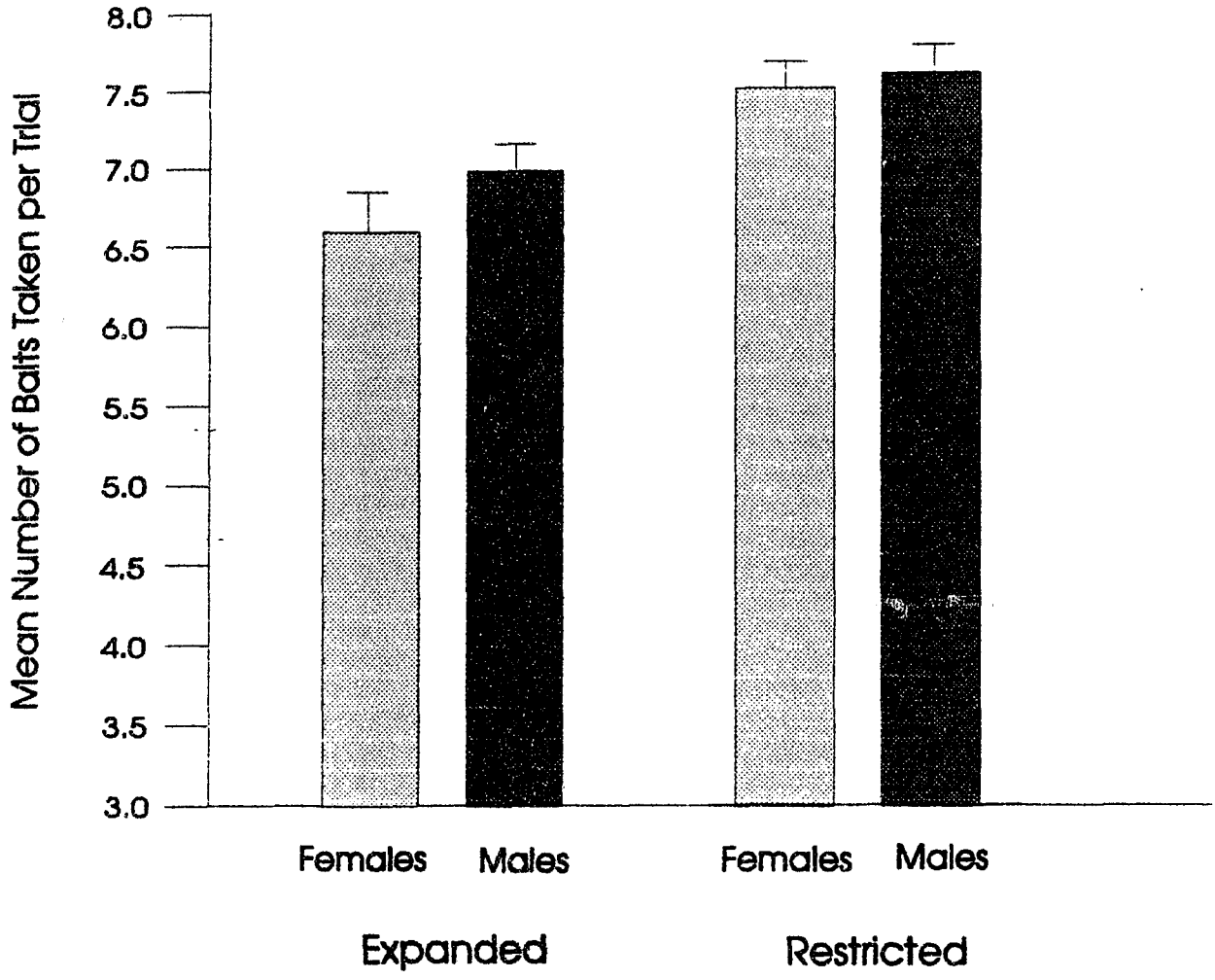

Figure 8. Average number of baits taken per trial for interaction of rearing by gender. Error bars equal Standard Error of the Mean.

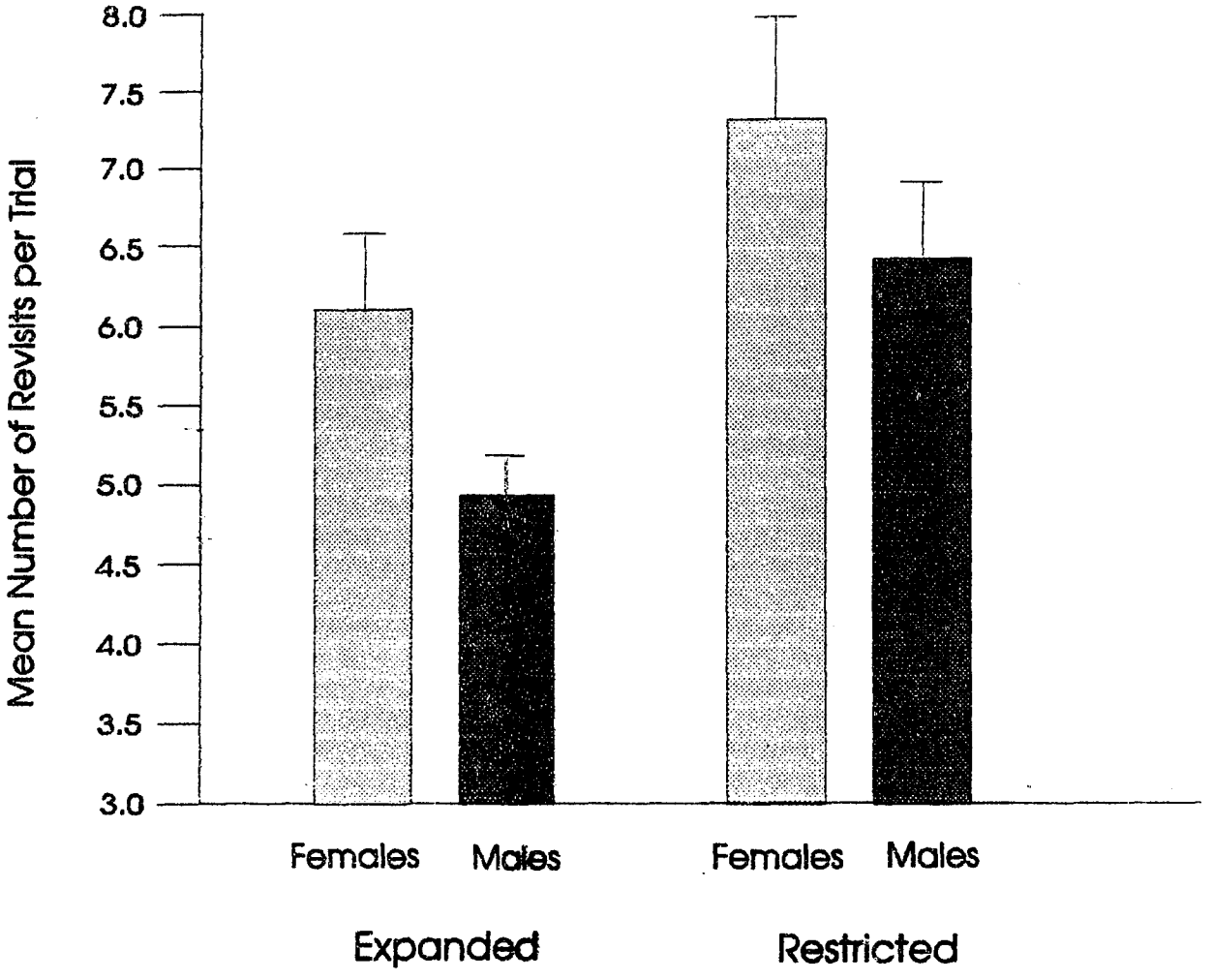

Figure 9. Average number of revisits per trial for interaction of rearing by gender. Error bars equal Standard Error of the Mean.

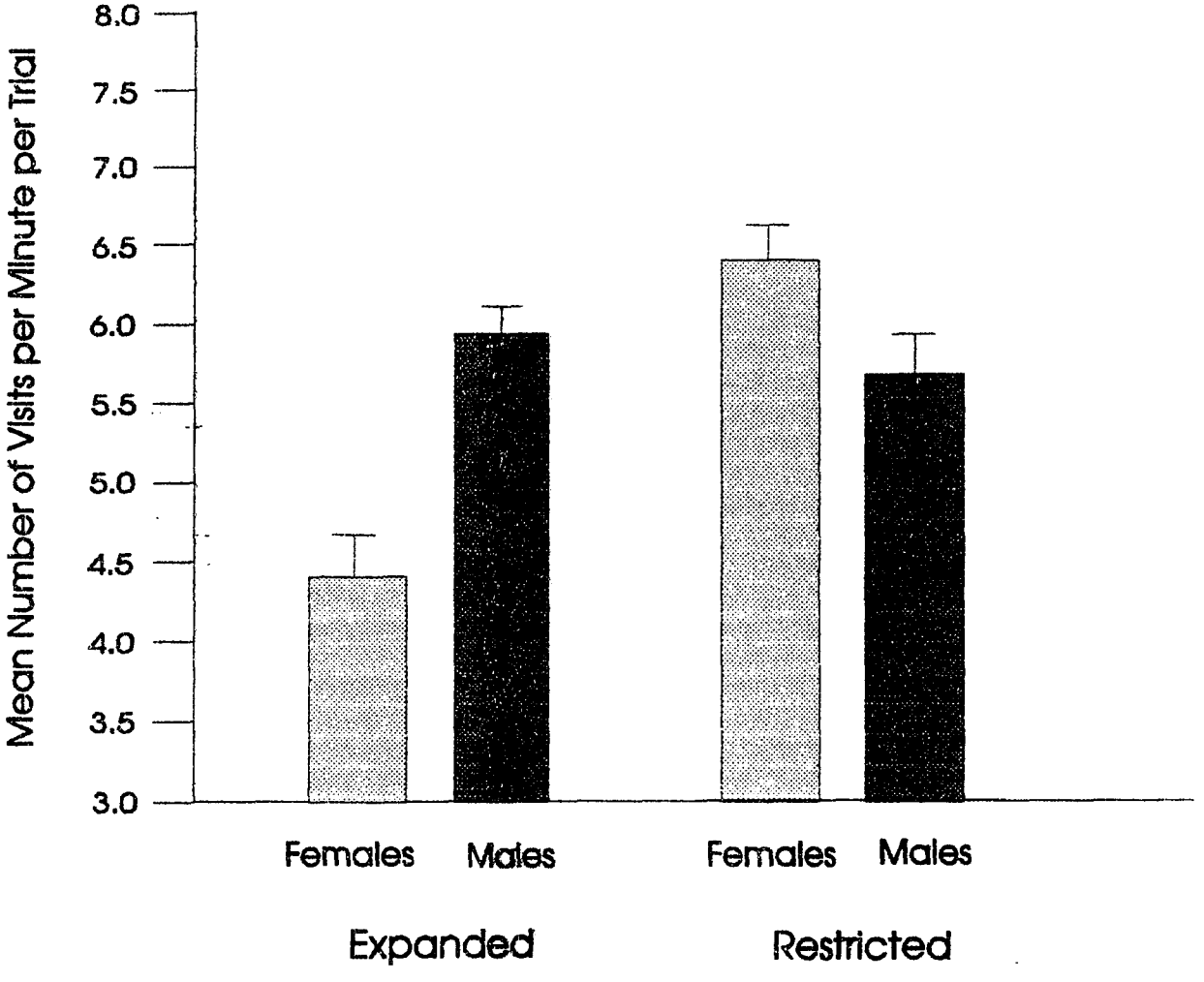

Figure 10. Average number of visits per minute per trial for interaction of rearing by gender. Error bars equal Standard Error of the Mean.

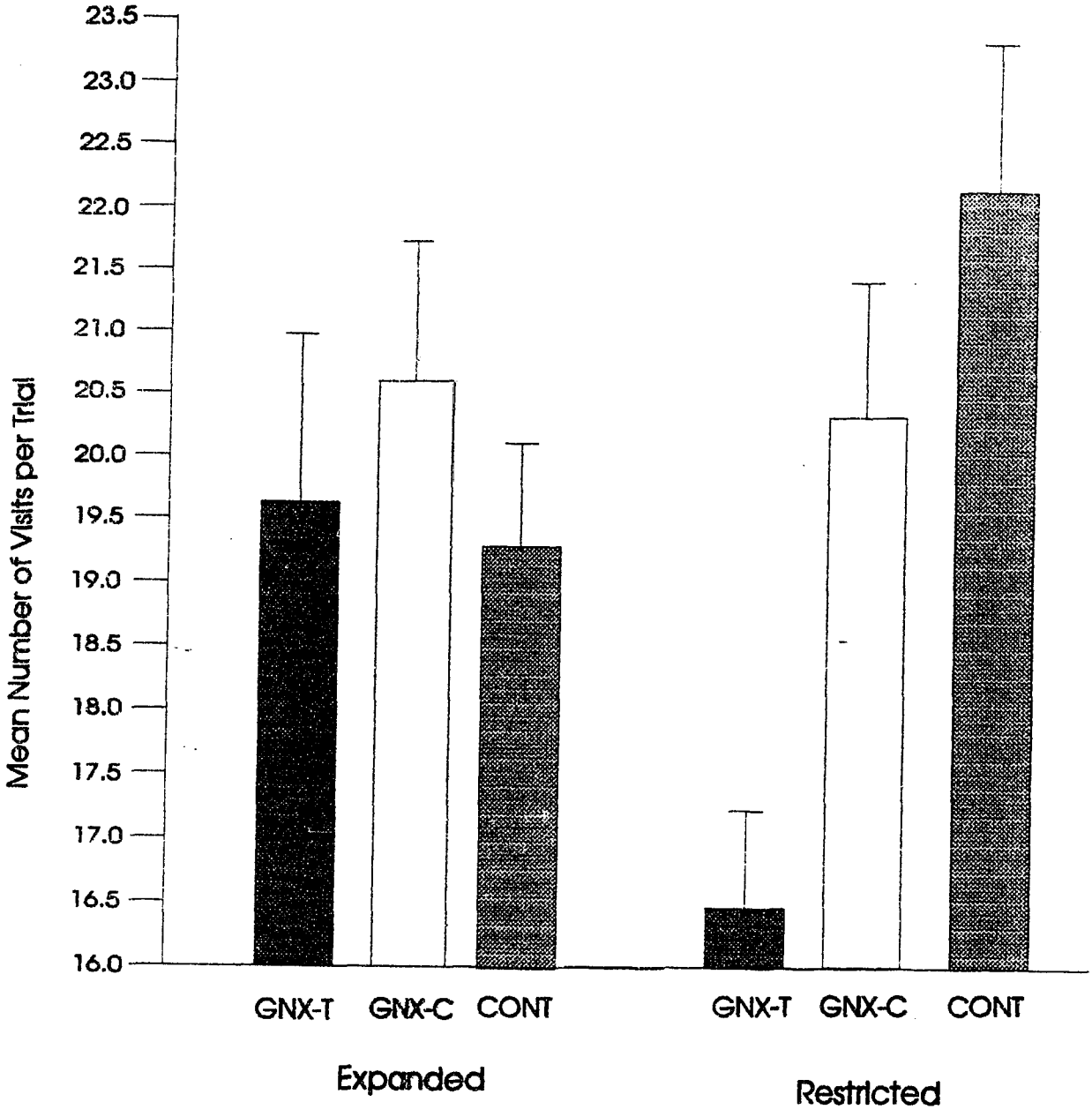

Figure 11. Average number of visits per trial for interaction of rearing by hormone. Error bars equal Standard Error of the Mean.

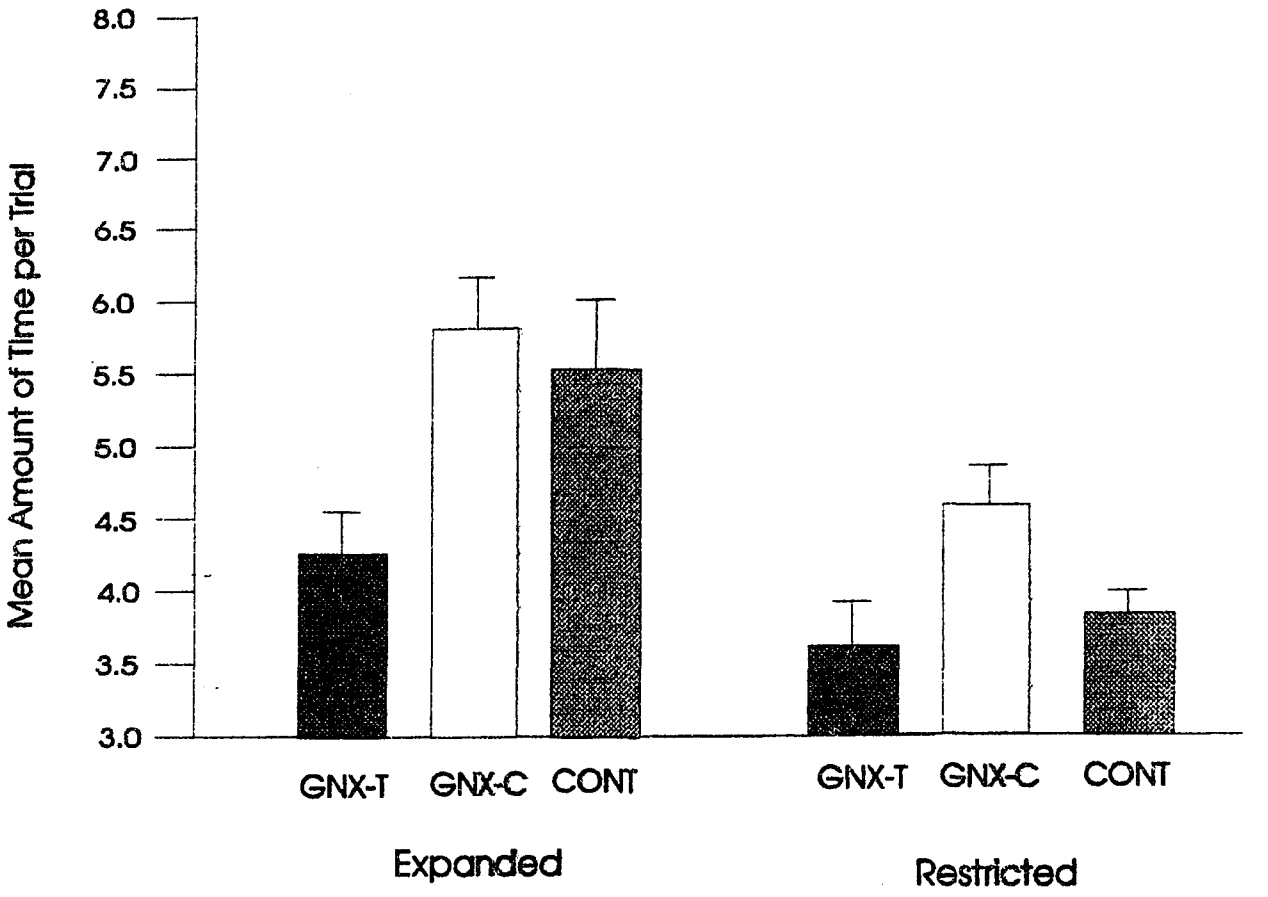

Figure 12. Average amount of time per trial for interaction of rearing by hormone. Error bars equal Standard Error of the Mean.

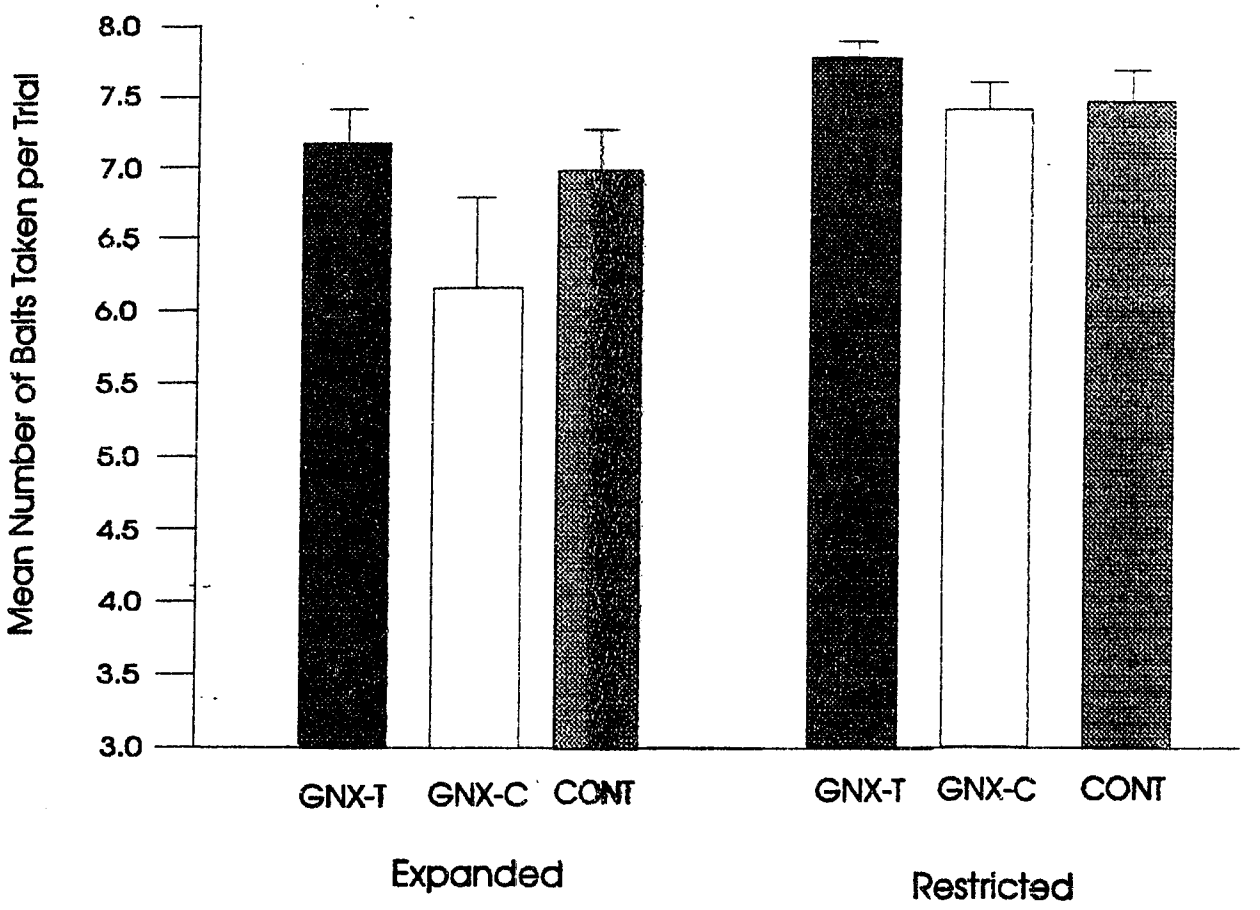

Figure 13. Average number of baits taken per trial for interaction of rearing by hormone. Error bars equal Standard Error of the Mean.

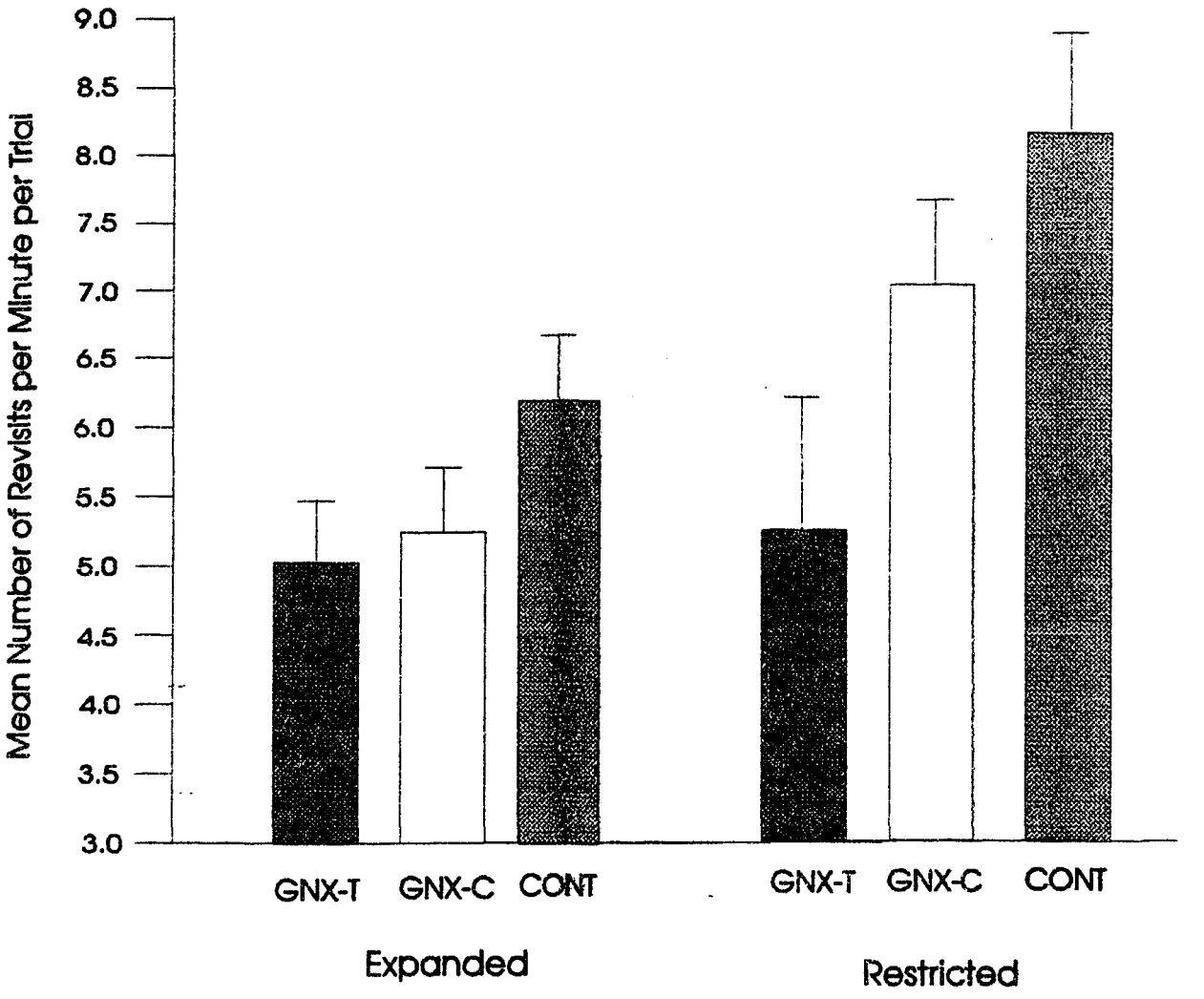

Figure 14. Average number of revisits per trial for interaction of rearing by hormone. Error bars equal Standard Error of the Mean.

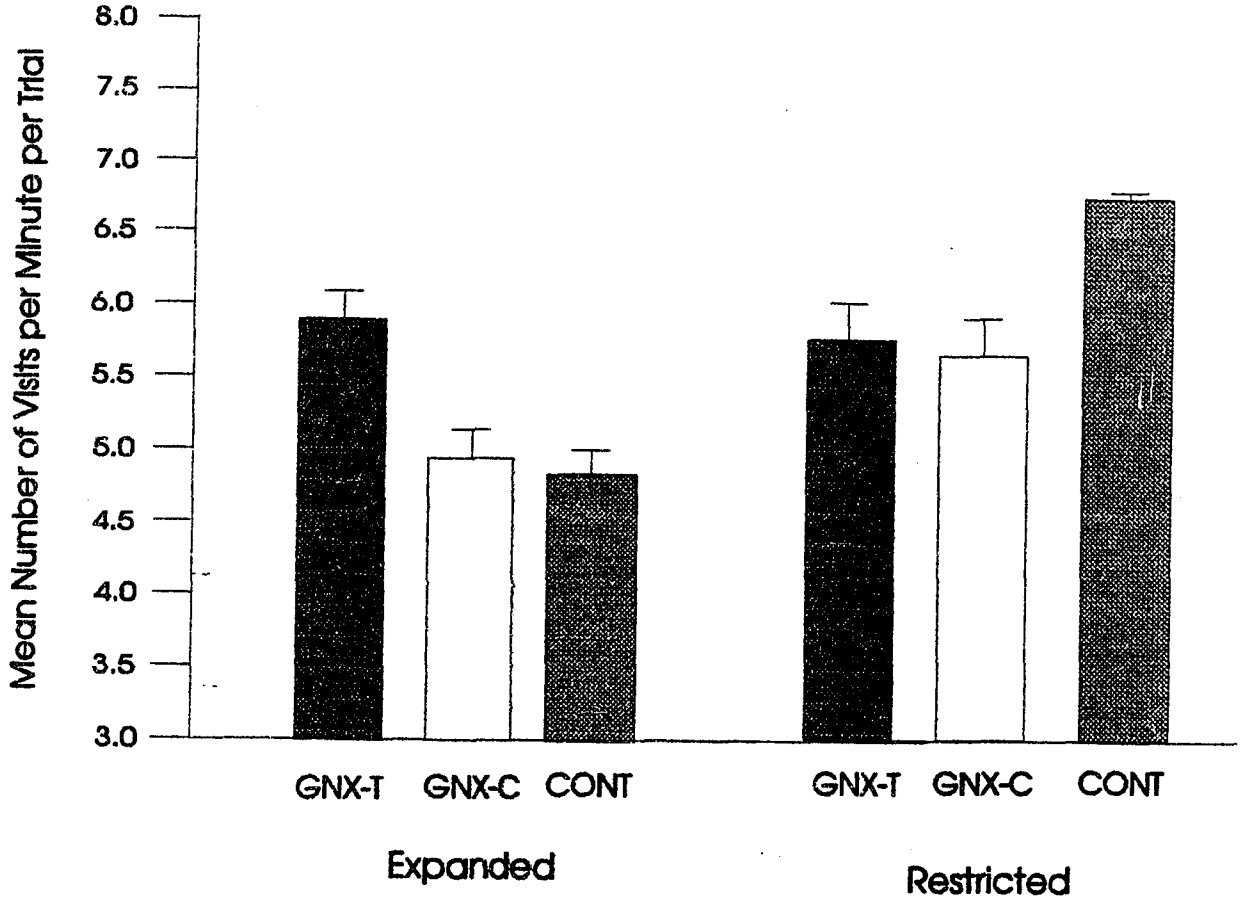

Figure 15. Average number of visits per minute per trial for interaction of rearing by hormone. Error bars equal Standard Error of the Mean.