SEXUALLY DIMORPHIC SPATIAL LEARNING IN MEADOW VOLES *MICROTUS PENNSYLVANICUS* AND DEER MICE *PEROMYSCUS MANICULATUS*

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Summary

A number of studies examining developmental, neural and hormonal aspects of sexually dimorphic spatial learning (Morris water-maze) in meadow voles (*Microtus pennsylvanicus*) and deer mice (*Peromyscus maniculatus*) are described. We found that, in adult deer mice, female spatial performance decreased during the breeding season relative to the non-breeding season, whereas the reverse pattern was observed in male performance. There was a sex difference favouring males in spatial learning during the breeding season, but not during the non-breeding season. In adult meadow voles, females with low levels of oestradiol and males performed better in the water-maze than females with high levels of oestradiol. Postweaning voles (20 and 25 days after birth) acquired the water-maze task more quickly than preweaning voles (day 10). No sex difference in water-maze performance was evident at any of these juvenile ages. When these same voles were tested again as adults to investigate retention and re-acquisition of the water-maze, both males and females from male-biased litters re-acquired the task better than males and females from female-biased litters. Together, the results of these studies indicate that sexually dimorphic spatial ability is dependent on the organizational (*in utero*) and activational effects of gonadal hormones. These studies provide the first demonstration of the influence of *natural* changes in reproductive status on spatial learning of deer mice and meadow voles. The results also demonstrate that spatial performance of males and females is differentially affected by changes in reproductive status and that group differences in the laboratory are associated with group differences in space utilization in the wild. These findings help to clarify previous apparently contradictory findings about sex differences in spatial ability.

Key words: sex differences, spatial learning, meadow voles, deer mice, gonadal hormone levels, space use, home range size, *Microtus pennsylvanicus*, *Peromyscus maniculatus*.

Introduction

Sex differences in spatial ability/learning have been well documented in past research in both humans (Linn and Peterson, 1986; McGee, 1979) and rodents (Beatty, 1992; Williams and Meck, 1991). In humans, many paper-and-pencil tests of spatial ability have found a male superiority in performance (Harshman *et al.* 1983). There are also sex differences favouring males in more 'real-world' tasks such as route-learning through a map (Galea and Kimura, 1993). In laboratory rats, males out-perform females on tasks such as the radial-arm maze (when a subset of arms is baited) and the Morris water-maze (Roof and Havens, 1990; Williams and Meck, 1991). In the Morris water-maze, an animal must learn to navigate to a hidden submerged platform in a circular maze filled with opaque water (Morris, 1984). This task has been successfully used to examine the influence of various neural and hormonal mechanisms on spatial abilities (McNamara and Skelton, 1993).

Gaulin and FitzGerald (1986) proposed that sex differences in spatial ability would evolve through sexual selection if the amount of spatial information to be processed was greater in one sex than the other. They tested this hypothesis using two species of voles that exhibited different uses of space: one polygynous (the meadow vole *Microtus pennsylvanicus*) and the other monogamous (the pine vole *M. pinetorum*). In the polygynous meadow vole, males range widely during the breeding season, presumably to gain access to mates, while females hold territories. In the monogamous pine vole, in contrast, pair-bonds are formed during the breeding season and these pairs cohabit a single territory. Gaulin and FitzGerald (1986) found that males of the polygynous meadow vole had larger home ranges than females, whereas there were no sex differences in home-range size of the monogamous pine vole. They proposed that sexually dimorphic home-range size could be thought of as a reproductive tactic in the meadow vole because the dimorphism disappears outside the breeding season and does not appear until the animal attains puberty. They hypothesized that sexually dimorphic spatial learning may have evolved in the polygynous species in response to the

increased spatial requirements of the widely ranging polygynous males, while there was little selection pressure for the evolution of sexually dimorphic spatial learning in the monogamous species. The results of tests conducted during the breeding season indicated that food-reinforced performance on a spatial maze was sexually dimorphic, favouring males in the polygynous meadow voles with no significant sex differences evident in the monogamous pine vole (Gaulin and FitzGerald, 1986). Their findings suggested that sexually dimorphic spatial ability is related to differential reproductive strategies.

Sex differences in reproductive behaviours are mediated by exposure to gonadal hormones, which may also mediate sex differences in non-reproductive behaviours. Both organizational (pre- or perinatal effects) and activational (adult) effects of gonadal hormones may influence sexually dimorphic spatial ability in both humans (Hampson, 1990; Kimura and Hampson, 1994) and rodents (Galea et al. 1995; for a review, see Williams and Meck, 1991).

Thus, there appear to be at least two factors, one functional and the other proximate, that are potentially important for the expression of sexually dimorphic spatial learning in rodents. The functional or evolutionary factor appears to be space use in the wild (such as home-range size) and the causal or proximate factor appears to be the level of organizational and activational gonadal hormones. We have carried out a series of studies using meadow voles and deer mice to help elucidate the relationships between these two factors and sexually dimorphic spatial ability.

If, as Gaulin and FitzGerald (1986) suggested, sexually dimorphic spatial ability evolved through sex differences in reproductive strategies, one might expect to see sexually dimorphic spatial ability in the seasonal breeder *only* when an animal is reproductively active. During periods of reproductive quiescence (during the non-breeding season and during juvenile ages), when there are minimal reproductive behaviours evident (e.g. no sex differences in space use), there should be no evidence for sex differences in spatial learning. The expression of sexually dimorphic spatial learning may depend on the reproductive status of the animal. According to this argument, sexually dimorphic spatial learning should be evident in reproductive or 'breeding' adult animals and not in non-reproductive 'non-breeding' animals. Higher circulating levels of gonadal hormones are also associated with 'breeding' adult animals, whereas lower circulating levels of gonadal hormones are associated with 'non-breeding' animals.

We investigated the effects of reproductive status on sexually dimorphic spatial learning in three naturally occurring contexts: (1) the breeding and non-breeding conditions; (2) different stages of juvenile development; and (3) individuals exposed prenatally to different organizational levels of gonadal hormones caused by different litter sex-ratios.

General water-maze procedure

Measurement of space use in the wild by voles and deer mice may vary greatly. Some researchers use home-range size as an indication of space use, which is usually defined as the area traversed by an individual in a 24–48 h period. Dispersal is another measurement that is characterized by a long-distance movement in which the animal does not return to the initial home-range. For a more complete description of these terms and a listing of the different types of movements in the wild (including home-range size), the reader is referred to Madison (1985). In the present paper, past research in both home-range size and dispersal characteristics in the wild will be considered. In our laboratory studies, however, we used performance in the water-maze as our operational definition of space use.

The spatial navigation task chosen was the Morris water-maze for a number of reasons: it does not require food deprivation; it is typically considered to be less stressful than a food-rewarded maze; and both meadow voles and deer mice are known to swim in the wild (Dagg and Windsor, 1972; Kant et al. 1988).

Animals were typically given four learning trials per day for six consecutive days in the training period. Each trial consisted of a release from one of the four quadrants surrounding the circular maze. The quadrant release point was chosen quasi-randomly so that for any given day each release point was used once. Each animal was given 60 s to find the platform. At the end of 60 s, if the animal had not found the platform, it was placed on the platform for 15 s. Latency (time taken) to reach the platform was recorded as the dependent variable.

Breeding status and spatial learning in adult deer mice

Most muroid rodents are seasonal breeders and show an annual pattern of reproduction. A number of reproductive characteristics, including sexually dimorphic home-range size and higher gonadal hormone levels, are expressed during the breeding season but not during the non-breeding season (Bronson and Perrigo, 1987). For example, in both deer mice and meadow voles, sex differences favouring males in home-range size are evident only during the breeding season and disappear during the non-breeding season (Madison and McShea, 1987; Wolff, 1989). In males housed under long-day cycles, testes mass and testosterone levels increase relative to those of males housed under short-day cycles (Bronson and Perrigo, 1987). Similarly, female deer mice are cyclic during the breeding season but become acyclic during the non-breeding season (Sadlier, 1974).

Thus, reproductive tactics, sexually dimorphic home-range size and gonadal hormone levels change across the seasons in deer mice. Accordingly, we examined the spatial learning performance of photoperiodically induced breeding (reproductive) and non-breeding (non-reproductive) adult male and female deer mice in a version of the Morris water-maze task (Galea et al. 1994a).

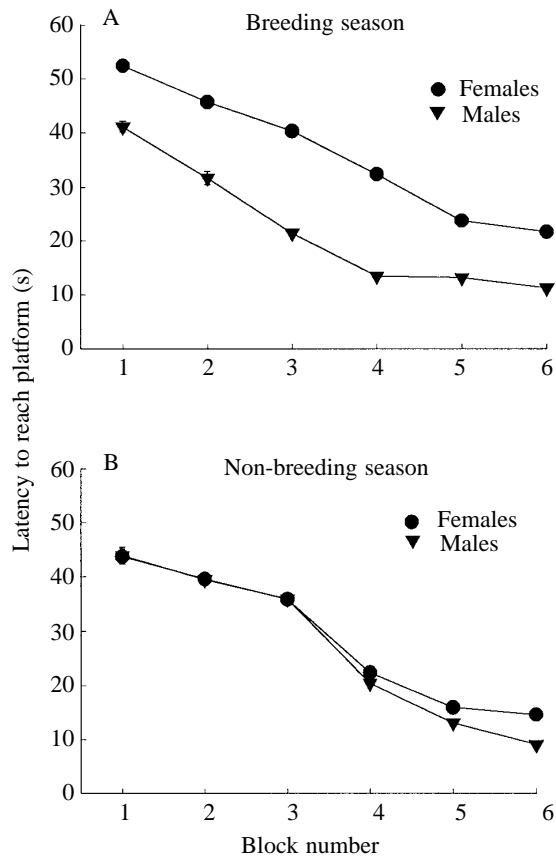Deer mice were tested either during the breeding season (summer; long-day photoperiod) or during the non-breeding

Fig. 1. Group mean latencies to reach the hidden submerged platform in the Morris water-maze task across all six blocks (*N*=8 in each group) (A) during the breeding season and (B) during the non-breeding season. There was a significant sex by season interaction, with sex differences favouring males present only during the breeding season. Error bars where visible are S.E.M. Reprinted from Galea *et al.* (1994*a*) with permission.
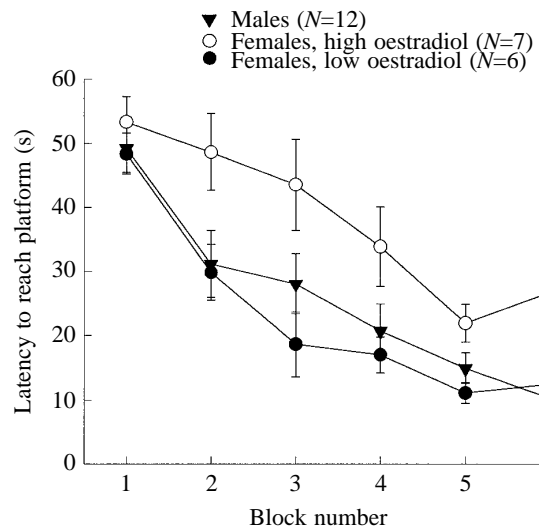

Fig. 2. Group mean acquisition of a Morris water-maze task over six blocks of trials. There were four trials per block and two blocks per day. Females with high levels of oestradiol (based on a median split) performed significantly worse than females with low levels of oestradiol and males. Error bars are S.E.M. Reprinted from Galea *et al.* (1995) with permission.

season (winter; short-day photoperiod) with a total of six blocks of four trials conducted in a single day. During the breeding season, spatial task acquisition was better in males than in females (Fig. 1). In contrast, during the non-breeding season there were no significant sex differences in spatial task acquisition. This seasonal change in sexually dimorphic spatial learning was primarily due to the female spatial-performance levels decreasing from the non-breeding to the breeding season. This result suggests that changes in the levels of sex hormones, which vary across the season in deer mice, may account for the seasonal fluctuations in spatial acquisition and that these changes differentially influence the spatial learning of male and female mice.

Space use as measured by home-range size seems to be well correlated with spatial learning in the Morris water-maze in deer mice across the seasons. During the breeding season, when there are sex differences favouring males in home-range size (Wolff, 1989), there are also corresponding sex differences in spatial learning, whereas during the non-breeding season both sex differences disappear.

Effects of reproductive status on spatial learning in female meadow voles

Female meadow voles are induced ovulators and require the presence of a male to enter behavioural oestrus (Sawrey and Dewsbury, 1985). After being paired with a male, oestrogen levels in female voles are increased by approximately 200 % (Cohen-Parsons and Carter, 1987). Researchers have also found that reproductively active females (presumably high-oestrogen females) have smaller home-range sizes than non-reproductive females (see Madison, 1985). We examined spatial performance levels of adult male and female meadow voles in relation to gonadal hormone levels and reproductive status. 'Reproductively activated' females were considered to be those females that had high circulating levels of plasma oestradiol.

Spatial learning

We found that there were significant differences in spatial performance in the Morris water-maze that were related to the levels of oestradiol in adult female voles (Galea *et al.* 1995). Female voles with high levels of oestradiol (based on a median split) performed worse than female voles with low levels of oestradiol and males (Fig. 2). Oestradiol levels were positively correlated with spatial learning in females (r=0.6566, P<0.05).

A positive relationship seems to exist between home-range size in the wild and spatial learning in the laboratory in the female meadow vole. 'Reproductive' females show poorer spatial learning in the laboratory and smaller home-range sizes in the wild compared with 'non-reproductive' females.

Effect of juvenile age

Sex differences in home-range size do not become evident until adulthood (at approximately 60 days of age), as there are no apparent sex differences in home-range size or dispersal in juvenile meadow voles (Baird and Birney, 1982).

Spatial learning

We examined levels of spatial learning in preweaning (day 10 after birth) and postweaning (day 15, 20 and 25) juvenile meadow voles (Galea *et al.* 1994*b*). We found no evidence for a sex difference in spatial performance in any of the juvenile age groups. There was, however, a significant effect of age, with older animals acquiring the task faster than younger animals (Fig. 3). The age differences in task acquisition were not accounted for by age differences in swimming speed. Interestingly, by day 25, juvenile voles were performing as well as adult males. This was the same period (subadult) during which meadow voles exhibit the greatest dispersal in the wild (Baird and Birney, 1982). In addition, the juvenile ages (days 10–20) that exhibited poorer spatial learning in the laboratory are associated with little movement of any type in the wild (Baird and Birney, 1982).

Once again, there is evidence for a relationship between movement in the wild and spatial learning in the laboratory. Moreover, the age group that exhibits the greatest dispersal in the wild also exhibits the best spatial performance in the laboratory.
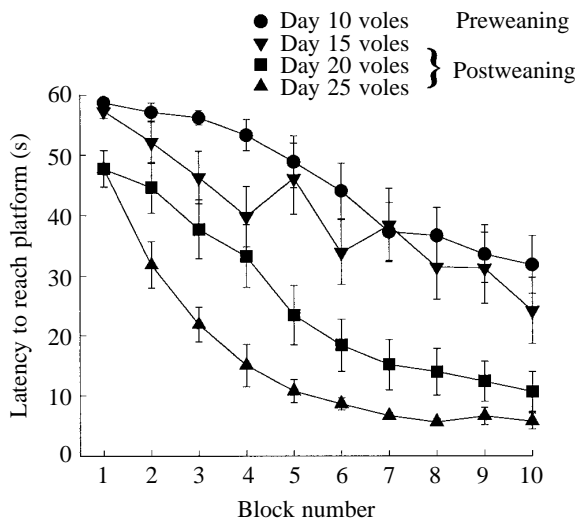

Fig. 3. Group mean latencies to reach the hidden platform in the Morris water-maze task across 10 blocks per juvenile age group (N=16 on days 10, 15 and 20, and N=19 on day 25). Males and females are grouped together because there were no significant differences in spatial performance. There were four trials per block and two blocks per day. There was a significant age effect, with older animals exhibiting shorter latencies to reach the platform than younger animals, despite accounting for age differences in swimming speed. Error bars are S.E.M. Reprinted from Galea *et al.* (1994*b*) with permission.

Effect of litter sex-ratio: organizational effects

One way in which to investigate the natural organizational effects of sex steroids on spatial ability is to examine the effect of *in utero* position or litter sex-ratio. vom Saal and Bronson (1980) found that animals located between two males (2M) *in utero* were exposed to higher levels of androgens than animals located between two females (0M) *in utero*. Litter sex-ratio may also be used to examine the organizational effects of hormones because females born into a male-biased litter will come from a population of mainly 2Ms.

Ims (1989) found that the litter sex-ratio accounted for 79 % of the variance in the amount of dispersal of the adult female grey-sided vole (*Clethrionomys rufocanus*) in an isolated population. He found that females from a male-biased population had a greater tendency to disperse than did females from a female-biased population. Furthermore, 2M female house mice were found to exhibit larger home-range sizes than 0M females (Zielinski *et al.* 1992).

Spatial learning

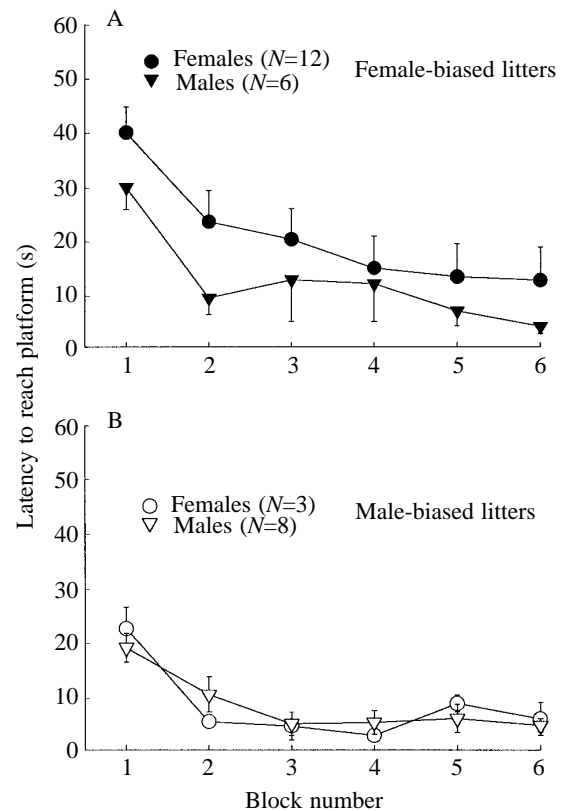We examined spatial performance levels in the water maze

Fig. 4. Group mean acquisition of a Morris water-maze task across six blocks in male and females from either female-biased (A) or male-biased (B) litters. There were four trials per block and two blocks per day. There was a significant effect of litter sex-ratio, with voles from male-biased litters outperforming voles from female-biased litters. A significant sex difference favouring males was observed in the female-biased litters only. Error bars are S.E.M. Reprinted from Galea *et al.* (1994*c*) with permission.

of male and female meadow voles born into either male- or female-biased litters. We found that both adult males and females from male-biased litters exhibited better water-maze performance than both males and females from female-biased litters (Galea *et al.* 1994*c*). In fact, sex differences favouring males were seen only in the female-biased litters and not in the male-biased litters (Fig. 4). However, the amount of variance in spatial learning that was accounted for by litter sex-ratio was approximately 8%.

There is evidence, therefore, that androgens or their metabolites influence both home-range size and spatial learning in the vole. Specifically, higher levels of androgens *in utero* are associated with an increase in home-range size, dispersal in the wild and spatial learning in the Morris water-maze in the laboratory.

Conclusions

The expression of sexually dimorphic spatial ability in rodents appears to depend on the reproductive status of the animal. In all instances, increased space use, as measured by home-range size (or dispersal), in the wild corresponded with better spatial learning in the Morris water-maze in the laboratory. When sex differences in home-range size have been shown to occur in the wild (i.e. during the breeding season in deer mice, or in reproductively activated female meadow voles), sex differences in spatial learning performance have also been observed in the laboratory. Furthermore, the reproductive state of females and, to a lesser extent, of males seems to be related to both home-range size and spatial learning.

There was also evidence for an organizational effect of testosterone or its metabolites on sexually dimorphic spatial ability and an activational effect of oestradiol in females on sexually dimorphic spatial ability. The results of the studies reviewed here suggest that sexually dimorphic spatial ability is related to both organizational (*in utero*) and activational influences of gonadal hormones. These studies provide the first clear demonstrations of a relationship between *natural* changes in reproductive status and spatial learning.

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