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Research report

Sex difference and laterality in the volume of mouse dentate gyrus granule cell layer

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Abstract

Sex differences in spatial learning have been reported in both humans and rodents. Correspondingly, there have been reports of sexual dimorphism in the morphology of the hippocampal formation (HF), a brain structure implicated in spatial cognition. In Experiment 1, we confirmed earlier reports that the overall volume of the granule cell layer (GCL) of the dentate gyrus (DG) of A/J mice is larger in males than in females. We also found that male A/J mice have a larger GCL volume in the right hemisphere than the left. Female A/J mice displayed no such laterality. A similar pattern of laterality, favoring the right HF, had been reported previously in male, but not female, rats. In Experiment 2, we examined mice with a defective structural gene for androgen receptors (*testicular feminization mutant*, or *tfm* mice) on a C57/BL6J background. The C57/J strain had not previously been examined for hippocampal sexual dimorphism. We found no sexual dimorphism in the left, right, or total volume of the GCL in C57/BL6J mice whether they were wildtype or *tfm*. However, the right GCL volume was greater than the left in wildtype C57/BL6J mice of either sex. No lateralization of GCL volume was found in the androgen-insensitive *tfm*-affected males or the partially androgen-insensitive *tfm*-carrier females. These findings confirm earlier reports that sexual dimorphism in mouse HF is found in some inbred strains but not others, and indicate for the first time that mouse HF structures are lateralized. The absence of lateralization in partially or wholly androgen-insensitive mice suggests that androgen receptors may play a role in development of laterality in the GCL independently of any sexual dimorphism in this structure.

Keywords: Sexual dimorphism; Hippocampus; Dentate gyrus; Androgen; Androgen insensitivity; *tfm*

1. Introduction

Studies of neural sexual dimorphism enhance our understanding of sex differences in behavior. By relating neural systems and hormones to behavior, sexual dimorphism studies may one day afford a better understanding of the underlying cellular mechanisms of human behavioral sex differences. There are many reports of neural sexual dimorphisms in various animals, including humans (see Ref. [5] for review). Some of these sexual dimorphisms are observed along with corresponding behavioral sex differences, and sometimes both the behavioral and the morphological differences can be reversed through hormone manipulation [2,8,11,14]. A sexually dimorphic behavior that has repeatedly been related to specific neuroanatomical regions is spatial cognition.

Sex differences in spatial cognition have been reported in a variety of mammals including humans [1,14,20]. Since spatial cognition performance has been attributed to the hippocampal formation (HF) [9,12], one might expect to find morphological sexual dimorphisms in the hippocampus of species that display sex differences in spatial cognition. Indeed, there have been several reports of sexual dimorphism in the HF, including dimorphisms in the overall volume of the HF in rats [4,10] and in the number, size, and density of cells in the dentate gyrus (DG) of the HF in mice [17–19].

Wimer and Wimer (1984) reported that male mice have a higher cell density in the granule cell layer (GCL) of the DG than do females in some inbred strains. Three of the six strains examined had a high granule cell number and three had a low number of cells in the GCL. Within the high cell number strains, males had significantly more granule cells than did females. However, the low cell number strains displayed a sexually monomorphic number of granule cells.

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In a follow-up study, Wimer et al. (1988) reported that the sex difference in granule cell number appears around postnatal days 20 and 27, when both sexes begin to exhibit a reduction in granule cell number. Since mouse testosterone levels decline immediately after birth and begin to rise in males at about post-natal day 20, and since androgen concentrating neurons are abundant in the mouse hippocampus [15], the authors hypothesized that androgens may play a role in mediating the development of the dimorphism in the GCL of these mice.

The purpose of the present study was to confirm the sex dimorphism that Wimer and Wimer found in the GCL of mice, to examine whether lateralization of HF structure found in rats is also present in mice, and to explore the role of androgens in the development of the sex difference in the GCL. In Experiment 1, we measured the GCL volume of A/J mice, a strain of mice found to have a high number of granule cells and a sex dimorphism in granule cell number previously. We confirmed the sexual dimorphism of this structure in this strain and report, for the first time, that the GCL is lateralized in male mice. In Experiment 2, we examined C57BL/J mice, which were not examined by Wimer and Wimer. We found that mice of this strain have a relatively small GCL volume and, as with the low GCL cell number strains studied by Wimer and Wimer, there was no sexual dimorphism of GCL volume in this strain. Mutants with a defective gene for the androgen receptor (*testicular feminization mutant*, or *tfm*) are available on the C57/BL6J background, so we examined these mutants to investigate the role of androgen receptors on GCL morphology. We found that the laterality of the GCL seen in wildtype C57/BL6J mice is absent in mice of this strain carrying the *tfm* mutation.

2. Materials and methods

2.1. Experiment 1

Four male and four female A/J mice from Jackson Laboratory 296–409 days of age were weighed, overdosed with pentobarbital, and perfused with saline and buffered formalin. The brains were post-fixed in buffered formalin for at least 1 week, and frozen-sectioned coronally in 60-μm thick sections, after overnight infiltration in a 20% sucrose solution. Every third section was mounted and stained with thionin. The slices were examined under a dissecting microscope, and the images were captured by a digital camera. The images were digitized with Adobe Photoshop software, the cross-sectional area of the GCL measured in the NIH-Image software (Fig. 1), and GCL volume was reconstructed as cubic millimeter. Borders of the GCL were determined with a final magnification of approximately 200×. At this magnification, GCL borders appear relatively even (Fig. 1). Brain weights were obtained individually after post-fixation, prior to sectioning (Table 1). All measures were made by an observer 'blind' to group membership of the tissues examined. All GCL measures were made by the same observer.

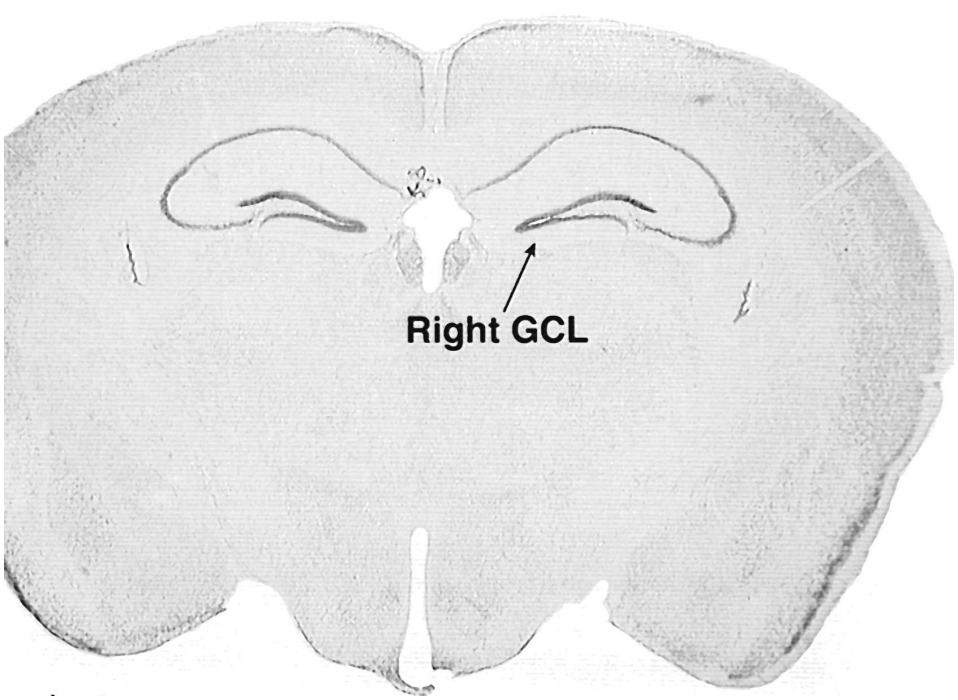

Fig. 1. Thionin-stained cross-section of a female *tfm* carrier C57/BL6J mouse brain, indicating the right granule cell layer (GCL).

Table 1
Mean brain and body weights (±S.E.) of male and female C57/BL6J mice carrying either the wildtype or the *tfm* allele for the androgen receptor

	C57 wildtype female	C57 wildtype male	C57 *tfm* female	C57 *tfm* male
Brain weight (mg)	510.3 ± 20 (3)	457.5 ± 29 (4)	453.5 ± 19 (4)	472.2 ± 23 (5)
Body weight (g)	24.3 ± 1.2 (3)	23.6 ± 2.3 (4)	24.6 ± 2.1 (4)	21.69 ± 2.6 (5)

Note that C57 *tfm* females are heterozygous, carrying one wildtype and one *tfm* copy of the androgen receptor gene. There was no significant effect of either sex or genotype on either brain weight or body weight. The number of mice in each group is presented in parentheses.

2.2. Experiment 2

A total of 16 C57/BL6J mice from Jackson Laboratory were examined as above. Of these 16, four were wildtype males, three wildtype (non-carrier) females, five *tfm*-affected males, and four *tfm*-carrier females.

In each experiment, statistical analysis consisted of a mixed two-way ANOVA with sex as an independent factor and laterality as a repeated measure. In cases where a significant interaction was seen, independent *t*-tests were used as post-hoc tests for sex differences, and dependent *t*-tests were used as post-hoc tests for laterality.

3. Results

3.1. Experiment 1

As depicted in Fig. 2, total volume of the GCL in A/J mice was greater in males than in females ($p < 0.05$; main effect of sex in two-way ANOVA), confirming the report of Wimer et al. [18]. The sex difference in GCL volume was also seen when left and right measures were combined ($p < 0.05$ two-tailed; independent *t*-test). There was no significant main effect of laterality ($p > 0.10$), but there was a significant interaction between laterality and sex ($p < 0.04$). The interaction appeared to be due to the fact that in males, the right GCL volume was significantly greater than the left ($p < 0.04$; dependent *t*-test), but no such laterality was found in females. Likewise, the right GCL volume was greater in males than in females ($p < 0.01$), but the left GCL was not sexually dimorphic. Thus, the GCL volume of A/J mice was sexually dimorphic in two ways: (1) in terms of total volume—males had greater overall and greater right GCL volume than females, and (2) in terms of laterality—males were lateralized but females were not. Among A/J mice, males had heavier bodies than did females (mean ± S.E.M.: 36.5 ± 1.96 g in males vs. 27.8 ± 0.50 in females), but there was no sex difference in whole brain weight (427.3 ± 7.9 mg in males vs. 429.3 ± 9.7 in females).

3.2. Experiment 2

As Fig. 3 reveals, the GCL volume of C57/BL6J mice was lateralized in wildtypes, consisting of unaffected males and non-carrier females. Analysis of variance revealed a significant main effect of laterality ($p < 0.05$), but no significant main effect of sex nor any significant interaction of the two factors. Post-hoc analysis revealed that the right GCL volume was greater than the left in wildtypes of either sex ($p < 0.05$; dependent *t*-tests). In contrast to A/J mice, the C57/BL6J mice demonstrated no sexual dimorphisms in either total GCL volume nor lateralization of GCL volume. However, *tfm*-affected males and *tfm*-carrier females, unlike wildtype mice of this strain, did not display any lateralization of GCL volume (Fig. 4). Thus, no sexual dimorphism was found in the GCL volume of any

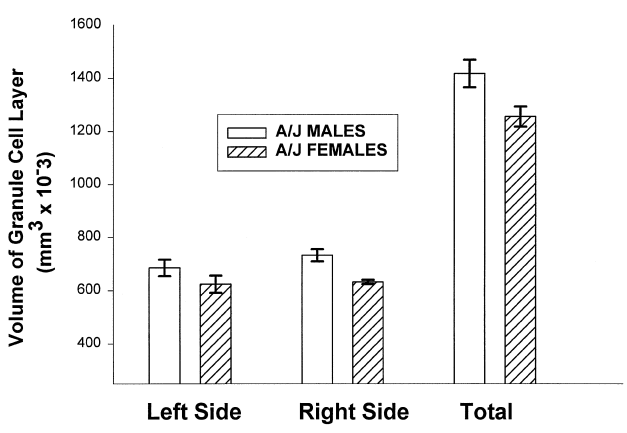

Fig. 2. Among A/J mice, the volume of the granule cell layer (GCL) was greater in males than in females. Furthermore, the right GCL was larger than the left in males but not in females. The left GCL was not significantly larger in males than in females. Means and standard errors are depicted.

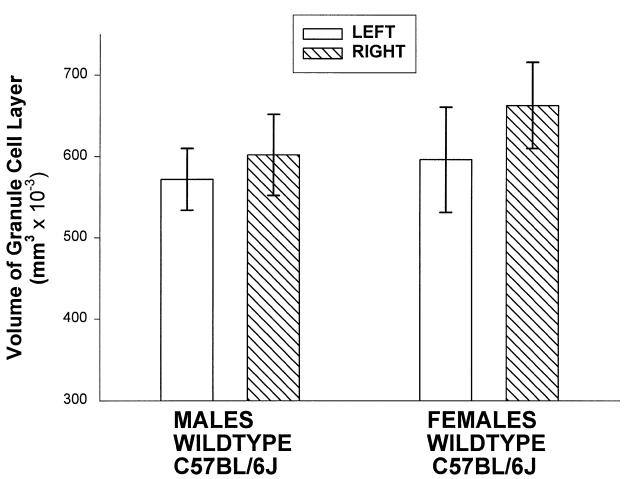

Fig. 3. The GCL volume of C57 mice was smaller than that of A/J mice (compare with Fig. 2). Among C57 mice, there was no sexual dimorphism in the volume of the GCL. However, the right was significantly larger than the left in both sexes (dependent *t*-tests).

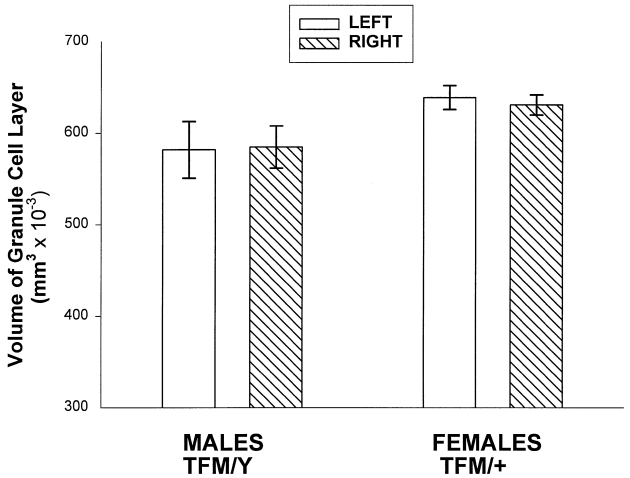

Fig. 4. C57 mice carrying the *tfm* mutation, which renders the animals either completely androgen-insensitive (in XY males) or partially androgen-insensitive (in XX females), display no laterality in the volume of the GCL. In wildtype mice of this strain, the right GCL is larger than the left (see Fig. 3). As there is no sexual dimorphism in GCL volume in the background C57/BL6J strain, these *tfm* animals shed no light on the role of androgen receptors in sexual differentiation of the GCL in other mouse strains.

of the C57/BL6J mice, in terms of total volume or pattern of laterality. Laterality of GCL volume was seen only among C57/BL6J mice carrying a wildtype version of the androgen receptor gene, not among mice carrying the *tfm* allele for the androgen receptor.

4. Discussion

In Experiment 1, we confirmed past reports of a sexual dimorphism in the number of GCL cells in A/J mice [18] by finding that the overall GCL volume in this strain is greater in males than in females. In addition, we also found that the GCL volume in A/J mice is lateralized in males but not in females; more specifically, the right GCL volume is greater than the left in males only. Although Wimer and Wimer did not report any laterality of GCL volume or cell number, previous research from several laboratories has found the same pattern of laterality (right larger than left) in the male rat hippocampus [4,10,14] and in the human hippocampus of both sexes [7]. Because it is difficult to account for overall body size differences between the sexes, it is difficult to determine whether a sex difference in the size of a brain structure is related to a sex difference in brain function or just a correlate of overall body size. However, when the sex difference is in asymmetry rather than in size, it is unlikely that the sex difference is a simple reflection of the sex difference in body size.

In Experiment 2, we found that the GCL volume in C57/BL6J mice is not sexually dimorphic. To our knowledge, this is the first time that sex differences in the GCL have been examined in this strain. Our data indicate that the GCL volume of C57/BL6J mice is less than that of A/J mice. So the absence of sexual dimorphism in C57/BL6J mice may be due to low GCL volume, conforming to Wimer and Wimer's conclusion that sexual dimorphism is seen only in strains with a large number of granule cells. This result was a disappointment, because if the GCL had been sexually dimorphic in this strain, then examination of androgen receptor mutations on this background strain might have indicated whether androgen receptors play a role in the sexual differentiation of the rodent HF.

Nonetheless, we did replicate the A/J laterality of the GCL in the C57/BL6J mice, since the right GCL volume was significantly larger than the left, among wildtype males and females. Thus, the laterality of the GCL may be a general phenomenon among rodents, even if sex differences in GCL laterality are not. Interestingly, no such laterality was seen among genetic male (XY) or female (XX) C57/BL6J mice carrying the *tfm* allele for the androgen receptor, which renders them completely or partially androgen-insensitive, respectively. This finding suggests that androgen receptors may be involved in the development of lateralization in the HF. Because the gene for the androgen receptor is found on the X chromosome, *tfm*-affected males lack androgen receptors. The *tfm*-carrier females are mosaic for the expression of the *tfm* gene (because each somatic cell expresses either the X carrying the mutated gene or the X carrying the wildtype androgen receptor gene). However, the wildtype males and females, who have intact androgen receptors, had significantly lateralized GCL volumes.

Because no sexual dimorphism was found in the GCL of the C57/BL6J mice which provide the background for the *tfm* mutation, we cannot exploit *tfm* mice to determine whether androgen receptors mediate the sexual dimorphism of the GCL in mice. However, it is worth noting that since laterality, especially in the HF, tends to be sexually dimorphic [4,10,14], the fact that androgen receptors seem to have some effect on the development of laterality in the C57/BL6J strain provides a hint that these receptors may also be involved in the development of sexual dimorphism in sexually dimorphic strains. However, evidence in the literature for this hypothesis is still limited.

Wimer and Wimer (1989) proposed that the interaction of testosterone with mitochondrial and nuclear DNA in hippocampal neurons may lead to a higher rate of neuron survival in developing male mice. Early treatment of females with androgen has been shown to prevent cell death in motoneurons [2], possibly by enhancing the production of trophic factors [3]. On the other hand, it is possible that androgens affect neurogenesis in this system, since neurogenesis continues into adulthood in the rodent GCL.

Further evidence for the involvement of testosterone in the sex dimorphism of the HF comes from the study of

Sprague–Dawley rats. Control rats displayed three sexual dimorphisms: (1) the male GCL had a larger width than the female GCL; (2) male GCLs were lateralized (larger right than left) while female GCLs were not; and (3) males learned the Morris water maze task faster than did females [13]. Perinatal testosterone treatment improved the females' adult performance on the Morris water maze task and masculinized the GCL, both in width and in laterality. However, these results do not tell us whether testosterone is acting on androgen or estrogen receptors, since testosterone can be aromatized to interact with estrogen receptors. In fact, treatment of newborn females with estrogen has been shown to masculinize their spatial learning in adulthood [20]. Thus, it is possible that estrogen receptors are involved in the development of sexual dimorphism in the GCL, since estrogen receptors are relatively abundant in the DG [16].

More research is needed to elucidate the exact role of steroid receptors in the development of sexual dimorphism in the HF. To determine whether androgen or estrogen receptors are responsible for the masculinization of the GCL in females, future studies could examine early treatment of sexually dimorphic strains of mice with the non-aromatizable androgen dihydrotestosterone (DHT). Since both androgens and estrogens have been shown to reverse the HF sexual dimorphism [6,14,20], the effect of early estrogen treatment on the HF morphology is also worth exploring.

Acknowledgements

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