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X-chromosome dosage affects male sexual behavior

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ABSTRACT

Sex differences in the brain and behavior are primarily attributed to dichotomous androgen exposure between males and females during neonatal development, as well as adult responses to gonadal hormones. Here we tested an alternative hypothesis and asked if sex chromosome complement influences male copulatory behavior, a standard behavior for studies of sexual differentiation. We used two mouse models with non-canonical associations between chromosomal and gonadal sex. In both models, we found evidence for sex chromosome complement as an important factor regulating sex differences in the expression of masculine sexual behavior. Counter intuitively, males with two X-chromosomes were faster to ejaculate and display more ejaculations than males with a single X. Moreover, mice of both sexes with two X-chromosomes displayed increased frequencies of mounts and thrusts. We speculate that expression levels of a yet to be discovered gene(s) on the X-chromosome may affect sexual behavior in mice and perhaps in other mammals.

Introduction

The original studies on mammalian sexual differentiation of behavior identified androgen, produced in the developing testes, as the critical factor responsible for differences between adult male and female behavior (Phoenix et al., 1959). Testosterone, acting both directly, and after aromatization to estradiol, binds to its receptors in the brain and modifies neural circuits. In adults, these modifications lead to increased expression of behaviors more typical of males, and decreased display of behaviors more often shown by females. However, in addition to androgen differences, there are genetic differences between males and females caused by unequal dosage of sex chromosome genes. In mammals, and many other species, sex is determined by genetic inheritance of sex chromosomes. Normal female mammals (XX) have two X-chromosomes while males (XY) have a single X- and a Y-chromosome. The X-chromosome encodes hundreds of genes with no direct paralogs on Y, whereas the Y-chromosome encodes many fewer genes, including the testis-determining factor *Sry* (Ellegren, 2011; Koopman et al., 1991). In addition to *Sry*, genetic differences between XX and XY individuals are now recognized as a source of variation that shapes sex differences in brain and behaviors (Arnold, 2009).

Over the last decade, several mutant mice with atypical sex chromosome arrangements have been developed and used to test the effects of sex chromosome complement on sexual differentiation (Arnold, 2009). Here we employ the Four Core Genotypes (FCG) and Y* models, which are described in Table 1. In FCG mice, males and females can have either XX or XY sex chromosomes (De Vries et al., 2002). *Sry* (testis determining gene) is deleted on the FCG Y-chromosome and a transgenic copy of *Sry* is located on an autosome, thereby unlinking differentiation of the gonads from the sex chromosomes. Autosomal inheritance of the *Sry* transgene causes testes development in both XX and XY mice (gonadal males), and ovaries develop in mice without the autosomal transgene (gonadal females). Since same gonadal sex FCG mice differ by XX and XY genotypes, sex chromosome effects revealed by the FCG can be attributed to one of three major mechanisms: genes on Y, genes that escape X-inactivation, and paternally imprinted X-genes.

The Y* model is used to determine whether sex chromosome effects present in the FCG are due to dose of X- or Y-chromosome genes. The Y*-chromosome was generated by a spontaneous translocation and inverted duplication of the pseudoautosomal region (PAR) of Y (Eicher et al., 1991). During meiosis, the altered PAR of Y* recombines aberrantly with the X-chromosome and generates male gametes with four sex chromosomes: non-recombined X and Y*, and recombined Y^{*X} (PAR without unique X and Y genes) and X^{Y*} (an X attached to Y chromosome). Gonadal male and female Y* mice can have one or two copies of the X-chromosome, whereas only males have a Y-chromosome. Therefore, while FCG XX and XY genotypes differ in both dose of X and presence of Y, Y* mice of the same gonadal sex only differ in dose of X (Table 1). If dosage of X-chromosome genes is important for the observed differences in FCG, we expect to see the same, or perhaps more pronounced, sex chromosome effects in Y* mice.

Using these mouse models, herein we examined the sex chromosome hypothesis by studying two highly sexually dimorphic behaviors: masculine sexual behavior and aggression (Bonthuis et al.,

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Table 1
Genotype and sex chromosome complement of FCG and Y* mice. The gonadal sex and dose of sex chromosomes are shown among genotypes of the Four Core Genotypes (FCG) and Y* models. In the genotypes column, the first X represents the maternally inherited sex chromosome, and the second sex chromosome is paternally inherited. Y^-, *Sry* deleted Y-chromosome. *Sry*, sex-determining region of Y (FCG *Sry* is a transgene within an autosome). Copies of X, dose of X-chromosome specific genes. Copies of Y, dose of Y-chromosome specific genes.

Composition of sex chromosome regions					
	Genotype	Gonads	*Sry*	Copies of Y	Copies of X
FCG					
XYM	XY^-*Sry*	Testes	1	1	1
XXM	XX*Sry*	Testes	1	0	2
XYF	XY^-	Ovaries	0	1	1
XXF	XX	Ovaries	0	0	2
*Y**					
1XM	XY*	Testes	1	1	1
2XM	XX^{Y*}	Testes	1	1	2
1XF	XY^{*X}	Ovaries	0	0	1
2XF	XX	Ovaries	0	0	2

2010). We found a strong effect of X-chromosome number on several aspects of masculine sexual behavior; counter intuitively individuals with two X-chromosomes displayed more behavior than mice with one X-chromosome. The X-chromosome effect did not generalize to sexually dimorphic resident–intruder aggression, or dimorphic vasopressin (AVP) density in the lateral septum of Y* mice. Our data indicate that two X-chromosomes increase male sexual behavior in mice, but the Y-chromosome may increase aggression and AVP immunoreactivity in the lateral septum.

Methods

Animals

All animal care and procedures were performed in accordance with and approved by the University of Virginia Animal Care and Use Committee. Adult (55–75 days old) FCG and Y* mice in the C57BL/6J background strain (see Table 1) were gonadectomized and implanted (SC) with Silastic tubing (1.02 mm inner diameter × 2.16 mm outer diameter; Dow Corning) filled with 1 cm of crystalline testosterone while under general isoflurane anesthesia. The mice were housed alone on a 12 h reverse light cycle with 2400 light onset and 1200 dark onset (EST), and given food (diet # 7912; Harlan Teklad, Indianapolis, IN) and water ad libitum. In Experiment 1, 12 mice per genotype (total n = 48) were used for FCG male sex behavior tests. Our Y* breeding colony was originally started with B6Ei.LT-Y^*/EiJ males and C57BL/6JEiJ females purchased from The Jackson Laboratory (Bar Harbor, ME; stock numbers 002021 and 000924). The mice in our lab were maintained in the B6Ei substrain. For Y* male sex behavior tests we used n = 8 XY^{*X}, n = 12 XX, n = 18 XY*, and n = 16 XX^{Y*} mice. In Experiment 3 (aggression) we used n = 14 XY^{*X}, n = 14 XX, n = 20 XY*, and n = 20 XX^{Y*} mice. FCG mice were genotyped by PCR of the YMT2/B-related *Ssty* family on the Y-chromosome (Gatewood et al., 2006), and Y* mice by amplification of reverse transcription of RNA and PCR amplification of *Xist* (Park et al., 2008) as previously described.

Anogenital distance

Anogenital distance (AGD) was measured in a separate cohort of 96 Y* mice (n = 20 1XM, 24 2XM, 24 1XF, and 28 2XF) on postnatal day 10. Using dial calipers with 0.1 mm precision, the distance was measured between the most ventral aspect of the anus to the dorsal aspect of the genital tubercle.

Testosterone radioimmunoassay

Immediately before gonadectomy, blood was collected from each anesthetized mouse by a suborbital puncture, spun and serum frozen. After behavioral testing, serum was collected from trunk blood at time of euthanasia. Testosterone measurements were performed in singlet reactions using Diagnostic Products Corporation testosterone RIA with a detectible range of 0.138–7.487 ng/ml. The intra-assay coefficient of variation (CV) for these assays was 4.2 +/− 0.91 (mean +/− SEM) and the inter-assay CV was 10%. The University of Virginia Ligand Core laboratory performed the assays (supported by NICHD (SCCPRR) Grant U54-HD28934).

Male sex behavior

Male sex behavior was tested once a week for four consecutive weeks with hormone primed, sexually experienced, ovariectomized, stimulus C57BL/6J females as previously described (Park et al., 2009). Trials were video recorded in the dark under red-light illumination between 1200 and 1800 h EST. An observer blind to genotypes scored the following behaviors: time to first mount, time to first intromission, number of mounts, number of deep thrusts in each mount, and time to ejaculation.

Resident–intruder aggression

One to two weeks after surgery, mice were given 3 days of social exposure (Sipos and Nyby, 1998). Beginning 2 days later, each mouse was tested on three consecutive days for resident–intruder aggression. Tests were conducted in the dark under red-light illumination between 1200 and 1700 h (Gatewood et al., 2006). The intruders were anosmic C57BL/6J males. Anosmia was induced with Dichlobenil (2,6 Dichlorobenzonitrile) and verified by a hidden cookie test (Brandt et al., 1990). Tests were 10 min in duration or ended when the resident first attacked the intruder, and attack latencies were scored.

Tissue preparation and vasopressin immunocytochemistry

Y* mice from the previous behavioral experiments were deeply anesthetized with isoflurane inhalant, and brains were extracted and fixed by submersion in 4% acrolein in PBS for 4 h. Brains were removed, placed in 30% sucrose overnight, and frozen on dry ice. Tissue was cut into 30 μm coronal sections, collected into four vials, and then stored in antifreeze (30% w/v sucrose, 1% w/v PVP 40, 30% v/v ethylene glycol in 0.02 M TBS) at −20 °C until processing. Immunocytochemical staining for vasopressin was performed as previously described (Scordalakes and Rissman, 2004). The density of vasopressin immunoreactive fibers (AVP-IR) in the lateral septum was determined using microscopy and MetaMorph (Molecular Devices, Sunnydale, CA) image analysis software of a standard area that surrounded the region. The defined region for the rostral lateral septum was a rectangle of about $4.6 \times 10^5 \mu m^2$, while the caudal septum was defined by a triangle of approximately $5.2 \times 10^5 \mu m^2$. The regions were aligned on the images with the edge just medial to the lateral ventricles. A researcher blind to sex and genotype quantified both left and right sides of one rostral section corresponding to figure 27, and one caudal section corresponding to figure 29, in the mouse brain atlas of Franklin and Paxinos (2008), in a similar manner as described (Rood et al., 2008). The average area stained was then calculated.

Quantitative real-time PCR

Y* mice were anesthetized as above and brains were frozen on crushed dry ice. Fresh frozen tissue was cut into 120 μM coronal