



Steroid-independent male sexual behavior in B6D2F2 male mice



Christine M. McInnis ^{*,1}, Samitha Venu, Jin Ho Park

Psychology Department, University of Massachusetts, Boston, Boston, MA 02125, United States

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ABSTRACT

It is well established that male sexual behavior (MSB) is regulated by gonadal steroids; however, individual differences in MSB, independent of gonadal steroids, are prevalent across a wide range of species, and further investigation is necessary to advance our understanding of steroid-independent MSB. Studies utilizing B6D2F1 hybrid male mice in which a significant proportion retain MSB after long-term orchidectomy, identified as steroid-independent-maters (SI-maters), have begun to unravel the genetic underpinnings of steroid-independent MSB. A recent study demonstrated that steroid-independent MSB is a heritable behavioral phenotype that is mainly passed down from B6D2F1 hybrid SI-maters when crossed with C57BL6J female mice. To begin to uncover whether the strain of the dam plays a role in the inheritance of steroid-independent MSB, B6D2F1 hybrid females were crossed with B6D2F1 hybrid males. While the present study confirms the finding that steroid-independent MSB is a heritable behavioral phenotype and that SI-mater sires are more likely to pass down some components of MSB than SI-non-maters to their offspring, it also reveals that the B6D2F2 male offspring that were identified as SI-maters that displayed the full repertoire of steroid-independent MSB had the same probability of being sired from either a B6D2F1 SI-mater or SI-non-mater. These results, in conjunction with previous findings, indicate that the specific chromosomal loci pattern that codes for steroid-independent MSB in the B6D2F2 male offspring may result regardless of whether the father was a SI-mater or SI-non-mater, and that the maternal strain may be an important factor in the inheritance of steroid-independent MSB.

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1. Introduction

Strong selective pressure drives the evolution of mating behaviors, and genetic differences in mating ability that increase reproductive fitness become more prevalent in the general population. In many mammalian species, male sexual behavior (MSB) is highly dependent upon gonadal steroids; orchidectomy reduces masculine sexual behaviors in mammals, but the extent of the reduction varies both inter- and intra-specifically [reviewed in (Hart, 1974; Hull et al., 2006)]. However, in several mammalian species, including humans, striking inter-individual variation in the response to orchidectomy has been documented [reviewed in (Park and Rissman, 2010)]. Gonadal steroid-independent MSB (SI-MSB) has been documented in several rodent models studied in the laboratory, including one hybrid strain of mouse, B6D2F1, (Clemens et al., 1988; McGill and Manning, 1976; Thompson et al., 1976), Siberian hamsters (Park et al., 2004), and Damaraland mole-rats (Carter et al., 2014).

B6D2F1 hybrid male mice are produced by crossing C57BL6J (B6) dams with DBA sires, and ~30% of the male mice retain the full repertoire of MSB up to 25 weeks after castration (herein after referred to as 'SI-maters', previously referred to as maters (McInnis et al., 2016)). Males of both the inbred parent B6 and DBA strains cease to demonstrate MSB shortly after orchidectomy [reviewed in (Burns et al., 2000)], and B6D2F1 hybrid males demonstrate more robust SI-MSB than the offspring of the reciprocal cross (D2B6F1 hybrids; (McGill and Manning, 1976). Identifying an underlying mechanism regulating SI-MSB in these hybrid mice has been difficult; hormonal characterization comparing SI-maters vs. SI-non-maters has revealed nothing exceptional (Clemens et al., 1988; Park et al., 2009; Sinchak et al., 1996). Recent bioinformatic analyses have begun to characterize the genetic underpinnings of SI-MSB, and SI-MSB was recently found to be a heritable behavioral phenotype passed down from B6D2F1 hybrid 'SI-maters' when crossed with B6 female mice (Bharadwaj et al., 2013; McInnis et al., 2016; Park et al., 2010). Therefore, a specific chromosomal loci distribution pattern may be responsible for the expression of SI-MSB. However, whether the maternal strain was an important factor in the inheritance of SI-MSB remained unanswered. Thus, to assess the potential genetic contributions of SI-MSB from B6D2F1 hybrid dams and sires, we identified B6D2F1 male mice and their F2 male offspring as either SI-maters or SI-non-maters. We hypothesized that SI-MSB may result from

* Corresponding author at: Department of Pathology, Brigham and Women's Hospital, 77 Avenue Louis Pasteur, Boston, MA 02115, United States.

E-mail address: cmcinnis1@partners.org (C.M. McInnis).

¹ Current Address: Department of Pathology, Brigham and Women's Hospital Boston, MA 02115, United States.

the B6D2F1 hybrid female X B6D2F1 hybrid male cross, regardless of whether the B6D2F1 hybrid male was a SI-mater or not because the resulting chromosomal loci distribution pattern for SI-MSB could be influenced by genetic contributions by either the B6D2F1 hybrid dam or sire.

2. Methods

2.1. Animals

Animals were bred and raised in the animal facility at the University of Massachusetts, Boston. Male B6D2F1 hybrid mice ($n = 24$; *Mus musculus*) were paired with a cohort of sexually naïve B6D2F1 hybrid females to produce male offspring (F2). Male mice were weaned at 20–21 days and singly housed until the onset of the experiment (between 50 and 80 d of age). Animals were given food (Prolab RMH 3000; PMI International, Brentwood, MO) and water ad libitum and maintained on a 12:12 light:dark cycle, with lights going off at 1200 h EST. All procedures were performed according to the AALAC guidelines and approved by the University of Massachusetts, Boston IACUC. All surgeries and sacrifices were performed while the animals were anesthetized with isoflurane, and all efforts were made to minimize suffering.

2.2. Sexual experience prior to orchidectomy and mating with B6D2F1 hybrid females

B6D2F1 hybrid males were provided sexual experience with hormonally primed stimulus female B6 mice on at least three separate occasions prior to being paired with B6D2F1 hybrid females for one week before undergoing orchidectomy. Of the 24 B6D2F1 dams paired with B6D2F1 males, 23 produced litters. All of the F2 males were also provided sexual experience with hormonally primed stimulus female B6 mice on at least three separate occasions prior to being orchidectomized.

2.3. Behavioral testing after orchidectomy

Behavioral tests were conducted until all of the mice could be reliably identified as SI-maters, or SI-non-maters. Thus, B6D2F1 hybrid males were tested for MSB every two weeks for 23 weeks after orchidectomy, and F2 males were tested for MSB every two weeks for 15 weeks after they were provided sexual experience and underwent orchidectomy. All males were considered SI-maters if they demonstrated the ejaculation reflex on either of the last two tests, SI-non-maters if they did not display mounting behavior on the last two tests, and “intermediates” if they did not meet the criterion for either group. Testing for MSB was conducted as previously described in (Bharadwaj et al., 2013). Mount latency (ML), intromission latency (IL), and ejaculation latency (EL) were recorded.

2.4. Statistical analyses

One-way ANOVAs were used to analyze differences in composition of litters sired by the female B6D2F1 X male B6D2F1 hybrid cross. Differences in the proportion of B6D2F1 hybrid males and their male offspring demonstrating SI-MSB, the proportion of intermediate F2 offspring from either SI-mater or SI-non-mater sires were assessed with the Chi-square measure. Effect sizes were further estimated by calculating eta-squared (η^2) for ANOVAs and Phi (ϕ) for Chi-square tests. Post-hoc comparisons were conducted using the Fisher Protected Least Significant Difference test where appropriate. Observed differences were considered significant if $p < 0.05$. Statistical tests were run using the Statview program (Statview 5; SAS Institute, Cary, NC, USA).

3. Results

Of the 24 orchidectomized B6D2F1 hybrid males paired with B6D2F1 hybrid females, 13 were categorized as SI-maters (54.2%), 6 were categorized as SI-non-maters (25%), and 5 displayed behavior that did not meet either criteria (Fig. 1A). Between the B6D2F1 hybrid male sires that were SI-maters and SI-non-maters, there were no significant differences in litter composition. There were no differences in the number of F2 male offspring [*F-value* (Amateau and McCarthy, 2004; Park et al., 2004) = 0.49, $p = 0.1$, $\eta^2 = 0.05$], F2 female offspring [*F-value* (Amateau and McCarthy, 2004; Park et al., 2004) = 0.2, $p = 0.07$, $\eta^2 = 0.02$] or litter size [*F-value* (Amateau and McCarthy, 2004; Park et al., 2004) = 0.46, $p = 0.1$, $\eta^2 = 0.01$] between the two groups.

Of the 40 F2 males born to the B6D2F1 SI-maters in this experiment, 7 were SI-maters (18%) and 15 were SI-non-maters (38%), and 18 (45%) did not fit either criterion. Of the 20 F2 male offspring born to the SI-non-maters, 3 were SI-maters (15%), 16 were SI-non-maters (80%), and 1 (5%) could not be identified as either. F2 males classified as SI-maters had F1 sires that were equally distributed between the SI-mater and SI-non-mater groups (31% and 50%, respectively; $\chi^2(1) = 0.65$, $p = 0.42$, $\phi = 0.19$; Fig. 1B). Additionally, there was no significant difference in the number of F2 SI-maters sired between the male B6D2F1 hybrids that were SI-maters or SI-non-maters [*F-value* (Amateau and McCarthy, 2004; Park et al., 2004) = 0.18, $p = 0.07$, $\eta^2 = 0.01$]. However, F1 SI-mater sires, relative to SI-non-maters, produced significantly more F2 ‘intermediate’ male offspring (45% versus 5%; $\chi^2(1) = 9.86$, $p = 0.002$, $\phi = 0.41$), as well as significantly more SI-‘mounters’ (male offspring that demonstrated at least one mount: F2 SI-maters plus intermediates; 62.5% versus 20%; $\chi(1) = 9.64$, $p = 0.002$, $\phi = 0.40$).

There were no differences in ejaculation, intromission, or mount latencies on the last MSB test session between the F2 SI-maters that were sired from either B6D2F1 hybrid SI-maters or SI-non-maters (all p -values > 0.05 for all comparisons; $\eta^2 = 0.03$, 0.12, and 0.21 for ejaculation, intromission, or mount latencies, respectively; data not illustrated).

4. Discussion

A proportion of orchidectomized male F2 offspring derived by crossing female and male B6D2F1 hybrid mice demonstrate SI-MSB, and our results provide further support that this behavioral phenotype is a heritable trait. Heritability of SI-MSB has been demonstrated previously in select B6D2F1 recombinant inbred strains (Coquelin, 1991) and in male BXB1 mice, which are derived by crossing C57BL/6J female mice and B6D2F1 hybrid male mice (McClinnis et al., 2016). Furthermore, whether orchidectomized BXB1 males display SI-MSB was highly dependent upon whether their sires also exhibited SI-MSB. Our findings that F1 SI-maters, relative to SI-non-maters, were significantly more likely to sire F2 “intermediate” males also indicate that the behavioral phenotype of the B6D2F1 sires significantly influences whether SI-MSB is expressed in their male offspring when paired with B6D2F1 females. The F2 “intermediates” displayed some, but not all, components of SI-MSB, which prevented them from being classified as an SI-mater. Thus, it seems only “partial” SI-MSB is passed on since the paternal behavioral phenotype of the F1 sire does not seem to predict whether the F2 male offspring would demonstrate the full repertoire of SI-MSB, including the ejaculatory pattern, as both F1 sires that were classified as SI-maters or SI-non-maters were equally likely to sire F2 SI-maters (Fig. 1).

The genetic mechanism underlying the inheritance of SI-MSB may be based upon allelic heterozygosity between multiple loci, and a specific chromosomal loci allelic distribution pattern with B6 alleles at some loci and DBA/2J alleles at other loci may regulate inheritance of SI-MSB (Coquelin, 1991). Fig. 2 depicts a schematic of the resulting heterozygous loci of B6D2F1 hybrid male mice that have inherited B alleles

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