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Inheritance of steroid-independent male sexual behavior in male offspring of B6D2F1 mice

Christine M. McInnis [a,*,1], Paul J. Bonthuis [b,2], Emilie F. Rissman [b,3], Jin Ho Park [a,b]

[a] Psychology Department, University of Massachusetts, Boston, Boston, MA 02125, United States
[b] Department of Biochemistry & Molecular Genetics, University of Virginia School of Medicine, Charlottesville, VA 22908, United States

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ABSTRACT

The importance of gonadal steroids in modulating male sexual behavior is well established. Individual differences in male sexual behavior, independent of gonadal steroids, are prevalent across a wide range of species, including man. However, the genetic mechanisms underlying steroid-independent male sexual behavior are poorly understood. A high proportion of B6D2F1 hybrid male mice demonstrates steroid-independent male sexual behavior (identified as "maters"), providing a mouse model that opens up avenues of investigation into the mechanisms regulating male sexual behavior in the absence of gonadal hormones. Recent studies have revealed several proteins that play a significant factor in regulating steroid-independent male sexual behavior in B6D2F1 male mice, including amyloid precursor protein (APP), tau, and synaptophysin. The specific goals of our study were to determine whether steroid-independent male sexual behavior was a heritable trait by determining if it was dependent upon the behavioral phenotype of the B6D2F1 sire, and whether the differential expression of APP, tau, and synaptophysin in the medial preoptic area found in the B6D2F1 sires that did and did not mate after gonadectomy was similar to those found in their male offspring. After adult B6D2F1 male mice were bred with C57BL/6J female mice, they and their male offspring (BXB_1) were orchidectomized and identified as either maters or "non-maters". A significant proportion of the BXB_1 maters was sired only from B6D2F1 maters, indicating that the steroid-independent male sexual behavior behavioral phenotype of the B6D2F1 hybrid males, when crossed with C57BL/6J female mice, is inherited by their male offspring. Additionally, APP, tau, and synaptophysin were elevated in in the medial preoptic area in both the B6D2F1 and BXB_1 maters relative to the B6D2F1 and BXB_1 non-maters, respectively, suggesting a potential genetic mechanism for the inheritance of steroid-independent male sexual behavior.

Introduction

An individual's fitness and contribution of genes to the next generation depends on sexual behavior, which itself has been subject to strong selection throughout the course of evolution. In most rodents, male sexual behavior is strictly dependent upon the presence of gonadal steroids [reviewed in (Hart, 1974; Hull et al., 2006)]. In contrast, other vertebrates, particularly larger mammals including humans, do not have an absolute requirement for gonadal steroids. Individual variation in response to castration is one of the defining characteristics of several hormone-dependent social behaviors. It is not unusual for men to demonstrate male sexual behavior years after surgical or chemical castration (Heim and Hursch, 1979).

Unlike most laboratory rodents, a significant proportion (~30%) of B6D2F1 hybrid male mice (offspring of C57BL/6 dam X DBA/2 sire F0 cross) retains copulatory behavior for as many as 25 weeks after orchidectomy (herein after referred to as maters; (Clemens et al., 1988; McGill and Manning, 1976; Thompson et al., 1976). Several studies have utilized the hybrid B6D2F1 mouse to investigate potential neuroendocrine mechanisms which may govern gonadal steroid-independent male sexual behavior. A comparison of the hormonal profile of maters vs. non-maters revealed that there were no differences in plasma T, estradiol (E2, which is converted from T by aromatase in the brain), and dihydrotestosterone (DHT; non-aromatizable metabolite of T) concentrations – all of which were at low or undetectable measures in circulation (Clemens et al., 1988; Sinchak et al., 1996). mRNA levels of estrogen receptor-alpha (ERα), androgen receptor (AR), and aromatase enzyme in the medial preoptic area (MPOA), an area critical for male sexual behavior, were also equivalent between maters and non-maters (Park et al., 2009). Finally, maters administered flutamide (androgen receptor antagonist), letrozole (aromatase inhibitor), or ICI

* Corresponding author at: Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115, United States.
E-mail address: cmcinnis1@partners.org (C.M. McInnis).
[1] Department of Pathology, Brigham and Women's Hospital Boston, Harvard Medical School, Massachusetts 02115.
[2] Department of Neurobiology and Anatomy, University of Utah, School of Medicine, Salt Lake City, UT 84112.
[3] Department of Biological Sciences, North Carolina State University, Raleigh, NC 27695-7614.

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182,780 (an ER antagonist), continued to copulate, demonstrating that any remaining levels of non-gonadal steroids were not responsible for the persistent copulation in the maters (Park et al., 2009). The difficulty in determining whether neuroendocrine differences regulate persistent copulation in these males has led to studies investigating alternative mechanisms.

A gene expression analyses of the MPOA between B6D2F1 hybrid maters and non-maters revealed over 500 differentially expressed genes (Park et al., 2010). We further investigated two genes in particular, amyloid beta (A4) precursor protein (*App*, located on chromosome 16 in the mouse) and microtubule associated protein tau (*Mapt*, located on chromosome 11 in the mouse), which are normally associated with Alzheimer's Disease. Expression of these genes and proteins was significantly higher in maters relative to the non-maters (Park et al., 2010). Furthermore, transgenic male mice that overexpressed either APP or tau, the two protein products of *App* and *Mapt*, respectively, displayed enhanced sexual behavior after orchidectomy compared to control wildtype littermates, demonstrating that the relationship of APP and tau with male sexual behavior was beyond correlational (Bharadwaj et al., 2013; Park et al., 2010). Because the normal function of both genes have been associated with synaptic plasticity, this led to an investigation that revealed that relative to B6D2F1 non-maters, the maters exhibited higher levels of synaptophysin, a protein generally enriched in synapses and associated with synaptic connectivity (synaptophysin gene located on the X chromosome) (Bharadwaj et al., 2013).

One assumption underlying any genetic mechanisms of steroid-independent male sexual behavior is that behavioral qualities of sires should pass to male offspring, and thus we designed a study to test the hypothesis that the behavioral phenotype of male offspring would be more closely related to that of the sire's behavioral phenotype. In addition we assessed levels of APP, tau and synaptophysin in the MPOA of both the sires and sons to determine the relationship between the behavioral trait of steroid-independent male sexual behavior and the protein levels of these candidate genes.

Methods

Animals

Male B6D2F1 hybrid mice ($n = 36$; *Mus musculus*) were bred by crossing C57BL/6J (B6) females with DBA/2J males. Adult hybrid male mice were crossed with another cohort of B6 females to produce male BXB_1 offspring. All male mice were produced at the University of Virginia, Charlottesville, weaned at 20–21 days and housed singly until the onset of the experiment (between 50 and 80 days of age). All of the mice were maintained on a 12:12 light:dark cycle (light off at 1200 h EST) and received food (Harlan Diet 8604; Harlan Teklad, Madison, WI) and water ad libitum. All procedures were performed according to the AALAC guidelines and approved by the University of Virginia Animal Use and Care Committee. All surgeries were performed while the animals were anesthetized with isoflurane.

Sexual experience prior to orchidectomy and mating with B6 females

Although prior mating experience is not necessary for the expression of steroid-independent male sexual behavior in B6D2F1 hybrid male mice, it does increase the proportion of males that demonstrate male sexual behavior after long-term orchidectomy (Manning and Thompson, 1976). Thus, B6D2F1 hybrid males were provided sexual experience with hormonally primed stimulus female B6 mice on at least three separate occasions prior to being paired overnight with an ovary-intact B6 female mouse. The next day, all B6D2F1 hybrid males underwent orchidectomy. Of the 36 B6 dams paired with B6D2F1 males, 29 produced litters. When the male BXB_1 offspring reached adulthood, they were also provided sexual experience with hormonally primed stimulus female B6 mice on at least three separate occasions prior to being orchidectomized.

Behavioral testing after orchidectomy

B6D2F1 hybrid males were tested for male sexual behavior every two weeks for 8 weeks after orchidectomy. Males were considered to be maters if they demonstrated mounts, intromissions and the ejaculation reflex on the last behavioral test ($n = 8$). Males were considered non-maters ($n = 7$) if they did not display any of the components of male sexual behavior during the last test. Any males that did not demonstrate consistent behavior and did not fulfill either of the criteria were classified as behavioral 'intermediates' and were excluded from analyses in this study.

Orchidectomized male BXB_1 offspring were paired every two weeks with a stimulus B6 female in their home cage for two hours. Eight weeks after orchidectomy, BXB_1 males were tested for gonadal steroid-independent male sexual behavior. Males were considered to be maters if they demonstrated mounts, intromissions and the ejaculation reflex ($n = 10$) and non-maters ($n = 27$) if they did not display any of the components of male sexual behavior. Similar to their sires, any males that did not meet either criteria were classified as behavioral intermediates and were not included in the analyses of this study.

Testing for male sexual behavior was conducted as previously described in (Park et al., 2009). All tests were conducted under dim red lights during the dark phase of the light/dark cycle. All males were placed singly into Plexiglas arenas (17.8 cm w × 17.8 cm h × 25.4 cm l) with their home cage bedding which had not been changed for 2 weeks. After the males habituated to the arena for one hour, tests began with the introduction of a hormone-treated stimulus B6 female mouse into the arena. Stimulus females were ovariectomized and injected subcutaneously with 5 μg estradiol benzoate (dissolved in sesame oil) 48 h prior to testing. Three to five hours prior to testing, stimulus females were injected subcutaneously with 5 μg progesterone. Once the male mounted, the test continued to a criterion of a successful ejaculatory reflex or for 120 min, whichever occurred first. If the stimulus female became unreceptive during testing she was replaced with a receptive female.

All tests were videotaped and scored by an observer blind to the classification of the individuals. During each behavioral test, the behavioral components recorded were: mount latency (ML; time from the introduction of a receptive female to the first mount), intromission latency (IL; time from the introduction of a receptive female to the first intromission), and ejaculation latency (EL; interval between the first intromission and ejaculation).

Western blot analyses

At the completion of the sexual behavior tests, all males were sacrificed. Brains were dissected, rapidly frozen, and then stored at −80 °C. Frozen brains were shipped to the University of MA, Boston, and all procedures were conducted in accordance with our animal protocol, approved by the University of MA, Boston IACUC. Brains dissected from B6D2F1 maters that ejaculated on both behavioral tests conducted on weeks 6 and 8 post-castration ($n = 5$), along with brains from one of their BXB_1 sons that were identified as maters were chosen to analyze protein levels in the MPOA and frontal cortex. Additionally, brains from B6D2F1 non-maters along with brains from one of their BXB_1 sons that were identified as non-maters were chosen for Western blot analyses ($n = 5$/group; B6D2F1 non-maters were randomly chosen using a random number generator to match the sample size of the maters). All brains were cut into 100 μm thick coronal sections with a Leica cryostat. Based on the Franklin and Paxinos mouse brain atlas (Franklin and Paxinos, 1997), the MPOA and frontal cortex were dissected and homogenized in Thermo Scientific Tissue Protein