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Age, sex, and gonadal hormones differently influence anxiety- and depression-related behavior during puberty in mice

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ABSTRACT

Anxiety and depression symptoms increase dramatically during adolescence, with girls showing a steeper increase than boys after puberty onset. The timing of the onset of this sex bias led us to hypothesize that ovarian hormones contribute to depression and anxiety during puberty. In humans, it is difficult to disentangle direct effects of gonadal hormones from social and environmental factors that interact with pubertal development to influence mental health. To test the role of gonadal hormones in anxiety- and depression-related behavior during puberty, we manipulated gonadal hormones in mice while controlling social and environmental factors. Similar to humans, we find that mice show an increase in depression-related behavior from pre-pubertal to late-pubertal ages, but this increase is not dependent on gonadal hormones and does not differ between sexes. Anxiety-related behavior, however, is more complex during puberty, with differences that depend on sex, age, behavioral test, and hormonal status. Briefly, males castrated before puberty show greater anxiety-related behavior during late puberty compared to intact males, while pubertal females are unaffected by ovariectomy or hormone injections in all assays except the marble burying test. Despite this sex-specific effect of pubertal hormones on anxiety-related behavior, we find no sex differences in intact young adults, suggesting that males and females use separate mechanisms to converge on a similar behavioral phenotype. Our results are consistent with anxiolytic effects of testicular hormones during puberty in males but are not consistent with a causal role for ovarian hormones in increasing anxiety- and depression-related behavior during puberty in females.

1. Introduction

Anxiety and depression symptoms increase in early adolescence, particularly in girls (Altemus et al., 2014; Costello et al., 2011; Silberg et al., 1999). Gonadal hormones and sexual maturation of the body are hypothesized to play a role in this increase, but the causal route by which this effect occurs is debated (Graber, 2013). Multiple studies have found that pubertal status in girls is a better predictor of anxiety and depression symptoms than age (Angold et al., 1998; Reardon et al., 2009), and girls who start puberty earlier than their peers have higher risk of various negative mental health outcomes, including anxiety and depression symptoms (Graber, 2013; Mendle et al., 2007). Data in boys is less consistent, with some studies demonstrating associations between pubertal status/timing and mental health outcomes, but the direction of these relationships differs across studies (Graber, 2013; Mendle and Ferrero, 2012). Importantly, in both boys and girls, social and environmental factors interact with pubertal status and timing to impact mental health outcomes, making it difficult to distinguish direct effects of hormones from other factors (Caspi and Moffitt, 1991; Deardorff et al., 2013; Ge et al., 2002; Ge et al., 1996, 2001; Obeidallah et al., 2004; Rudolph and Troop-Gordon, 2010).

Given the difficulty of disentangling multiple variables in humans, it is valuable to turn to animal models. In rodents, gonadal hormones can be manipulated through gonadectomy and hormone injection to test for causal relationships between hormones and anxiety- and depression-related behavior. In most studies in adult female rodents, estradiol, progesterone, and androgens reduce anxiety- and depression-related behavior (Bitran et al., 1995; Frye and Wawrzycki, 2003; Hilakivi-Clarke, 1996; Lund et al., 2005; Martinez-Mota et al., 1999; Mora et al., 1996; Okada et al., 1997; Walf et al., 2009). A smaller number of studies, again in adult female rodents, have found anxiogenic effects of progesterone (Galeeva and Tuohimaa, 2001) and estradiol (Morgan and Pfaff, 2002). In adult male rodents, both androgens and estrogens reduce anxiety- and depression-related behavior (Chen et al., 2014; Frye

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et al., 2008; Frye and Wawrzycki, 2003; Hilakivi-Clarke, 1996; Wainwright et al., 2016).

Despite the large number of studies on the role of gonadal hormones in anxiety- and depression-related behavior in adult animals, data in peripubertal animals is lacking. It is critical to test hormone effects specifically during the developmental window surrounding puberty, because gonadal hormones can elicit starkly different effects at different ages (Sisk and Zehr, 2005). For example, certain gonadal hormone metabolites can influence anxiety-related behavior in opposite directions during puberty compared to adulthood (Shen et al., 2007), suggesting that results from adult animals cannot simply be extrapolated to pubertal animals. Anxiety- and depression-related behavior also change across adolescence in male mice, but the role of gonadal hormones and potential sex differences in this process are unknown (Hefner and Holmes, 2007). Finally, puberty is thought to be a sensitive period for hormone-related circuit reorganization (Byrne et al., 2016; Cunningham et al., 2002; Peper and Dahl, 2013; Piekarski et al., 2017a, 2017b; Romeo, 2003; Schulz et al., 2009; Sisk and Zehr, 2005), underscoring the importance of understanding how pubertal hormones interact with age and sex to influence the development of brain and psychopathology.

In the current project, our goal was to determine if gonadal hormones play a causal role in the development of anxiety- and depression-related behavior at puberty in both males and females. To this end, we manipulated peripubertal gonadal hormone exposure and measured anxiety-related behavior, depression-related behavior, and repetitive/compulsive behavior with the elevated plus maze (EPM), open field test, forced swim test (FST), and the marble-burying test. We found that gonadal hormones alter anxiety-related behavior in a sex-specific manner during puberty. Males castrated before puberty showed greater anxiety-related behavior than intact males, while females were unaffected by pre-pubertal ovariectomy or hormone injections in all assays except the marble burying test. In contrast, depression-related behavior increased from pre-pubertal to late-pubertal ages but was unaffected by pre-pubertal gonadectomy and did not differ between sexes. Interestingly, despite the sex-specific effect of gonadectomy on anxiety-related behavior during puberty, we found no sex differences in intact male and female mice tested in young adulthood. This pattern indicates that a similar adult behavioral phenotype may be achieved by different mechanisms in male and female mice (De Vries and Panzica, 2006). In conclusion, our data show that an increase in depression-related behavior at puberty can be modeled in mice. Furthermore, our mouse models suggest anxiolytic effects of testicular hormones during puberty but do not support a causal role for ovarian hormones in the etiology of anxiety and depression symptoms during puberty. We discuss alternate factors that may explain sex differences observed in humans.

2. Methods

2.1. Animals

Male and female C57BL/6N mice (Charles River Laboratories, Wilmington, MA) were bred in our animal facility and were housed on a 12 h/12 h reverse light-dark cycle (lights on at 10pm). Mice were weaned at postnatal day (P) 21 and housed in groups of 2–5 same-sex siblings with nesting material and a paper hut. All mice had *ad libitum* access to food and water in their home cages. All procedures were approved by the UC Berkeley Animal Care and Use Committee.

2.2. Experimental groups

In the first set of experiments, peripubertal changes in anxiety- and depression-related behavior were studied in separate groups of male and female mice tested either before puberty (first day of testing on P24) or during late puberty (first day of testing between P40 and P47) (Fig. 1A).

To test if gonadal hormones mediate peripubertal changes in anxiety- and depression-related behavior, male and female mice were gonadectomized or sham gonadectomized prior to puberty (details in Section 2.3) and were tested for anxiety- and depression-related behavior during late pubertal ages (first day of testing between P40 and P47) (Fig. 2A).

To test if early-onset puberty could alter anxiety- and depression-related behavior (Fig. 3), female mice were injected with estradiol and progesterone or oil vehicle to induce early puberty onset (details in Section 2.4) and were tested for anxiety- and depression-related behavior at ages when vehicle control animals were still pre-pubertal (first day of testing on P27) (Fig. 3A).

To test whether sex differences in anxiety- and depression-related behavior would emerge or persist into early adulthood, male and female mice with intact gonads were tested in young adulthood (first day of testing between P69 and P83) (Fig. 4A).

2.3. Gonadectomies

Surgeries took place on P24 or P25, before puberty onset. Prior to surgery, mice were injected with 0.05 mg/kg buprenorphine and 10 mg/kg meloxicam subcutaneously. During surgery, animals were anesthetized with 1–2% isoflurane. The incision area was shaved and scrubbed with ethanol and betadine. Ophthalmic ointment was placed over the eyes to prevent drying. A 1 cm incision was made with a scalpel in the lower abdomen across the midline to access the abdominal cavity. For ovariectomies, the ovaries were clamped off from the uterine horn with locking forceps and ligated with sterile sutures. After ligation, ovaries were excised with a scalpel. For castrations, the blood supply to each testis was clamped with locking forceps, after which the testes were ligated with sterile sutures and excised with a scalpel. The muscle and skin layers were then sutured, and wound clips were placed over the incision for 7–10 days to allow the incision to heal. An additional injection of 10 mg/kg meloxicam was given 12–24 h after surgery. Sham control surgeries were identical to ovariectomies and castrations except that the ovaries/testes were simply visualized and were not clamped, ligated or excised. Mice were allowed to recover on a heating pad until ambulatory and were post-surgically monitored for 7–10 days to check for normal weight gain and signs of discomfort/distress. No mice were eliminated from study due to surgical complications.

2.4. Mouse model of early female puberty

To advance age at puberty onset, gonadally intact females were injected with 17 beta-estradiol (0.01 mg/kg subcutaneous) or vehicle at P24. At P26, mice were injected with progesterone (20 mg/kg subcutaneous) or vehicle (Piekarski et al., 2017a). This treatment advances first peripubertal exposure to gonadal steroids and is sufficient to induce endogenous puberty (Ramirez and Sawyer, 1965; Smith and Davidson, 1968).

Vehicle- and hormone-treated mice were visually assessed for vaginal opening, an indicator of puberty onset in female mice, after the last behavior test was completed on P28. All hormone-treated mice had undergone vaginal opening on P28, while all but one vehicle-treated mouse had not yet undergone vaginal opening at P28. The one vehicle-treated mouse that had undergone vaginal opening by P28 was excluded from all analyses.

2.5. Behavioral test battery

Mice were gently handled for 1 min each day for 2 days before the start of behavioral testing to habituate them to handling.

All groups of mice experienced the same behavior test battery. Behavior testing took place over 2 consecutive days. On the first day,