Title

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

Authors

Boivin, Josiah R
Piekarski, David J
Wahlberg, Jessica K
et al.

Publication Date

2017-11-01

DOI

10.1016/j.psyneuen.2017.08.009

Peer reviewed

HHS Public Access

Author manuscript

Psychoneuroendocrinology. Author manuscript; available in PMC 2019 June 27.

Published in final edited form as:

Psychoneuroendocrinology. 2017 November ; 85: 78–87. doi:10.1016/j.psyneuen.2017.08.009.

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

Josiah R. Boivin[#1], **David J. Piekarski**[#2], **Jessica K. Wahlberg**[2], and **Linda Wilbrecht**[2,3,4]

[1.]UC San Francisco, Neuroscience Graduate Program, 1550 4th St., San Francisco, CA 94158, USA

[2.]UC Berkeley, Department of Psychology, 16 Barker Hall, Berkeley, CA 94720, USA

[3.]UC Berkeley, Helen Wills Neuroscience Institute, 16 Barker Hall, Berkeley, CA 94720, USA

[#] These authors contributed equally to this work.

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 at 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.

[4.]Address for correspondence: Linda Wilbrecht, PhD, 16 Barker Hall, UC Berkeley, Berkeley, CA 94720, (510) 600-3560, wilbrecht@berkeley.edu.

[*]Contributed equally

Conflicts of interest: none

Keywords

Puberty; anxiety; depression; gonadal hormones; sex differences; psychopathology

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 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; Piekarski et al., 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 cause 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 12h/12h 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 0.05mg/kg buprenorphine and 10mg/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 10mg/kg meloxicam was given 12–24 hours 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 (0.01mg/kg subcutaneous) or vehicle at P24. At P26, mice were injected with progesterone (20mg/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 minute 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, mice were tested on the elevated plus maze (EPM) and then immediately transferred to the open field test. On the second day, mice were tested on the marble burying test and then immediately transferred to the forced swim test (FST). All testing took place during the last 3 hours of the light cycle (7am-10am). On each testing day, mice were allowed to habituate to the testing room in their home cages for 30 minutes before testing began.

2.5.1. Elevated plus maze (EPM)—The mouse was placed in the center of an elevated plus maze and allowed to explore freely for 10 minutes. The EPM was made of opaque white acrylic consisting of 2 open arms (30cm long by 6cm wide), 2 closed arms (30cm long by 6cm wide, with 20.5cm high walls on the sides and end of each arm), and a center square (6cm by 6cm). The closed arms of the EPM were attached to a stable platform raised 66cm from the floor. The number of entries into each zone (i.e. center, open, or closed) and the total time spent in each zone was analyzed using EthoVision software (Noldus; Sacramento, CA). The chamber was cleaned with 70% ethanol and allowed to dry between mice. The EPM was performed with room lights on (260 lux).

2.5.2. Open field test: Immediately after finishing the elevated plus maze, the mouse was transferred to a clear acrylic open field arena (42cm by 42cm floor dimensions, with 4 walls that were each 30.5cm high, and no ceiling) for 15 minutes. The acrylic open field arena was located inside a sound-attenuated chamber (Med Associates; Fairfax, VT) with lights on (40 lux inside the chamber). Locomotion was monitored using infrared beam breaks (Versamax, AccuScan Instruments; Columbus, OH). Total distance covered and percent of time spent in the center (defined as >7.875cm from the edges of the chamber, i.e. 3 grid squares in Versamax analysis software) were analyzed. The chamber was cleaned with 70% ethanol and allowed to dry between mice. Mice were returned to the home cage immediately after the open field test.

2.5.3. Marble burying test—After 30 minutes of habituation to the testing room, each mouse was moved separately from its home cage to a clean cage containing 3cm deep fresh bedding and 20 marbles arranged in 5 evenly spaced rows on top of the bedding. A lid was placed over the cage, and the mouse was left undisturbed for 30 minutes. The test was performed with room lights on (45 lux inside the cage with the lid on). The number of buried marbles was recorded by the experimenter. “Buried” was defined as >50% covered by bedding. A fresh cage with fresh bedding was used for each mouse.

2.5.4. Forced swim test—Immediately after completing the marble burying test, each mouse was placed in a 2L glass beaker (13cm diameter, 18cm height) containing 10cm deep, room temperature water (21 degrees C). Mice were tested individually. The beaker was located inside a sound-attenuated chamber (Med Associates; Fairfax, VT) with lights on (40

lux inside the chamber). The mouse was left in the beaker for 6 minutes and was monitored by video (Microsoft LifeCam; Redmond, WA). After 6 minutes, the mouse was removed from the water, gently patted with paper towel, and placed in a warmed cage lined with paper towel until dry. Glass beakers were cleaned with 70% ethanol and allowed to dry between mice. Fresh room temperature (21 degrees C) water was used for each mouse. Time spent immobile, defined as the absence of movements except those necessary for balancing the mouse and keeping its head above water (e.g. paddling of a paw for balance) was calculated for the last 4 minutes of each 6-minute video. Each video was scored manually by 2 experimenters blind to the mouse's experimental group. The 2 raters maintained an inter-rater correlation of R=0.96 and a mean difference of 12.45 +/− 8.16 seconds. The mean of the 2 raters' scores was used for each mouse.

It is possible that the behavior of the mice in the open field and FST was affected by their experience in the preceding EPM and marble burying tests. We therefore chose to perform the less anxiogenic test first on each day. We performed the EPM before the open field test because the EPM offers closed arms that are considerably more sheltered than any portion of the open field, and we performed the marble burying test before the FST because we expect that exploring a cage containing marbles is less anxiogenic than swimming in water.

2.6. Statistical comparisons

All statistical comparisons were performed using GraphPad Prism (GraphPad, San Diego, CA). Data were tested for normality using a D'Agostino and Pearson omnibus normality test, and groups that were directly compared to each other were tested for equal variance using an F test. For comparisons between 2 groups, a t test was used when data were normally distributed, and Welch's correction was applied when variance was unequal. Groups that were not normally distributed were compared using a Mann Whitney U test. When more than 2 groups were considered simultaneously (Fig. 1 and Supplemental Fig. S3), a 2-way ANOVA was performed with age and sex as factors (Fig. 1) or with sex and gonadal status as factors (Supplemental Fig. S3). Post-hoc comparisons were then performed as described above (i.e. using a t test or Mann Whitney U test) with Bonferroni corrections for multiple comparisons.

For gonadally intact P40 mice, unmanipulated animals (i.e. those that received no surgery) did not differ from sham-operated mice in any measure tested (Supplemental Fig. S1). Unmanipulated and sham animals were therefore combined into a single gonadally intact P40 male group and a single gonadally intact P40 female group for all analyses.

For the analysis shown in Fig. 2, planned comparisons were performed to ask 3 *a priori* questions: 1) Does castration alter anxiety- and depression-related behavior in males, 2) Does ovariectomy alter anxiety- and depression-related behavior in females, and 3) Does the effect of gonadectomy differ between males and females. As these were separate *a priori* questions, multiple comparisons corrections were not applied to these tests.

One animal was excluded from the open field analysis due to equipment failure that resulted in data loss. One animal was excluded from EPM analysis due to failure to record the video.

One female mouse was excluded from all analyses due to precocious puberty onset, as described in section 2.4. The final n for each group is listed in Supplemental Table S1.

3. Results

3.1. Peripubertal changes in anxiety- and depression-related behavior in mice undergoing natural puberty

We first asked whether anxiety- and depression-related behavior changes with age from pre-pubertal (P24) to late-pubertal (P40) ages in male and female mice undergoing natural puberty. To this end, separate groups of mice were tested at P24 or P40 on the elevated plus maze (EPM), open field, forced swim test (FST), and marble burying test (Fig. 1A). We found a main effect of sex ($F_{1,72}$=4.92, p=0.03) but no main effect of age ($F_{1,72}$=1.49, p=0.23) or age by sex interaction ($F_{1,72}$=0.07, p=0.79) for the percent of time spent in the open arms of the EPM (Fig. 1B). Post-hoc comparisons revealed that males spent more time in the open arms than females at P40 (t_{37}=2.36, uncorrected p=0.02) but not at P24 (U=51, uncorrected p=0.25). For the percent of time spent in the center of the open field (Fig. 1C), we found no main effect of sex ($F_{1,72}$=0.03, p=0.86), age ($F_{1,72}$=0.14, p=0.71), or interaction ($F_{1,72}$=0.85, p=0.36). In the FST (Fig. 1D), P40 mice spent more time immobile than P24 mice ($F_{1,72}$=9.32, p=0.003), but male and female mice did not differ from each other ($F_{1,72}$=0.11, p=0.74), and there was no age by sex interaction ($F_{1,72}$=1.76, p=0.19). Post-hoc comparisons of P24 to P40 within each sex did not remain significant after Bonferroni corrections for multiple comparisons (t_{12}=2.29, uncorrected p=0.04 for males; U=140, uncorrected p=0.19 for females). Similarly, in the marble burying test (Fig. 1E), P40 mice buried more marbles than P24 mice ($F_{1,72}$=7.60, p=0.007), but there was no effect of sex ($F_{1,72}$=0.37, p=0.55) and no interaction ($F_{1,72}$=0.12, p=0.74). Post-hoc comparisons revealed that P40 males buried more marbles than P24 males (t_{29}=2.80, uncorrected p=0.009), while a comparison of P40 to P24 females did not reach significance (U=136, uncorrected p=0.15). Effects of age or sex on performance in the EPM, FST, and marble burying test were not explained by generalized locomotor differences, as there was no effect of age, sex, or age by sex interaction in locomotor distance covered in the open field (Supplemental Fig. S2A; $F_{1,72}$=0.33, p=0.57 for age; $F_{1,72}$=1.28, p=0.26 for sex; $F_{1,72}$=1.80, p=0.18 for interaction).

3.2. Effects of pre-pubertal gonadectomy on anxiety- and depression-related behavior at late-pubertal ages

We next asked whether gonadal hormone exposure during puberty affects anxiety- and depression-related behavior. Male and female mice underwent gonadectomy or sham gonadectomy before puberty onset (P24) and were tested for behavior at P40, when gonadally intact mice were in late puberty (Fig. 2A). Analysis was designed to answer 3 *a priori* questions: 1) Does pre-pubertal castration alter anxiety- and depression-related behavior in P40 male mice, 2) Does pre-pubertal ovariectomy alter anxiety- and depression-related behavior in P40 female mice, and 3) Does the effect of gonadectomy on anxiety- and depression-related behavior differ between males and females. To enable direct comparison of the effect of gonadectomy in males to that in females, values for each sex were normalized to the mean intact value for that sex (Fig. 2). As a complement to the analysis of

normalized data presented here, analysis of raw data using a 2-way ANOVA is presented in Supplemental Fig. S3.

3.2.1. Effects of castration—Compared to intact males, castrated males spent less time in the open arms of the EPM (Fig. 2B; t_{32}=4.23, p=0.0002) and showed a trend toward spending less time in the center of the open field (Fig. 2C; t_{33}=1.78, p=0.08). Effects of castration on performance in the EPM and open field were not explained by generalized locomotor effects, because castrated males did not differ from intact males in locomotor distance covered in the open field (Supplemental Fig. S2C; U=103, p=0.23). Castration did not affect the percent of time spent immobile in the FST (Fig. 2D; U=94.00, p=0.13) or the percent of marbles buried in the marble burying test (Fig. 2E; t_{33}=0.34, p=0.73).

3.2.2. Effects of ovariectomy: Ovariectomized (OVX) females did not differ from intact females in the percent of time spent in the open arms of the EPM (Fig. 2B; t_{42}=1.06, p=0.30) or in the center of the open field (Fig. 2C; t_{42}=0.38, p=0.70). OVX also did not affect the percent of time spent immobile in the FST (Fig. 2D; U=210.0, p=0.86). In the marble burying test, OVX females buried fewer marbles than intact females (Fig. 2E; U=128.5, p=0.02). This effect cannot be explained by generalized locomotor effects of ovariectomy, as OVX females did not differ from intact females in locomotor distance covered in the open field (Supplemental Fig. S2B; t_{42}=0.46, p=0.65).

3.2.3. Comparison of gonadectomy effect in males versus females: Normalizing the values for gonadectomized mice to those of intact mice within each sex enabled direct comparison of the effect of gonadectomy between males and females. The effect of gonadectomy differed between males and females in the EPM (Fig. 2B; t_{20}=4.88, p<0.0001) and marble-burying test (Fig. 2E; U=37.00, p=0.0089), but the effect of gonadectomy did not differ between males and females in the open field test (Fig. 2C; t_{25}=1.51, p=0.14) or the FST (Fig. 2D; U=66.00, p=0.25).

3.3. Effects of pre-pubertal hormone exposure in females

Due to the association between early-onset puberty and anxiety and depression in girls (see Introduction), we used a mouse model of early puberty (Piekarski et al., 2017a) to ask if earlier exposure to gonadal hormones alters anxiety- and depression-related behavior in females. Female mice were injected with 0.01mg/kg 17 beta-estradiol (subcutaneous) on P24 and 20mg/kg progesterone (subcutaneous) on P26 (Fig. 3A). This treatment regimen advances first peripubertal exposure to gonadal steroids and is sufficient to induce endogenous puberty (Ramirez and Sawyer, 1965; Smith and Davidson, 1968). Control littermates were injected with equivalent volumes of vehicle on P24 and P26. Hormone- and vehicle-treated mice were then tested on the EPM, open field, FST, and marble-burying test on P27-P28 (Fig. 3A). Hormone- and vehicle-treated mice did not differ in performance on the EPM (Fig. 3B; t_{25}=0.28, p=0.78), open field (Fig. 3C; t_{24}=0.02, p=0.98), FST (Fig. 3D; t_{25}=0.0074, p=0.99), or marble burying test (Fig. 3E; t_{25}=0.06, p=0.95).

3.4. Sex comparison in young adult animals

To test whether the sex difference we observed in the EPM performance of gonadally intact mice at P40 (Fig. 1B) would persist into adulthood, and to test whether additional sex differences would emerge post-pubertally, we tested gonadally intact male and female mice in young adulthood (P70) (Fig. 4A). We looked not only for a difference in means, but also for a difference in variance between the sexes, hypothesizing that females would show greater variance based on previous literature showing fluctuations in anxiety- and depression-related behavior across estrous cycle in rodents under some conditions (Diaz-Veliz et al., 1997; Frye et al., 2000; Frye and Walf, 2002; Galeeva and Tuohimaa, 2001; Marvan et al., 1997; Mora et al., 1996; Walf et al., 2009). We found no difference between young adult male and female mice in their performance on the EPM (Fig. 4B; U=57.00, p=0.46), open field (Fig. 4C; t_{23}=0.33, p=0.74), FST (Fig. 4D; t_{23}=0.78, p=0.44), or marble burying test (Fig. 4E; t_{23}=0.27, p=0.79). There were also no sex differences in the amount of within-group variance on the EPM ($F_{9,13}$=1.62, p=0.41), open field ($F_{9,14}$=1.19, p=0.74), FST ($F_{9,14}$=2.52, p=0.12), or marble burying test ($F_{9,14}$=1.37, p=0.58).

4. Discussion

We were motivated by age and sex differences in human mental health to use a mouse model to test for direct effects of gonadal hormones on anxiety- and depression-related behavior during puberty. Based on a striking increase in anxiety and depression symptoms in girls just after puberty onset (Graber, 2013), we were particularly interested in testing the hypothesis that the increase in ovarian hormones at puberty onset plays a causal role in increasing anxiety- and depression-related behavior. Our data do not support this hypothesis. We found that depression-related behavior did increase in mice from pre-pubertal to late-pubertal ages (Fig. 1D) but that this increase did not depend on gonadal hormones (Fig. 2D) and did not show a significant sex effect (Fig. 1D). In contrast, gonadal hormones did alter anxiety-related behavior in a sex-specific manner during puberty. Males castrated before puberty showed greater anxiety-related behavior on the EPM and open field during late puberty compared to intact males, while ovariectomy had no effect on these tests but decreased marble burying in females (Fig. 2). These 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 anxiety- and depression-related behavior during puberty in females. Injection of ovarian hormones to elicit earlier puberty in females did not affect anxiety- and depression-related behavior (Fig. 3). Finally, we observed no sex difference in anxiety- and depression-related behavior in gonadally intact animals tested in young adulthood (Fig. 4), suggesting that a similar adult behavioral phenotype may be achieved by different mechanisms in male and female mice (De Vries and Panzica, 2006).

4.1. Testicular hormones are anxiolytic during puberty

Our results suggest that testicular hormones are anxiolytic during puberty. Anxiolytic effects of testicular hormones during puberty may be critical for promoting the approach/exploratory behaviors necessary for foraging and navigating the transition to adult social behaviors. Future work could test whether puberty in males is a sensitive period for the anxiolytic effects of testicular hormones, or whether hormone replacement in adulthood can

rescue the effect of prepubertal castration. It is possible, for example, that testicular hormones influence learning processes related to approach/avoidance behaviors throughout puberty rather than simply having an acute, activational effect on anxiety-related behavior at the time of testing. This outcome would support the hypothesis that there is a sensitive period for gonadal hormones to influence the development of limbic circuitry (Cunningham et al., 2002; Peper and Dahl, 2013; Piekarski et al., 2017a; Piekarski et al., 2017b; Romeo, 2003; Schulz et al., 2009; Sisk and Zehr, 2005).

4.2. Effects of pubertal ovarian hormones

Ultimately, both male and female mice must take risks during puberty to successfully navigate the transition from the natal nest to independent foraging and adult social behavior. While our results suggest that testicular hormones may facilitate exploratory and approach behaviors, as shown by reduced exploration of the 'risky' open arms of the EPM and center of the open field after castration, no change in these behaviors was observed in females after ovariectomy or injection of ovarian hormones. It is possible that in female mice, androgens also influence behavior, but the source of these androgens in females may be adrenal rather than gonadal. To examine this possibility, future studies could test the effects of adrenalectomy and androgen administration, or androgen receptor blockade after ovariectomy, on anxiety-related behavior in pubertal female mice.

While OVX did not alter performance on the EPM or open field, OVX did reduce marble burying in late-pubertal females (Fig. 2E). Marble burying models some aspects of anxiety and/or compulsive behavior (Njung'e and Handley, 1991; Thomas et al., 2009), but interpreting changes in marble burying is complex. While the OVX-induced decrease in marble burying could be interpreted as a reduction in anxiety-related or compulsive behavior, it is noteworthy that 9 out of the 15 OVX mice in our study buried zero marbles (compared to 8 out of 29 intact P40 females; $p=0.04$ for chi-square test for zero vs non-zero burying). An absence of burying, as opposed to a reduction in burying, may indicate an avoidant phenotype that is distinct from the reduction in marble burying observed during proestrus (i.e. a high estradiol/progesterone state) and with SSRI treatment in previous literature (Schneider and Popik, 2007). We speculate that both extremes of performance on the marble burying test, i.e. burying no marbles or burying all marbles, may represent anxiety-like phenotypes of either avoidant or compulsive responses to the marbles. In this interpretation, OVX may have induced an avoidant phenotype in our study, and ovarian hormones may promote active/approach responses to the potentially anxiogenic marbles. It is interesting to note, however, that ovarian hormone injection that elicited earlier puberty onset in females had no significant effect on marble burying (Fig. 3E).

4.3. Hormone-independent age effects and hormone by age interaction should both be considered

In our dataset, gonadectomy altered anxiety-related behavior, while depression-related behavior increased with age independent of gonadal hormone status. These results suggest that age and pubertal hormones may have dissociable effects on anxiety versus depression-related behavior at puberty.

However, we should be careful about concluding no effect of gonadal hormones at any stage in depression-related behavior. In adult male and female rodents, gonadectomy increases depression-related behavior in the FST (Frye and Wawrzycki, 2003; Hilakivi-Clarke, 1996; Okada et al., 1997). The contrast between our results in peripubertal animals and those in adults could reflect age differences in the effects of gonadal hormones. There are multiple examples in the literature of hormones affecting neural or behavioral outputs differently during puberty compared to adulthood (Schulz et al., 2009; Shen et al., 2007; Sisk and Zehr, 2005). Age differences in the effects of hormones in animal models are underscored by human studies showing different risk of anxiety and depression symptoms depending on the timing or tempo of puberty (Graber, 2013; Mendle and Ferrero, 2012; Mendle et al., 2007). Collectively, these data emphasize the importance of considering age, sex, and the current and 'historical' hormonal/pubertal status when considering the role of gonadal hormones in psychiatric disease states.

4.4. Interactions between biological and environmental factors: early puberty, sex differences and mental health risk

4.4.1. Early puberty and mental health risk—In contrast to the human literature showing greater risk of anxiety and depression in girls who experience early puberty (Graber, 2013; Mendle et al., 2007), we saw no effect of prepubertal hormone treatment on anxiety- and depression-related behavior in female mice (Fig. 3). Several human studies have highlighted the importance of environmental and social factors that mediate or moderate the relationship between pubertal timing and mental health outcomes. For example, recent stressful life events, pre-existing psychiatric symptoms, and harsh family/neighborhood conditions amplify the degree to which early puberty predicts psychiatric/behavioral symptoms (Deardorff et al., 2013; Ge et al., 2002; Ge et al., 2001; Obeidallah et al., 2004; Rudolph and Troop-Gordon, 2010). In some studies, early puberty does not confer risk of psychiatric and behavioral symptoms unless specific environmental conditions are met. For example, in a racially diverse sample of adolescent girls, early puberty conferred risk of externalizing behavior only in subjects living in neighborhoods characterized by high concentrated disadvantage (Obeidallah et al., 2004). Similarly, in two illuminating studies, associating primarily with other girls rather than mixed-sex peer groups abolished the relationship between early puberty and later psychiatric/behavioral problems (Caspi and Moffitt, 1991; Ge et al., 1996). In support of these human studies, a large rodent literature indicates that the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes interact with each other and that the behavioral and neural effects of a variety of stressors differ by age, sex, and gonadal hormone status (Burke et al., 2017; Novais et al., 2017; Romeo, 2013; Romeo et al., 2016). Our current results complement prior rodent and human work and suggest that the relationship between early puberty and psychiatric symptoms in girls is not likely to be deterministically driven by gonadal hormones alone. Future rodent studies examining interactions between environmental stressors and gonadal hormones during puberty might reveal effects of pre-pubertal hormone exposure not shown in our data. For example, pre-pubertal hormone exposure may alter the effects of specific stressors on anxiety-like behavior in female mice, although the direction of these effects is difficult to predict based on data from adult rodents indicating that the effects of ovarian hormones on stress responses depend on the specific combination and doses of hormones administered

(Viau, 2002). While future rodent work can further illuminate the interactions between stress and gonadal hormone exposure during puberty, we speculate that negative mental health outcomes associated with early puberty in girls may also be related to the complex, gendered adversity that girls and women navigate.

4.4.2. Sex differences in post-pubertal mice and humans—While rates of anxiety and depression are greater in women than in men (Altemus et al., 2014), our study and the majority of previous rodent studies do not show greater anxiety- and depression-related behavior in adult females compared to males (Fig. 4) (Diaz-Veliz et al., 1997; Frye et al., 2000; Frye and Walf, 2002; Frye and Wawrzycki, 2003). Despite sex-specific effects of gonadal hormones on anxiety-related behavior during puberty (Fig. 2), males and females in our study converged on the same behavioral phenotype in young adulthood. It is possible that a latent sex difference in young adult mice could be revealed by the addition of specific environmental factors (McCarthy et al., 2012). For example, stress amplifies fluctuations in FST performance across the estrous cycle in adult female rats (Marvan et al., 1997), and similar interactions between stress and gonadal hormones could occur in relation to sex differences in anxiety- and depression-related behavior. The idea that stressors might unmask sex differences in anxiety- and depression-related behavior in rodents is supported by literature indicating that testosterone can suppress and estradiol can amplify stress responses under some conditions (Romeo et al., 2016; Viau, 2002). As in the case of mental health risks associated with early puberty, it is possible that human sex differences in anxiety and depression are not an inevitable result of the differing hormonal milieu between males and females, but may depend on gender socialization and environmental stressors that are modifiable.

4.5. Relevance to public health: hormone manipulation during puberty in humans

Several human phenomena highlight the importance of understanding the role of pubertal hormones in adolescent brain maturation. For example, the prevalent use of hormonal contraception by adolescent girls calls for research on the neural effects of these alterations in hormonal milieu during a potential sensitive period of brain maturation. Similarly, the growing practice of gender-affirming hormone treatment in transgender adolescents calls for research on the neurodevelopmental implications of delaying pubertal hormone exposure and/or administering exogenous hormones during puberty. Finally, the advancing age of puberty in girls (Herman-Giddens, 2006) calls for research on the neurodevelopmental implications of gonadal hormone exposure at ages that are typically pre-pubertal, particularly in light of the negative mental health outcomes associated with early puberty in girls. Animal research can not only reveal direct effects of hormones on brain maturation, but can also help to disentangle interactions between environmental stressors and gonadal hormone exposure.

4.6. Conclusion

To better understand how gonadal hormones at puberty onset impact depression and anxiety in humans, we manipulated gonadal hormones and tested male and female mice on anxiety- and depression-related behavior assays. We were particularly interested in determining if ovarian hormones enhanced anxiety- and depression-related behavior after puberty onset in a

standard laboratory environment where exposure to stressors was controlled. We found no evidence to support an independent, causal role for ovarian hormones in anxiety- and depression-related behavior at puberty.

We did observe an increase in depression-related behavior from pre-pubertal to late-pubertal ages, but this increase did not depend on gonadal hormones during puberty or show a main effect of sex. In contrast, anxiety-related behavior was sensitive to gonadal hormones during puberty in a sex-specific manner. Testicular hormones were anxiolytic during puberty on classic tests for anxiety-related behavior, while pubertal ovarian hormones did not affect performance on any test except marble burying. Interestingly, gonadally intact males and females converged on the same performance in all behaviors by young adulthood despite sex-specific effects of prepubertal gonadectomy.

Our findings highlight the importance of investigating the effects of gonadal hormones in both males and females specifically during puberty, where results may differ from those obtained from adult animals. Our results also invite further investigation of potential interactions between environmental stressors and pubertal development, as these interactions may be critical for shaping mental health outcomes in human adolescents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Julianna Deardorff, Ron Dahl, and Ahna Suleiman for valuable insights and discussion in the preparation of this manuscript.

Funding Sources

This research was funded by F32MH110107 (D.J.P.), a seed grant from the UC Consortium on the Developmental Science of Adolescence, and NSF 1640885 (L.W.).

References

Altemus M, Sarvaiya N, Neill Epperson C, 2014 Sex differences in anxiety and depression clinical perspectives. Frontiers in neuroendocrinology 35, 320–330. [PubMed: 24887405]

Angold A, Costello EJ, Worthman CM, 1998 Puberty and depression: the roles of age, pubertal status and pubertal timing. Psychological medicine 28, 51–61. [PubMed: 9483683]

Bitran D, Shiekh M, McLeod M, 1995 Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain GABAA receptors. Journal of neuroendocrinology 7, 171–177. [PubMed: 7606242]

Burke AR, McCormick CM, Pellis SM, Lukkes JL, 2017 Impact of adolescent social experiences on behavior and neural circuits implicated in mental illnesses. Neuroscience and biobehavioral reviews 76, 280–300. [PubMed: 28111268]

Byrne ML, Whittle S, Vijayakumar N, Dennison M, Simmons JG, Allen NB, 2016 A systematic review of adrenarche as a sensitive period in neurobiological development and mental health. Developmental cognitive neuroscience.

Caspi A, Moffitt TE, 1991 Individual differences are accentuated during periods of social change: the sample case of girls at puberty. Journal of personality and social psychology 61, 157–168. [PubMed: 1890586]

Chen CV, Brummet JL, Lonstein JS, Jordan CL, Breedlove SM, 2014 New knockout model confirms a role for androgen receptors in regulating anxiety-like behaviors and HPA response in mice. Hormones and behavior 65, 211–218. [PubMed: 24440052]

Costello EJ, Copeland W, Angold A, 2011 Trends in psychopathology across the adolescent years: what changes when children become adolescents, and when adolescents become adults? Journal of child psychology and psychiatry, and allied disciplines 52, 1015–1025.

Cunningham MG, Bhattacharyya S, Benes FM, 2002 Amygdalo-cortical sprouting continues into early adulthood: implications for the development of normal and abnormal function during adolescence. The Journal of comparative neurology 453, 116–130. [PubMed: 12373778]

De Vries GJ, Panzica GC, 2006 Sexual differentiation of central vasopressin and vasotocin systems in vertebrates: different mechanisms, similar endpoints. Neuroscience 138, 947–955. [PubMed: 16310321]

Deardorff J, Cham H, Gonzales NA, White RM, Tein JY, Wong JJ, Roosa MW, 2013 Pubertal timing and Mexican-origin girls' internalizing and externalizing symptoms: the influence of harsh parenting. Developmental psychology 49, 1790–1804. [PubMed: 23231686]

Diaz-Veliz G, Alarcon T, Espinoza C, Dussaubat N, Mora S, 1997 Ketanserin and anxiety levels: influence of gender, estrous cycle, ovariectomy and ovarian hormones in female rats. Pharmacology, biochemistry, and behavior 58, 637–642.

Frye CA, Edinger K, Sumida K, 2008 Androgen administration to aged male mice increases anti-anxiety behavior and enhances cognitive performance. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 33, 1049–1061. [PubMed: 17625503]

Frye CA, Petralia SM, Rhodes ME, 2000 Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3alpha, 5alpha-THP. Pharmacology, biochemistry, and behavior 67, 587–596.

Frye CA, Walf AA, 2002 Changes in progesterone metabolites in the hippocampus can modulate open field and forced swim test behavior of proestrous rats. Hormones and behavior 41, 306–315. [PubMed: 11971664]

Frye CA, Wawrzycki J, 2003 Effect of prenatal stress and gonadal hormone condition on depressive behaviors of female and male rats. Hormones and behavior 44, 319–326. [PubMed: 14613726]

Galeeva A, Tuohimaa P, 2001 Analysis of mouse plus-maze behavior modulated by ovarian steroids. Behavioural brain research 119, 41–47. [PubMed: 11164524]

Ge X, Brody GH, Conger RD, Simons RL, Murry VM, 2002 Contextual amplification of pubertal transition effects on deviant peer affiliation and externalizing behavior among African American children. Developmental psychology 38, 42–54. [PubMed: 11806701]

Ge X, Conger RD, Elder GH Jr., 1996 Coming of age too early: pubertal influences on girls' vulnerability to psychological distress. Child development 67, 3386–3400. [PubMed: 9071784]

Ge X, Conger RD, Elder GH Jr., 2001 Pubertal transition, stressful life events, and the emergence of gender differences in adolescent depressive symptoms. Developmental psychology 37, 404–417. [PubMed: 11370915]

Graber JA, 2013 Pubertal timing and the development of psychopathology in adolescence and beyond. Horm Behav 64, 262–269. [PubMed: 23998670]

Hefner K, Holmes A, 2007 Ontogeny of fear-, anxiety- and depression-related behavior across adolescence in C57BL/6J mice. Behavioural brain research 176, 210–215. [PubMed: 17098297]

Herman-Giddens ME, 2006 Recent data on pubertal milestones in United States children: the secular trend toward earlier development. International journal of andrology 29, 241–246; discussion 286–290. [PubMed: 16466545]

Hilakivi-Clarke L, 1996 Role of estradiol in alcohol intake and alcohol-related behaviors. Journal of studies on alcohol 57, 162–170. [PubMed: 8683965]

Lund TD, Rovis T, Chung WC, Handa RJ, 2005 Novel actions of estrogen receptor-beta on anxiety-related behaviors. Endocrinology 146, 797–807. [PubMed: 15514081]

Martinez-Mota L, Contreras CM, Saavedra M, 1999 Progesterone reduces immobility in rats forced to swim. Archives of medical research 30, 286–289. [PubMed: 10573629]

Marvan ML, Santana S, Chavez Chavez L, Bertran M, 1997 Inescapable shocks accentuate fluctuations of forced swimming immobility in different phases of the rat estrous cycle. Archives of medical research 28, 369–372. [PubMed: 9291632]

McCarthy MM, Arnold AP, Ball GF, Blaustein JD, De Vries GJ, 2012 Sex differences in the brain: the not so inconvenient truth. The Journal of neuroscience : the official journal of the Society for Neuroscience 32, 2241–2247. [PubMed: 22396398]

Mendle J, Ferrero J, 2012 Detrimental psychological outcomes associated with pubertal timing in adolescent boys. Developmental Review 32, 49–66.

Mendle J, Turkheimer E, Emery RE, 2007 Detrimental Psychological Outcomes Associated with Early Pubertal Timing in Adolescent Girls. Developmental review : DR 27, 151–171. [PubMed: 20740062]

Mora S, Dussaubat N, Diaz-Veliz G, 1996 Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. Psychoneuroendocrinology 21, 609–620. [PubMed: 9044444]

Morgan MA, Pfaff DW, 2002 Estrogen's effects on activity, anxiety, and fear in two mouse strains. Behavioural brain research 132, 85–93. [PubMed: 11853861]

Njung'e K, Handley SL, 1991 Evaluation of marble-burying behavior as a model of anxiety. Pharmacology, biochemistry, and behavior 38, 63–67.

Novais A, Monteiro S, Roque S, Correia-Neves M, Sousa N, 2017 How age, sex and genotype shape the stress response. Neurobiology of stress 6, 44–56. [PubMed: 28229108]

Obeidallah D, Brennan RT, Brooks-Gunn J, Earls F, 2004 Links between pubertal timing and neighborhood contexts: implications for girls' violent behavior. Journal of the American Academy of Child and Adolescent Psychiatry 43, 1460–1468. [PubMed: 15564815]

Okada M, Hayashi N, Kometani M, Nakao K, Inukai T, 1997 Influences of ovariectomy and continuous replacement of 17beta-estradiol on the tail skin temperature and behavior in the forced swimming test in rats. Japanese journal of pharmacology 73, 93–96. [PubMed: 9032138]

Peper JS, Dahl RE, 2013 Surging Hormones: Brain-Behavior Interactions During Puberty. Current directions in psychological science 22, 134–139. [PubMed: 26290625]

Piekarski DJ, Boivin JR, Wilbrecht L, 2017a. Ovarian Hormones Organize the Maturation of Inhibitory Neurotransmission in the Frontal Cortex at Puberty Onset in Female Mice. Current Biology Epub Date 06 6 2017.

Piekarski DJ, Johnson CM, Boivin JR, Thomas AW, Lin WC, Delevich K, E MG, Wilbrecht L, 2017b Does puberty mark a transition in sensitive periods for plasticity in the associative neocortex? Brain research 1654, 123–144. [PubMed: 27590721]

Ramirez VD, Sawyer CH, 1965 Advancement of puberty in the female rat by estrogen Endocrinology 76, 1158–1168. [PubMed: 14301523]

Reardon LE, Leen-Feldner EW, Hayward C, 2009 A critical review of the empirical literature on the relation between anxiety and puberty. Clinical psychology review 29, 1–23. [PubMed: 19019513]

Romeo RD, 2003 Puberty: a period of both organizational and activational effects of steroid hormones on neurobehavioural development. Journal of neuroendocrinology 15, 1185–1192. [PubMed: 14636181]

Romeo RD, 2013 The Teenage Brain: The Stress Response and the Adolescent Brain. Current directions in psychological science 22, 140–145. [PubMed: 25541572]

Romeo RD, Patel R, Pham L, So VM, 2016 Adolescence and the ontogeny of the hormonal stress response in male and female rats and mice. Neuroscience and biobehavioral reviews 70, 206–216. [PubMed: 27235079]

Rudolph KD, Troop-Gordon W, 2010 Personal-accentuation and contextual-amplification models of pubertal timing: predicting youth depression. Dev Psychopathol 22, 433–451. [PubMed: 20423552]

Schneider T, Popik P, 2007 Attenuation of estrous cycle-dependent marble burying in female rats by acute treatment with progesterone and antidepressants. Psychoneuroendocrinology 32, 651–659. [PubMed: 17561352]

Schulz KM, Molenda-Figueira HA, Sisk CL, 2009 Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. Hormones and behavior 55, 597–604. [PubMed: 19446076]

Shen H, Gong QH, Aoki C, Yuan M, Ruderman Y, Dattilo M, Williams K, Smith SS, 2007 Reversal of neurosteroid effects at alpha4beta2delta GABAA receptors triggers anxiety at puberty. Nature neuroscience 10, 469–477. [PubMed: 17351635]

Silberg J, Pickles A, Rutter M, Hewitt J, Simonoff E, Maes H, Carbonneau R, Murrelle L, Foley D, Eaves L, 1999 The influence of genetic factors and life stress on depression among adolescent girls. Archives of general psychiatry 56, 225–232. [PubMed: 10078499]

Sisk CL, Zehr JL, 2005 Pubertal hormones organize the adolescent brain and behavior. Frontiers in neuroendocrinology 26, 163–174. [PubMed: 16309736]

Smith ER, Davidson JM, 1968 Role of estrogen in the cerebral control of puberty in female rats. Endocrinology 82, 100–108. [PubMed: 5634983]

Thomas A, Burant A, Bui N, Graham D, Yuva-Paylor LA, Paylor R, 2009 Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. Psychopharmacology 204, 361–373. [PubMed: 19189082]

Viau V, 2002 Functional cross-talk between the hypothalamic-pituitary-gonadal and -adrenal axes. Journal of neuroendocrinology 14, 506–513. [PubMed: 12047726]

Wainwright SR, Workman JL, Tehrani A, Hamson DK, Chow C, Lieblich SE, Galea LA, 2016 Testosterone has antidepressant-like efficacy and facilitates imipramine-induced neuroplasticity in male rats exposed to chronic unpredictable stress. Hormones and behavior 79, 58–69. [PubMed: 26774465]

Walf AA, Koonce C, Manley K, Frye CA, 2009 Proestrous compared to diestrous wildtype, but not estrogen receptor beta knockout, mice have better performance in the spontaneous alternation and object recognition tasks and reduced anxiety-like behavior in the elevated plus and mirror maze. Behavioural brain research 196, 254–260. [PubMed: 18926853]

Highlights

- Depression-related behavior increased in mice after puberty onset
- Pre-pubertal gonadectomy did not affect the increase in depression-related behavior
- Ovarian hormones in females did not enhance anxiety- or depression-related behavior
- Pre-pubertal castration enhanced anxiety-related behavior in males
- Males and females showed similar anxiety-related behavior in young adulthood

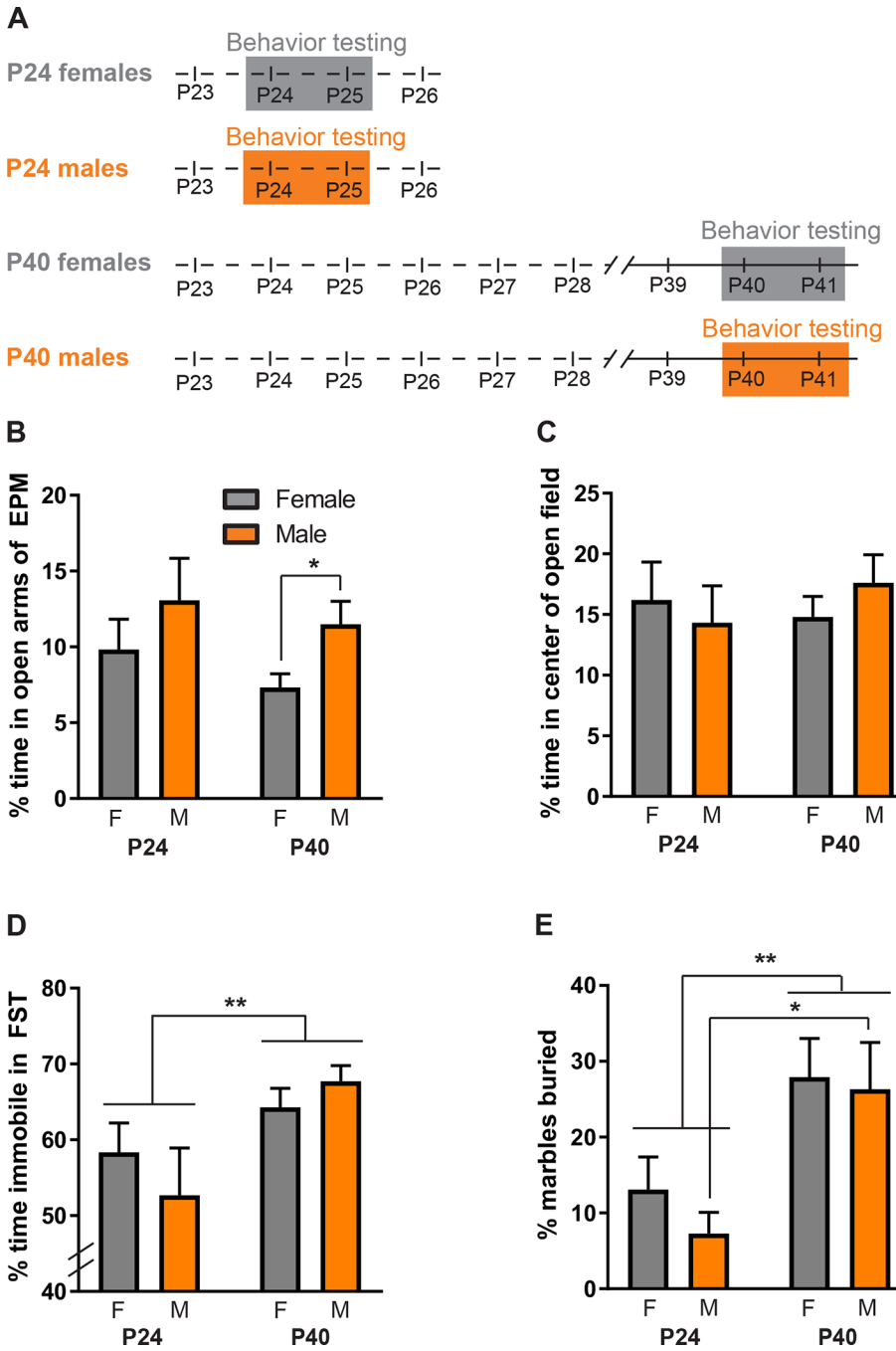

Fig. 1. Peripubertal changes in anxiety- and depression-related behavior.

All bars are mean ± SEM. A) Separate groups of male and female mice were tested for anxiety- and depression-related behavior prior to puberty (P24–25) and during late puberty (P40–47). n=13 P24 females; n=11 P24 males; n=29 P40 females; n=23 P40 males. B) Time spent on the open arms of the EPM did not change with age, but P40 males spent more time in the open arms than P40 females. C) Time spent in the center of the open field did not change with age or differ between males and females. D) P40 mice spent more time immobile in the FST compared to P24 mice, with no difference between males and females.

E) P40 mice buried more marbles than P24 mice, with no difference between males and females. **p<0.01 for main effect of age. *p<0.05 after Bonferroni correction for multiple comparisons.

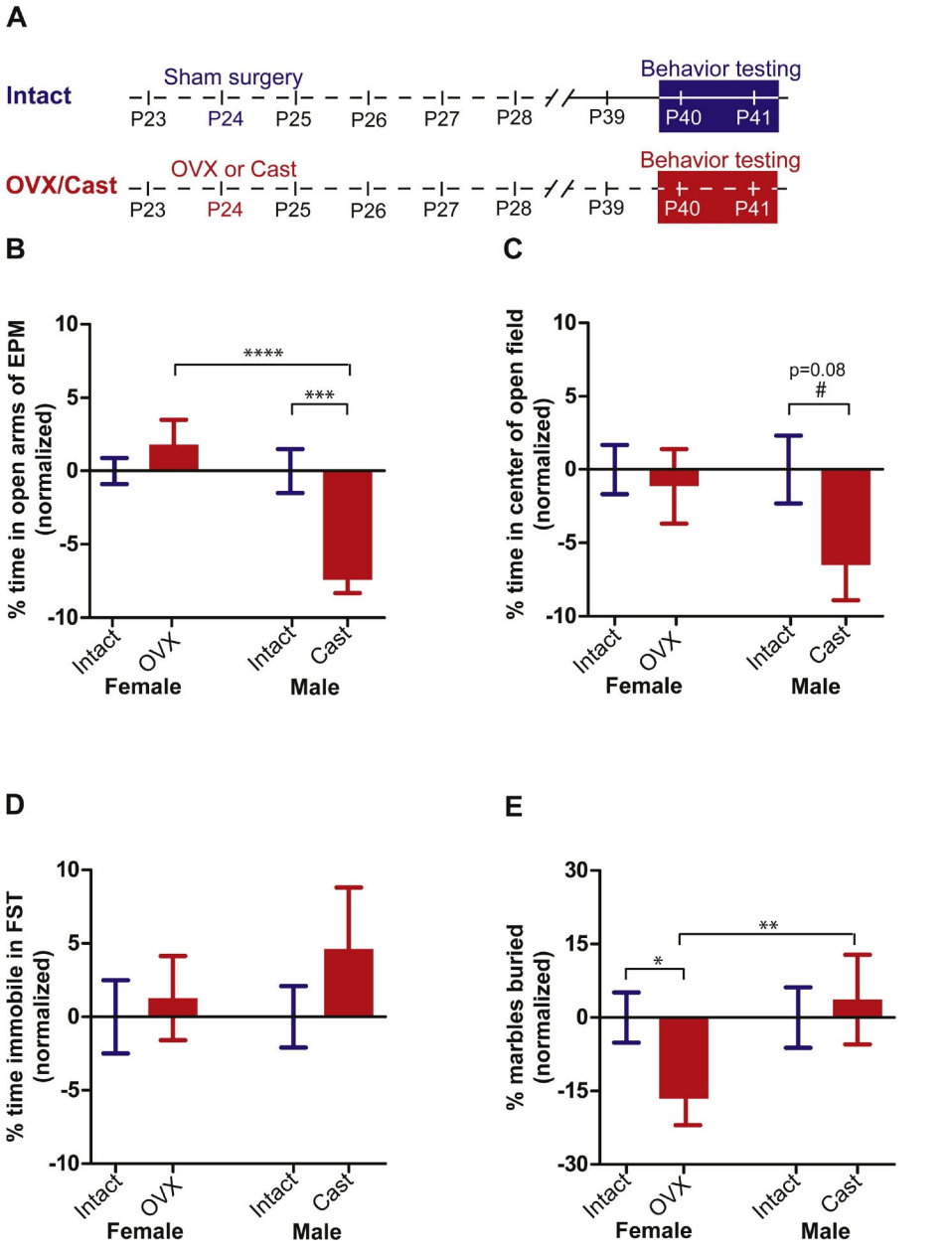

Fig. 2. Effects of pre-pubertal gonadectomy on anxiety- and depression-related behavior in late-pubertal mice.

All bars are mean ± SEM. A) Male and female mice underwent gonadectomy or sham surgery at P24, before puberty, and were tested for anxiety-related behavior at P40–47, during late puberty. n=15 ovariectomized (OVX) females; n=29 intact females; n=12 castrated (CAST) males; n=23 intact males. B) OVX did not affect EPM performance, but CAST males spent less time in the open arms compared to intact males, which resulted in a significant difference in the effect of gonadectomy between males and females. C) OVX did not affect open field performance, but CAST males showed a trend toward spending less time in the center of the open field compared to intact males. D) Gonadectomy did not affect FST performance in either sex. E) OVX decreased marble burying in females, while CAST

had no effect, resulting in a significant difference in the effect of gonadectomy between males and females. #p<0.1; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

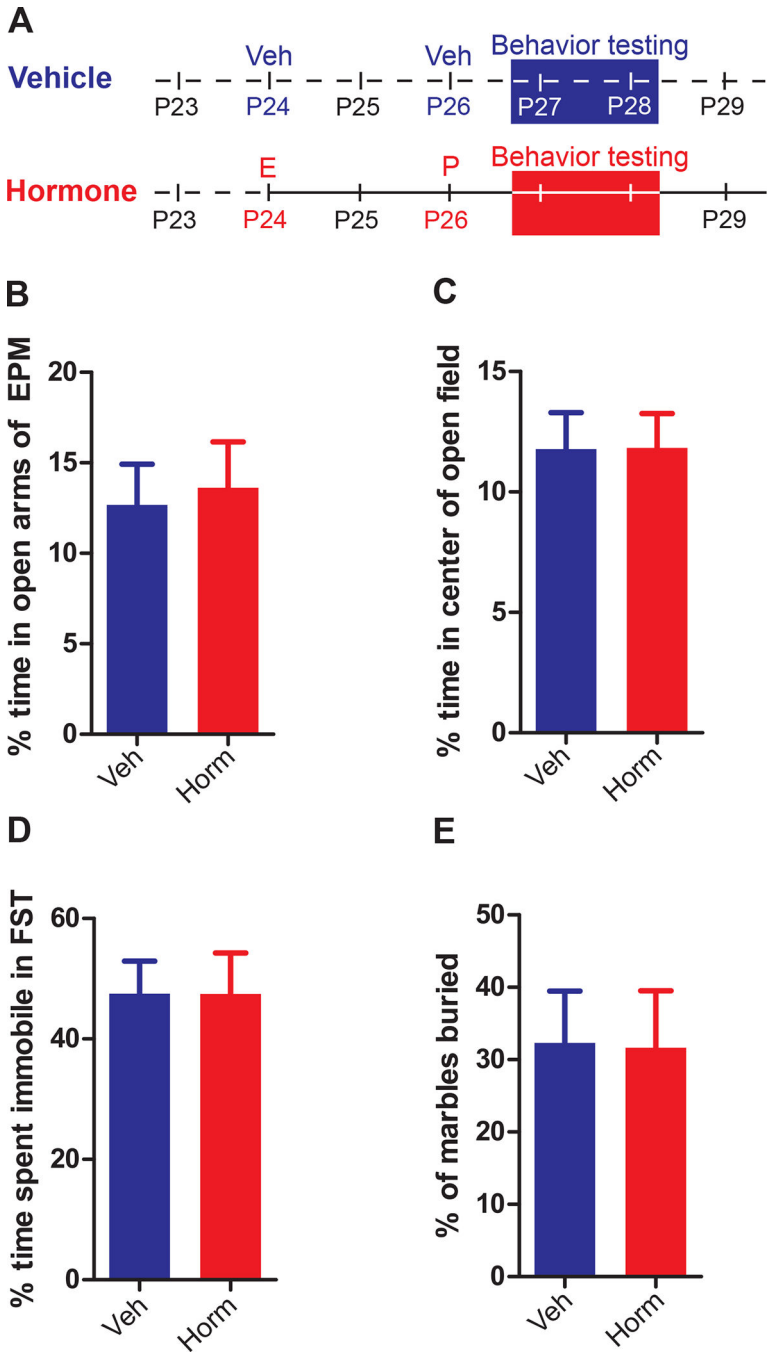

Fig. 3. Induction of early-onset puberty in female mice does not alter anxiety- and depression-related behavior.

All bars are mean ± SEM. A) Female mice were injected with estradiol or vehicle on P24 and progesterone or vehicle on P26, a treatment that disinhibits the HPG axis and induces endogenous ovarian hormone release (Ramirez and Sawyer, 1965; Smith and Davidson, 1968). Hormone- and vehicle-treated mice were tested for anxiety- and depression-related behavior at P27–28, an age when vehicle-treated mice were still pre-pubertal. n=15 vehicle-treated mice; n=12 hormone-treated mice. Hormone treatment did not alter performance on the EPM (B), open field (C), FST (D), or marble burying test (E).

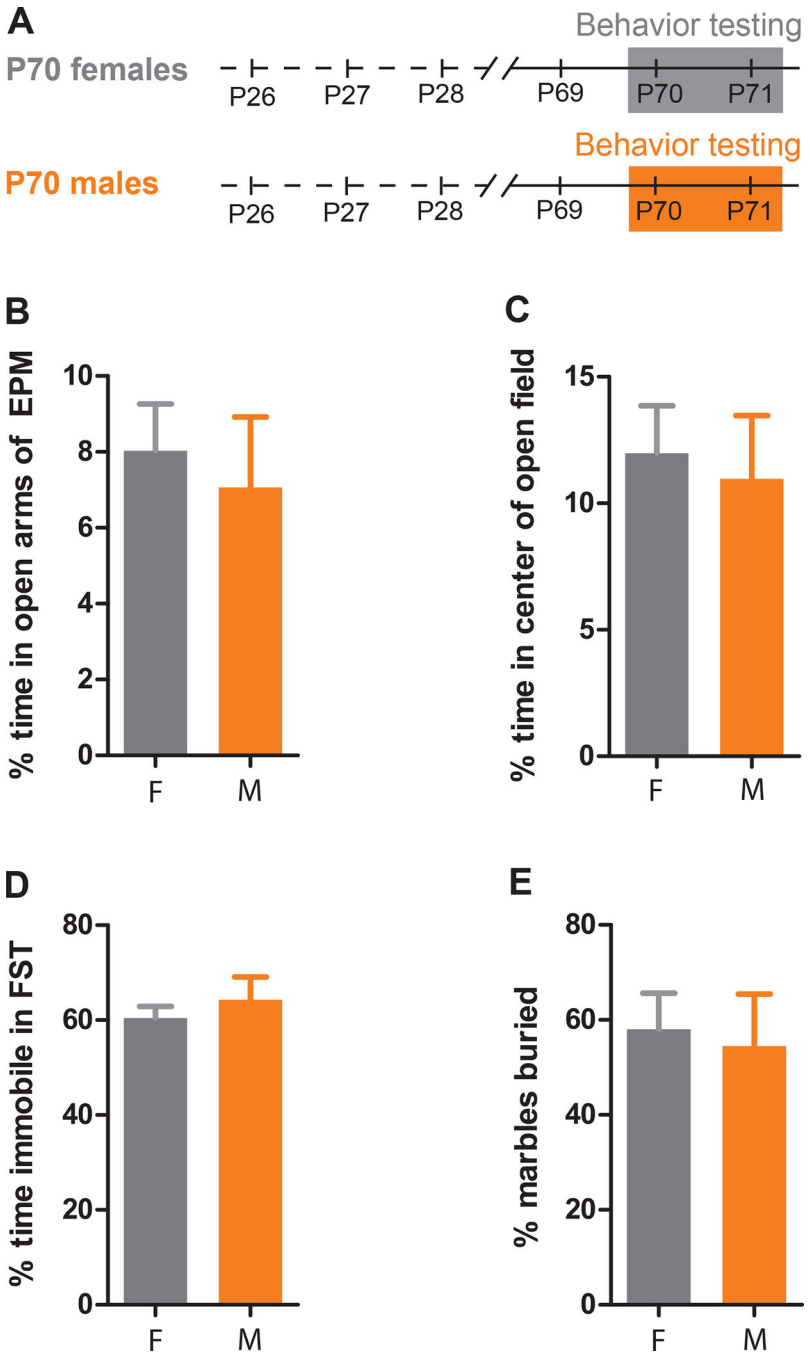

Fig. 4. Young adult males and females do not differ in their performance on any behavior tested.

All bars are mean ± SEM. A) Gonadally intact, hormonally unmanipulated mice were tested for anxiety- and depression-related behavior post-pubertally (P69–83). n=15 females; n=10 males. Young adult male and female mice did not differ in their performance on the EPM (B), open field (C), FST (D), or marble burying test (E).