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Gonadectomy prior to puberty decreases normal parental behavior in adult mice

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ABSTRACT

Sex steroid hormones secreted by gonads influence development and expression of many behaviors including parental behaviors. The capacity to display many behaviors develops under the influence of sex steroid hormones; it begins with gonadal differentiation and lasts through puberty. The timing of gonadectomy may have important and long lasting effects on the organization and activation of neural circuits regulating the expression of different behaviors. The present study investigated the importance of exposure to endogenous gonadal steroid hormones during pubertal period/adolescence on parental behavior in adult mice. Male and female WT mice were gonadectomized either before puberty (25 days of age) or after puberty (60 days of age) and tested for parental behavior with and without estradiol benzoate (EB) replacement in adulthood. Additional groups of mice were gonadectomized at P25 and supplemented with estradiol (females) or testosterone (males) during puberty. Female mice gonadectomized after puberty or gonadectomized before puberty and supplemented with estradiol during puberty, displayed better pup directed parental behaviors in comparison to mice gonadectomized at 25 days of age regardless of treatment with estradiol in adulthood. However, mice treated with EB in adulthood displayed better non-pup directed nest building behavior than when they were tested without EB treatment regardless of sex and time of gonadectomy. To examine whether the sensitivity to sex steroid hormones was altered due to differences in time without gonads prior to the testing, mice were also tested for female sex behavior and there were no differences between mice gonadectomized at P25 or P60, although this could not completely rule out the possibility that parental behavior is more sensitive to prolonged absence of steroid hormones than female sex behavior. These results suggest that the absence of gonads and thereby the absence of appropriate gonadal steroid hormones during puberty/adolescence may have a profound effect on pup directed parental behaviors in adult mice.

Introduction

Gonadal steroid hormones influence the development of brain and consequently behavior. Steroid hormones affect brain development and activity through two relatively separable processes characterized as organizational versus activational (Arnold and Breedlove, 1985; Schulz et al., 2009; Sisk and Foster, 2004). Usually, more permanent organizational effects occur perinatally, while activational effects occur later in life when gonadal hormones act on specific neural circuits to trigger specific aspects of physiology or the expression of various adult behaviors (reviewed in (Arnold, 2009; Majdic and Tobet, 2011; McCarthy and Arnold, 2011)). A growing number of studies also show that gonadal hormones can have "organizational" effects on the brain later in life, during puberty and possibly in adult life (Ahmed et al., 2008; De Lorme et al., 2012; Romeo, 2003; Romeo et al., 2000; Schulz and Sisk, 2006; Schulz et al., 2009). Puberty is a period during which the hypothalamic–pituitary–gonadal axis reactivates with the elevated secretion of gonadal steroid hormones (Sisk and Zehr, 2005). Many brain regions are sensitive to the action of gonadal steroid hormones, including parts of the brain that are thought to be involved in the regulation of parental behavior (Kalinichev et al., 2000).

Parental behavior is broadly defined as any behavior performed in the relation to one's offspring, or as any behavior that contributes directly to the survival of fertilized eggs or newborns. In mammals, maternal care is more common than paternal, although paternal behavior is also present in several mammalian species including mice and humans (Nelson, 2005). In many species, maternal behavior is triggered by the exposure to steroid hormones during pregnancy. However, nulliparous

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(virgin) female rats and mice exhibit parental behavior when presented with foster pups even without circulating gonadal steroids (Numan and Insel, 2003). By contrast, male rats and mice are commonly infanticidal (Lonstein and De Vries, 2000), although a recent study by Tachikawa et al. (2013) demonstrated that male mice are not aggressive towards pups if cohabited with female mice for two weeks after mating. In this study, it was suggested that pheromonal cues are responsible for aggressive behavior of males towards pups and this response is diminished if fathers are cohabited with pregnant females or the vomeronasal organ in sexually naïve males has been surgically removed. Many studies of parental behavior in mice and rats, and influences of gonadal steroid hormones on parental behavior, have been done in parturient rodents (e.g. (Lonstein et al., 1999; Stolzenberg and Rissman, 2011)) or in virgin, gonadally intact or gonadectomized rodents in adulthood (e.g. (Gatewood et al., 2006; Koch and Ehret, 1989; Lonstein et al., 1999; Ogawa et al., 1998a; Okabe et al., 2013; Okabe et al., 2010; Stolzenberg and Rissman, 2011)). Gonadectomy in adult life seems to have little effect on parental behavior in adult virgin female mice (Ogawa et al., 1998a) and rats (Lonstein et al., 1999) while in male mice, published results differ with one study reporting improvement of paternal behavior after gonadectomy (Okabe et al., 2013) and another finding no influence of adult gonadectomy on paternal behavior (Gatewood et al., 2006). Although pup retrieval behavior can improve with gonadectomy (or social experience) in adulthood in mice of both sexes (Okabe et al., 2010), we were not aware of reports concerning the possible consequences of juvenile gonadectomy on the development of parental behavior in mice. Therefore, the present study examined parental behavior in mice of both sexes that were gonadectomized at 25 days of age (before puberty) or at 60 days of age (after puberty) to explore whether the prolonged absence of gonads during pubertal/adolescent period can impact parental behaviors directed towards foster pups in adult mice. To check whether exposure to sex steroid hormones during puberty is necessary for the development of parental behavior directed towards foster pups in adult mice, some mice were also gonadectomized before puberty and supplemented with sex steroid hormones during puberty.

Materials and methods

Animals

C57BL/6J male and female mice were originally obtained from Harlan, Italy and bred at the University of Ljubljana, Veterinary Faculty, in standard conditions with 12–12 LD cycle (lights on at 3 am and off at 3 pm) and food (phytoestrogen free diet; Harlan Teklad Diet 2016, Harlan, Milan, Italy) and water ad libitum. Mice were weaned at 21 days of age and mice from the same litters were group-housed (3 mice of same sex per cage). Mice were housed in 15 cm high cages with floor area of 37.5 × 22 cm. First groups of male (n = 11) and female mice (n = 10) were gonadectomized (GDX) at 25 days of age (before puberty), the second groups of male (n = 5) and female (n = 8) mice were gonadectomized at 60 days of age (after puberty) and additional groups of male (n = 4) and female mice (n = 4) were gonadectomized at 25 days of age (before puberty) and had inserted testosterone (T) or estradiol benzoate (EB) silastic implants, respectively, from 25 to 60 days of age (during puberty).

Foster pups for assessing parental behavior were obtained from the mice of the same strain, bred in the same conditions.

Sexually experienced stimulus males (C57BL/6J), previously used for mating (at least 3 successful mating with weaned litters), were used for assessing sexual behavior in female mice. Stimulus males were housed individually in 13 cm high cages with 28.5 × 10.5 cm floor area and bred also in the same conditions.

All animal experiments were approved by Veterinary Administration of Slovenia (license no. 34401-32/2012/8) and were done according to ethical principles, EU Directive 2010/63/EU and NIH guidelines.

Surgery and hormone replacement

Male and female mice were gonadectomized bilaterally at 25 or 60 days of age to remove endogenous gonadal steroids. Mice were anesthetized with the mixture of ketamine (Vetoquinol Biowet, Gorzowie, Poland; 100 μg/g BW), acepromazine (Fort Dodge Animal Health, Fort Dodge, IA, USA; 2 μg/g BW) and xylazine (Chanelle Pharmaceuticals Ltd., Loughrea, Ireland; 10 μg/g BW) and gonads were excised through small incisions. Incisions were stitched and mice received two injections of butorfanol (Turbogesic, Fort Dodge Animal Health, Fort Dodge, IA, USA; 2 μg/g BW) after surgery to ease any potential pain. The additional group of males and females, gonadectomized at 25 days of age, received during gonadectomy subcutaneous silastic implants (1.02 mm inner diameter, 2.16 mm outer diameter) filled with sex steroid hormones according to sex. Males received implants filled 10 mm in length with crystalline testosterone (T; Sigma, Taufkirchen, Germany; (Gatewood et al., 2006)) and females received implants filled with 5 mm in length with crystalline 17β-estradiol 3-benzoate (EB; Sigma, Taufkirchen, Germany) diluted 1:1 with cholesterol (Wersinger et al., 1999) and closed on both ends by medical silastic adhesive (Dow Corning, Lakeside, AZ, USA). Implants were inserted subcutaneously in the midscapular region. Mice from this group had silastic implants removed in general anesthesia at 60 days of age, after 35 days of hormone exposure. Mice were left to recover for at least 10 days (Seitz et al., 2010) before behavior assessments were performed. Mice were tested as hormonally naïve first and then later after priming with estradiol benzoate (EB; only mice without gonadal hormones priming during puberty). All mice were initially tested twice on two consequent days for parental behavior between 70 and 75 days of age. Two to 3 days after the second test, mice without gonadal hormone priming during puberty received subcutaneous implants of EB and were tested again 10 days after implantation. Silastic implants (1.02 mm inner diameter, 2.16 mm outer diameter) were again filled with 5 mm in length with crystalline 17β-estradiol 3-benzoate (EB; Sigma, Taufkirchen, Germany) (diluted 1:1 with cholesterol) (Wersinger et al., 1999) and closed on both ends by medical silastic adhesive (Dow Corning, Lakeside, AZ, USA). Implants were inserted subcutaneously in the midscapular region under anesthesia. Similar implants filled with 17β-estradiol have yielded plasma estradiol levels within the physiological range in female mice (Bakker et al., 2002) and rats (Seale et al., 2004). For female sex behavior tests, implants inserted in adulthood were left in situ and mice were injected subcutaneously with 0.8 mg of progesterone (P; Sigma) approximately 4 to 8 h before each test. All mice were initially tested for female sexual behavior after conclusion of parental behavior tests between 90 and 100 days of age.

Parental behavior test

Each mouse was tested in a standard parental behavior test 4 times, twice without hormone supplement and twice primed with EB (only mice without gonadal hormones priming during puberty) to examine the potential effect of estradiol on parental behavior directed towards foster pups, although this is not mimicking physiological situation during pregnancy where mice are exposed first to high levels of progesterone followed by high estradiol exposure. First two (without hormones) and last two trials (with hormones) were performed on two consecutive days; on the first day the first trial lasted 20 min and the next day the second trial lasted for 15 min as mice were experienced and had shorter latencies to initiate parental behavior. Mice were transferred to smaller test-cages (14 cm high with 35 × 15 cm floor area) with at least three day-old bedding from their home cage 24 h prior to behavioral testing. After the first and last two trials, mice were returned to their larger home cages in their respective social groups.

Parental behavior tests were performed during the light cycle 1 to 2 h before the start of the dark cycle as described by Gatewood et al. (2006). This is supported by previously published study reporting that