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An increase in estradiol facilitates the onset of paternal behavior in the dwarf hamster (*Phodopus campbelli*)

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ABSTRACT

In the dwarf hamster (*Phodopus campbelli*), activational effects of testosterone (T) and estradiol (E_2) in the regulation of paternal behavior have been repeatedly rejected because peripheral concentrations of E_2 do not change across the reproductive cycle of males. Further, castration no affected paternal behavior despite that both T and E_2 concentrations decreased significantly. However, the role of these hormones has not been evaluated in models of castration and hormonal replacement in virgin males. Here, we analysed the effects of E_2 and T in paternal behavior in virgin male dwarf hamster (*Phodopus campbelli*). Thirty paternal (PAT) males were bilaterally castrated; of them, 10 were implanted with T, 10 with E_2 and 10 males received no treatment. Other 10 PAT males underwent sham-castration. Seventeen aggressive (AGG) males were also bilaterally castrated; of these, 10 AGG received E_2 replacement, 7 were not treated. Other 7 AGG males were submitted to sham-castration. Following treatments, paternal behavior tests were conducted again. T and E_2 levels in plasma were quantified by radioimmunoassay (RIA). The results showed that the treatments did not affect the paternal behavior of males that were initially paternal. Neither castration nor sham-castration surgery affected the behavior of AGG males. However, when these males were treated with E_2 and the concentrations of this hormone increase significantly they became paternal. Our data suggest that an increase in E_2 levels shifted infanticidal behavior to paternal behavior in dwarf hamster.

1. Introduction

Most male rodents of biparental species must inhibit their infanticidal behavior and become paternal before the birth of the pups to avoid damaging their offspring (Hrdy, 1979; Huck et al., 1982; Hausfater and Hrdy, 1984; Vom Saal and Howard, 1982). The transition from non-paternal male to paternal male includes hormonal changes that may suppress infanticidal behavior and trigger the onset paternal behavior through the activation of the neural circuit involved in regulating this behavior (Brown, 1993; Brown et al., 1995). Hormones such as prolactin, progesterone, testosterone (T), and its metabolites estradiol (E_2) and dihydrotestosterone, have been associated with the regulation of paternal behavior (Reburn and Wynne-Edwards, 1999; Wynne-Edwards and Reburn, 2000; Trainor et al., 2003; Schum and Wynne-Edwards, 2005; Wynne-Edwards and Timonin, 2007; Wynne-Edwards, 2010; Lonstein et al., 2015). However, only studies manipulating T, E_2, dihydrotestosterone and progesterone levels support a causal link between these hormones and paternal behavior.

In California mouse (*Peromyscus californicus*), in fathers castration reduces the degree of paternal care, whereas castrated males that receive exogenous T replacement display an increase in paternal behavior (Trainor and Marler, 2001). In this rodent, T regulates paternal behavior *via* conversion to E_2; castrated males with sexual experience treated with T or E_2 provide more paternal care than males that received dihydrotestosterone or an empty implant. Further, the inhibition of aromatase, an enzyme that converts T to E_2, blocks the positive effect of T on paternal behavior (Trainor and Marler, 2002). In the Mexican volcano mouse (*Neotomodon alstoni*), T replacement induces paternal behavior in males that were aggressive or indifferent toward pups (Luis et al., 2012). In the Mongolian gerbil (*Meriones unguiculatus*), a first study reported that virgin castrated males that remain with a female of the species from pregnancy to parturition displayed higher levels of

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paternal care than castrated males with T replacement (Clark and Galef, 1999). However, a subsequent investigation showed that virgin castrated males that received T, E_2 or dihydrotestosterone replacement shifted their infanticidal behavior to paternal behavior, while castrated males without hormone replacement and sham-castrated males continued exhibiting aggression toward pups (Martínez et al., 2015). This study was the first to report that dihydrotestosterone, a non-aromatizable androgen, is involved in the mechanisms that facilitate the onset of paternal behavior in a rodent.

In the dwarf hamster (*Phodopus campbelli*), the role of T and E_2 in the regulation of paternal behavior has been repeatedly rejected, because peripheral concentrations of E_2 do not change across the reproductive cycle of males. Further, castration no affected paternal behavior despite that both T and E_2 concentrations decreased significantly. Additionally, the administration of an aromatase inhibitor did not alter paternal responsiveness, although the aromatization of T to E_2 in specific brain areas contributes to the onset of paternal behavior in other rodent species (Hume and Wynne-Edwards, 2005, 2006; Schum and Wynne-Edwards, 2005). However, the role of T and E_2 in paternal behavior of dwarf hamsters has not been evaluated away from the influence of stimuli from female and the pups. Adapting one of the approaches used to establish hormonal basis of maternal behavior, the "ovariectomy and hormonal replacement in virgin females", (Siegel and Rosenblatt, 1975; Bridges and Russel, 1981; Bridges, 1984; Numan et al., 2006), and with the aim to expand upon the previous findings in the California mouse, the Mexican volcano mouse and the Mongolian gerbil (Trainor and Marler, 2001; Luis et al., 2012; Martínez et al., 2015), we analysed the effects of T and E_2 on the onset of paternal behavior in dwarf hamster (*Phodopus campbelli*). Males of this biparental rodent species display extensive paternal behavior under wild and laboratory conditions (Wynne-Edwards, 2003). The males provide their offspring the same level of care as females, except suckling (Wynne-Edwards, 1987, 1995; Jones and Wynne-Edwards, 2001). They also act as "midwives" during delivery (Jones and Wynne-Edwards, 2000). These rodents also engage in alloparental care, where juvenile males provide care for their younger siblings by grooming, sniffing and pup retrieval (Vella et al., 2005).

2. Materials and methods

2.1. Animals

This study was performed using virgin male dwarf hamsters, aged 90 to 120 days. These animals were obtained from a breeding colony maintained at the Facultad de Estudios Superiores Iztacala, UNAM. The animals were housed under an inverted photoperiod of 12:12 h light–dark cycle (light onset at 1800 h) at an ambient temperature of 17–21 °C. The hamsters were fed with pellets of Lab Chow 5001 (Nutrimentos Purina, México) and tap water *ad libitum*. The males were weaned between 17 and 20 days of age to avoid potential interaction with their siblings from a second litter. Adult age is reached in this species around to 90 days old (Vella et al., 2005; Timonin and Wynne-Edwards, 2008). Two or three hamsters of the same sex were housed in a polycarbonate cage with sawdust bedding. Before experimental treatments, the hamsters were submitted to a test to screen paternal behavior to characterize their behavior toward pups as either paternal or aggressive. We define "paternal males" as adult virgin males that give care to unrelated pups. Allopaternal males, in contrast, are usually juveniles that provide care to their siblings (Woodroffe and Vincent, 1994). The classification criteria for males included the following: paternal males showed sniffing (touching the pup with the nose), grooming (male holds and moves the pup with forelegs, while it licks its body) and may retrieve pups. The aggressive males showed sniffing and attacked the pups. When a male attacked the pup, the cage was tapped immediately to disrupt the aggression.

For the screen of paternal behavior, each male was placed in a polycarbonate cage (27 × 21 × 14 cm) with clean sawdust bedding, and after 10 min, two 2- to 3-day-old pups were introduced into the cage. When males were paternal, the observation period lasted 30 min.

The paternal behavior screening was performed 8 to 12 days before the beginning of surgery. Eighty-seven male dwarf hamsters were submitted to paternal behavior tests; of these, 66 (73.34%) males were paternal (PAT) and 24 (26.66%) males were aggressive toward pups (AGG). Thirty PAT males were bilaterally castrated; of them, 10 were implanted with T, 10 with E_2 and 10 males received no treatment. Other 10 PAT males underwent sham-castration surgery. Seventeen AGG males were also bilaterally castrated; of these, 10 received E_2 replacement, 7 males were not treated. Other 7 AGG males were submitted to sham-castration surgery. Due to the small proportion of AGG males, only the role of estradiol was determined. We chose E_2 because of the causal relationship between this hormone, and paternal behavior has been observed in rodents such as California mouse and Mongolian gerbil (Trainor and Marler, 2002; Martínez et al., 2015). In addition, this hormone has an important role in the regulation of maternal behavior (Lonstein et al., 2015). Although a high percentage of virgin male dwarf hamsters were paternal, we decided to continue with this study because it was possible that some aspect of paternal behavior would change with castration or with castration followed by T or E_2 replacement in PAT hamsters. Further, it was very interesting to determine the effect of estradiol in AGG males.

All the experiments were performed in accordance with the ethical guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023) and the ethical guidelines and technical specifications of the Mexican Official Norm for the Production, Care and Use of Laboratory Animals (Sikes and The Animal Care and Use Committee of the American Society of Mammalogists, 2016).

2.2. Surgeries and implants

Hamsters were anaesthetised with 10 mg/kg xylazine and 80 mg/kg ketamine before surgery and placement of T and E_2 implants. Only sterile surgical instruments were used. Here, benzalkonium chloride was applied to disinfect the scrotum, and a small midline incision was made, through which the testes were exteriorised. The spermatic vessels were tied with 6.0 cat-gut sutures, and the testes were removed. The incision was closed with 6.0 silk sutures. In sham-castrated hamsters, the skin of the scrotum was incised to draw out the testes, which were then put back, followed by closure of the incision with sutures. Acetylsalicylic acid (~100 mg/kg) was administered orally as an analgesic during the first 12 h following surgery. At 24 h after surgery, the hamsters were returned to their home cages. Implants were made of Silastic tube (Silastic Laboratory Tubing, i.d. 1.47 o.d., 1.96 mm) packed with 10 mm of testosterone propionate or β-oestradiol 3-benzoate (Sigma Aldrich, St. Louis, MO, USA), and the ends were sealed with silicone. In the California mouse and the Mongolian gerbil, 10 mm (~0.1 mg) implants of these steroid hormones successfully exerted positive effects on paternal behavior (Trainor and Marler, 2001; Martínez et al., 2015).

2.3. Paternal behavior test

PAT and AGG males were submitted to paternal behavior testing 8 to 10 days after surgery. Each male was placed in a polycarbonate cage following the method described above for screening paternal behavior. Four pups were bitten and immediately euthanised with an anaesthetic overdose. Paternal behavior was recorded for 30 min. A single observer recorded the onset latency of paternal behavior (the length of time it took for the male to groom the pup) and the time spent grooming. In addition, the time spent sniffing or in contact with pups was recorded. Behavioral observations were carried out between 11 and 14 h of the dark phase, under red light illumination.