Physiology & Behavior 118 (2013) 159–164

Contents lists available at SciVerse ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Testosterone inhibits facilitating effects of parenting experience on parental behavior and the oxytocin neural system in mice

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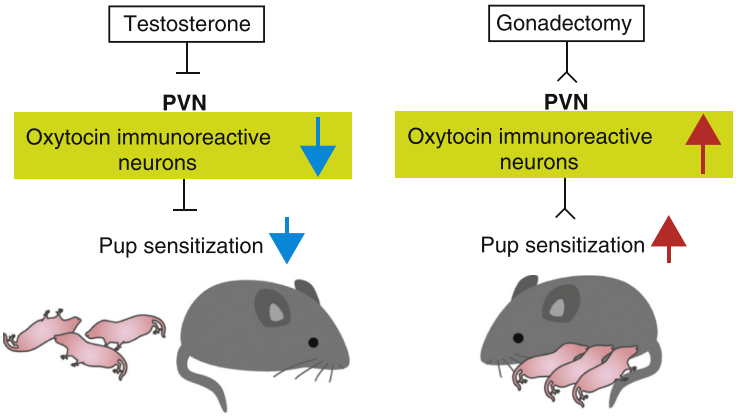
HIGHLIGHTS

- Pup sensitization occurred only in virgin female mice.
- Gonadecomy induced the pup sensitization in virgin male mice.
- Testosterone treatment impaired pup sensitization in both sexes.
- Testosterone suppressed the oxytocin (OT) system in both sexes.
- Sex differences in pup sensitization were caused by testosterone via the OT system.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 March 2013
Accepted 6 May 2013
Available online 14 May 2013

Keywords:
Parental behavior
Gonadal hormones
Oxytocin
Social experience

ABSTRACT

Parental behavior in mammals is facilitated by sensory experiences from infant, and by endocrine hormones. However, the interactions between these factors in the parental behavior of nonreproductive adults are not understood. We examined the interactive effects of gonadal hormones and the experience of repeated pup exposure on parental behavior in sexually naive mice. We also compared oxytocin (OT) expression levels in the paraventricular nucleus of the hypothalamus to behavioral outcomes. Clear sex differences were observed in retrieving tests; initial retrieving latency was shorter in females than in males, and 5-time pup exposure shortened retrieving latency in females only. Gonadectomy influenced neither initial retrieving latency nor pup sensitization in females. In contrast, gonadectomy shortened initial retrieving latency and caused pup sensitization in males. Estrogen implants given simultaneously with gonadectomy further shortened the initial retrieving latency in males, but pup sensitization was not affected and occurred in both sexes. In contrast, simultaneous testosterone implants impaired pup sensitization in both sexes. Similar to the results for responsiveness to pups, the number of OT neurons was increased by gonadectomy in males only. In comparison to gonadectomy only, OT neurons were decreased by simultaneous testosterone implants, but were not influenced by estrogen in either sex. Considering the parallel inhibitory effects of testosterone on both pup sensitization and number of OT neurons, we postulate that sex differences in parental responsiveness facilitated by repeated pup exposure were caused by an inhibitory effect of testosterone via the OT neural system in mice.

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0031-9384/$ – see front matter
http://dx.doi.org/10.1016/j.physbeh.2013.05.017

1. Introduction

Parental behavior is universal in mammals, and differences in behavior according to sex are common to many species. Females show a greater degree of parental behavior compared to males. For example, when adult virgin female mice were exposed to pups, they showed spontaneously highly parental responsiveness even at the first experience. In contrast, virgin male mice usually showed escape or aggressive behavior toward pups. These sex differences in initial responsiveness to pups are based on endocrine hormones; androgen suppresses, while estrogen enhances, initial parental responsiveness in mice [1–5].

Social experiences play a fundamental role in parental responsiveness, especially in male mice. We recently demonstrated that mating, as well as parental experiences, enhances pup retrieval and approach behavior toward pup vocalization in male mice, similar to maternal behaviors observed in mother mice [6]. These findings indicate that male mice have a changing mechanism from aggression to parenting that is mediated by social experience. An important question is whether these social experiences in males can themselves facilitate the parenting behavior, or whether social experience can change hormonal levels and induce parenting behavior as a consequence. To address this question, it is necessary to examine changes in parental behavioral induced by social experience under constant levels of gonadal hormones. Pup sensitization is a well-characterized behavioral transmission model in which constant exposure to pups can facilitate parental responsiveness [7]. Using this model, we elucidated the effects of gonadal hormones on facilitation of parental behavior by social experiences in virgin male and female mice.

Oxytocin is a nanopeptide hormone that is synthesized in the hypothalamic paraventricular nuclei (PVN) and supraoptic nuclei, and is released into general circulation via the posterior pituitary. Oxytocin receptors are distributed in various brain regions and oxytocin acts on these receptors. Oxytocin is required for the milk-ejection reflex [8], and has been implicated in various social behaviors, such as reproductive and parental behaviors [9]. Oxytocin antagonists inhibit the induction of maternal behavior [10], and oxytocin-receptor knockout mice display abnormal maternal pup-retrieval latency [11]. These findings suggest that oxytocin plays an important role in the onset or sensitization of parental behavior. In addition, the oxytocin system in rats is enhanced by estrogen [12,13], suggesting that this system is affected by gonadal hormones. However, few studies have made a direct comparison between parental behavior of virgin mice and the oxytocin system in uniform paradigm. It is also unknown whether gonadal hormones modulate the oxytocin system when parental behavior is enhanced by social experience.

The second purpose of this study was to examine the target neural molecules of gonadal hormones that are related to facilitation of parental behavior by social experience. We assessed the number of oxytocin-immunoreactive (OT-ir) neurons in the PVN to compare the OT neural system and the facilitation of parental responsiveness by pup sensitization under different gonadal hormone statuses.

2. Materials and methods

2.1. Animals and housing

C57BL/6J (B6) mice, originally obtained from Japan Clea Co. Ltd. (Japan Clea, Yokohama, Japan), were bred in our laboratory. Food and water were provided ad libitum, and animals were maintained under a standard 12 L:12 D cycle (lights on at 0600 h) in a breeding cage (17.5 cm × 24.5 cm × 12.5 cm). The environment was maintained at constant temperature (24 ± 1 °C) and humidity (50 ± 5%). The study was approved by the Ethic Committee of Azabu University (#09-01), Japan.

Three to 5 mice that had no experience with mating or pup care were housed together in same-sex groups, and designated as "virgin intact mice" (intact male and intact female). At 7–10 weeks, virgin mice of both sexes were anesthetized with isoflurane; males were castrated (Cast) and females were ovariectomized (Ovx). In the gonadal hormone-treated groups, virgin mice were gonadectomized and simultaneously implanted with either an estrogen (E) or a testosterone (T) tube in the back of their neck (Cast E; Ovx E; Cast T; Ovx T). The tubes (Dow Corning Silastic tubing: 2-mm outer dia, 1-mm inner dia.) were 7 mm in length. Estrogen diluted to 30% with cholesterol, or T diluted to 75% with cholesterol (Wako Pure Chemicals, Osaka, Japan), was packed into the tube to 5 mm, and tubes were sealed on both ends by silastic adhesive. The blood consistency of each hormone treatment was reported in our previous study [14]. After surgery, the mice were again housed 3–5 per breeding cage; the experiments began 3 weeks later.

2.2. Protocol for pup sensitization and retrieving test

Each subject mice (intact female, n = 8; intact male, n = 7) were individually moved from the breeding cage to each new test cage of the same size. After a habituation period of 2 days, during which time a nest was built, three 4- to 5-day-old pups with no relation to the subjects were placed in separate corners of the cage over a 20-min period, and the latency until all 3 pups were retrieved was measured (the "initial retrieving latency"). During this period, if at least 1 pup was attacked by the subject, pups were immediately removed from the test cage and the latency was assigned a value of 1200 s for statistical purposes. The latency duration was also assigned a value of 1200 s if a subject did not retrieve all 3 pups. For induction of pup sensitization, the same pups were repeatedly introduced into the test cage an additional 5 times at 3-min intervals. In cases where a pup was attacked, all pups were immediately removed from the test cage until the next exposure period. After the repeated pup exposure, subject mice were left alone in the test cage. Four days after the pup exposure, 3 new 4- to 5-day-old pups were placed in separate corners of the test cage, and retrieving latency was measured for 20 min (the "4 days after retrieve latency"). The latency of retrieving of all pups was compared between the initial and 4 days after retrieving latencies among each group. To investigate the effect of gonadal hormones on facilitation of parental responsiveness by repeated pup exposure, another set of subject mice (Intact male, n = 11; Intact female, n = 12; Cast, n = 11; Ovx, n = 11; Cast E, n = 8; Ovx E, n = 12; Cast T, n = 15; Ovx T, n = 13) were used in an experiment with the same design as that described above, and the latency of retrieving pups was compared before and after repeated pup exposure.

2.3. Immunohistochemistry

Another set of subject mice (Intact male, n = 8; Intact female, n = 8; Cast, n = 8; Ovx, n = 10; Cast E, n = 10; Ovx E, n = 10; Cast T, n = 7; Ovx T, n = 10) was prepared, to analyze the number of OT-ir neurons. Three weeks after surgery, subject mice were individually moved from its breeding cage to a new cage of the same size. After a habituation period of 2 days, all subjects were sacrificed for immunohistochemistry.

Subjects were deeply anesthetized with sodium pentobarbital (50 mg/kg) and perfused with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB) at pH 7.4. The brains were removed and immersed in the same fixative at 4 °C overnight, then placed in 0.1 M phosphate buffer saline (PBS) containing 30% sucrose at 4 °C until they sank. The brains were cut coronally at 30 μm on a freezing microtome. Every third section from the serial sections was washed with PBS, and blocked with 3% Normal Goat Serum (Vector, Burlingame, CA) in 1% bovine serum albumin in PBS with Triton X-100