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Age-related changes in sexual function and steroid-hormone receptors in the medial preoptic area of male rats

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ABSTRACT

Testosterone is the main circulating steroid hormone in males, and acts to facilitate sexual behavior via both reduction to dihydrotestosterone (DHT) and aromatization to estradiol. The mPOA is a key site involved in mediating actions of androgens and estrogens in the control of masculine sexual behavior, but the respective roles of these hormones is not fully understood. As males age they show impairments in sexual function, and a decreased facilitation of behavior by steroid hormones compared to younger animals. We hypothesized that an anatomical substrate for these behavioral changes is a decline in expression and/or activation of hormone receptor-sensitive cells in the mPOA. We tested this by quantifying and comparing numbers of AR- and ERα-containing cells, and Fos as a marker of activated neurons, in the mPOA of mature (4–5 months) and aged (12–13 months) male rats, assessed one hour after copulation to one ejaculation. Numbers of AR- and ERα cells did not change with age or after sex, but the percentage of AR- and ERα-cells that co-expressed Fos were significantly up-regulated by sex, independent of age. Age effects were found for the percentage of Fos cells that co-expressed ERα (up-regulated in the central mPOA) and the percentage of Fos cells co-expressing AR in the posterior mPOA. Interestingly, serum estradiol concentrations positively correlated with intromission latency in aged but not mature animals. These data show that the aging male brain continues to have high expression and activation of both AR and ERα in the mPOA with copulation, raising the possibility that differences in relationships between hormones, behavior, and neural activation may underlie some age-related impairments.

1. Introduction

Sexual behavior in many species of male mammals undergoes marked declines during aging for both motivational and copulatory behaviors, starting in middle age and becoming more severe with more advanced aging (Amstislavskaia et al., 2010; Smith et al., 1992). These behaviors are highly dependent on appropriate secretion patterns and concentrations of steroid hormones, especially androgens and estrogens. Castrated males exhibit impaired copulatory behavior, but will exhibit behavior similar to that of intact animals if given testosterone replacement (McGinnis and Dreifuss, 1989; Park et al., 2007). Both the androgenic and estrogenic metabolites of testosterone are required for the full manifestation of these behaviors. Castrated males administered hormone replacement using the non-aromatizable androgen 5α-dihydrotestosterone (DHT) alone, or those given testosterone with an aromatase inhibitor, still show behavioral impairments, underscoring the importance of estradiol for copulation (Hull et al., 2006; Putnam et al., 2003). In fact, administration of estradiol to castrated males restores certain components of the sexual behavior repertory, including both motivational behaviors, such as anticipatory levels changes and mounting, and consummatory behaviors, such as intromissions (Attila et al., 2009; Roselli et al., 2003). During aging, serum testosterone concentrations decline, but interestingly, this does not correlate with declines in sexual behavior (Smith et al., 1992; Wu and Gore, 2009; Chambers and Phoenix, 1984; Chambers and Phoenix, 1986). Regarding estradiol, replacement of this hormone to aging male rats does not fully restore copulatory measures to those seen in young animals (Chambers and Phoenix, 1986). Thus, both classes of hormones are needed, but their exact roles still require elucidation.

In the neural network of brain nuclei that underlies sexual behavior in males, the medial preoptic area (mPOA) plays a key role in both sexual motivation and copulatory performance (Yeh et al., 2009; Hull and Dominguez, 2007). Lesions to the mPOA impair, while electrical stimulation facilitates, male sexual behavior (Liu et al., 1997;

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Rodríguez-Manzo et al., 2000). The mPOA is a site of hormone action in the control of sexual behavior (Russell et al., 2012; Wood and Williams, 2001), with a high density of steroid hormone receptors, including estrogen receptor alpha (ERα) and androgen receptor (AR) (Dellovade et al., 1995; Pérez et al., 2003; Wu and Gore, 2009; Wu and Gore, 2010; Simerly et al., 1990). These ERα and AR sensitive cells in the mPOA are both activated by copulation (Gréco et al., 1998).

Despite this understanding of the importance of the mPOA, testosterone and estradiol in male sexual behavior, relatively little is known about this interplay during reproductive aging. We hypothesize that age-related impairments in sexual behavior are due at least in part to impairments in the hormone responsiveness of the mPOA through age-related changes in both the expression and activation of the AR and ERα in this region. To test this, we quantified ERα-positive, AR-positive and Fos-positive cells in the mPOA of mature and aged rats. This work was conducted in the framework of both age and prior sexual experience, the latter exerting a strong influence on sexual behavioral outcomes (Hull and Dominguez, 2006; Wu and Gore, 2010).

2. Materials and methods

2.1. Animals and husbandry

Sprague-Dawley male rats (Harlan, Indianapolis, IN; 3 months (n = 24) or 12 months (n = 13) at arrival) were pair housed in large plastic cages, in a climate-controlled room, on a 14:10 h light/dark cycle, with lights off at 10:00H and off at 20:00H. Food and water were freely available. Conspecific females (n = 16) were purchased as young adults, and ovariectomized under ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (4 mg/kg) anesthesia. They were brought into behavioral estrus with an injection of 4 μg estradiol benzoate (s.c.), followed 44 h later by an injection of 400 μg (s.c.) progesterone. Testing took place 4 h later. Sexual receptivity of females was confirmed by placing her into a cage with a separate stud male shortly before the test began and watching for lordosis. All procedures were done in accordance with the National Institutes of Health's Guidelines for the Care and Use of Animals and were approved by the Institutional Animal Care and Use Committee at the University of Texas at Austin.

2.2. Behavioral testing

Males were used at two ages: mature adult (MAT) and aged (AG). To match sexual experience, MAT males (3 months) were allowed to mate with a sexually receptive female for 90 min, every other day, for 14 days, for a total of 7 experience sessions. On an 8th day animals were observed to confirm that they achieved at least two ejaculations during the final experience session. Two males failed and were excluded from further testing. Aged males (12 months) were retired breeders at purchase and were not given further experience sessions in the lab.

Sexual behavioral data were obtained on the test day, which took place at least 2 days after the last sexual behavioral experience for the MAT males. Animals of both ages in the sex groups were allowed to copulate to one ejaculation. One MAT animal failed to copulate after 1 h and was excluded from further analysis. A no-sex control group of each age was handled, but females were not introduced into their home cage. This resulted in four groups: MAT males (approximately 4 months at euthanasia) given sex (MAT-S, n = 16), MAT males with no sex (MAT-NS, n = 5), and the AG counterparts (approximately 13 months at euthanasia) with (AG-S, n = 8) or without (AG-NS, n = 5) copulation. We note that while the number of animals in the AG groups is small due to difficulties in attaining animals at the appropriate age, the study was adequately powered. All animals were euthanized with an overdose of sodium pentobarbital (100 mg/kg), 1 h after ejaculation (sex groups) or handling (no-sex groups).

2.3. Immunohistochemistry

Rats were perfused transcardially with saline under pentobarbital anesthesia, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (0.1 M PB; pH = 7.35). Brains were removed, postfixed for 1 h in the same fixative at room temperature, and stored in 30% sucrose at 4 °C. Coronal sections were cut at 35 μm into four equal series through the mPOA and stored in cryoprotectant (30% ethylene glycol, 30% sucrose, 0.00002% sodium azide in 0.1 M PB) at − 20 °C until use.

Sections underwent immunohistochemical staining for either ERα or AR, with each nuclear receptor double-labeled with Fos, the immediate early gene product and an indicator of transcriptional activation (Morgan and Curran, 1991). Washes in 0.1 M PB, 4 × for 5 min, preceded all incubations. Sections underwent the following incubations: 1% H_2O_2 in 0.1 M PB, and then blocked for 60 min in 2% normal goat serum and 0.04% Triton-X in 0.1 M PB (blocking solution); then rabbit anti-ERα primary antibody (1: 8000; EMD Millipore 04–820, RRID: AB_1587018, Massart et al., 2015) or rabbit anti-AR (1: 400; Santa Cruz Biotechnology sc-816, RRID: AB_1563391, Picot et al., 2014) in blocking solution, overnight at room temperature. Control sections run in parallel had omission of the primary antibody. Although we did not conduct pre-adsorption of the antibodies in this study, all of the primary antibodies are well-characterized (Massart et al., 2015; Picot et al., 2014; Ladron de Guevara-Miranda et al., 2017), and expression patterns were entirely consistent with the literature (i.e., labeling was nuclear, with expected distribution). The following day, sections were incubated in anti-rabbit biotinylated secondary antibody (1:500 in blocking solution; Vector Labs, Burlingame, CA, USA) before avidin-biotin conjugate (1:000 in 0.1 M PB; Vectastain ABC Elite kit; Vector Laboratories, Burlingame, CA). Sections were then incubated with biotinylated tyramine (1:1000 in 0.1 M PB; Perkin Elmer, Waltham, MA) for 10 min, and visualized with Alexa 488-tagged streptavidin (1:400 in 0.1 M PB; Life Technologies, Grand Island, NY). After washing thoroughly with 0.1 M PB, sections were then incubated with mouse anti-Fos primary antibody (1:600; Santa Cruz Biotechnology sc-271,243, RRID: 1,563,391, Ladron de Guevara-Miranda et al., 2017) in blocking solution, overnight at room temperature. The following day, sections were incubated for 60 min with Alexa 555 goat-anti mouse secondary (1:400 in 0.1 M PB; Life Technologies, Grand Island, NY). Sections were then mounted and coverslipped. For negative controls, sections underwent the same immunostaining procedure in parallel, with primary antibodies excluded.

Immunofluorescence was detected on a Zeiss Axio Scope.A1 microscope equipped with fluorescence channels. To determine the number of cells containing ERα or AR, Fos, and double-labeled cells, the mPOA was identified using the anterior commissure and optic chiasm as landmarks at 20 × magnification. All immunolabeled cells were counted bilaterally in a 300 × 400 μm area in the middle of the POA in six sections across the mPOA from rostral to caudal (Fig. 1). Counts were performed manually using ImageJ. Cell counts were averaged across both hemispheres. We use the terminology anterior (2 most rostral sections), central (2 middle sections) and posterior (2 most caudal sections) mPOA.

2.4. Serum estradiol

At euthanasia, a terminal blood sample was collected and placed on ice until it was centrifuged at 4 °C 1500 × g for 10 min. Serum was collected and stored at − 80 °C until analysis. All hormone assay protocols were identical to those published previously (Yin et al., 2015). Estradiol concentrations were analyzed in duplicate samples (200 μL) in a single assay, using an estradiol RIA kit (Cat. No. DSL-4800, Beckman Coulter, Webster, TX); assay sensitivity was 6 pg/mL and intra-assay CV was 4.3%. We were unable to measure serum testosterone due to a shortfall in serum volumes.