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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 ER α -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 ER α cells did not change with age or after sex, but the percentage of AR- and ER α -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 ER α (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 ER α in the mPOA with copulation, raises the possibility that differences in relationships between hormones, behavior, and neural activation may underlie some age-related impairments.

Keywords

Aging; Male; Estrogen receptor alpha; Androgen receptor; Preoptic Area; Estradiol

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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 (Amstislavskaja 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 5 α -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, Rodriguez-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 (ER α) and androgen receptor (AR) (Ottinger et al. 1995, Perez et al. 2003, Wu and Gore 2009, Wu and Gore 2010, Simerly et al. 1990). These ER α and AR sensitive cells in the mPOA are both activated by copulation (Greco 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 ER α in this region. To test this, we quantified ER α -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).

Materials and Methods

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 μ g estradiol benzoate (s.c.), followed 44 hours later by an injection of 400 μ g (s.c.) progesterone. Testing took place 4 hours 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.

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 1h 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 hour after ejaculation (sex groups) or handling (no-sex groups).

Immunohistochemistry

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

Sections underwent immunohistochemical staining for either ER α 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 0.1M PB, 4 $\times$ for 5 min, preceded all incubations. Sections underwent the following incubations: 1% H₂O₂ in 0.1M PB, and then blocked for 60 min in 2% normal goat serum and 0.04% Triton-X in 0.1M PB (blocking solution); then rabbit anti-ER α primary antibody (1: 8,000; 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 0.1M PB; Vectastain ABC Elite kit; Vector Laboratories, Burlingame, CA). Sections were then incubated with biotinylated tyramine (1:1000 in 0.1M PB; Perkin Elmer, Waltham, MA) for 10 min, and visualized with Alexa 488-tagged streptavidin (1:400 in 0.1M PB; Life Technologies, Grand Island, NY). After washing thoroughly with 0.1M PB, sections were then incubated with mouse anti-Fos primary antibody (1:600; Santa Cruz Biotechnology sc-271243, RRID: 1563391, Ladron de Guevara-Miranda et al. 2017) in blocking solution, overnight at room temperature. The following day, sections were incubated for 60 minutes with Alexa 555 goat-anti mouse secondary (1:400 in 0.1M 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 ER α or AR, Fos, and double-labeled cells, the mPOA was identified using the anterior commissure and optic chiasm as landmarks at 20 $\times$ magnification. All immunolabeled cells were counted bilaterally in a 300 $\times$ 400 μ m 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.

Serum Estradiol

At euthanasia, a terminal blood sample was collected and placed on ice until it was centrifuged at 4°C 1500 $\times$ g for 10 minutes. 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 μ l) 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.

Statistical Analysis

The mPOA is highly heterogeneous from rostral to caudal, with different levels playing differing roles in the regulation of appetitive or consummatory sexual behaviors (Balthazart and Ball 2007). Therefore, analysis was performed independently for the anterior, central, and posterior subsections of the mPOA, each represented by 2 sections per rat. First, a one-way ANOVA was used to determine if the number of ER α and AR cells differed across these mPOA subsections; Tukey's post hoc tests were used for follow up when appropriate. For subsequent analyses, two-way ANOVA was used to determine main effects of age and sex, and their interactions, followed by post hoc tests when appropriate. This method was used to analyze differences in the number of cells immunopositive for ER α , AR, Fos, and the percent of Fos-positive cells that expressed AR or ER α , as well as serum estradiol. ANOVAs were performed with R (version 3.2.2) and significance was set at $p < 0.05$. Effect sizes were calculated in excel based on ANOVA results. Correlations between estradiol and behaviors were done using Sigma Stat, independently on anterior, central and posterior mPOA subsections, and separately for the MAT and AG males.

Results

Representative micrographs are presented in Figure 1, along with a map of the anterior, central, and posterior mPOA areas that were analyzed. First, we used one-way ANOVA to determine whether ER α , AR, or Fos cell numbers varied from anterior to posterior. For ER α , there was a significant difference in the number of ER α containing cells across the 3 subregions of the mPOA ($F_{(2,96)} = 90.32$, $\eta^2 = 0.653$, $p < 0.001$; Figure 2). Tukey's post hoc tests demonstrated the central mPOA had significantly more ER α -positive cells than both the posterior mPOA and the anterior mPOA. For AR, oneway ANOVA revealed there was a significant difference in the number of cells across subregions of the mPOA ($F_{(2,90)} = 188.6$, $\eta^2 = 0.807$, $p < 0.001$; Figure 2), with the posterior mPOA having significantly more AR-positive cells than the central mPOA, which in turn had significantly more AR-positive cells than the anterior mPOA. For Fos there was a significant difference across subregions ($F_{(2,90)} = 90.32$, $\eta^2 = 0.179$, $p < 0.001$; Figure 2). Tukey's post hoc tests demonstrated the central and posterior mPOA both had significantly more Fos-positive cells than the rostral mPOA. Because of these region-specific differences, subsequent analyses are presented from anterior to posterior across the mPOA.

Anterior mPOA

Numbers of ER α , AR and Fos-positive cells—There were no significant main effects of sex, treatment, nor an age by treatment interaction, on the number of ER α - (Fig. 2A) or AR-positive cells in the anterior mPOA (Fig. 2D). For Fos, there was a significant main effect of sex ($F_{(1, 30)} = 14.31$, $\eta^2 = 0.394$, $p < 0.001$) on the number of Fos-positive cells in the anterior mPOA (Fig. 2G), such that subjects that had sex prior to sacrifice had significantly more Fos-positive cells than their no-sex counterparts. However, there was no effect of age nor was there an age by sex interaction on Fos cell numbers.

Percentages of double-labeled cells—The percentage of ER α -positive cells that co-expressed Fos in the anterior mPOA showed a significant main effect of sex ($F_{(1, 26)} =$

23.19, $\eta^2 = 0.435$, $p < 0.001$) but no effect of age, nor was there an age by sex interaction (Fig. 3A). By contrast, the percentage of Fos-positive cells that co-expressed ER α was unaffected by age, sex, or their interaction (Fig. 4A).

The percentage of AR-positive cells that co-expressed Fos was also significantly affected by sex ($F_{(1, 26)} = 6.41$, $\eta^2 = 0.196$, $p < 0.018$), but there was no effect of age, nor an age by sex interaction (Fig. 3D). For the percentage of Fos-positive cells that co-expressed AR, there were no main effects of age or sex, nor a significant interaction (Fig 4D).

Central mPOA

Numbers of ER α , AR and Fos-positive cells—The numbers of ER α -positive cells in the central mPOA were unaffected by sex, age, or their interaction (Fig. 2B). This was also the case for AR-positive cell numbers (Fig. 2E). However, there was a significant main effect of sex ($F_{(1, 29)} = 89.99$, $\eta^2 = 0.754$, $p < 0.001$) on the number of Fos-positive cells. Specifically, male rats that had sex prior to euthanasia had significantly more Fos-positive cells than the no-sex males (Fig. 2H). There was not a main effect of age nor an age by sex interaction on Fos-positive cell numbers.

Percentages of double-labeled cells—The percentage of ER α -positive cells that co-expressed Fos had a significant effect of sex ($F_{(1, 29)} = 160.46$, $\eta^2 = 0.824$, $p < 0.001$) as well as a significant age by sex interaction ($F_{(1, 29)} = 5.28$, $\eta^2 = 0.027$, $p < 0.05$; Fig. 3B). For the interaction, post hoc analysis revealed that AG-S animals had significantly more ER α -Fos cells than all other groups. MAT-S animals also had more ER α -Fos than MAT-NS. For the percent of Fos-positive cells that co-expressed ER α , there was a significant age by sex interaction ($F_{(1, 29)} = 11.63$, $\eta^2 = 0.194$, $p < 0.01$; Fig. 4B). Specifically, post hoc analysis revealed that AG-S animals had significantly more Fos-ER α cells than all other groups.

The percentage of AR-positive cells that co-expressed Fos in the central mPOA had a significant main effect of sex ($F_{(1, 27)} = 6.41$, $\eta^2 = 0.196$, $p < 0.05$; Fig. 3E). Males that had sex had significantly more cells than those that did not copulate. However, there was no main effect of age, nor an age by sex interaction (Fig. 3E). For the percentage of Fos-positive cells that co-expressed AR, there was a significant main effect of sex ($F_{(1, 27)} = 8.48$, $\eta^2 = 0.239$, $p < 0.01$; Fig. 4E), such that animals that had sex had significantly more cells than those that did not copulate prior. However, there was not a main effect of age nor an age by sex interaction on this endpoint.

Posterior mPOA

Numbers of ER α , AR and Fos-positive cells—For ER α and AR in the posterior mPOA, there were no significant main effects of either sex or age on immunopositive cells, nor was there an age by sex interaction (Fig. 2C, 2F). For Fos, there was a significant main effect of sex ($F_{(1, 28)} = 36.71$, $\eta^2 = 0.557$, $p < 0.001$), with male rats that had sex prior to euthanasia having significantly more Fos-positive cells (Fig 2I). However, there was not a main effect of age, nor was there an age by sex interaction.

Percentages of double-labeled cells—The percentage of ER α -positive cells that co-expressed Fos had no main effect of sex or age, nor was there an age by sex interaction (Fig. 3C). The percent of Fos-positive cells that co-expressed ER α was significantly affected by sex ($F_{(1, 28)} = 5.80$, $\eta^2 = 0.171$, $p < 0.05$), but there was no age effect, nor an age by sex interaction (Fig. 4C).

For the percentage of AR-positive cells that co-expressed Fos, there was a significant main effect of sex ($F_{(1, 26)} = 6.41$, $\eta^2 = 0.196$, $p < 0.05$), but no main effect of age or an age by sex interaction (Fig. 3F). Results on the percentage of Fos-positive cells that co-expressed AR revealed a significant main effect of sex ($F_{(1, 28)} = 18.36$, $\eta^2 = 0.355$, $p < 0.01$) whereby animals that had sex had significantly more such double-labeled cells than animals that did not copulate prior to euthanasia (Fig. 4F). Additionally, there was a significant main effect of age ($F_{(1, 28)} = 4.50$, $\eta^2 = 0.087$, $p < 0.05$), with mature animals having significantly more Fos-AR cells than aged animals. However, there was not an age by sex interaction.

Sex Behavior

Animals that had sex prior to euthanasia had their behaviors scored for numbers of mounts, numbers of intromissions, mount latency, intromission latency and ejaculation latency. The only significant difference between MAT-S and AG-S animals was in the number of mounts (Fig. 5A, $d = 1.015$, $p < 0.05$), for which mature animals had a greater number of mounts than aged animals. Numbers of intromissions, and behavioral latencies, were similar between the mature and aged rats (Fig. 5B).

Serum Estradiol Concentrations, and Correlations with Behaviors

A two-way ANOVA revealed there were main effects of both sex ($F_{(1, 28)} = 6.023$, $\eta^2 = 0.151$, $p < 0.05$) and age ($F_{(1, 28)} = 7.952$, $\eta^2 = 0.200$, $p < 0.05$; Fig. 6) on serum estradiol. The AG animals had higher estradiol concentrations than MAT animals, and estradiol concentrations were higher in the sex than the non-sex groups. Correlations were run between serum estradiol concentrations separately in the mature and aged males. Estradiol did not correlate with any behavioral measures in the mature animals. A significant positive correlation was found between estradiol and intromission latency only in aged animals ($r = 0.873$, $p < 0.01$; Fig. 7B). Aged animals also had a nonsignificant positive correlation between estradiol concentrations and mount latency ($r = .723$, $p = 0.066$; Fig. 7A).

Discussion

This study examined the activation of steroid hormone receptors in the mPOA after copulation in aging males, and relationships among receptor activation, sex behavior, and peripheral serum estradiol. The sexual impairments in our aged males were relatively modest based on previous studies (Chamber and Phoenix 1984, Wu and Gore 2010, Smith et al. 1992), with the only behavioral difference a decreased number of mounts in aged males. However, the age of our older males at testing, 13 months, was younger than those in most other studies.

Regarding hormone receptors, we found no age-related differences in cell number for either ER α , AR or Fos in any of the sub-regions examined, but an up-regulation in Fos-

immunolabeled cells in animals that copulated. When the percentage of Fos cells that co-expressed ER α or AR were examined, though, several differences with aging emerged that we believe may relate to the behavioral changes. There was also an age-related increase in the %Fos cells that co-expressed ER α in the central mPOA, and a decrease of %Fos-AR cells with age. Serum estradiol was higher both in aged animals, and animals that had sex. Estradiol did not correlate with ER α or AR cell number in either age group, or with any behavioral measures in young animals. It did correlate with mount and intromission latencies in aged animals.

It is important to note a possible confound with our experiment, namely, that an exact match in the timing of the sexual experience was not possible with two different age groups. We had to choose between giving experience to all animals when they were young adults and having a larger gap between the final experience day and the day of testing in the aged group, or giving experience to all groups in the two weeks prior to testing, and we opted for the first option as the better model for our experimental question. However, we also note that aging impairs behavior without regard for recency of sexual experience. It is notable that experienced aged males show impaired sexual behavior whether there was a gap between their initial sexual behavior and time of testing (Wu and Gore 2010), or if the males were tested continuously from a young age (Smith et al. 1992). Despite this possible confound, we believe that our data show a particular importance for estradiol in sexual behavior in aging males.

Sexual Experience, Steroid Hormones and Behavior

Sexual experience changes male sexual behavior, serum hormone concentrations, and hormone receptor expression. Experience alters both appetitive and consummatory aspects of sexual behavior, increasing ultrasonic vocalizations and fos expression in the MPOA, and decreasing the number of intromissions before ejaculation and post-ejaculatory intervals (Bialy et al. 2000, Lumley and Hull 1999, Larsson 1959). Testosterone, and sometimes AR, but not estradiol or ER α , is also increased with experience (Wu and Gore 2009, Swaney et al. 2012, Hull and Dominguez 2006).

Castration eliminates sexual behavior in males, which is restored by either testosterone or estradiol plus DHT (McGinnis and Dreifuss 1989). DHT alone does not result in behavior significantly different from castrates, and estradiol alone facilitates some measures of sexual behavior, but usually does not restore a full copulatory sequence, emphasizing the important of having both an androgen and an estrogen in circulation (Attila et al. 2009, McGinnis and Dreifuss 1989, Wu and Gore 2009, Goya et al. 1990). There is evidence that there are different hormone-dependent mechanisms controlling different components of sexual behavior (reviewed in Hull and Dominguez 2007). Estradiol is particularly important in partner preference, sexual motivation, and mounting (Hamson et al. 2008, Yeh et al. 2009, Bakker et al. 2002, Roselli et al. 2003). In fact, mice with testicular feminization (TFM) that lack the androgen receptor still prefer estrous over non-estrous females, and have normal mount latencies (Zuloaga et al. 2008). This indicates that a functional androgen receptor may not be required for these appetitive behaviors. Consistent with this role of estrogens, aromatase knockout mice also showed increased latencies to mount, intromit or ejaculate,

and showed no partner preference at all (Bakker et al. 2002). By contrast, androgen receptor activation is much more strongly implicated in ejaculation and satiety (Romano-Torres et al. 2007, Yeh et al. 2009), and erection is also more dependent on circulating androgens than estradiol (Hull and Dominguez 2007).

Steroid Hormones, Sex, and Aging

Testosterone and sexual behavior both decline during aging, but the concentration of testosterone does not predict behavior in aged animals, and testosterone administration to aged castrates does not restore behavior in a similar manner to young males (Wu and Gore 2009, Smith et al. 1992, Goya et al. 1990, Roselli et al. 1993, Chambers and Phoenix 1986). However, previous research has also found facilitation of mount latency by testosterone only in aged animals (Wu and Gore 2010). We would have liked to have been able to measure serum testosterone in the current study (not possible due to inadequate serum), but we predict it would not have changed drastically between our mature and aged groups due to the lack of differences in the number of intromissions with age.

Reports of serum estradiol concentrations during aging have been inconsistent, with results showing them increasing, decreasing, or remaining the same (Luine et al. 2007, Herath et al. 2001, Wu and Gore 2009, Goya et al. 1990, Smith et al. 1992, Herrera-Perez et al. 2008). An age-related elevation in estradiol may be due to increased aromatization in adipose tissue (Gautier et al. 2013). We also found increased estradiol in males of both ages after sexual behavior. Measuring estradiol immediately after sexual behavior is not common in males, but it has been found that they may experience a reflexive release of LH and testosterone, both upon the introduction of a female and after ejaculation (Nyby 2008, Shulman and Spritzer 2014, Kamel and Frankel 1978). Increased serum testosterone would be available for aromatization in adipose tissue, or more estradiol may be secreted directly by the testes in response to the LH surge (Winters and Troen 1986). In these studies, reflexive testosterone release did not correlate with any measure of male sexual behavior, except that it was absent in males failing to copulate, leading some to suggest its involvement in sexual satiety (Kamel and Frankel 1978, Nyby 2008). The purpose of reflexive release is not known, as lower fixed concentrations of testosterone are sufficient for copulation, but that it is highly conserved indicates it does have some function (Bronson and Desjardins 1982, Maruniak and Bronson 1976, Nyby 2008).

AR and ER α Activation in Sexual Behavior and Aging

We found no age-related changes in either ER α or AR in any of the subregions in the mPOA. This is consistent with previous studies of ER α in males showing no age-related changes in ER α mRNA or protein (Madeira et al. 2000, Bottner et al. 2007, Wu and Gore 2010). AR is less consistent, with previous studies reporting either increased (Wu and Gore 2009), decreased (Chambers et al. 1991), or no change (Wu and Gore 2010) in this receptor.

Work in young animals has showed about 30% of Fos cells that were activated by mating also expressed ER α (Greco et al. 1998). Although that study did not measure Fos coexpression with AR, they did examine colocalization of ER α and AR. Approximately 80-90% of ER α expressing cells also contained AR, making it likely that many ER α cells in

this study also contain AR. Although they did not examine mating induced activation, Wood and Newman (2008) also found AR and ER α colocalization in hamster nuclei that both have high steroid hormone concentrations and are important in male sexual behavior, including the mPOA, BnST, and medial amygdala. In the mPOA they found regional differences in cells expressing only ER α , which were increased in number in the rostral mPOA.

Conclusion

We initially hypothesized that decreased activation of ER α and AR, or changes in receptor expression, in the mPOA may be contributing to sexual impairments in aged males. Our results show that not only was receptor expression largely the same across the mPOA in mature and aged animals, but activation of AR and ER α was similar. The fact that impairments of sexual behavior in aged animals were limited to numbers of mounts suggests that our animals at 13 months are on the cusp of the transition into reproductive senescence. What is most interesting is that at this point in life, serum estradiol concentrations are increasing, motivational behaviors (mounting) are decreasing, and a relationship emerges between serum E2 and latencies to intromit (and a trend to mount). These results can also be related to findings of Fos cells that co-express ER α . In the central POA, there is a substantial and significant increase in %Fos-ER α cells only in the aged males, that when considered together with the serum hormone and behavior data, suggests this subpopulation of cells as a neuroanatomical substrate for this change.

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Highlights

- The percentage of AR- and ER α -cells that co-express Fos were significantly up-regulated by sex, independent of age.
- Age affects the percentage of Fos cells co-expressing ER α and the percentage of Fos cells co-expressing AR in the mPOA.
- Estradiol concentrations positively correlated with intromission latency in aged but not mature animals.

Fig 1.

A) Representative micrograph of double-labeling of ERα- and Fos- in the mPOA. ERα-immunoreactive cells are labeled with a green fluorophore as indicated with a green arrow. Fos-immunoreactive cells are labeled with a red fluorophore as indicated with a red arrow. Colocalized ERα and Fos immunoreactive cells appear yellow, and an example shown with the orange arrow. B) Representative micrograph of double-labeling of AR (green) and Fos (red), similarly labeled as in A. Counting of single- and double-labeled cells was done in $30 \times 400 \mu\text{m}^2$ sections of the mPOA at anterior (C, Bregma 0.12mm), central (D, Bregma -0.36mm), and posterior (E, Bregma -0.96) levels. Coordinates are shown with respect to Bregma from Paxinos and Watson, 2007. Scale bar = 10 μm.

Fig 2.

Total numbers of ER α (A-C), AR (D-F) and Fos (G-I) immunoreactive cell counts are shown in mature and aged male rats from the sex and no-sex groups in the three mPOA sub-regions. There were no main effects or interactions for ER α or AR in any of the sub-regions. Fos-positive cells were higher in all of the mPOA sub-regions of animals that had sex. Abbreviations here and in other figures are: MAT-S, mature-sex; MAT-NS, mature-no sex; AG-S, aged-sex; AG-NS, aged-no sex. Data here and subsequently are mean + SEM.

Fig 3.

The percentage of ER α or AR cells that co-express Fos are shown from anterior to posterior. For ER α -Fos double labeling (A-C), there were main effects of sex (increased compared to no sex) in the anterior and central mPOA ($p < 0.001$). In the central mPOA (B) there was also a significant interaction of age and sex, with AG-S group having a greater percentage of double labeled cells than any other group. In addition, the MAT-S group was greater than the MAT-NS for this endpoint. AR-Fos double labeling (D-F) was significantly higher in sex than no-sex males across the mPOA. P-values for interactions are indicated as: **, $p < 0.01$, *** $p < 0.001$.

Fig 4.

The percentage of Fos-positive cells also expressing ER α or AR are shown from anterior to posterior. For ER α -Fos double labeling, the central mPOA (B) had a significant interaction of age and sex, with the AG-S group having more double-labeled cells than any other groups. In the posterior mPOA (C), there was significant main effect of sex, with fewer cells in the sex than the no-sex groups. For AR-Fos double labeling, in the central mPOA (E) animals that had sex had more double-labeled cells compared to no-sex animals. In the posterior mPOA (F), main effects of both age and sex were found, with higher numbers in sex vs. no-sex rats, and an age-related decrease. P-values for interactions are indicated as: *, $p < 0.05$; **, $p < 0.01$.

Fig 5. Behavioral measures are shown for MAT-S and AG-S groups. A) Numbers of mounts were significantly higher in MAT-S than AG-S males ($p < 0.05$). Numbers of Intromissions, and latencies to mount, intromit, or ejaculate (B) were unaffected.

Fig 6.

Serum estradiol concentrations are shown, measured 1 hour post-copulation or handling. There was a main effect of both sex and age ($p < 0.05$), with AG animals having higher estradiol concentrations than MAT animals, and animals that had had sex prior to sacrifice having higher estradiol concentrations than those that did not.

Fig 7.

Correlations between serum estradiol and mount latency (A) and intromission latency (B) are shown separately for MAT-S and AG-S male rats. In AG-S males, mount latency had a non-significant trend for a positive correlation with estradiol ($r = 0.723$, $p = 0.066$). Intromission latency had a significant positive correlation with estradiol ($r = 0.873$, $p < 0.01$) in the AG-S males.