Published in final edited form as:

Brain Res. 2011 March 16; 1379: 137–148. doi:10.1016/j.brainres.2010.11.004.

I. Levels of 5 α -reduced progesterone metabolite in the midbrain account for variability in reproductive behavior of middle-aged female rats

Alicia A. Walf^d, Jason J. Paris^a, Danielle C. Llaneza^a, and Cheryl A. Frye^{a,b,c,d,*}

^aDepartment of Psychology, The University at Albany-SUNY, Albany, NY, USA

^bDepartment of Biological Sciences, The University at Albany-SUNY, Albany, NY, USA

^cCenter for Neuroscience Research, The University at Albany-SUNY, Albany, NY, USA

^dCenter Life Sciences Research, The University at Albany-SUNY, Albany, NY, USA

Abstract

At middle-age, the reproductive capacity of female rats begins to decline. Whether there are consequences for social and reproductive behaviors related to changes in estradiol (E₂), progesterone (P₄) and its 5 α -reduced metabolites, dihydroprogesterone (DHP) and 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP), is of interest. In Experiment 1, 1-year-old female breeder rats that had “maintained their reproductive status” (having 4–5 days estrous cycles, > 60% successful pregnancies after mating, > 10 pups/litter) or their age-matched counterparts with “declining reproductive status” were assessed in social interaction, standard mating, and paced mating when in proestrus. Rats that maintained reproductive status tended to have higher levels of proceptivity, and significantly reduced aggression, towards males, compared to rats with declining reproductive status. Basal midbrain E₂ and DHP levels accounted for a significant proportion of variance in lordosis. In Experiment 2, 1-year-old, age-matched, female breeders that had maintained reproductive status or were in reproductive decline were compared to three-month old, nulliparous females that had regular (4–5 days) or irregular estrous cycles. Age did not influence paced mating but younger rats had greater diencephalon E₂ than did middle-aged rats. After mating, rats with declining/irregular reproductive status had higher P₄ and DHP levels in midbrain than did rats with maintaining/regular reproductive status, albeit differences in midbrain 3 α ,5 α -THP were not seen. Middle-aged rats that maintained reproductive function had greater 3 α ,5 α -THP formation in diencephalon compared to other groups. Thus, age-related changes in central progestogen formation in midbrain or diencephalon may contribute to some variability in expression of reproductive behaviors.

Keywords

Aging; Estropause; Lordosis; Progesterone; Social interaction

1. Introduction

Reproductive senescence of women is characterized by decline in ovarian function occurring concomitant with aging. The onset and manifestation of reproductive senescence, as with

other behaviors and sequelae that are sensitive to aging (e.g. cognition, affect/quality-of-life, libido, cardiovascular changes, etc.), is highly variable among individuals (Henderson, 2009; Hodis and Mack, 2009; Ryan et al., 2009). As such, it can be a challenge to determine the contribution of ovarian versus aging effects on reproductive function, but this information may be critical for treatment of symptomology associated with menopausal decline in ovarian steroids. To begin to discern the brain targets and mechanisms for these effects, a typical approach is to remove the main endogenous source of steroid hormones, the ovaries (i.e. ovariectomy), in young rodents and determine outcomes on behavioral indices. Although this approach models low levels of estradiol (E_2) and progesterone (P_4) among post-menopausal women, ovariectomy is dissimilar to the menopausal transition of women, which can be characterized by low and/or fluctuating E_2 and P_4 levels, as well as other changes in the body associated with aging alone. Thus, a naturalistic approach that can be utilized to determine changes in steroids' functional effects is to investigate individual differences in reproductive status and sequelae among age-matched rats.

Changes in steroid hormones that occur with aging and decline in reproductive ability (often termed reproductive senescence, or estropause) of female rodents are typically inferred from changes in vaginal cytology and/or hypothalamic–pituitary–ovarian axis function. Estropause in rats typically occurs during middle-age (9–12 months old) and neuroendocrine changes in the female at this time comprise a sequence of reproductive states, which can be classified with vaginal cytology: regular estrous cycles, irregular estrous cyclicity, persistent estrous, persistent diestrous, and anestrus (Anzalone et al., 2001; Butcher and Page, 1982; Clemens and Meites, 1971; Dudley, 1982; Finch et al., 1984; Huang et al., 1978; LeFevre and McClintock, 1988; Matt et al., 1986). Rodents typically enter these stages in the order described above and do not revert back to a previous state, but there is some individual variability in this pattern. In support, only about 50% of rats enter the persistent estrous state (LeFevre and McClintock, 1988). Estropause is associated with changes in hypothalamic–pituitary–ovarian axis feedback and variability in E_2 and P_4 levels, particularly during transitioning states (Huang et al., 1978; Lu et al., 1979; Miller and Riegler, 1980; Page and Butcher, 1982; Wise and Ratner, 1980). Given reports of great variability in these neuroendocrine measures, the neuroendocrine mechanisms and behavioral consequences for reproduction needs further examination.

There is some suggestion of changes in reproductive behavior with aging of rodents. For instance, middle-aged rats in persistent estrus display lordosis (i.e. the typical rodent mating posture) when mounted by a sexually-vigorous male, or with manual palpation by the experimenter (Cooper, 1977; Lefevre and McClintock, 1988) on several consecutive days, compared to what is observed among young rats that only display lordosis when in proestrus (i.e. every 4–5 days). It is unclear, but of interest, whether differences are based, in part, upon the type of mating experience the female has. For example, in the laboratory, rats are typically tested for reproductive behavior by placing female rodents in a small chamber, such as an aquarium, with a sexually-experienced male. In this standard mating situation, it often appears that the male controls the timing of sexual contacts because he can chase and/or corner the female to initiate a sexual contact. However, in larger arenas, following an intromission, females typically withdraw from, and then approach, males to receive additional sexual contacts. In this sense, the female self-regulates, or paces, her sexual contacts with the male. In the laboratory, it is possible to use a semi-natural paced mating behavioral task, in which female rodents are mated in a chamber wherein the female, but not the male, can roam freely and temporally-control, or “pace,” her sexual contacts (Cameron et al., 2008; Erskine et al., 2004). This ability to pace contacts alters neuroendocrine function to enhance fertility and fecundity (Frye and Erskine, 1990; Frye et al., 1996). As well, pacing, rather than other less socially-challenging behavioral tasks, has significant effects to increase levels of the P_4 metabolite, and neurosteroid, 5 α -pregnan-3 α -ol-20-one

(3 α ,5 α -THP, a.k.a. allopregnanolone) in the midbrain (Frye and Rhodes, 2006; Frye et al., 2007). 3 α ,5 α -THP is formed from ovarian P₄'s metabolism to dihydroprogesterone (DHP) by 5 α -reduction and followed by 3 β -hydroxylation (Frye, 2009). 3 α ,5 α -THP can also be formed *de novo* in brain regions with high levels of biosynthesizing enzymes, such as in the midbrain ventral tegmental area (Frye, 2009). Of interest in this context is that 3 α ,5 α -THP can modulate the output and feedback of the hypothalamic–pituitary–adrenal/stress axis response (Barbaccia et al., 1996; Patchev et al., 1996). As well, 3 α ,5 α -THP clearly influences behaviors that are not traditionally considered to be reproduction-related, and are mediated by the hippocampus and cortex, such as cognition and affect (Frye and Rhodes, 2006; Frye et al., 2009, 2010a,b). A good example of how 3 α ,5 α -THP may integrate neuroendocrine function, reproduction-related behaviors, and cognitive/affective behaviors to promote lifelong neural plasticity is parity. Recent studies suggest that rats that have had litters, compared to virgin rats, may have greater responses to 3 α ,5 α -THP, have reduced anxiety- and fear-like responding, perform better on a variety of cognitive tasks when young and demonstrate less decline with aging (Byrnes and Bridges, 2006; Gatewood et al., 2005; Kinsley et al., 1999; Macbeth and Luine, 2010; Paris and Frye, 2008; Pawluski et al., 2006a; Walf and Frye, 2008). Thus, the role of P₄ metabolites, such as 3 α ,5 α -THP, in reproductive behaviors with aging is of interest.

In the present study, it was of interest to investigate whether individual variability in reproductive status during aging and social and reproductive behaviors may be related to P₄ and/or its metabolism in the midbrain. In Experiment 1, middle-aged (12 months old) female rats, with a comparable breeding and environmental history, were determined to be maintaining reproductive status (regular 4–5 days estrous cycles, > 60% successful pregnancies when mated, > 10 pups/litter) or declining in reproductive status (irregular estrous cycles, < 60% successful pregnancies when mated, < 10 pups/litter) and were assessed for non-sexual social interaction behavior, sexual behavior in a standard mating paradigm, and sexual behavior in a paced mating paradigm. Concentrations of E₂, P₄ and its metabolites were later assessed in midbrain, diencephalon, and cerebellum when all rats were in the estrus phase. We anticipated that there would be variability in how reproductive status may alter social and reproductive behaviors in the standard and paced mating paradigms and that levels of DHP and/or 3 α ,5 α -THP in the midbrain may partly account for observed behavioral differences. In Experiment 2, middle-aged (12 months old) female rats that were maintaining or had declining reproductive status were compared to nulliparous young adult (3 months old) rats that were characterized by having regular 4–5 days, or irregular, estrous cycles. All rats were tested in the paced mating paradigm when in the proestrous phase of their cycle. After mating, midbrain, diencephalon, and cerebellum tissues were collected for assessment of E₂, P₄ and its metabolites. We anticipated that changes in reproductive status among middle-aged or young adult rats would influence the neurosteroidogenic response to mating in midbrain and/or diencephalon.

2. Results

2.1. Reproductive status characterization

In the present study, the social and reproductive behavior of age-matched, experimentally-naïve female rats, with a lifetime history of breeding (on average approximately 4–6 litters each), that began to demonstrate differences with aging in reproductive status were compared (see Paris et al., 2011). In the past, we have noticed that at approximately 10 to 12 months of age, breeder female rats in our colony begin to show decline in reproductive status/breeding capacity, characterized by reduced likelihood to become pregnant following mating and, if pregnancy is successful, the number of pups per litter is lower than younger breeders. As such, in the present study, between 10 and 12 months of age, rats were assessed

for estrous cyclicity, fertility, fecundity and categorized as maintaining or declining reproductive function based upon selection parameters described below.

2.1.1. Estrous cycle assessment—Vaginal cytology was analyzed, under a microscope, and the proportion of leukocytes, and cornified, and nucleated epithelial cells in the vaginal smears were used to classify rats as being in proestrus, estrus, metestrus, or diestrus. Rather than a precipitous decline in ovarian hormones with aging/estropause among rats, a decline in reproductive status is more so characterized by changes in endocrine feedback and distinct abnormal estrous cyclicity (Clemens and Meites, 1971; Dudley, 1982; Huang et al., 1978). As such, we first determined whether rats had normal 4–5 days estrous cycles or irregular cycles (greater than 5 days on average), as assessed by examination of vaginal cytology. In each cohort, rats were assessed for daily estrous cyclicity between 10 and 12 months of age (except when obviously pregnant or lactating). The average length of a cycle, as defined by days between two proestrous phases, was determined over 30 days of monitoring as per previously published methods (Frye and Bayon, 1999; Long and Evans, 1922). No cycle assessments were based on times when rats were pregnant or lactating. We then compared the average cycle length between regular and irregular cyclers using an unpaired *t*-test ($t_{44}=-8.60$, $p<0.01$) and confirmed that the regularly cycling rats had a lower cycle length (4.6 ± 0.1 days) than did rats with irregular cycles (7.5 ± 0.3 days).

2.1.2. Fertility assessment—The number of successful pregnancies was determined in these rats following their last mating and this information was analyzed as an index of fertility. Rats were mated with a sexually-experienced male when they were sexually-receptive (i.e., proestrous–estrous phase). There was a trend towards a difference, as assessed with an unpaired *t*-test ($t_{44}=1.80$, $p<0.07$), in the percentage of successful pregnancies based upon whether rats had regular ($64.7\pm4.6\%$) or irregular ($51.5\pm5.6\%$) cycles. We then categorized rats as having normative (> 60% successful pregnancies) or low fertility (< 60% successful pregnancies). These two groups were analyzed using an unpaired *t*-test ($t_{44}=-7.23$, $p<0.01$), and found that normative fertility was ($84.3\pm3.8\%$ successful pregnancies) and low fertility was ($45.9\pm3.3\%$ successful pregnancies).

2.1.3. Fecundity Assessment—In rats that had litters following their last mating, the number of pups per litter was utilized as an index of fecundity of the rat. Differences in fecundity were noted between regularly cycling rats (9.0 ± 0.7 pups per litter) and those that had irregular cycles (4.4 ± 1.1 pups per litter), as analyzed with an unpaired *t*-test ($t_{44}=3.77$, $p<0.01$). We then categorized rats as having normative fecundity, which was considered when rats had >10 pups per litter, and low fertility was considered when rats had < 10. These two groups were analyzed using an unpaired *t*-test ($t_{44}=-6.08$, $p<0.01$), and found that normative fecundity was (12.4 ± 0.6 pups per litter) and low fertility was (5.2 ± 0.7 pups per litter).

2.2. Experiment 1—reproductive status affects social and sexual behavior of middle-aged rats

2.2.1. Cyclicity, fertility, and fecundity—Among 12-month-old rats that had been breeders for their lifetime, there were differences in the average length of the estrous cycle based upon reproductive status [$F(1,44)=19.91$, $p<0.01$]. Rats that were considered to be maintaining reproductive status had shorter cycles than did rats that had decline in reproductive status (Fig. 1, left).

Fertility was different among rats with maintaining or declining status [$F(1,44)=8.68$, $p<0.01$]. Rats maintaining reproductive status had a greater percentage of successful

pregnancies following their last mating than did rats with declining reproductive status (Fig. 1, middle).

Fecundity, or average litter size, differed among rats selected for reproductive status [$F(1,44)=24.83, p\leq 0.01$]. Rats maintaining reproductive status had larger litters than did rats with declining reproductive function (Fig. 1, right).

2.2.2. Social interaction—Reproductive status did not significantly alter social interaction with a conspecific. Rats maintaining reproductive status, or those with declining reproductive status, spent a similar duration interacting with a conspecific in this task (Table 1).

2.2.3. Standard sex testing—Reproductive status did not significantly alter lordosis in a standard mating sex task. Rats maintaining reproductive status had similar lordosis quotients and lordosis ratings as did those in reproductive decline (Fig. 2, top left and right). However, there was a tendency for rats maintaining reproductive status to have greater proceptivity quotients than did those rats with reproductive status in decline [$F(1,44) = 2.94, p = 0.09$] (Fig. 2, bottom left). As well, rats maintaining reproductive status had significantly lower aggression quotients than did rats with declining reproductive status [$F(1,44) = 7.66, p < 0.01$] (Fig. 2, bottom right).

2.2.4. Paced mating—Reproductive status influenced some measures of sexual behavior in the paced mating task. For lordosis quotients and ratings, rats maintaining reproductive status were similar to rats with declining reproductive status (Fig. 3, top left and right). For proceptivity, rats maintaining reproductive status had higher proceptivity quotients than rats with declining reproductive status, but this effect was a statistical tendency [$F(1,41) = 2.77, p = 0.10$] (Fig. 3, bottom left). For aggression, rats maintaining reproductive status had significantly lower aggression quotients than did rats with declining reproductive status [$F(1,44) = 5.49, p < 0.02$] (Fig. 3, bottom right). Irrespective of reproductive status, pacing of sexual contacts (indicated by the percent of exits from the male compartment following a sexual contact) was maintained among sexually-receptive rats that maintained reproductive status ($26.2\pm 3.8\%$) compared to those that were experiencing reproductive decline ($23.9\pm 4.2\%$).

2.2.5. Endocrine milieu—As with the behavioral measures discussed above, there was a considerable amount of individual variability in endocrine milieu of rats. In the midbrain, diencephalon, or cerebellum, mean concentrations of neither E_2 and P_4 , nor the P_4 metabolites, DHP and $3\alpha,5\alpha$ -THP, differed significantly between groups (Table 2). Nevertheless, DHP levels in the midbrain accounted for a significant proportion of variance in lordosis quotients and ratings, and tended to contribute to proceptivity and aggression in the standard mating task (Table 3). As well, $3\alpha,5\alpha$ -THP levels in the midbrain accounted for variance in lordosis quotients and ratings in the paced mating task, and lordosis quotients in the standard mating task. There was no indication of significant contributions to variance in these measures for E_2 or P_4 in the midbrain, or any steroids in the diencephalon or cerebellum.

2.3. Experiment 2—reproductive status affects neurosteroidogenesis in response to mating among young adult and middle-aged rats

2.3.1. Paced mating—In smaller control groups, young adult rats that had a regular 4–5 days estrous cycle, or irregular estrous cycles, were compared to middle-aged rats that were maintaining reproductive status or experiencing reproductive decline. There were some apparent, but modest, differences in reproductive measures; however, groups were largely

similar in response to mounting stimuli. Neither lordosis quotients, lordosis ratings, nor proceptivity quotients significantly differed among these smaller control groups (Table 4). Aggression quotients did not significantly differ but were notably reduced among middle-aged rats that maintained reproductive status compared to all other groups (Table 4). Irrespective of age, rats with regular/maintained reproductive status tended to pace their mating contacts less than rats with irregular/declining reproductive status [$F(1,18) = 3.32, p = 0.09$].

2.3.2. Endocrine milieu—Despite similarities in reproductive behavior, the neuroendocrine response to paced mating was significantly different with reproductive status. Midbrain concentrations of P_4 [$F(1,18) = 9.12, p < 0.01$] and DHP [$F(1,18) = 6.18, p < 0.05$] were greater among mated rats with irregular/declining reproductive status compared to those with regular/maintaining reproductive status (Table 4, see *). Midbrain $3\alpha,5\alpha$ -THP was notably high among all groups, but neither $3\alpha,5\alpha$ -THP, nor E_2 , significantly differed in this region (Table 4). In diencephalon, there was a significant interaction [$F(1,18) = 5.26, p < 0.05$], wherein $3\alpha,5\alpha$ -THP was significantly greater among mated middle-aged rats maintaining reproductive status compared to that of mated middle-aged rats with declining reproductive status ($p = 0.05$) or mated young adult rats with regular estrous cycles ($p = 0.02$; Table 4, see ‡). Levels of other steroids in the midbrain did not significantly differ (Table 4).

Age influenced neuroendocrine milieu among mated rats. Middle-aged rats had significantly lower levels of E_2 in diencephalon than did young-adult rats [$F(1,18) = 8.59, p < 0.01$] (Table 4, see †). In cerebellum, there were no significant differences in concentrations of any steroids.

3. Discussion

3.1. Individual differences in estradiol and progesterone metabolites may mediate aspects of mating among middle-aged rats

The current findings partially supported our first hypothesis that changes in reproductive status would concur with reproductive behavior and basal concentrations of P_4 metabolites in the brain. Behavioral responses of rats with different reproductive viability (selected based on cyclicity, fertility, and fecundity at the time of testing), but with comparable breeding histories, were highly variable. The group differences found in the social interaction task were modest, but were similar to previous reports on socially-relevant behaviors. For example, in a study comparing social interaction of young (3–6 months old) and aged (20–30 months old) Wistar rats, similar duration was spent interacting with a novel conspecific in both age groups (Hunt et al., in press). Minimal between-groups differences were also observed in the frequency of lordosis displayed in mating tasks. However, there were differences noted for proceptivity and aggression behavior towards males in the standard and paced mating tasks, with rats that had maintained reproductive status having greater proceptivity and lower aggression than rats with decline in reproductive status. As well, substantial individual variability in basal steroid levels (assessed in the estrous phase of the cycle) in midbrain, diencephalon, and cerebellum were observed within groups. Basal concentrations of midbrain E_2 or DHP accounted for a significant proportion of variance in lordosis in the standard mating task, and DHP tended to contribute to proceptivity and aggression, in this task. Midbrain E_2 also significantly contributed to variance in lordosis expressed in the paced mating task and tended to contribute to proceptivity in this task. Furthermore, $3\alpha,5\alpha$ -THP levels contributed to the variance in lordosis in either the standard or paced mating tasks and tended to influence aggression on the paced mating task. Together, these data suggest that individual differences in P_4 metabolite formation in the

midbrain may contribute to some variability in reproductive behaviors among middle-aged rats.

3.2. Reproductive status contributes to the neurosteroidogenic response to paced mating among middle-aged and young adult rats

In Experiment 2, our hypothesis that reproductive status of middle-aged or young adult rats would influence the neurosteroidogenic response to paced mating was partially-supported. It is notable that Experiment 1 was limited in that subjects were tested in measures of cognitive performance prior to testing in social and sexual measures. As well, subjects were tested in affective and cognitive tasks prior to tissue collection. It is possible that these experiences may have influenced later behavioral, or endocrine, responses. Experiment 2 assessed rats that were tested only once in paced mating and tissues were collected immediately after testing. As in Experiment 1, behavioral differences among middle-aged and young adult rats when tested in proestrus for paced mating were minimal. However, when tissues were collected immediately after mating, it was apparent that those rats that had declining or irregular reproductive status had greater concentrations of P_4 and DHP in the midbrain, but did not differ in their $3\alpha,5\alpha$ -THP levels, compared to those with regular or maintaining reproductive status. These data may indicate dysregulation in neurosteroid formation from central vs. peripheral sources. In support, central formation of $3\alpha,5\alpha$ -THP in diencephalon was significantly greater among middle-aged rats maintaining reproductive status compared to those with declining reproductive function or young adults that were regularly cycling. As such, an interesting future direction will be to consider the influence of changes in peripheral glands (ovaries/adrenals) or central enzymatic processes that may account for changes in neurosteroid formation related to age and/or reproductive function.

3.3. Age-related changes in factors that promote neurosteroidogenesis in the VTA or VMH may partly underlie observed effects

The present study extends previous findings by addressing the extent to which age-related neuroendocrine fluctuations account for variability in reproductive capacity. Previous work has demonstrated that some of the individual differences in whether aged rats display constant lordosis are related to circulating P_4 levels during the estrous cycle (Gans et al., 1995). Although we were not able to measure steroid levels of all rats in the present study, we observed great variability in steroid levels with aging among a subset of rats assessed. It may be that some of the variabilities in the behavioral changes that are observed with reproductive decline are modulated by P_4 metabolites. In another study, compared to cycling 4-month old female, Wistar rats, 16-month old female rats have lower levels of $3\alpha,5\alpha$ -THP in serum, hippocampus, hypothalamus, and anterior pituitary gland (Genazzani et al., 2004). Indeed, in the present basal levels of midbrain E_2 , DHP, and $3\alpha,5\alpha$ -THP were shown to influence variability in reproductive measures. When measured at the time of mating, midbrain levels of P_4 and DHP, as well as diencephalon levels of $3\alpha,5\alpha$ -THP, were associated with changes in age and reproductive status. Among young rodents, central biosynthesis of $3\alpha,5\alpha$ -THP can be induced by E_2 (Cheng and Karavolas, 1973; Frye et al., 1998; Micevych et al., 2008). As such, mating-induced basal levels of E_2 in midbrain may account for enhancements in $3\alpha,5\alpha$ -THP in response to mating.

The subtle differences noted in the present study for progestogen levels in the midbrain and diencephalon suggest that with aging and reproductive senescence, decline throughout the brain may not be uniform. A question to be addressed is whether there are site-specific effects of progestogens to mediate subtle changes in behavior. Indeed, we have observed that only $3\alpha,5\alpha$ -THP's actions in the VTA (and not other areas of the midbrain, including central grey and substantia nigra; Frye and Rhodes, 2006; Frye et al., 2008) influence lordosis. Others have demonstrated that muscimol (a $GABA_A$ agonist like $3\alpha,5\alpha$ -THP) is

rewarding when infused to the posterior (but not anterior) VTA (Ikemoto et al., 1998), suggesting site-specificity for naturally-rewarding nuclei within this region. Within the diencephalon, the ventromedial hypothalamus is a known site that is important in modulating lordosis. Classically, the VMH is thought to influence initiation of lordosis via actions of E₂; however, recent work demonstrates that the non-genomic Src-kinase signaling pathway that can be mediated by 3 α ,5 α -THP, acts to promote lordosis in this nucleus (González-Flores et al., 2010). As such, enzymatic/neurosteroidogenic factors that promote 3 α ,5 α -THP formation in the VTA and VMH may be future targets of study in reproductive decline associated with age and endocrine status.

3.4. Mating-related behavioral/endocrine changes may have implications for the perimenopausal transition and affective/cognitive decline

Although the present study suggests that there may be some subtle differences in reproductive behavior with aging and reproductive decline, it may be that the magnitude of difference are modest in these measures compared to measures of cognitive and affective function, which seem to be even more sensitive to these changes. Indeed, we and others, have demonstrated that there are differences in other behavioral measures, such as affect, cognition, and pain sensitivity, with changes in reproductive status of female rats (Berkley et al., 2007; Paris et al., 2011; Walf and Frye, 2008; Walf et al., 2009). It is of interest whether some of these subtle changes in reproductive responses demonstrated herein are related to changes in these non-reproductive measures. For instance, defensive aggression, normally exhibited by female rodents towards males, as well as anxiety/fear responses to novel or aversive stimuli, must be dampened for mating to occur. We have demonstrated greater levels of anxiety-like behavior in rats with declining reproductive status, compared to those that have maintained reproductive status, following exposure to novel and aversive situations (i.e. elevated plus maze, zero maze, mirror maze, and Vogel punished drinking; Walf et al., 2009). As well, we have recently reported that rats maintaining reproductive status outperformed rats with decline in reproductive status on several cognitive tasks (object recognition, Y-maze, and water maze), and levels of P₄ and 3 α ,5 α -THP in the medial prefrontal cortex and hippocampus contributed to these behaviors (Paris et al., 2011). Together with the results of the present study, these findings suggest that there is heterogeneity in aging- and reproductive decline-related changes in the brain, and functional effects.

Perimenopause may be a time of challenge and opportunity. During the perimenopausal transition, physical and psychological change associated with the climacteric can occur. In support, some women may experience more subtle differences in sexual responding than other physical and/or psychological symptoms of perimenopause (Freeman et al., 2007). It has been proposed that some hormone-based therapeutics may be most efficacious when administered during the years proximate to the perimenopause. As such, it is important to have animal models to investigate this transition period. The model described in the present study may be particularly useful in lieu of more invasive manipulations, such as ovariectomy or 4-vinylcyclohexene diepoxide given the face, construct, and predictive validity that accompanies a natural model of reproductive senescence. Using this ethologically-relevant approach, variations in hormones (estrogen and progestogens) and behaviors (appetitive aspects of sexual behavior) that change with perimenopause are evident. This model can be used to assess co-morbid changes in affective and cognitive functions. Of note, the incidence of sexual desire disorder among women is overestimated when current depression is accounted for, suggesting that these changes in sexual function may be secondary to affective/cognitive responses (Johannes et al., 2009). Further, the predictive validity of this approach has been demonstrated. Hormone replacement therapies that are used with some success among women can remediate changes in behavior or

physical symptoms of the reproductive transition, which are foremost concerns among women that seek such therapies (Santen et al., 2010). We have observed that conjugated equine estrogens, in combination with medroxyprogesterone, can improve affective behavior of middle-aged rats utilizing the present model of natural reproductive transition (Frye et al., 2010a,b). Indeed, such models are needed, given that it is not just hormone-based therapies that are on the rise during the perimenopause, but prescriptions for anti-depressants (Schmidt and Rubinow, 2006, 2009). Thus, the present model of natural reproductive transition may be useful in assessing therapeutics that target the “window of opportunity” that is the perimenopausal transition.

3.5. Social and environmental factors may influence age-related behavioral/endocrine outcomes

Another consideration to make in interpreting the present results is the background and social environment of rats in the present study. The present experiments came from the observation in our laboratory that rats that were breeders and from the same source (Taconic Farms) and age (10 months old) rats began to show different levels of decline in standard reproductive indices (cyclicity, fertility, and fecundity) which were utilized as selection parameters for experimental assessment. This pattern was replicated in a second cohort from Taconic Farms as well as two smaller cohorts derived from in-house breeding. In the present study, rats were group-housed and were multiparous, experiencing near constant pregnancies and lactation throughout their adult lives until estropause. As well, rats were housed in a brand-new facility that had some renovations being completed at the time, which may have influenced subjects, but also had a similar breeding history and comparable successful pregnancies prior to inclusion in this study. The pattern in which rats enter estropause can be altered by environmental or experiential factors, such as social isolation and parity (Hermes and McClintock, 2008; Lefevre and McClintock, 1991, 1992). Some of the subtle differences that were noted in the present study for reproductive function may be due, in part, to rats being group-housed, which is typical to their natural pattern of living with conspecifics in burrows, as well as their high degree of multiparity. In younger rodents, it is clear that these social parameters can alter neuroendocrine function and behavior, such that isolation reduces fertility and fecundity (Calhoun, 1962). Social isolation is used as a model of stress-related disorders, and decreases $3\alpha,5\alpha$ -THP levels, and increases aggressive behavior (Guidotti et al., 2001). Conversely, parity enhances cognitive function and affective behavior (Kinsley and Lambert, 2008; Paris and Frye, 2008; Pawluski et al., 2006a,b). Indeed, further examination of the reflexive nature of neuroendocrine function and behavior during maintenance and decline of reproductive capacity with aging is of great interest.

3.6. Conclusions

In summary, the present data confirm what has been previously reported about reproductive capacity changes with aging and extend these findings to demonstrate that there are subtle differences in some aspects of mating, but not social interaction. Moreover, the variability in these reproductive measures may be related to the influence of basal levels of E_2 and DHP in the midbrain, to catalyze neurosteroidogenesis in response to mating. Reproduction requires neuroendocrine stimuli to elicit appropriate behavioral responses for pregnancy, parturition, and maternal behaviors. Together, these data suggest that P_4 metabolites in the midbrain may contribute to variability in reproductive behaviors of middle-aged rats.

4. Experimental procedures

All procedures utilized in the present study involving animal subjects were done with the approval of The University at Albany Institutional Animal Care and Use Committee and in

accordance with The National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

4.1. Animals and housing

For Experiment 1, middle-aged adult (12 months old) female Long–Evans rats ($n = 46$) were obtained from Taconic Farms (Germantown, NY- Cohorts 1 and 2, $n = 30$) at 2 months of age and/or from in-house breeding (breeders originally obtained from Taconic Farms- Cohorts 3 and 4, $n = 16$). Rats from Cohorts 1 and 2 were purchased from Taconic Farms at the same time to begin the rat colony in the Laboratory Animal Care Facility core in The Life Sciences Research Building, which was completing construction, at The University at Albany-SUNY. Rats in Cohorts 3 and 4 were offspring of breeders in Cohorts 1 and 2, and were also age-matched breeders in this facility, which was subject to renovations associated with this new facility. As such, all rats were age-matched and had a commensurate breeding history and environment. Indeed, there were no clear differences between cohorts for behavioral measures and, thus, data from the four cohorts were combined for analyses. Rats were group-housed (3–4 per cage) in polycarbonate cages (45×24×21 cm) in a temperature-controlled room (21 ± 1 °C), under reversed-lighting conditions (lights off 08:00–20:00 h), with *ad libitum* access to rodent chow and water in their cages.

For Experiment 2, adult (12 months old) female Long–Evans rats ($n = 11$) were obtained from in-house breeding (original stock from Taconic Farms, Germantown, NY) as were nulliparous young adult (3 months old) female Long–Evans rats ($n = 11$). Rats were housed in the Laboratory Animal Care Facility at the University at Albany-SUNY via the housing parameters described above. Middle-aged rats in Experiment 2 had a commensurate breeding history to that of middle-aged rats in Experiment 1.

4.2. Experimental groups

Using the characteristics of cyclicity, fertility, and fecundity, age-matched rats with very similar environmental exposures could be assessed for differences in reproductive function, social and sexual responding, and endocrine milieu. Testing was conducted 3–5 days after assessment in three cognitive tasks (reported in Paris et al., 2011) and prior to testing in two cognitive tasks (reported in Paris et al., 2011) and several affective measures (reported in Walf et al., 2009). The criteria used to determine their conditions (i.e. maintaining reproductive status or declining reproductive status) were based upon cyclicity, fertility, and fecundity. Rats were considered to be maintaining reproductive status when they had regular 4–5 days estrous cycles, > 60% successful pregnancies following last matings, and/or normal fecundity (> 10 pups/litter; Paris et al., 2011). Conversely, rats considered to have declining reproductive status had longer than 5-day estrous cycles, < 60% successful pregnancy rate, and/or smaller litter sizes (< 10 pups/litter). These measures were analyzed between these two groups using multiple one-way analyses of variance (ANOVA) and are described in the Results section. Data are depicted in Fig. 1.

4.3. Behavioral testing

Rats in Experiment 1 were tested for social and sexual behavior in three tasks and rats in Experiment 2 were tested for sexual behavior in paced mating only. All rats were tested 1–4 weeks after weaning of their last litter so that they were not lactating at the time of testing. Rats were tested in one task per day when in proestrus. Given that all rats in this experiment were age-matched, retired breeders from our colony, with the same prior breeding and environmental experiences, behavioral differences on the day of proestrus could be assessed as a function of present ovarian/reproductive status. Data were collected by trained experimenters that were unaware of the hypothesis of the study, and the condition of the rats during testing.

4.3.1. Social interaction—The social interaction task assesses responses of rats to situations when they can interact with a novel conspecific (Frye et al., 2000). In this task, a novel conspecific is placed in one corner of an open field (made of white melamine; 76 cm×57 cm×35 cm). The experimental rat is then immediately placed in the open field in the opposite corner than the novel conspecific is placed. The time that the experimental rat engages in social interaction with the novel conspecific is recorded. Social interaction consists of the experimental rat touching, licking, grooming, crawling over and/or under, sniffing, genital investigation, following with contact, tumbling, and boxing with the novel conspecific. The novel conspecific that is utilized in this task is an ovariectomized rat to avoid the possibility of vaginocervical stimulation (and subsequent endocrine responses) of experimental rats by a male, if male rats were to be utilized as the novel conspecific.

4.3.2. Standard mating—Responses of experimental rats following mounting by a sexually experienced male conspecific in a standard mating paradigm were assessed. Rats were tested in a clear glass 50×25×30 cm mating chamber with approximately 5 cm of bedding on the floor of the chamber. The frequency (quotient=incidence/number of mounts×100) female rats exhibit lordosis, proceptivity, or aggression in response to mounting by a male during a 10-min test period, or after ten mounts were made, whichever came first, was used to quantify sexual receptivity of female rats (Frye and Vongher, 1999). Lordosis is the characteristic mating posture of rats, and many other four legged mammals, where there is ventral arching of the spine, and the tail is moved to one side. Proceptivity prior to sexual contact was noted when female rats engaged in behaviors, such as hopping, darting, and/or ear wiggling, which occurs in the courtship phase of mating when the female is demonstrating willingness to engage in mating with the male. Defensive aggression, referred to as aggression in this report, was considered when female rats engaged in vocalizations or defensive postures towards the male (kicking, boxing). Experimental rats were vaginally-masked to minimize possible mating-induced changes in sexual behavior, neuroendocrine function, and pregnancy (Frye et al., 2007).

4.3.3. Paced mating—Paced mating was utilized in addition to standard mating because it can be considered more ethologically-relevant than standard mating. In Experiments 1 and 2, methods used were as modified from Frye et al. (2008) in a white melamine chamber (the chamber used for Experiment 1 was 37.5 cm×75 cm×30 cm; the chamber used for Experiment 2 was 30 cm×40 cm×40 cm in order to accommodate testing of both young-adult- and middle-aged-sized rats), which was equally divided by a partition that had a small (5 cm in diameter) hole in the bottom center. This hole allowed the female, but not the sexually-experienced male used in this task, to enter both sides of the chamber. Females were placed in the side of the chamber opposite the male and then rats were tested for an entire ejaculatory series. Behaviors recorded were the same as described above for standard mating, but were determined in rats for each type of copulatory stimuli (i.e. mounts, intromission, or ejaculation by the male). Pacing measures included the percentage of times the female exit the compartment where she received a particular copulatory stimuli from a male after this stimuli. Clear differences were not noted in the present study, so the mean across the three types of copulatory stimuli were analyzed across conditions. Experimental rats were vaginally-masked. Three rats (1 that was maintaining reproductive status and 2 that had declining reproductive status) did not pace in the chamber and, thus, behavioral data for paced mating could not be included for these subjects.

4.4. Radioimmunoassay for steroid concentrations

In Experiment 1 (Cohorts 1 and 2), some rats were euthanized by rapid decapitation and had tissues collected when in the estrous phase of their cycle, between 17 and 20 days after sex testing, to assess differences in basal steroid levels. Age-matched, middle-aged breeders

with a commensurate breeding and environmental history, are a very limited resource. As such, cohorts 3 and 4 were not able to be used for tissue collection for this project. In Experiment 2, rats were euthanized by rapid decapitation and had tissues collected in the proestrous phase of their cycle, immediately after testing. Following decapitation, whole brains were collected from the skull and flash frozen, and trunk blood was collected into chilled test tubes. Blood was spun at 4 °C, and plasma was decanted into clean 1.5 ml tubes and stored at -20 °C. Immediately before the radioimmunoassay, plasma and brains were thawed slowly on ice. From the thawed brains, the midbrain, diencephalon, and cerebellum were dissected out (Frye and Rhodes, 2006; Frye et al., 2007). Dissected regions were prepared and analyzed with radioimmunoassay for assessment of E₂ and progestogens using previously described methods (Frye and Rhodes, 2006; Frye et al., 2007). Of note, levels of plasma corticosterone and E₂/progestogens were determined in medial prefrontal cortex, and hippocampus of these rats and reported (Paris et al., 2011).

4.5. Statistical analyses

Cyclicity, fertility, and fecundity, and Experiment 1 behavioral measures (social interaction, standard mating, and paced mating), and Experiment 1 steroid levels, were analyzed with multiple one-way ANOVA with reproductive status as the between-subjects variable. Despite high variability in groups for the measures assessed, there was homogeneity of variance as assessed with Levene's test and, therefore, the assumptions of the ANOVA were not violated. Main effects were further analyzed with Fisher PLSD post hoc comparisons to determine group differences. Experiment 2 behavioral measures (paced mating) and steroid levels were assessed via two-way ANOVA with age (young adult or middle-aged) and reproductive status (regular/maintaining reproductive status or irregular/declining reproductive status) as factors. Interactions were followed up with one-way ANOVAs with alpha level corrected for multiple comparisons (results of post-hoc tests are listed in parentheses following effects). To determine the extent that steroid levels could account for variance in behaviors examined, simple linear regressions were utilized. Main effects were considered significant when $p \leq 0.05$ and a trend when $p \leq 0.10$.

Acknowledgments

The technical assistance provided by Dr. M.E. Rhodes and A. Babson, I. Chin, and S. Youmans is greatly appreciated. This research was supported, in part, by funding from the National Science Foundation, the National Institute of Mental Health, and the Department of Defense CDMRP Breast Cancer Research Program.

References

- Anzalone CR, Hong LS, Lu JK, LaPolt PS. Influences of age and ovarian follicular reserve on estrous cycle patterns, ovulation, and hormone secretion in the Long-Evans rat. *Biol Reprod.* 2001; 64:1056–1062. [PubMed: 11259250]
- Barbaccia ML, Roscetti G, Trabucchi M, Mostallino MC, Concas A, Purdy RH, Biggio G. Time-dependent changes in rat brain neuroactive steroid concentrations and GABA_A receptor function after acute stress. *Neuroendocrinology.* 1996; 63:166–172. [PubMed: 9053781]
- Berkley KJ, McAllister SL, Accius BE, Winnard KP. Endometriosis-induced vaginal hyperalgesia in the rat: effect of estropause, ovariectomy, and estradiol replacement. *Pain.* 2007; 132:s150–s159. [PubMed: 17959309]
- Butcher, RL.; Page, RD. Role of the aging ovary in cessation of reproduction. In: Schwartz, NB.; Hunziker-Dunn, M., editors. *Dynamics of Ovarian Function*. Raven Press; New York: 1982. p. 253-271.
- Byrnes EM, Bridges RS. Reproductive experience alters anxiety-like behavior in the female rat. *Horm Behav.* 2006; 50:70–76. [PubMed: 16524576]

- Calhoun, J. U.S. Dept. of Health, Education, and Welfare. The Ecology and Sociology of the Norway Rat. Bethesda, MD: 1962.
- Cameron NM, Fish EW, Meaney MJ. Maternal influences on the sexual behavior and reproductive success of the female rat. *Horm Behav.* 2008; 54:178–184. [PubMed: 18417127]
- Cheng YJ, Karavolas HJ. Conversion of progesterone to 5 α -pregnane-3, 20-dione and 3 α -hydroxy-5 α -pregnan-20-one by rat medial basal hypothalamus and the effects of estradiol and stage of estrous cycle on the conversion. *Endocrinology.* 1973; 93:1157–1162. [PubMed: 4591653]
- Clemens JA, Meites J. Neuroendocrine status of old constant-estrous rats. *Neuroendocrinol.* 1971; 7:249–256.
- Cooper RL. Sexual receptivity in aged female rats. Behavioral evidence for increased sensitivity to estrogen. *Horm Behav.* 1977; 9:321–333. [PubMed: 611081]
- Dudley SD. Responsiveness to estradiol in central nervous system of aging female rats. *Neurosci Biobehav Rev.* 1982; 6:39–45. [PubMed: 7041015]
- Erskine MS, Lehmann ML, Cameron NM, Polston EK. Co-regulation of female sexual behavior and pregnancy induction: an exploratory synthesis. *Behav Brain Res.* 2004; 153:295–315. [PubMed: 15265625]
- Finch CE, Felicio LS, Mobbs CV, Nelson JF. Ovarian and steroidal influences in neuroendocrine aging processes in female rodents. *Endocrine Rev.* 1984; 8:335–344.
- Freeman EW, Sammel MD, Lin H, Gracia CR, Pien GW, Nelson DB, Sheng L. Symptoms associated with menopausal transition and reproductive hormones in midlife women. *Obstet Gynecol.* 2007; 110:230–240. [PubMed: 17666595]
- Frye, CA. Neurosteroids—from basic research to clinical perspectives. In: Rubin, RT.; Pfaff, DW., editors. *Hormones/Behavior Relations of Clinical Importance.* Academic Press; San Diego: 2009. p. 395–416.
- Frye CA, Bayon LE, Pursnani NK, Purdy RH. The neurosteroids, progesterone and 3 α , 5 α -THP, enhance sexual motivation, receptivity, and proceptivity in female rats. *Brain Res.* 1998; 808:72–83. [PubMed: 9795145]
- Frye CA, Bayon LE. Mating stimuli influence endogenous variations in the neurosteroids 3 α , 5 α -THP and 3 α -Diol. *J Neuroendocrinol.* 1999; 11:839–847. [PubMed: 10520134]
- Frye CA, Erskine MS. Influence of time of mating and paced copulation on induction of pseudopregnancy in cyclic female rats. *J Reprod Fertil.* 1990; 90:375–385. [PubMed: 2250236]
- Frye CA, Koonce CJ, Walf AA. Mnemonic effects of progesterone to mice require formation of 3 α , 5 α -THP. *NeuroReport.* 2010a; 21:590–595. [PubMed: 20431492]
- Frye CA, McCormick CM, Coopersmith C, Erskine MS. Effects of paced and non-paced mating stimulation on plasma progesterone, 3 α -diol and corticosterone. *Psychoneuroendocrinol.* 1996; 21:431–439.
- Frye CA, Paris JJ, Rhodes ME. Engaging in paced mating, but neither exploratory, anti-anxiety, nor social behavior, increases 5 α -reduced progestin concentrations in midbrain, hippocampus, striatum, and cortex. *Reproduction.* 2007; 133:663–674. [PubMed: 17379660]
- Frye CA, Paris JJ, Rhodes ME. Exploratory, anti-anxiety, social, and sexual behaviors of rats in behavioral estrus is attenuated with inhibition of 3 α , 5 α -THP formation in the midbrain ventral tegmental area. *Behav Brain Res.* 2008; 193:269–276. [PubMed: 18590775]
- Frye CA, Paris JJ, Rhodes ME. Increasing 3 α , 5 α -THP following inhibition of neurosteroid biosynthesis in the ventral tegmental area reinstates anti-anxiety, social, and sexual behavior of naturally receptive rats. *Reproduction.* 2009; 137:119–128. [PubMed: 18818272]
- Frye CA, Petralia SM, Rhodes ME. Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3 α , 5 α -THP. *Pharmacol Biochem Behav.* 2000; 67:587–596. [PubMed: 11164090]
- Frye CA, Rhodes ME. Progestin concentrations are increased following paced mating in midbrain, hippocampus, diencephalon, and cortex of rats in behavioral oestrus, but only in midbrain of diestrous rats. *Neuroendocrinology.* 2006; 83:336–347. [PubMed: 17028418]
- Frye CA, Vongher JM. 3 α , 5 α -THP in the midbrain ventral tegmental area of rats and hamsters is increased in exogenous hormonal states associated with estrous cyclicity and sexual receptivity. *J Endocrinol Investigation.* 1999; 22:455–464.

- Frye CA, Walf AA, Paris JJ. Conjugated equine estrogen, with medroxyprogesterone acetate, enhances formation of 5 α -reduced progestogens and reduces anxiety-like behavior of middle-aged rats. *Behav Pharmacol*. 2010b; 21:530–539. [PubMed: 20679892]
- Gatewood JD, Morgan MD, Eaton M, McNamara IM, Stevens LF, Macbeth AH, Meyer EA, Lomas LM, Kozub FJ, Lambert KG, Kinsley CH. Motherhood mitigates aging-related decrements in learning and memory and positively affects brain aging in the rat. *Brain Res Bull*. 2005; 66:91–98. [PubMed: 15982524]
- Gans SE, Stamper JL, Butler T, McClintock MK. Endocrine basis for two types of individual differences in lordosis reflex intensity. *Horm Behav*. 1995; 29:367–391. [PubMed: 7490011]
- Genazzani AR, Stomati M, Bernardi F, Luisi S, Casarosa E, Puccetti S, Genazzani AD, Palumbo M, Luisi M. Conjugated equine estrogens reverse the effects of aging on central and peripheral allopregnanolone and beta-endorphin levels in female rats. *Fertil Steril*. 2004; 81:757–766. [PubMed: 15019806]
- González-Flores O, Beyer C, Gómora-Arrati P, García-Juárez M, Lima-Hernández FJ, Soto-Sánchez A, Etgen AM. A role for Src kinase in progestin facilitation of estrous behavior in estradiol-primed female rats. *Horm Behav*. 2010; 58:223–229. [PubMed: 20307541]
- Guidotti A, Dong E, Matsumoto K, Pinna G, Rasmusson AM, Costa E. The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5 α -dihydroprogesterone in psychiatric disorders. *Brain Res Brain Res Reviews*. 2001; 37:110–115.
- Henderson VW. Menopause, cognitive ageing and dementia: practice implications. *Menopause Int*. 2009; 15:41–44. [PubMed: 19237622]
- Hermes GL, McClintock MK. Isolation and the timing of mammary gland development, gonadarche, and ovarian senescence: implications for mammary tumor burden. *Develop Psychobiol*. 2008; 50:353–360.
- Hodis HN, Mack WJ. Coronary heart disease and hormone replacement therapy after the menopause. *Climacteric*. 2009; 12:71–75. [PubMed: 19811246]
- Huang HH, Steger RW, Bruni JF, Meites J. Patterns of sex steroid and gonadotropin secretion in aging female rats. *Endocrinology*. 1978; 103:1855–1859. [PubMed: 570913]
- Hunt GE, Van Nieuwenhuijzen PS, Chan-Ling T, McGregor IS. ‘When an old rat smells a cat’: A decline in defense-related, but not accessory olfactory, Fos expression in aged rats. *Neurobiol Aging*. in press. 10.1016/j.neurobiolaging.2009.03.014
- Ikemoto S, Murphy JM, McBride WJ. Regional differences within the rat ventral tegmental area for muscimol self-infusions. *Pharmacol Biochem Behav*. 1998; 61:87–92. [PubMed: 9715810]
- Johannes CB, Clayton AH, Odom DM, Rosen RC, Russo PA, Shifren JL, Monz BU. Distressing sexual problems in United States women revisited: prevalence after accounting for depression. *J Clin Psychiatry*. 2009; 70:1698–1706. [PubMed: 20141709]
- Kinsley CH, Madonia L, Gifford GW, Tureski K, Griffin GR, Lowry C, Williams J, Collins J, McLearn H, Lambert KG. Motherhood improves learning and memory. *Nature*. 1999; 402:137–138. [PubMed: 10647003]
- Kinsley CH, Lambert KG. Reproduction-induced neuroplasticity: natural behavioural and neuronal alterations associated with the production and care of offspring. *J Neuroendocrinol*. 2008; 20:515–525. [PubMed: 18266940]
- LeFevre J, McClintock MK. Reproductive senescence in female rats: a longitudinal study of individual differences in estrous cycles and behavior. *Biol Reproduction*. 1988; 38:780–789.
- LeFevre J, McClintock MK. Isolation accelerates reproductive senescence and alters its predictors in female rats. *Horm Behav*. 1991; 25:258–272. [PubMed: 2066084]
- LeFevre JA, McClintock MK. Social modulation of behavioral reproductive senescence in female rats. *Physiol Behav*. 1992; 52:603–608. [PubMed: 1409927]
- Long JA, Evans HM. Estrus cycle in the rat and its associated phenomena. *Mem Univ Calif*. 1922; 6:1–146.
- Lu KH, Hopper BR, Vargo TM, Yen SS. Chronological changes in sex steroid, gonadotropin and prolactin secretions in aging female rats displaying different reproductive states. *Biol Reprod*. 1979; 21:193–203. [PubMed: 573635]

- Macbeth AH, Luine VN. Changes in anxiety and cognition due to reproductive experience: a review of data from rodent and human mothers. *Neurosci Biobehav Rev.* 2010; 34:452–467. [PubMed: 19761791]
- Matt DW, Lee J, Sarver PL, Judd HL, Lu JK. Chronological changes in fertility, fecundity and steroid hormone secretion during consecutive pregnancies in aging rats. *Biol Reprod.* 1986; 34:478–487. [PubMed: 3697463]
- Micevych P, Soma KK, Sinchak K. Neuroprogesterone: key to estrogen positive feedback? *Brain Res Rev.* 2008; 57:470–480. [PubMed: 17850878]
- Miller AE, Riegler GD. Temporal changes in serum progesterone in aging female rats. *Endocrinol.* 1980; 106:1579–1583.
- Page RD, Butcher RL. Follicular and plasma patterns of steroids in young and old rats during normal and prolonged estrous cycles. *Biol Reprod.* 1982; 27:383–392. [PubMed: 6215069]
- Paris JJ, Frye CA. Estrous cycle, pregnancy, and parity enhance performance of rats in object recognition or object placement tasks. *Reproduction.* 2008; 136:105–115. [PubMed: 18390689]
- Paris JJ, Walf AA, Frye CA. II. Cognitive performance of middle-aged female rats is influenced by capacity to metabolize progesterone in the prefrontal cortex and hippocampus. *Brain Res.* 2011; 1379:149–163. [PubMed: 21044614]
- Patchev VK, Hassan AH, Holsboer DF, Almeida OF. The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. *Neuropsychopharmacology.* 1996; 15:533–540. [PubMed: 8946427]
- Pawluski JL, Walker SK, Galea LA. Reproductive experience differentially affects spatial reference and working memory performance in the mother. *Horm Behav.* 2006a; 49:143–149. [PubMed: 15992800]
- Pawluski JL, Vanderbyl BL, Ragan K, Galea LA. First reproductive experience persistently affects spatial reference and working memory in the mother and these effects are not due to pregnancy or “mothering” alone. *Behav Brain Res.* 2006b; 175:157–165. [PubMed: 17011053]
- Ryan J, Burger HG, Szoek C, Leher P, Ancelin ML, Henderson VW, Dennerstein L. A prospective study of the association between endogenous hormones and depressive symptoms in postmenopausal women. *Menopause.* 2009; 16:509–517. [PubMed: 19169164]
- Santen RJ, Allred DC, Ardoin SP, Archer DF, Boyd N, Braunstein GD, Burger HG, Colditz GA, Davis SR, Gambacciani M, Gower BA, Henderson VW, Jarjour WN, Karas RH, Kleerekoper M, Lobo RA, Manson JE, Marsden J, Martin KA, Martin L, Pinkerton JV, Rubinow DR, Teede H, Thiboutot DM, Utian WH. Postmenopausal hormone therapy: an endocrine society scientific statement. *J Clin Endocrinol Metab.* 2010; 95:s1–s66. [PubMed: 20566620]
- Schmidt PJ, Rubinow DR. Reproductive ageing, sex steroids and depression. *J Br Menopause Soc.* 2006; 12:178–185. [PubMed: 17178020]
- Schmidt PJ, Rubinow DR. Sex hormones and mood in the perimenopause. *Ann NY Acad Sci.* 2009; 1179:70–85. [PubMed: 19906233]
- Walf AA, Frye CA. Parity and estrogen-administration alter affective behavior of ovariectomized rats. *Physiol Behav.* 2008; 93:351–356. [PubMed: 17936862]
- Walf AA, Paris JJ, Frye CA. Nociceptive and anxiety-like behavior in reproductively competent and reproductively senescent middle-aged rats. *Gend Med.* 2009; 6:235–246. [PubMed: 19406372]
- Wise PM, Ratner A. LHRH-induced LH and FSH responses in the aged female rat. *J Gerontology.* 1980; 35:506–511.

Fig. 1. Cyclicity, fertility, and fecundity: Middle-aged rats whose reproductive status was maintained ($n=20$) had shorter estrous cycles (left panel), greater percentage of successful pregnancies (middle panel), and larger litters (right panel), as indicated by mean bar $\pm$ SEM bars, compared to same-aged rats that whose reproductive status was in decline ($n=26$). * indicates that rats maintaining reproductive status were different from rats that had a decline in reproductive status ($p\leq 0.05$).

Fig. 2.

Standard mating behavior: Middle-aged rats whose reproductive status was maintained ($n=20$) had similar lordosis quotients (top, left panel) and lordosis ratings (top, right panel), and tended to have higher proceptivity (bottom, left panel), and had significantly lower aggression (bottom, right panel) quotients, as indicated by mean bar $\pm$ SEM bars, compared to same-aged rats that whose reproductive status was in decline ($n=26$). * indicates that rats maintaining reproductive status were different from rats that had a decline in reproductive status ($p \leq 0.05$). # indicates that rats maintaining reproductive status tended to be different from rats that had a decline in reproductive status ($p \leq 0.10$).

Fig. 3.

Paced mating behavior: Middle-aged rats whose reproductive status was maintained ($n=19$) had similar lordosis quotients (top, left panel) and lordosis ratings (top, right panel), and tended to have higher proceptivity (bottom, left panel), and had significantly lower aggression (bottom, right panel) quotients, as indicated by mean bar $\pm$ SEM bars, compared to same-aged rats that whose reproductive status was in decline ($n=24$). * indicates that rats maintaining reproductive status were different from rats that had a decline in reproductive status ($p \leq 0.05$). # indicates that rats maintaining reproductive status tended to be different from rats that had a decline in reproductive status ($p \leq 0.10$).

Table 1

Time spent engaging in social interaction with a conspecific (secs $\pm$ SEM) among middle-aged rats whose reproductive status was maintained ($n = 20$) or was in decline ($n = 26$).

Time engaging in social interaction	
Maintaining reproductive status ($n = 20$)	32 ± 2
Declining reproductive status ($n = 26$)	34 ± 2

Table 2

Brain concentrations (mean±SEM) of estradiol (E₂), progesterone (P₄), dihydroprogesterone (DHP), and 5α-pregnan-3α-ol-20-one (3α,5α-THP) in the midbrain, diencephalon, and cerebellum of middle-aged rats that had maintained reproductive status (*n* = 14) or had a decline in reproductive status (*n* = 12).

Measure	Reproductive status	
	Maintaining reproductive status (<i>n</i> = 14)	Declining reproductive status (<i>n</i> = 12)
<i>Midbrain</i>		
E ₂ (pg/g)	4.9 ± 1.9	7.8 ± 2.4
P ₄ (ng/g)	4.9 ± 1.3	4.9 ± 1.1
DHP (ng/g)	1.9 ± 0.5	1.5 ± 0.2
3α,5α-THP (ng/g)	10.2 ± 0.2	10.0 ± 0.4
<i>Diencephalon</i>		
E ₂ (pg/g)	9.9 ± 3.4	21.6 ± 5.8
P ₄ (ng/g)	6.6 ± 1.8	9.9 ± 2.3
DHP (ng/g)	3.8 ± 1.6	2.1 ± 0.5
3α,5α-THP (ng/g)	17.9 ± 1.3	20.6 ± 2.5
<i>Cerebellum</i>		
E ₂ (pg/g)	0.5 ± 0.2	1.2 ± 0.4
P ₄ (ng/g)	1.2 ± 0.3	1.6 ± 0.4
DHP (ng/g)	0.4 ± 0.1	0.5 ± 0.2
3α,5α-THP (ng/g)	1.8 ± 0.1	1.8 ± 0.1

Table 3

Regression analyses depicting the extent to which midbrain estradiol (E_2), progesterone (P_4), dihydroprogesterone (DHP), or 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP) contributed to standard mating and paced mating measures of middle-aged rats that had maintained reproductive status ($n = 14$) or had a decline in reproductive status ($n = 12$). * indicates significant amount of variance in behavioral measure is accounted for by steroid concentrations ($p \leq 0.05$). # indicates significant amount of variance in behavioral measure is accounted for by steroid concentrations ($p \leq 0.10$).

Regressions utilizing midbrain steroid concentrations				
	Standard mating		Paced mating	
	E_2			
	$R^2, F(df)$	p	$R^2, F(df)$	p
Lordosis	$R^2 = 0.16,$	$p = 0.05^*$	$R^2 = 0.26,$	$p = 0.01^*$
quotients	$F(1,24) = 4.42$		$F(1,24) = 8.07$	
Lordosis	$R^2 = 0.26,$	$p = 0.01^*$	$R^2 = 0.31,$	$p = 0.01^*$
ratings	$F(1,24) = 7.90$		$F(1,24) = 10.40$	
Proceptivity	$R^2 = 0.02,$	$p = 0.54$	$R^2 = 0.12,$	$p = 0.10\#$
quotients	$F(1,24) = 0.38$		$F(1,24) = 3.01$	
Aggression	$R^2 = 0.08,$	$p = 0.17$	$R^2 = 0.01,$	$p = 0.67$
quotients	$F(1,24) = 2.02$		$F(1,24) = 0.18$	
	P_4			
	$R^2, F(df)$	p	$R^2, F(df)$	p
Lordosis	$R^2 = 0.07,$	$p = 0.22$	$R^2 = 0.07,$	$p = 0.21$
quotients	$F(1,24) = 1.59$		$F(1,24) = 1.68$	
Lordosis	$R^2 = 0.08,$	$p = 0.19$	$R^2 = 0.07,$	$p = 0.19$
ratings	$F(1,24) = 1.87$		$F(1,24) = 1.79$	
Proceptivity	$R^2 = 0.01,$	$p = 0.61$	$R^2 < 0.00,$	$p = 0.94$
quotients	$F(1,24) = 0.26$		$F(1,24) = 0.01$	
Aggression	$R^2 = 0.01,$	$p = 0.65$	$R^2 = 0.05,$	$p = 0.31$
quotients	$F(1,24) = 0.21$		$F(1,24) = 1.09$	
	DHP			
	$R^2, F(df)$	p	$R^2, F(df)$	p
Lordosis	$R^2 = 0.16,$	$p = 0.05^*$	$R^2 = 0.09,$	$p = 0.15$
quotients	$F(1,24) = 4.24$		$F(1,24) = 2.25$	
Lordosis	$R^2 = 0.25,$	$p < 0.01^*$	$R^2 = 0.11,$	$p = 0.12$
ratings	$F(1,24) = 8.97$		$F(1,24) = 2.66$	
Proceptivity	$R^2 = 0.13,$	$p = 0.08\#$	$R^2 = 0.01,$	$p = 0.59$
quotients	$F(1,24) = 3.32$		$F(1,24) = 0.30$	
Aggression	$R^2 = 0.11,$	$p = 0.10\#$	$R^2 = 0.01,$	$p = 0.60$

Regressions utilizing midbrain steroid concentrations				
	Standard mating		Paced mating	
	E_2			
	$R^2, F(df)$	p	$R^2, F(df)$	p
quotients	$F(1,24) = 2.88$		$F(1,24) = 0.29$	
	3α,5α-THP			
	$R^2, F(df)$	p	$R^2, F(df)$	p
Lordosis	$R^2 = 0.11,$	$p = 0.09\#$	$R^2 = 0.11,$	$p = 0.10\#$
quotients	$F(1,24) = 3.09$		$F(1,24) = 2.86$	
Lordosis	$R^2 = 0.08,$	$p = 0.16$	$R^2 = 0.09,$	$p = 0.14$
ratings	$F(1,24) = 2.09$		$F(1,24) = 2.37$	
Proceptivity	$R^2 = 0.04,$	$p = 0.34$	$R^2 = 0.01,$	$p = 0.71$
quotients	$F(1,24) = 0.94$		$F(1,24) = 0.14$	
Aggression	$R^2 = 0.01,$	$p = 0.56$	$R^2 = 0.14,$	$p = 0.06\#$
quotients	$F(1,24) = 0.34$		$F(1,24) = 3.79$	

Table 4

Paced mating performance and concentrations (mean $\pm$ SEM) of estradiol (E2), progesterone (P4) dihydroprogesterone (DHP), and 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP) in the midbrain, diencephalon, and cerebellum of mated young rats that had regular ($n = 3$) or irregular ($n = 8$) estrous cycles or mated middle-aged rats that had maintained ($n = 3$) or had a decline ($n = 8$) in reproductive status. * indicates significant difference between irregular/declining rats compared to regular/maintain rats. † indicates significant difference between young compared to middle-aged rats. ‡ indicates significant interaction wherein mated middle-aged rats that maintained reproductive status are different compared to mated regularly cycling adult, and middle-aged declining rats ($p \leq 0.05$).

Age	Young adult		Middle-aged	
Reproductive status	Regular reproductive status ($n = 3$)	Irregular reproductive status ($n = 8$)	Maintaining reproductive status ($n = 3$)	Declining reproductive status ($n = 8$)
Lordosis quotients	100 $\pm$ 0%	98 $\pm$ 2%	100 $\pm$ 0%	100 $\pm$ 0%
Lordosis ratings	3.0 $\pm$ 0.0	2.6 $\pm$ 0.2	2.9 $\pm$ 0.1	3.0 $\pm$ 0.0
Proceptivity quotients	100 $\pm$ 0%	89.5 $\pm$ 7%	100 $\pm$ 0%	100 $\pm$ 0%
Aggression quotients	6 $\pm$ 6%	9 $\pm$ 9%	0 $\pm$ 0%	10 $\pm$ 10%
% exits from male compartment	44 $\pm$ 4%	67 $\pm$ 10%	40 $\pm$ 20%	66 $\pm$ 11%
Midbrain E ₂ (pg/g)	5.0 $\pm$ 4.4	4.6 $\pm$ 2.5	8.2 $\pm$ 4.5	4.3 $\pm$ 2.3
Midbrain P ₄ (ng/g)	0.7 $\pm$ 0.3	2.8 $\pm$ 0.6*	1.1 $\pm$ 0.4	3.1 $\pm$ 0.5*
Midbrain DHP (ng/g)	4.1 $\pm$ 1.4	7.0 $\pm$ 0.8*	3.8 $\pm$ 1.2	5.9 $\pm$ 0.6*
Midbrain 3 α ,5 α -THP (ng/g)	9.8 $\pm$ 2.7	13.4 $\pm$ 2.5	12.2 $\pm$ 4.4	20.3 $\pm$ 6.0
Diencephalon E ₂ (pg/g)	18.3 $\pm$ 10.3	8.4 $\pm$ 3.4	1.4 $\pm$ 0.5†	1.6 $\pm$ 0.3†
Diencephalon P ₄ (ng/g)	0.9 $\pm$ 0.1	3.7 $\pm$ 1.3	1.5 $\pm$ 0.6	2.0 $\pm$ 0.4
Diencephalon DHP (ng/g)	8.4 $\pm$ 2.3	9.6 $\pm$ 1.3	8.9 $\pm$ 1.8	9.0 $\pm$ 1.9
Diencephalon 3 α ,5 α -THP (ng/g)	11.1 $\pm$ 4.1	24.2 $\pm$ 4.5	45.4 $\pm$ 19.0‡	23.9 $\pm$ 4.3
Cerebellum E ₂ (pg/g)	1.0 $\pm$ 0.9	0.2 $\pm$ 0.1	1.1 $\pm$ 1.0	0.8 $\pm$ 0.4
Cerebellum P ₄ (ng/g)	0.6 $\pm$ 0.2	0.7 $\pm$ 0.3	0.3 $\pm$ 0.1	1.0 $\pm$ 0.2
Cerebellum DHP (ng/g)	1.2 $\pm$ 0.3	1.2 $\pm$ 0.3	0.7 $\pm$ 0.2	1.5 $\pm$ 0.1
Cerebellum 3 α ,5 α -THP (ng/g)	3.2 $\pm$ 0.3	3.5 $\pm$ 0.9	2.8 $\pm$ 0.8	8.0 $\pm$ 2.0