



Published in final edited form as:

Reproduction. 2008 July ; 136(1): 105–115. doi:10.1530/REP-07-0512.

Estrous cycle, pregnancy, and parity enhance performance of rats in object recognition or object placement tasks

Jason J Paris and Cheryl A Frye

The University at Albany, SUNY, Life Sciences Research Building Room 01058, 1400 Washington Avenue, Albany, New York 12222, USA

Abstract

Ovarian hormone elevations are associated with enhanced learning/memory. During behavioral estrus or pregnancy, progestins, such as progesterone (P_4) and its metabolite 5α -pregnan- 3α -ol-20-one ($3\alpha,5\alpha$ -THP), are elevated due, in part, to corpora luteal and placental secretion. During 'pseudopregnancy', the induction of corpora luteal functioning results in a hormonal milieu analogous to pregnancy, which ceases after about 12 days, due to the lack of placental formation. Multiparity is also associated with enhanced learning/memory, perhaps due to prior steroid exposure during pregnancy. Given evidence that progestins and/or parity may influence cognition, we investigated how natural alterations in the progestin milieu influence cognitive performance. In Experiment 1, virgin rats (nulliparous) or rats with two prior pregnancies (multiparous) were assessed on the object placement and recognition tasks, when in high-estrogen/ P_4 (behavioral estrus) or low-estrogen/ P_4 (diestrus) phases of the estrous cycle. In Experiment 2, primiparous or multiparous rats were tested in the object placement and recognition tasks when not pregnant, pseudopregnant, or pregnant (between gestational days (GDs) 6 and 12). In Experiment 3, pregnant primiparous or multiparous rats were assessed daily in the object placement or recognition tasks. Females in natural states associated with higher endogenous progestins (behavioral estrus, pregnancy, multiparity) outperformed rats in low progestin states (diestrus, non-pregnancy, nulliparity) on the object placement and recognition tasks. In earlier pregnancy, multiparous, compared with primiparous, rats had a lower corticosterone, but higher estrogen levels, concomitant with better object placement performance. From GD 13 until *post partum*, primiparous rats had higher $3\alpha,5\alpha$ -THP levels and improved object placement performance compared with multiparous rats.

Introduction

In the natural environment, sexually receptive female rodents will traverse complicated and potentially dangerous terrain in order to access mating partners. Among rats, the cyclical enhancement of estrogen (E_2) and progestins facilitate these reproductive behaviors in a number of ways. Actions of E_2 , progesterone (P_4), and the P_4 metabolite 5α -pregnan- 3α -ol-20-one ($3\alpha,5\alpha$ -THP) are important not only for copulation but also for an entire suite of behaviors relevant for successful reproduction, including cognition. When compared with non-receptive rats, naturally receptive females demonstrate increased exploratory and anti-anxiety behavior, as well as attenuated social neophobia and intersex aggression (Frye 2001, Frye *et al.* 2006). The systemic administration or central infusions of $3\alpha,5\alpha$ -THP to sexually non-receptive females can enhance the instatement of these behaviors (Frye & Rhodes 2006a, 2008). As well, performance in cognitive tasks can be better during behavioral estrus. Corticolimbic tasks, such as eye-blink-conditioning, passive avoidance, radial arm maze, and object recognition are enhanced among receptive, compared with non-receptive, female rodents (Shors *et al.* 1998,

Bowman *et al.* 2001, Rhodes & Frye 2004, Walf *et al.* 2006, Frye *et al.* 2007, Wood *et al.* 2001). Such enhancements may be important, considering the spatial and mnemonic requirements associated with finding mating partners and successfully returning to safe locations to rear pups.

Among both people and rodents, aging is associated with a decline in ovarian hormones, reproductive viability, and cognition. Reproductively senescent rats have decreased ovarian hormone levels, poorer reference, and working memory on both spatial and non-spatial tasks, and take longer to learn associations in a conditioning paradigm compared with reproductively viable rats (Pitsikas & Algeri 1992, Leblanc *et al.* 1996, Walf & Frye 2006). Among postmenopausal women, there are reports of age-associated decline in working memory (Zelinski *et al.* 1993, Small *et al.* 1999, Sherwin & Henry 2008). Thus, a natural decline in ovarian hormones is associated with poorer cognitive performance and decrements in learning.

Models of hormonal decline, such as removal of the ovaries, the primary source of endogenous hormones, can produce behavioral and/or cognitive deficits among rodents akin to those observed when cyclical hormone levels are low. Ovariectomy (OVX) attenuates mating, increases anxiety, and decreases social behavior (Frye 2001, Frye & Rhodes 2006b, Frye *et al.* 2006). The administration of E₂ alone or in conjunction with P₄ or 3 α ,5 α -THP reinstates these behaviors and can enhance sexual motivation in a mate-finding task (Frye 2001, Frye *et al.* 2006, Pazol *et al.* 2006, López *et al.* 2007). Further, E₂ alone or with P₄ can produce mnemonic effects in OVX rats (Gibbs 2000, Sandstrom & Williams 2001, Markham *et al.* 2002, Markowska & Savonenko 2002, Sato *et al.* 2004, Frye & Rhodes 2006a, Frye *et al.* 2006). In particular, oral or systemic E₂ administration can reinstate object recognition performance in OVX females to levels commensurate with that of sexually receptive rats (Luine *et al.* 2003, Fernandez & Frick 2004, Walf *et al.* 2006). Thus, behavioral and cognitive deficits associated with hormonal depletion can be overcome by E₂ and/or P₄ administration.

In contrast to the reduction in ovarian hormones, pregnancy is associated with increases in steroids for longer periods than are seen over the estrous cycle. In particular, circulating and central levels of progestins, including 3 α ,5 α -THP, are greatly elevated during pregnancy (Majewska *et al.* 1989, Concas *et al.* 1998, Frye & Walf 2004). This is due, in part, to the additional steroid secretion by the corpora lutea and placenta. These levels increase steadily throughout gestation and do not decline until parturition is imminent (Concas *et al.* 1998). The resulting endocrine milieu, as well as limbic morphology and function, are altered following pregnancy, parturition, and/or pup rearing (Featherstone *et al.* 2000, Tomizawa *et al.* 2003, Pawluski & Galea 2006). First, oxytocin associated with motherhood enhances long-term potentiation (LTP) of hippocampal tissue slices in culture and central infusions of oxytocin enhance long-term spatial memory in virgin mice (Tomizawa *et al.* 2003). Second, hippocampal spine density is increased in pregnant compared with non-pregnant rats and is also altered by postpartum mothering (Kinsley *et al.* 2006, Pawluski & Galea 2006). Third, neurogenesis in the subventricular zone of mice and rats is enhanced during pregnancy (Shingo *et al.* 2003, Furuta & Bridges 2005). Thus, plastic changes in substrates and/or regions relevant for cognition are associated with pregnancy.

Motherhood is associated with increased cognitive performance among rodents. For instance, rats that have had one or more litters outperform virgin rats in either probe or competitive trials of a dry land maze (Love *et al.* 2005) and make fewer errors in a radial arm maze than do virgins (Kinsley *et al.* 1999, Pawluski *et al.* 2006a, 2006b). These data are of particular interest because they suggest that a prolonged exposure to steroids can have long-lasting (even life-long) effects on cognition. Some of these effects may be due to trophic actions and/or morphological factors associated with hormonal milieu. 3 α ,5 α -THP is one such steroidogenic factor that is enhanced throughout pregnancy and has demonstrated neuroprotective and

neuroproliferative effects (Rhodes *et al.* 2004, Djebaili *et al.* 2005, Wang *et al.* 2005, VanLandingham *et al.* 2006, Leonelli *et al.* 2007, Wang *et al.* 2008). Of note, parity has been associated with reductions in age-related cognitive decrement and markers of neurodegeneration, as well as the increased expression of brain-derived neurotrophic factor (Gatewood *et al.* 2005, Pawluski & Galea 2007, Macbeth *et al.* 2008). Whether $3\alpha,5\alpha$ -THP may underlie some of the cognitive and/or trophic effects associated with reproductive experience has not been systematically investigated.

In order to further address the role that hormonal factors, such as E_2 and/or $3\alpha,5\alpha$ -THP, may play in cognitive enhancement associated with parity, we conducted three experiments utilizing natural variations of hormonal milieu. We hypothesized that rats with more reproductive experience would demonstrate greater cognitive performance compared with rats with less reproductive experience and that this enhancement may be related to an increased production of $3\alpha,5\alpha$ -THP. To test this, the following experiments were conducted: (1) cycling rats that either had two prior pregnancies (multiparous) or were virgin (nulliparous) were tested in the object placement (OP) and object recognition (OR) tasks when in the low-hormone phase of their cycle (diestrus) and in the high-hormone phase of their cycle (behavioral estrus); (2) multiparous and nulliparous rats were mated resulting in some rats becoming pregnant, some becoming pseudopregnant, and others not becoming pregnant. First-time (primiparous) or third-time (multiparous) dams that were pregnant, pseudopregnant, or not pregnant were assessed on the object placement and object recognition tasks between gestational days (GDs) 6–12 and (3) virgin and multiparous rats were mated and assessed on the object placement and object recognition tasks daily across every day of gestation until 2 days *post partum*. The plasma hormone levels of E_2 , $3\alpha, 5\alpha$ -THP, and the stress hormone, corticosterone (CORT), were also assessed daily.

Results

Experiment 1: endogenous hormonal variations in virgin versus multiparous rats

Object placement—Parity ($F(1,44) = 8.95, P < 0.05$) and estrous cycle phase ($F(1,44) = 4.42, P < 0.05$) influenced performance in the object placement task. Multiparous rats spent a greater percentage of time with the object in the novel location ($n = 24$; 3.6 ± 0.5 s with object in novel location versus 1.6 ± 0.3 s with object in familiar location) compared with nulliparous rats ($n = 24$; 3.4 ± 0.5 s with object in novel location versus 2.8 ± 0.4 s with object in familiar location). As well, rats in behavioral estrus spent a greater percentage of time with the object in the novel location (4.1 ± 0.5 s with object in novel location versus 2.0 ± 0.4 s with object in familiar location) compared to diestrus rats ($n = 24$; 2.9 ± 0.5 s with object in novel location versus 2.4 ± 0.4 s with object in familiar location) (Fig. 1(i)).

Object recognition—Parity ($F(1,44) = 5.38, P < 0.05$) and estrous cycle phase ($F(1,44) = 4.11, P < 0.05$) influenced performance in the object recognition task. Multiparous rats spent a greater percentage of time spent with the novel object ($n = 24$; 5.5 ± 1.0 s with novel object versus 2.0 ± 0.3 s with the familiar object) compared with nulliparous rats ($n = 24$; 3.5 ± 0.7 s with the novel versus 2.2 ± 0.4 s with the familiar object). In addition, rats in behavioral estrus spent a greater percentage of time with the novel object (5.7 ± 0.8 s with the novel object versus 2.3 ± 0.4 s with the familiar object) compared with diestrus rats ($n = 24$; 3.4 ± 0.8 s with the novel object versus 2.0 ± 0.3 s with the familiar object) (Fig. 1(ii)).

Experiment 2: variations in pregnancy status of primiparous versus multiparous rats

Object placement—Parity ($F(1,66) = 5.14, P < 0.05$) and pregnancy status ($F(2,66) = 7.70, P < 0.05$) influenced performance in the object placement task. Multiparous rats spent a greater percentage of time with the object in the novel location ($n = 36$; 4.3 ± 0.7 s with object in novel

location versus 2.3 ± 0.4 s with object in familiar location) than did primiparous rats ($n = 36$; 3.6 ± 0.4 s with object in novel location versus 2.5 ± 0.5 s with object in familiar location). As well, pregnant rats spent a greater percentage of time with the object in the novel location (4.1 ± 0.6 s with object in novel location versus 2.0 ± 0.4 s with object in familiar location) than did pseudopregnant rats ($n = 24$; 3.7 ± 0.9 s with object in novel location versus 2.4 ± 0.6 s with object in familiar location) and a significantly greater percentage of time than non-pregnant rats ($n = 24$; 3.9 ± 0.6 s with object in novel location versus 2.9 ± 0.5 s with object in familiar location) (Fig. 2(i)).

Object recognition—Parity influenced ($F(1,66) = 5.08, P < 0.05$), and pregnancy status tended to influence ($F(2,66) = 2.61, P < 0.10$), performance in the object recognition task. Multiparous rats spent a greater percentage of time with the novel object ($n = 36$; 6.5 ± 0.8 s with novel object versus 2.7 ± 0.3 s with familiar object) than did primiparous rats ($n = 36$; 4.0 ± 0.5 s with novel object versus 2.9 ± 0.7 s with familiar object). Additionally, pregnant rats ($n = 24$) spent a greater percentage of time with the novel object (6.4 ± 0.7 s with novel object versus 2.4 ± 0.4 s with familiar object) than did pseudopregnant rats ($n = 24$; 4.2 ± 0.8 s with novel object versus 3.3 ± 1.0 s with familiar object) or non-pregnant rats ($n = 24$; 5.2 ± 0.9 s with novel object versus 2.6 ± 0.3 s with familiar object) but this difference did not reach statistical significance (Fig. 2(ii)).

Experiment 3: hormonal variations across gestation in primiparous versus multiparous rats

Object placement—Parity ($F(1,180) = 21.30, P < 0.05$) and day of gestation ($F(10,180) = 2.69, P = 0.05$) influenced performance across pregnancy in the object placement task. The percentage of time spent with the object in the novel location was greater among multiparous rats compared with primiparous rats and significantly varied as a function of GD due to an increase in the performance of primiparous rats in mid- to late gestation (Fig. 3(i)).

Object recognition—Significant changes in performance that were dependent upon parity or the day of gestation were not observed when object recognition was examined across gestation of primiparous or multiparous dams (Fig. 3(ii)).

Endocrine measures—Overall, primiparous rats had significantly higher CORT levels than did multiparous rats ($F(1,378) = 4.50, P < 0.05$). Among rats in either condition, CORT levels varied significantly by the day of gestation with much lower concentrations on GD 14–21 ($F(21,378) = 5.00, P < 0.05$). Circulating E_2 levels were significantly higher, overall, among multiparous compared with primiparous rats ($F(1,378) = 4.65, P < 0.05$). Parity and day of gestation interacted to influence circulating $3\alpha,5\alpha$ -THP concentrations ($F(21,328) = 1.68, P < 0.05$) with primiparous rats having greater levels of plasma $3\alpha,5\alpha$ -THP on GD 13 of pregnancy until *post partum* compared with multiparous rats (Fig. 4).

Discussion

The present study examined the role of endogenous hormones on cognitive performance associated with reproductive experience. The hypothesis that reproductive experience would be indicative of greater cognitive performance in rats, in part, due to contributions of endogenous $3\alpha,5\alpha$ -THP, was upheld in the following ways. In the first experiment, female rats in the high endogenous E_2 /progesterin phase of their estrous cycle outperformed females in the low E_2 /progesterin phase of their cycle on both hippocampal- (object placement) and corticolimbic- (object recognition) mediated tasks. In the second experiment, pregnant rats, which were expected to have high endogenous E_2 and progesterin levels, tended to outperform non-pregnant rats expected to have lower E_2 /progesterin levels on both tasks. The performance of pseudopregnant rats fell between that of non-pregnant and pregnant rats. In both the

experiments, multiparous rats significantly outperformed rats with less reproductive experience. In the third experiment, performance on object placement was significantly altered across gestation with multiparous dams maintaining a high performance throughout pregnancy, and primiparous dams incrementally increasing to multiparous levels of performance. The object recognition performance did not significantly vary as a function of gestation. An examination of circulating steroids revealed that $3\alpha,5\alpha$ -THP levels were commensurate between groups until late gestation when first-time mothers were found to have a later and greater peak, in addition to a lesser decline, in circulating $3\alpha,5\alpha$ -THP compared with multiparous rats. As well, the largest increases in object placement performance among primiparous dams (GD 10–11 and GD 18–19) occurred following peak $3\alpha,5\alpha$ -THP increases and just after days when $3\alpha,5\alpha$ -THP was enhanced (GD 9 and GD 17) compared with multiparous dams. E_2 levels were relatively stable and consistently higher among multiparous compared with primiparous dams. When elevated, CORT levels were higher in first-time mothers compared with multiparous dams, but both groups fell to nadir concomitantly prior to parturition. These data support the hypothesis that $3\alpha,5\alpha$ -THP may underlie, in part, some of the cognitive-enhancing effects associated with reproductive experience.

The present investigation confirms prior reports that endogenous increases in E_2 and/or progestins are associated with enhanced cognitive task performance on hippocampally mediated tasks (Shors *et al.* 1998, Galea *et al.* 2000, Frick & Berger-Sweeney 2001, Wood *et al.* 2001, Rhodes & Frye 2004, Walf *et al.* 2006, Frye *et al.* 2007). This report extends these findings by assessing performance in object placement and object recognition among pseudopregnant dams, which are unique in that they have enhanced E_2 and progestin production but do not have the greatly exacerbated levels that are associated with placental secretion. Stepwise effects were observed with pregnant>pseudopregnant>non-pregnant in task performance, supporting the notion that ovarian hormones play an important role. This may be due, in part, to hippocampal remodeling. In support of this, natural enhancements of E_2 and P_4 that occur during behavioral estrus can modulate hippocampal dendritic spine density, such that density is greatest when rats are sexually receptive (Woolley *et al.* 1990, Woolley & McEwen 1993, McEwen & Woolley 1994, Kinsley *et al.* 2006). Further, enhancements in E_2 have also been shown to increase the amount of synaptic connections made to pyramidal cells in the CA1 region of the hippocampus (Woolley & McEwen 1992) as well as increase the number of cells to which synaptic connections are made (Yankova *et al.* 2001). While increased cell–cell signaling in hippocampus may, in part, underlie enhancements in learning, concomitant rapid actions of progestins at membrane receptors and downstream signal transduction factors may also play a role. E_2 and $3\alpha,5\alpha$ -THP have been shown to modulate the activity at glutamatergic *N*-methyl-D-aspartate receptors in culture (Weiland 1992, Park-Chung *et al.* 1994), which are important in hormone-facilitated hippocampal learning (Morris *et al.* 1986, El-Bakri *et al.* 2004, Frye *et al.* 2006, Wang *et al.* 2007). P_4 administration enhances cAMP in brain (Collado *et al.* 1985) and antagonism of second messenger systems, including G-proteins, protein kinase C, or cAMP-dependent protein kinase A, *in vitro*, attenuates effects of $3\alpha,5\alpha$ -THP to facilitate GABA_A activation (Fáncsik *et al.* 2000). Further, the MAPK pathway has been demonstrated to be integral to progestins' functional effects; blocking MAPK attenuates the intracellular actions of P_4 or membrane actions of $3\alpha,5\alpha$ -THP to enhance sexual behavior among rats (González-Flores *et al.* 2004, Acosta-Martínez *et al.* 2006). Tomizawa *et al.* (2003) have found that MAPK inhibition also attenuates LTP in multiparous mouse hippocampal slices. These data suggest that signal transduction factors are important in steroid actions and may, in part, underlie some functional cognitive performance changes associated with endogenous E_2 and progestin elevations.

Apart from cyclical changes, these factors may also play a role in parity-related cognitive enhancement. Dams having had at least one litter typically outperform virgins on tasks of cognitive performance (Kinsley *et al.* 1999, Love *et al.* 2005, Bodensteiner *et al.* 2006,

Pawluski *et al.* 2006a, 2006b, Macbeth *et al.* 2008). Primiparous or multiparous dams are found to have a greater spinal density on hippocampal dendrites compared with nulliparous dams (Pawluski & Galea 2006). Dendritic remodeling has also been detected in late-pregnant and postpartum rats (Kinsley *et al.* 2006) and may have functional effects due to actions of steroids. In support, Morris water maze performance was found to be associated with alterations in endogenous E₂ and progestins, with higher levels of P₄ indicating better performance among pregnant rats (Galea *et al.* 2000). The present study supports these findings, demonstrating a greater cognitive performance in multiparous, compared with nulliparous, dams. It cannot be ignored, however, that many studies to date have either found no cognitive and/or morphological differences between primiparous and multiparous dams, or have found differences that favor primiparous over multiparous dams (Wartella *et al.* 2003, Love *et al.* 2005, Pawluski & Galea 2006, Pawluski *et al.* 2006a, 2006b). One explanation may be that the beneficial effects of multiple pregnancies do not summate in a long-term manner. While parity appears to clearly be beneficial compared with no reproductive experience, benefits of more reproductive experience may not extend beyond the postpartum period. Another explanation is that the cognitive skills and associated neurobiology which are enhanced by multiple pregnancies may be more region- and task-specific. In the present study, performance in the object placement task was more divergent among primiparous and multiparous dams than object recognition performance. It may be the case that object recognition was more challenging since there were many different objects used as novel stimuli, whereas, in object placement, only two corners of the arena can be considered novel locations. As such, object placement may better lend itself to discriminating spatial differences than object recognition, which may be a primary difference between multiparous and primiparous rats. As well, there can be differences between studies utilizing virgin versus mated nulliparous rats as controls. However, we did not see large differences between virgin controls (Experiment 1) and mated nulliparous controls (Experiment 2) in these tasks.

In order to elucidate endocrine factors that may underlie performance differences and extend the present literature, circulating hormone levels of 3 α ,5 α -THP, E₂, and CORT were assessed in tail blood daily across gestation. The circulating levels of 3 α ,5 α -THP were higher in first-time mothers than those in multiparous dams in late gestation (GD 13 until *post partum*), coincident with enhanced primiparous performance. These data support the notion that elevated 3 α ,5 α -THP levels may alter the hippocampal plasticity to enhance cognitive performance. Past reports find that administration of P₄ with E₂ to OVX rats, increases hippocampal spine density (Woolley *et al.* 1996, Miranda *et al.* 1999) and some of these effects are likely due to actions through its metabolism to 3 α ,5 α -THP. In support, 3 α ,5 α -THP has trophic effects to enhance central and peripheral myelination, it is neuroprotective in traumatic brain injury and ischemic stroke, and improves functional outcome after trauma (Magnaghi *et al.* 2001, Schumacher *et al.* 2001, 2007, Ghomari *et al.* 2003, Sayeed *et al.* 2006, VanLandingham *et al.* 2007). As such, trophic effects of 3 α ,5 α -THP may underlie some of the neuroprotective aspects that have been reported with motherhood in aging (Gatewood *et al.* 2005, Pawluski & Galea 2007). As well, we have previously found that the administration of 3 α ,5 α -THP to OVX rats increases cognitive performance on the object placement task commensurate to P₄ administration alone (Frye *et al.* 2007). Thus, 3 α ,5 α -THP elevations in pregnancy may promote hippocampal remodeling in primiparous rats to enhance cognitive performance.

In the brain, E₂ can enhance the expression of the enzymes that convert P₄ to 3 α ,5 α -THP and can enhance 3 α ,5 α -THP production (Cheng & Karavolas 1973, Micevych *et al.* 2003, Frye & Rhodes 2005). As such, circulating levels of E₂ were assessed and were higher among multiparous compared with primiparous dams. In the present study, E₂ levels were largely stable in both groups and did not greatly fluctuate across gestation as did cognitive performance. While the trophic effects of E₂ and/or 3 α ,5 α -THP (such as morphological changes in dendritic spine density and neuroproliferation) were not assessed, alterations in

circulating E₂ concentrations did not appear to adequately account for cognitive improvement among primiparous dams when considered alone. The contribution of central 3 α ,5 α -THP biosynthesis should be considered in future investigations.

Tail blood collection is a mild stressor that was utilized in the present investigation to assess the function of the hypothalamic–pituitary–adrenal axis (HPA) in multiparous and primiparous rats. Overall, the concentrations of CORT among rats in both groups were scarcely elevated beyond what is normal during pregnancy. Commensurate with past reports, we observed an initial increase in circulating CORT levels followed by a gradual attenuation of the HPA response in later gestation (Neumann *et al.* 1998, Russell & Brunton 2006, Brunton & Russell 2007). Of interest, primiparous dams had significantly higher CORT levels than multiparous dams across early- to mid-gestation and the attenuation of primiparous CORT levels was somewhat delayed compared with multiparous dams in late-gestation. Despite this, it is not likely that stress accounts for differences in cognitive performance of primiparous and multiparous dams. On the contrary, investigations of interactions between stress and cognition reveal that stressed female rats typically perform better on radial arm maze, object recognition, and object placement tasks (Bowman *et al.* 2001, Beck & Luine 2002, Luine 2002), even when the stress is extended for 21 days, commensurate with the time course of the present investigation (Bowman *et al.* 2001, Beck & Luine 2002). In fact, alterations in circulating CORT levels are an indication of central 3 α ,5 α -THP function. In rats, 3 α ,5 α -THP plays a homeostatic role, increasing in the brain in response to enhanced glucocorticoid secretion to reinstate parasympathetic tone and dampen the HPA-stress response (Patchev *et al.* 1996, Bitran *et al.* 2000). The blockade of 3 α ,5 α -THP formation or actions at its GABAergic substrates prevents anti-anxiety behavior and stress-induced glucocorticoid secretion (Rhodes & Frye 2001, Reddy 2002). As such, one intriguing possibility is that central 3 α ,5 α -THP enhancement may underlie some of the cognitive effects of parity. These data support this notion, demonstrating that cognitive performance is best among all rats when CORT levels are attenuated, possibly due to the enhancement of central progestins. Thus, HPA attenuation may be indicative of 3 α ,5 α -THP enhancement to levels that can improve cognitive performance.

The present investigation brings up several clinically relevant points. First, among premenopausal women, differences in cognitive performance have been observed across the menstrual cycle (Rupp & Wallen 2008). In support of this, Wisconsin Card-Sorting performance is better during high-progestin, compared with low E₂/progestin, phases of the cycle (Solis-Ortiz *et al.* 2004). As well, among pregnant women, circulating 3 α ,5 α -THP levels are observed to mimic the steady elevation and sharp decline across gestation that is observed among rodents (Gilbert-Evans *et al.* 2005), although the endocrine effects of parity among women have not been systematically investigated. Lastly, among natural or surgery-related postmenopausal women, the cognitive decline associated with hormonal depletion is marked (Zelinski *et al.* 1993, Small *et al.* 1999, Sherwin & Henry 2008). The findings of the present study add support to an overwhelming literature that attests to the beneficial effects hormones can have upon cognitive performance (Hays *et al.* 2003, Smoller *et al.* 2003, Brunner *et al.* 2005).

Although the results of this study are intriguing, it had several limitations. First, multiparous rats were typically a little more than one month older than other groups at the time of testing. However, all rats experienced their first mating at the same approximate age and the data do not suggest that this influenced our results since multiparous animals outperformed primiparous and virgin rats. If age were a confounding factor, it would be expected that multiparous animals would demonstrate a decreased cognitive performance compared with other groups. Second, Experiment 2 utilized rats that were mated and became pregnant, pseudopregnant, or did not become pregnant. Although all multiparous rats had successful pregnancies prior to this experiment, we cannot rule out the possibility that there may have

been a genetic or physiological advantage for those that did become pregnant over those that did not become pregnant. However, this seems less likely considering evidence that pseudopregnant rats also increased performance over non-pregnant rats, despite not reproducing. Third, the contribution of other factors, such as alterations in anxiety and exploratory behavior, which are affected by parity and hormonal status (Byrnes & Bridges 2006, Frye *et al.* 2006), may underlie some of the cognitive performance effects and/or hormonal alterations indicated on these tasks. Future studies may aim to utilize a battery of anxiety measures in addition to object placement and recognition in order to assess this possibility.

These experiments demonstrate that utilizing natural hormone states associated with elevations in progestins (via cyclical- and pregnancy-related enhancement) yield differences in cognitive performance favoring high-progestin states. These data confirm past reports and suggest that some of the cognitive enhancement that is associated with reproductive experience may be related to elevations in circulating $3\alpha,5\alpha$ -THP. Future studies should aim to elucidate the trophic effects of $3\alpha,5\alpha$ -THP on hippocampal plasticity as well as its behavioral correlates throughout the estrous cycle and the reproductive experience.

Materials and Methods

These methods were pre-approved by the Institutional Animal Care and Use Committee at the University at Albany, SUNY.

Animals

Adult, female Long-Evans rats ($n = 140$), which were experimentally naïve, weighing between 250 and 325 g, were obtained from the breeding colony at The University at Albany, SUNY (stock originally purchased from Charles River, Raleigh, NC, USA). The rats were ear-tagged for identification and housed four per cage in polycarbonate cages (45×24×21 cm) in a temperature-controlled room (21±1 °C) in the Laboratory Animal Care Facility in The Life Sciences Research Building at SUNY, Albany. At the time of testing, multiparous (two pregnancies) rats were between 4 and 5 months of age and primiparous (one pregnancy) and nulliparous rats (no pregnancies) were over 3 months of age, but all rats had their first mating at approximately 50 days of age, irrespective of the experimental condition. All rats were maintained on a 12 h:12 h reversed-light cycle (lights off at 0800 h) with continuous access to Purina Rat Chow and tap water.

Object placement

The object placement task is a spatial memory task that primarily relies on hippocampal functioning (Ennaceur *et al.* 1997, Wallace *et al.* 2006). During training, the rats were placed in a white open field (76×57 cm with 35 cm high walls) in a brightly lit testing room. Two identical objects were used in this task. The rats were given 3 min to explore the open field which contained the two objects that were always placed in adjacent corners farthest from the rat starting location. These objects were colored and spherical in shape (plastic toys in shapes such as oranges, lemons, apples, hamburgers). After training, the rats were placed in a dark, sound-dampened room for 4 h. Following this interval, they were returned to the open field for testing. During testing, one of the spherical objects was moved to the opposing corner of the open field box. The rats were allowed to explore these objects for 3 min. They spent equal amounts of time exploring objects in this task (typically 4.7±1.0 s). Although they did not show a bias towards exploring objects on the right versus left side of the open field, placement of the novel object was counterbalanced such that, across all subjects, every corner of the open field was used. In this manner, the novel object location was counterbalanced across treatment groups and testing sessions to eliminate any possible effects of side preference. As well, the

open field box and objects were cleaned with Quatricide (15 g/l) between subjects to control for confounding olfactory stimuli between testing sessions. A greater percentage of the time spent exploring the object in the novel location as a function of the total amount of the time spent exploring both objects during testing (duration spent with object in novel location/(duration spent with object in novel location+duration spent with object in familiar location) ×100) is considered an index of enhanced cognitive performance in this task. Rats were not required to investigate the objects for any minimum amount of time during the training phase to be considered trained in this task for the following two reasons: (1) the lack of criterion for investigation allows this test to be suitable for repeated testing without test-decay effects (Luine *et al.* 2003) and (2) the objective of the present investigation was to assess the endocrine status that is changing over time. Thus, a rigorous maintenance of the test-timing was required in order to avoid confounds that could have shifted the time of day at which assessment occurred during gestation.

Object recognition

The object recognition task is a working memory task that primarily relies on cortical functioning and, to a lesser extent, hippocampal functioning (Ennaceur *et al.* 1997, Broadbent *et al.* 2004, Akirav & Maroun 2006). This task was used as modified from previously published methods (Ennaceur & Delacour 1988, Frye & Lacey 2001, Luine *et al.* 2003). Three objects were used in this task. The rats were trained as described above; however, during the testing trial, one of the spherical objects was replaced by a third (novel), cone-shaped object (a plastic toy in the shape of a buoy, a cartoon mouse, a soda bottle, or a pear) of a similar size. A greater percentage of time spent exploring the novel object as a function of the total amount of time spent exploring both objects during testing (duration spent with novel object/(duration spent with novel object+duration spent with familiar object)×100) is considered an index of enhanced cognitive performance in this task.

Procedure

Determination of estrous cycle phase—Estrous cycle phase was determined by daily examination of vaginal epithelium (between 0900 and 1000 h), as per previous methods (Long & Evans 1922, Frye *et al.* 2000). The rats were cycled through two normal estrous cycles (4–5 day cycle) prior to testing. All rats were tested on the morning of behavioral estrus, which is characterized by the presence of many nucleated epithelial cells, when E₂ levels are declining, but progesterin levels are high (Feder 1981, Frye & Bayon 1999).

Experiment 1: endogenous hormonal variations in nulliparous versus multiparous rats—Rats that were randomly assigned to be mated as multiparous (having had two prior births) or not mated as nulliparous (virgin) rats ($n = 12/\text{grp}$) were each assessed once in the object placement and once in the object recognition tasks on separate testing occasions when in behavioral estrus and diestrus.

Experiment 2: variations in pregnancy status of multiparous versus primiparous rats—Multiparous or nulliparous rats were mated and determined by daily examination of vaginal epithelium (between 0800 and 0900 h), to be pregnant (in anestrus by GD 6 continuing until parturition, $n = 12$ multiparous, $n = 12$ primiparous), pseudopregnant (in anestrus by GD 6 continuing at least until GD 12 without parturition, $n = 12$ multiparous, $n = 12$ primiparous), or not pregnant (regularly cycling without parturition, $n = 12$ multiparous, $n = 12$ primiparous). The rats were trained and tested between GDs 6–12 and each rat was assessed once on the object placement and object recognition task.

Experiment 3: hormonal variations across gestation in multiparous versus primiparous rats—Virgin or multiparous rats were mated and determined to be pregnant

by daily examination of vaginal epithelium, consistent weight gain and other observable criteria (presence of teats, palpable pups). Rats ($n = 10/\text{grp}$) were tested on each day of pregnancy from GD 1 (approximately 24 h after mating) to GD 21 on either the object placement or the object recognition task. After testing on GD 21, dams were single-housed in a polycarbonate cage (45×24×21 cm) for birthing. One postpartum test was performed in both tasks with object placement 2 days after parturition and object recognition 3 days after parturition or *vice versa*. Throughout pregnancy, the task-type was counterbalanced such that some rats in each group performed object placement on odd GDs and object recognition on even GDs, while others were tested on the opposite schedule. Since gestation lasts an odd amount of days in the rat (approximately 21 days) there were two days (GDs 1 and 2) wherein all rats performed in the same task (object recognition on GD 1 and object placement on GD 2) in order to maintain a counterbalanced design and the number of observations in each group throughout testing. Likewise, stimuli were counterbalanced across gestation such that no stimuli were used more than once within 7 GDs. Tail blood was collected at the end of each testing occasion.

Plasma collection

The rats were gently restrained by hand and blood was collected from a small nick at the tip of the tail. They were allowed limited ambulation while tail was massaged and blood was collected (~1 ml) in order to minimize stress and/or discomfort. Following collection, blood remained on ice until refrigerated centrifugation (4 °C at 3000 g for 10 min). Serum was immediately stored in a -80 °C freezer until analysis.

Radioimmunoassays

RIA for E₂, 3 α ,5 α -THP, and CORT was conducted on plasma by previously reported methods (Choi & Dallman 1999, Frye & Bayon 1999, Frye *et al.* 2007).

Radioactive probes

Tritiated [³H] 3 α ,5 α -THP (NET 1047, specific activity = 65.0 Ci/mmol), [³H] E₂ (NET 317, specific activity = 51.3 Ci/mmol), and [³H] CORT (NET 182, specific activity = 48.2 Ci/mmol) used for RIAs were purchased from Perkin–Elmer (Boston, MA, USA).

Antibodies

The 3 α ,5 α -THP antibody (purchased from Dr Robert Purdy, Veterans Medical Affairs, La Jolla, CA, USA) was used in a dilution of 1:5000, which binds between 40 and 60% of [³H] 3 α ,5 α -THP. The E₂ antibody (E#244, Dr G D Niswender, Colorado State University, Fort Collins, CO, USA) was used in a dilution of 1:40 000 and bound between 40 and 60% of [³H] E₂. The CORT antibody (Endocrine Sciences, Calabasas Hills, CA, USA: #B3-163) was used in a dilution of 1:20 000 and bound between 40 and 60% of [³H] CORT.

Analyses

In Experiments 1 and 2, behavioral data were analyzed using two-way ANOVAs to determine the effects of parity (Experiment 1, nulliparous or multiparous and Experiment 2, primiparous or multiparous) and hormone condition (Experiment 1, diestrus or behavioral estrus and Experiment 2, not pregnant, pseudopregnant, or pregnant) on performance in the object placement and recognition tasks. In Experiment 3, repeated measures ANOVAs were utilized to determine effects of parity and GD differences with parity condition (primiparous or multiparous) as the between-subjects factor and GD (1–21 and *post partum*) as the within-subjects factor. Planned comparison *t*-tests were used to determine significant differences between primiparous and multiparous rats on each GD. An α -level of $P < 0.05$ was used to determine statistical significance in all analyses. An α -level of $P < 0.10$ was determined to indicate a trend towards significance. When appropriate, significant analyses were assessed

using Fisher's protected least significant difference comparisons to determine group differences.

Acknowledgements

This research was supported by funding from the National Institute of Mental Health (MH06769801) and the National Science Foundation (IBN 95-14463, 98-96263, 03-16083). The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

- Acosta-Martínez M, Gonzalez-Flores O, Etgen AM. The role of progesterone receptors and the mitogen-activated protein kinase pathway in delta opioid receptor facilitation of female reproductive behaviors. *Hormones and Behavior* 2006;49:458–462. [PubMed: 16289112]
- Akirav I, Maroun M. Ventromedial prefrontal cortex is obligatory for consolidation and reconsolidation of object recognition memory. *Cerebral Cortex* 2006;16:1759–1765. [PubMed: 16421330]
- Beck KD, Luine VN. Sex differences in behavioral and neurochemical profiles after chronic stress: role of housing conditions. *Physiology and Behavior* 2002;75:661–673. [PubMed: 12020731]
- Bitran D, Foley M, Audette D, Leslie N, Frye CA. Activation of peripheral mitochondrial benzodiazepine receptors in the hippocampus stimulates allopregnanolone synthesis and produces anxiolytic-like effects in the rat. *Psychopharmacology* 2000;151:64–71. [PubMed: 10958118]
- Bodensteiner KJ, Cain P, Ray AS, Hamula LA. Effects of pregnancy on spatial cognition in female Hooded Long-Evans rats. *Hormones and Behavior* 2006;49:303–314. [PubMed: 16140300]
- Bowman RE, Zrull MC, Luine VN. Chronic restraint stress enhances radial arm maze performance in female rats. *Brain Research* 2001;904:279–289. [PubMed: 11406126]
- Broadbent N, Squire L, Clark R. Spatial memory, recognition memory, and the hippocampus. *Neuroscience* 2004;101:14515–14520.
- Brunner RL, Gass M, Aragaki A, Hays J, Granek I, Woods N, Mason E, Brzyski R, Ockene J, Assaf A, et al. Effects of conjugated equine estrogen on health-related quality of life in postmenopausal women with hysterectomy: results from the Women's Health Initiative Randomized Clinical Trial. *Archives of Internal Medicine* 2005;165:1976–1986. [PubMed: 16186467]
- Brunton PJ, Russell JA. Attenuated hypothalamo-pituitary–adrenal axis response to immune challenge during pregnancy: the neurosteroidopioid connection. *Journal of Physiology* 2007;586:369–375. [PubMed: 17991694]
- Byrnes EM, Bridges RS. Reproductive experience alters anxiety-like behavior in the female rat. *Hormones and Behavior* 2006;50:70–76. [PubMed: 16524576]
- 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]
- Choi S, Dallman MF. Hypothalamic obesity: multiple routes mediated by loss of function in medial cell groups. *Endocrinology* 1999;140:4081–4088. [PubMed: 10465279]
- Collado ML, Rodriguez-Manzo G, Cruz ML. Effect of progesterone upon adenylate cyclase activity and cAMP levels on brain areas. *Pharmacology Biochemistry and Behavior* 1985;23:501–504.
- Concas A, Mostallino MC, Porcu P, Follsea P, Barbaccia ML, Trabucchi M, Purdy RH, Grisenti P, Biggio G. Role of brain allopregnanolone in the plasticity of gamma-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. *PNAS* 1998;95:13284–13289. [PubMed: 9789080]
- Cutler SM, Vanlandingham JW, Stein DG. Tapered progesterone withdrawal promotes long-term recovery following brain trauma. *Experimental Neurology* 2006;200:78–85.
- Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG. The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. *Journal of Neurotrauma* 2005;22:106–118. [PubMed: 15665606]
- El-Bakri NK, Islam A, Zhu S, Elhassan A, Mohammed A, Winblad B, Adem A. Effects of estrogen and progesterone treatment on rat hippocampal NMDA receptors: relationship to Morris water maze performance. *Journal of Cellular and Molecular Medicine* 2004;8:537–544. [PubMed: 15601582]

- Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioral Brain Research* 1988;31:47–59.
- Ennaceur A, Neave N, Aggleton JP. Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research* 1997;113:509–519.
- Fáncsik A, Linn DM, Tasker JG. Neurosteroid modulation of GABA IPSCs is phosphorylation dependent. *Journal of Neuroscience* 2000;20:3067–3075. [PubMed: 10777770]
- Featherstone RE, Fleming AS, Ivy GO. Plasticity in the maternal circuit: effects of experience and partum condition on brain astrocyte number in female rats. *Behavioral Neuroscience* 2000;114:158–172. [PubMed: 10718271]
- Feder HH. Hormones and sexual behavior. *Annual Review of Psychology* 1984;35:165–200.
- Fernandez SM, Frick KM. Chronic oral estrogen affects memory and neurochemistry in middle-aged female mice. *Behavioral Neuroscience* 2004;118:1340–1351. [PubMed: 15598143]
- Frick KM, Berger-Sweeney J. Spatial reference memory and neocortical neurochemistry vary with the estrous cycle in C57BL/6 mice. *Behavioral Neuroscience* 2001;115:229–237. [PubMed: 11256446]
- Frye CA. The role of neurosteroids and non-genomic effects of progestins and androgens in mediating sexual receptivity of rodents. *Brain Research Reviews* 2001;37:201–222. [PubMed: 11744087]
- Frye CA, Bayon LE. Mating stimuli influence endogenous variations in the neurosteroids 3 α ,5 α -THP and 3 α -Diol. *Journal of Neuroendocrinology* 1999;11:839–847. [PubMed: 10520134]
- Frye CA, Lacey EH. Progestins influence performance on cognitive tasks independent of changes in affective behavior. *Psychobiology* 2001;28:550–563.
- Frye CA, Rhodes ME. Estrogen-priming can enhance progesterone's anti-seizure effects in part by increasing hippocampal levels of allopregnanolone. *Pharmacology Biochemistry and Behavior* 2005;81:907–916.
- Frye CA, Rhodes ME. Progestin concentrations are increased following paced mating in midbrain, hippocampus, diencephalon, and cortex of rats in behavioral estrus, but only in midbrain of diestrous rats. *Neuroendocrinology* 2006a;83:336–347. [PubMed: 17028418]
- Frye CA, Rhodes ME. Infusions of 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP) to the ventral tegmental area, but not the substantia nigra, enhance exploratory, anti-anxiety, social and sexual behaviours and concomitantly increase 3 α ,5 α -THP concentrations in the hippocampus, diencephalon and cortex of ovariectomised oestrogen-primed rats. *Journal of Neuroendocrinology* 2006b;18:960–975. [PubMed: 17076771]
- Frye CA, Rhodes ME. Infusions of 3 α ,5 α -THP to the VTA enhance exploratory, anti-anxiety, social, and sexual behavior and increase levels of 3 α ,5 α -THP in midbrain, hippocampus, diencephalon, and cortex of female rats. *Behavioral Brain Research* 2008;187:88–99.
- Frye CA, Walf AA. Hippocampal 3 α ,5 α -THP may alter depressive behavior of pregnant and lactating rats. *Pharmacology Biochemistry and Behavior* 2004;78:531–540.
- 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. *Pharmacology Biochemistry and Behavior* 2000;67:587–596.
- Frye CA, Rhodes ME, Petralia SM, Walf AA, Sumida K, Edinger KL. 3 α -Hydroxy-5 α -pregnan-20-one in the midbrain ventral tegmental area mediates social, sexual, and affective behaviors. *Neuroscience* 2006;138:1007–1014. [PubMed: 16324790]
- 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]
- Furuta M, Bridges BS. Gestation-induced cell proliferation in the rat brain. *Developmental Brain Research* 2005;156:61–66. [PubMed: 15862628]
- Galea LA, Ormerod BK, Sampath S, Kostaras X, Wilkie DM, Phelps MT. Spatial working memory and hippocampal size across pregnancy in rats. *Hormones and Behavior* 2000;37:86–95. [PubMed: 10712861]
- Gatewood JD, Morgan MD, Eaton M, McNamara IM, Stevens LF, Macbeth AH, Meyer EA, Lomas LM, Kozub FJ, Lambert KG, et al. Motherhood mitigates aging-related decrements in learning and

memory and positively affects brain aging in the rat. *Brain Research Bulletin* 2005;66:91–98. [PubMed: 15982524]

- Ghoumari AM, Ibanez C, El-Etr M, Leclerc P, Eychenne B, O'Malley BW, Baulieu EE, Schumacher M. Progesterone and its metabolites increase myelin basic protein expression in organotypic slice cultures of rat cerebellum. *Journal of Neurochemistry* 2003;86:848–859. [PubMed: 12887683]
- Gibbs RB. Long-term treatment with estrogen and progesterone enhances acquisition of a spatial memory task by ovariectomized aged rats. *Neurobiology of Aging* 2000;21:107–116. [PubMed: 10794855]
- Gilbert-Evans SE, Ross LE, Sellers EM, Purdy RH, Romach MK. 3 α -Reduced neuroactive steroids and their precursors during pregnancy and the postpartum period. *Gynecological Endocrinology* 2005;21:268–279. [PubMed: 16373246]
- González-Flores O, Shu J, Camacho-Arroyo I, Etgen AM. Regulation of lordosis by cyclic 3',5'-guanosine monophosphate, progesterone, and its 5 α -reduced metabolites involves mitogen-activated protein kinase. *Endocrinology* 2004;145:5560–5567. [PubMed: 15319353]
- Hays J, Hunt JR, Hubbell FA, Anderson GL, Limacher M, Allen C, Rossouw JE. The Women's Health Initiative recruitment methods and results. *Annals of Epidemiology* 2003;13:S18–S77. [PubMed: 14575939]
- 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, Trainer R, Stafisso-Sandoz G, Quadros P, Marcus LK, Hearon C, Meyer EA, Hester N, Morgan M, Kozub FJ, et al. Motherhood and the hormones of pregnancy modify concentrations of hippocampal neuronal dendritic spines. *Hormones and Behavior* 2006;49:131–142. [PubMed: 16005000]
- Leblanc P, Weyers MH, Soffié M. Age-related differences for duration discrimination in rats. *Physiology and Behavior* 1996;60:555–558. [PubMed: 8840918]
- Leonelli E, Bianchi R, Cavaletti G, Caruso D, Crippa D, Garcia-Segura LM, Lauria G, Magnaghi V, Roglio I, Melcangi RC. Progesterone and its derivatives are neuroprotective agents in experimental diabetic neuropathy: a multimodal analysis. *Neuroscience* 2007;144:1293–1304. [PubMed: 17187935]
- Long, JA.; Evans, HM. The oestrous cycle in the rat and its associated phenomena. In: Leuschner, AO., editor. *Memoirs of the University of California*. 6. Berkeley: University of California Press; 1922. p. 1-146.
- López HH, Wurzel G, Ragen B. The effect of acute bupropion on sexual motivation and behavior in the female rat. *Pharmacology Biochemistry and Behavior* 2007;87:369–379.
- Love G, Torrey N, McNamara I, Morgan M, Banks M, Hester NW, Gasper ER, Devries AC, Kinsley CH, Lambert KG. Maternal experience produces long-lasting behavioral modifications in the rat. *Behavioral Neuroscience* 2005;119:1084–1096. [PubMed: 16187836]
- Luine V. Sex differences in chronic stress effects on memory in rats. *Stress* 2002;5:205–216. [PubMed: 12186683]
- Luine VN, Jacome LF, Maclusky NJ. Rapid enhancement of visual and place memory by estrogens in rats. *Endocrinology* 2003;144:2836–2844. [PubMed: 12810538]
- Macbeth AH, Scharfman HE, Maclusky NJ, Gautreaux C, Luine VN. Effects of multiparity on recognition memory, monoaminergic neurotransmitters, and brain-derived neurotrophic factor (BDNF). *Hormones and Behavior*. 2008;10.1016/j.yhbeh.2007.08.011[in press]
- Magnaghi V, Cavarretta I, Galbiati M, Martini L, Melcangi RC. Neuroactive steroids and peripheral myelin proteins. *Brain Research Reviews* 2001;37:360–371. [PubMed: 11744100]
- Majewska MD, Falkay G, Baulieu EE. Modulation of uterine GABAA receptors during gestation and by tetrahydroprogesterone. *European Journal of Pharmacology* 1989;174:43–47. [PubMed: 2558901]
- Markham JA, Pych JC, Juraska JM. Ovarian hormone replacement to aged ovariectomized female rats benefits acquisition of the morris water maze. *Hormones and Behavior* 2002;42:284–293. [PubMed: 12460588]
- Markowska AL, Savonenko AV. Effectiveness of estrogen replacement in restoration of cognitive function after long-term estrogen withdrawal in aging rats. *Journal of Neuroscience* 2002;22:10985–10995. [PubMed: 12486194]

- McEwen BS, Woolley CS. Estradiol and progesterone regulate neuronal structure and synaptic connectivity in adult as well as developing brain. *Experimental Gerontology* 1994;29:431–436. [PubMed: 7925761]
- Micevych P, Sinchak K, Mills RH, Tao L, LaPolt P, Lu JK. The luteinizing hormone surge is preceded by an estrogen-induced increase of hypothalamic progesterone in ovariectomized and adrenalectomized rats. *Neuroendocrinology* 2003;78:29–35. [PubMed: 12869797]
- Miranda P, Williams CL, Einstein G. Granule cells in aging rats are sexually dimorphic in their response to estradiol. *Journal of Neuroscience* 1999;19:3316–3325. [PubMed: 10212291]
- Morris RG, Anderson E, Lynch GS, Baudry M. Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP5. *Nature* 1986;319:774–776. [PubMed: 2869411]
- Neumann ID, Johnstone HA, Hatzinger M, Liebsch G, Shipston M, Russell JA, Landgraf R, Douglas AJ. Attenuated neuroendocrine responses to emotional and physical stressors in pregnant rats involve adenohipophysial changes. *Journal of Physiology* 1998;508:289–300. [PubMed: 9490853]
- Park-Chung M, Wu FS, Farb DH. 3α -Hydroxy- 5α -pregnan-20-one sulfate: a negative modulator of the NMDA-induced current in cultured neurons. *Molecular Pharmacology* 1994;46:146–150. [PubMed: 7520124]
- 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, Galea LA. Hippocampal morphology is differentially affected by reproductive experience in the mother. *Journal of Neurobiology* 2006;66:71–81. [PubMed: 16216005]
- Pawluski JL, Galea LA. Reproductive experience alters hippocampal neurogenesis during the postpartum period in the dam. *Neuroscience* 2007;149:53–67. [PubMed: 17869008]
- Pawluski JL, Walker SK, Galea LA. Reproductive experience differentially affects spatial reference and working memory performance in the mother. *Hormones and Behavior* 2006a;49:43–49.
- 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. *Behavioral Brain Research* 2006b;175:157–165.
- Pazol K, Northcutt KV, Wilson ME, Wallen K. Medroxyprogesterone acetate acutely facilitates and sequentially inhibits sexual behavior in female rats. *Hormones and Behavior* 2006;49:105–113. [PubMed: 16095597]
- Pitsikas N, Algeri S. Deterioration of spatial and nonspatial reference and working memory in aged rats: protective effect of life-long calorie restriction. *Neurobiology of Aging* 1992;13:369–373. [PubMed: 1625765]
- Reddy DS. The clinical potentials of endogenous neurosteroids. *Drugs of Today* 2002;38:465–485. [PubMed: 12582464]
- Rhodes ME, Frye CA. Inhibiting progesterone metabolism in the hippocampus of proestrous rats decreases anxiolytic, and enhances exploratory and motor behaviors. *Cognitive, Affective & Behavioral Neuroscience* 2001;1:287–296.
- Rhodes ME, Frye CA. Estrogen has mnemonic enhancing effects in the passive avoidance task. *Pharmacology Biochemistry and Behavior* 2004;78:551–558.
- Rhodes ME, McCormick CM, Frye CA. $3\alpha,5\alpha$ -THP mediates progestins’ effects to protect against adrenalectomy-induced cell death in the dentate gyrus of female and male rats. *Pharmacology Biochemistry and Behavior* 2004;78:505–512.
- Rupp HA, Wallen K. Sex differences in response to visual sexual stimuli: a review. *Archives of Sexual Behavior* 2008;37:206–218. [PubMed: 17668311]
- Russell JA, Brunton PJ. Neuroactive steroids attenuate oxytocin stress responses in late pregnancy. *Neuroscience* 2006;138:879–889. [PubMed: 16310312]
- Sandstrom NJ, Williams CL. Memory retention is modulated by acute estradiol and progesterone replacement. *Behavioral Neuroscience* 2001;115:384–393. [PubMed: 11345963]

- Sato T, Tanaka K, Ohnishi Y, Teramoto T, Irifune M, Nishikawa T. Effects of estradiol and progesterone on radial maze performance in middle-aged female rats fed a low-calcium diet. *Behavioural Brain Research* 2004;150:33–42. [PubMed: 15033277]
- Sayed I, Guo Q, Hoffman SW, Stein DG. Allopregnanolone, a progesterone metabolite, is more effective than progesterone in reducing cortical infarct volume after transient middle cerebral artery occlusion. *Annals of Emergency Medicine* 2006;47:381–389. [PubMed: 16546625]
- Schumacher M, Guennoun R, Mercier G, Desarnaud F, Lacor P, Benavides J, Ferzaz B, Robert F, Baulieu EE. Progesterone synthesis and myelin formation in peripheral nerves. *Brain Research Reviews* 2001;37:343–359. [PubMed: 11744099]
- Schumacher M, Guennoun R, Stein DG, De Nicola AF. Progesterone: therapeutic opportunities for neuroprotection and myelin repair. *Pharmacology & Therapeutics* 2007;116:77–106. [PubMed: 17659348]
- Sherwin BB, Henry JF. Brain aging modulates the neuroprotective effects of estrogen on selective aspects of cognition in women: a critical review. *Frontiers in Neuroendocrinology* 2008;29:88–113. [PubMed: 17980408]
- Shingo T, Gregg C, Enwere E, Fujikawa H, Hassam R, Geary C, Cross JC, Weiss S. Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. *Science* 2003;299:117–120. [PubMed: 12511652]
- Shors TJ, Lewczyk C, Pacynski M, Mathew PR, Pickett J. Stages of estrous mediate the stress-induced impairment of associative learning in the female rat. *Neuroreport* 1998;9:419–423. [PubMed: 9512383]
- Small SA, Stern Y, Tang M, Mayeux R. Selective decline in memory function among healthy elderly. *Neurology* 1999;52:1392–1396. [PubMed: 10227623]
- Smoller JW, Pollack MH, Wassertheil-Smoller S, Barton B, Hendrix SL, Jackson RD, Dicken T, Oberman A, Sheps DS. Prevalence and correlates of panic attacks in postmenopausal women: results from an ancillary study to the Women's Health Initiative. *Archives of Internal Medicine* 2003;163:2041–2050. [PubMed: 14504117]
- Solis-Ortiz S, Guevara MA, Corsi-Cabrera M. Performance in a test demanding prefrontal functions is favored by early luteal phase progesterone: an electroencephalographic study. *Psychoneuroendocrinology* 2004;29:1047–1057. [PubMed: 15219656]
- Tomizawa K, Iga N, Lu YF, Moriwaki A, Matsushita M, Li ST, Miyamoto O, Itano T, Matsui H. Oxytocin improves long-lasting spatial memory during motherhood through MAP kinase cascade. *Nature Neuroscience* 2003;6:384–390.
- VanLandingham JW, Cutler SM, Virmani S, Hoffman SW, Covey DF, Krishnan K, Hammes SR, Jamnongjit M, Stein DG. The enantiomer of progesterone acts as a molecular neuroprotectant after traumatic brain injury. *Neuropharmacology* 2006;51:1078–1085. [PubMed: 16926035]
- VanLandingham JW, Cekic M, Cutler S, Hoffman SW, Stein DG. Neurosteroids reduce inflammation after TBI through CD55 induction. *Neuroscience Letters* 2007;425:94–98. [PubMed: 17826908]
- Walf AA, Frye CA. A review and update of mechanisms of estrogen in the hippocampus and amygdala for anxiety and depression behavior. *Neuropsychopharmacology* 2006;31:1097–1111. [PubMed: 16554740]
- Walf AA, Rhodes ME, Frye CA. Ovarian steroids enhance object recognition in naturally cycling and ovariectomized, hormone-primed rats. *Neurobiology of Learning and Memory* 2006;86:35–46. [PubMed: 16529958]
- Wallace M, Luine V, Arellanos A, Frankfurt M. Ovariectomized rats show decreased recognition memory and spine density in the hippocampus and prefrontal cortex. *Brain Research* 2006;1126:176–182. [PubMed: 16934233]
- Wang JM, Johnston PB, Ball BG, Brinton RD. The neurosteroid allopregnanolone promotes proliferation of rodent and human neural progenitor cells and regulates cell-cycle gene and protein expression. *Journal of Neuroscience* 2005;25:4706–4718. [PubMed: 15888646]
- Wang C, Marx CE, Morrow AL, Wilson WA, Moore SD. Neurosteroid modulation of GABAergic neurotransmission in the central amygdala: a role for NMDA receptors. *Neuroscience Letters* 2007;415:118–123. [PubMed: 17275189]

- Wang JM, Liu L, Irwin RW, Chen S, Brinton RD. Regenerative potential of allopregnanolone. *Brain Research Reviews* 2008;57:398–409. [PubMed: 17931704]
- Wartella J, Amory E, Lomas LM, Macbeth A, McNamara I, Stevens L, Lambert KG, Kinsley CH. Single or multiple reproductive experiences attenuate neurobehavioral stress and fear responses in the female rat. *Physiology and Behavior* 2003;79:373–381. [PubMed: 12954432]
- Weiland NG. Estradiol selectively regulates agonist binding sites on the *N*-methyl-D-aspartate receptor complex in the CA1 region of the hippocampus. *Endocrinology* 1992;131:662–668. [PubMed: 1353442]
- Wood GE, Beylin AV, Shors TJ. The contribution of adrenal and reproductive hormones to the opposing effects of stress on trace conditioning in males versus females. *Behavioral Neuroscience* 2001;115:175–187. [PubMed: 11256441]
- Woolley CS, McEwen BS. Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. *Journal of Neuroscience* 1992;12:2549–2554. [PubMed: 1613547]
- Woolley CS, McEwen BS. Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. *Journal of Comparative Neurology* 1993;336:293–306. [PubMed: 8245220]
- Woolley CS, Gould E, Frankfurt M, McEwen BS. Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *Journal of Neuroscience* 1990;10:4035–4039. [PubMed: 2269895]
- Woolley CS, Wenzel HJ, Schwartzkroin PA. Estradiol increases the frequency of multiple synapse boutons in the hippocampal CA1 region of the adult female rat. *Journal of Comparative Neurology* 1996;373:108–117. [PubMed: 8876466]
- Yankova M, Hart SA, Woolley CS. Estrogen increases synaptic connectivity between single presynaptic CA1 pyramidal cells: a serial electron-microscopic study. *PNAS* 2001;98:3525–3530. [PubMed: 11248111]
- Zelinski EM, Gilewski MJ, Schaie KW. Individual differences in cross-sectional and 3-year longitudinal memory performance across the adult life span. *Psychology and Aging* 1993;8:176–186. [PubMed: 8323722]

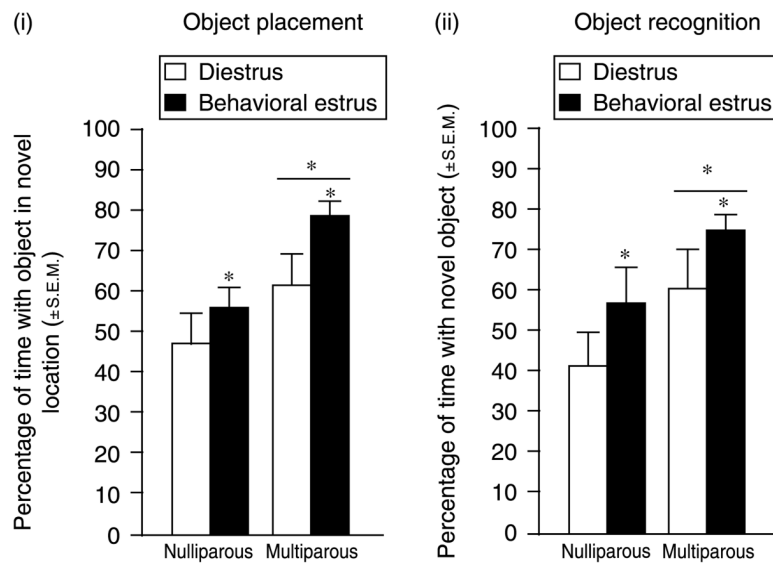


Figure 1.

(i) Multiparous rats ($n = 12/\text{grp}$) significantly outperform nulliparous rats ($n = 12/\text{grp}$) and rats in behavioral estrus significantly outperform diestrus rats on the OP task. (ii) Multiparous rats ($n = 12/\text{grp}$) significantly outperform nulliparous rats ($n = 12/\text{grp}$) and rats in behavioral estrus significantly outperform diestrus rats on the OR task. *Indicates significant difference, $P < 0.05$.

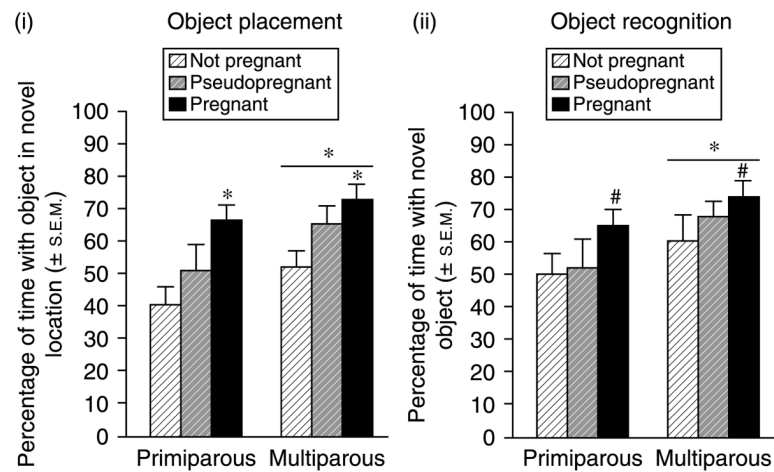


Figure 2.

(i) Multiparous rats ($n = 12/\text{grp}$) significantly outperform nulliparous rats ($n = 12/\text{grp}$) and pregnant rats significantly outperform non-pregnant rats on the OP task. (ii) Multiparous rats ($n = 12/\text{grp}$) significantly outperform nulliparous rats ($n = 12/\text{grp}$) on the OR task and pregnant rats tend to outperform non-pregnant rats on the OR task. *Indicates significant difference, $P < 0.05$. #Indicates trend towards difference, $P < 0.10$.

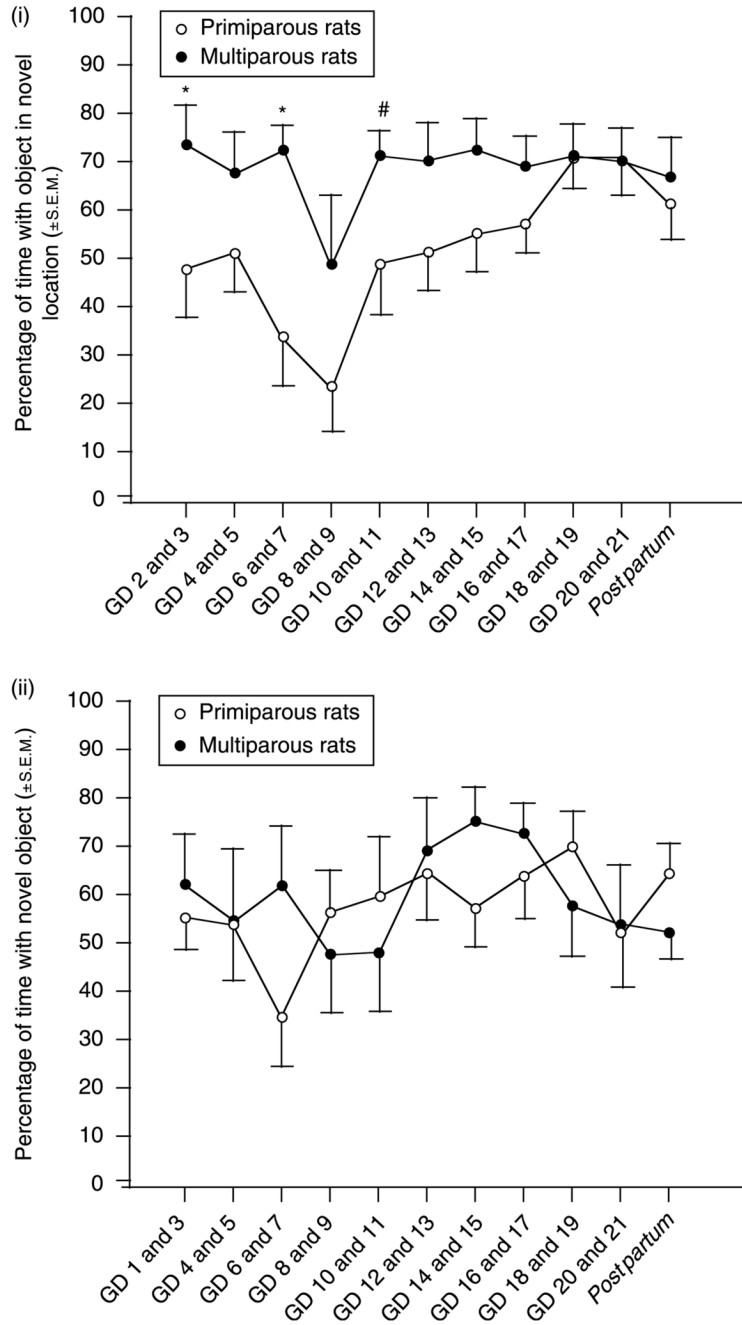


Figure 3. (i) Multiparous rats ($n = 10$) significantly outperform nulliparous rats ($n = 10$) on the OP task in a repeated measures analysis across gestation. (ii) Multiparous ($n = 10$) and nulliparous rats ($n = 10$) do not significantly differ on the OR task in a repeated measures analysis across gestation. *Indicates significant difference, $P < 0.05$. #Indicates trend towards difference, $P < 0.10$.

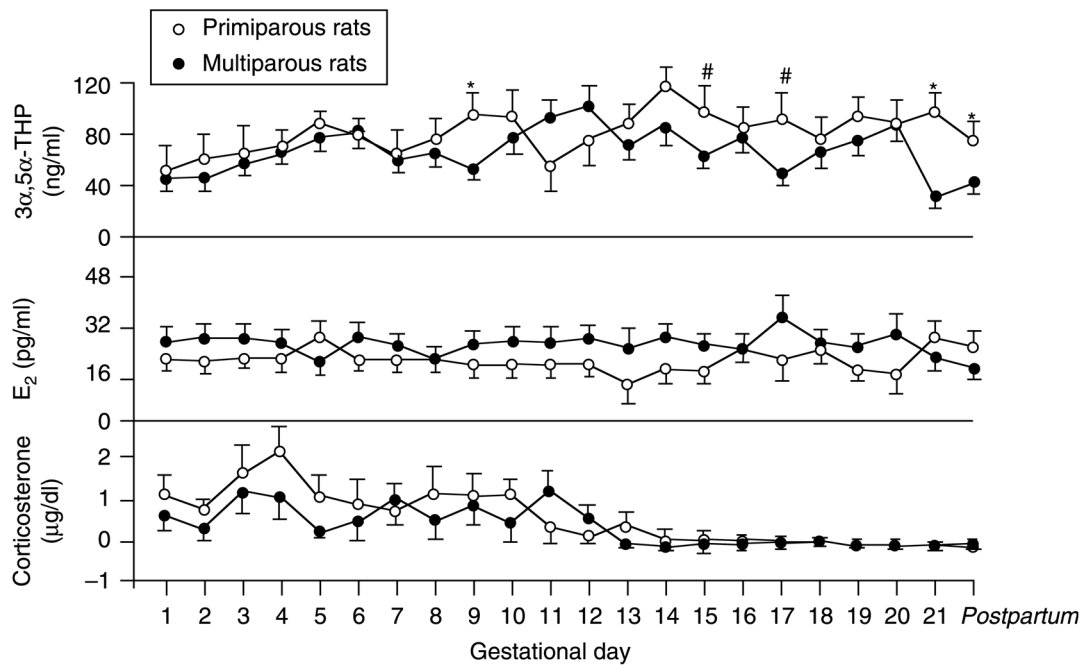


Figure 4. Depicts circulating concentrations of 3 α ,5 α -THP (top), estrogen (E₂, middle), and corticosterone (bottom) in tail blood of primiparous ($n = 10$) and multiparous ($n = 10$) rats across gestation. *Indicates significant difference, $P < 0.05$. #Indicates trend towards difference, $P < 0.10$.