Hippocampal learning, memory, and neurogenesis: effects of sex and estrogens across the lifespan in adults

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Abstract

There are sex differences in hippocampus-dependent cognition and neurogenesis suggesting that sex hormones are involved. Estrogens modulate certain forms of spatial and contextual memory and neurogenesis in the adult female rodent, and to a lesser extent male, hippocampus. This review focuses on the effects of sex and estrogens on hippocampal learning, memory, and neurogenesis in the young and aged adult rodent. We discuss how factors such as the type of estrogen, duration and dose of treatment, timing of treatment and type of memory influence the effects of estrogens on cognition and neurogenesis. We also address how reproductive experience (pregnancy and mothering) and aging interact with estrogens to modulate hippocampal cognition and neurogenesis in females. Given the evidence that adult hippocampal neurogenesis plays a role in long-term spatial memory and pattern separation, we also discuss the functional implications of regulating neurogenesis in the hippocampus.

Introduction

The integrity of the hippocampus is compromised in neurodegenerative diseases associated with cognitive decline such as Alzheimer's disease (Scheff et al., 2006; Selden et al., 1991; Snyder et al., 2005) and depression (McKinnon et al., 2009). Both of these diseases show a greater incidence in women (Baum, 2005; Gutierrez-Lobos et al., 2002). Any time sex differences are seen in a behavior or trait, this suggests that sex hormones are involved. Studies in rodents and humans have revealed sex differences favoring males in both hippocampus-dependent learning and memory (Jonasson, 2005; Maren et al., 1994; Postma et al., 2004) and hippocampus morphology (Galea et al., 2013; Ruigrok et al., 2014). The hippocampus is known to show dramatic plasticity in adulthood including the ability to produce new neurons in all mammalian species including humans (Christie and Cameron, 2006; Eriksson et al., 1998). One aspect that is not always taken into account when researching hippocampus-dependent memory and neurogenesis is that there are sex differences in adult neurogenesis in the hippocampus (reviewed in Galea et al., 2013) that may contribute to sex differences in learning and memory and vulnerability to neurological diseases involving the hippocampus.

The findings of sex differences suggest that sex hormones are involved in both hippocampus-dependent cognition and neuroplasticity. The majority of studies have investigated the effects of ovarian hormones, such as estradiol, on cognition and neuroplasticity. Hippocampal function and morphology are sensitive to changes in estrogens that occur across the reproductive cycle, pregnancy, motherhood, and aging in females (e.g., reviewed in Daniel, 2013; Pawluski et al., 2009). The purpose of this review is to summarize the effects of sex and estrogens on hippocampal learning and memory and neurogenesis throughout the lifespan and to discuss the functional implications of regulating neurogenesis in the hippocampus.

Hippocampus-dependent learning and memory

There are multiple memory systems in the brain (White and McDonald, 2002). Understanding the neural components of these memory systems and the factors that affect these different systems will lead to an advanced understanding of how learning and memory is represented in the brain. Two memory types are working and reference memory. Working memory can be defined as manipulation and retrieval of trial unique information to guide prospective action (Baddeley, 2003), while reference memory can be defined as a long-term memory for events or stimuli that stay stable over time (Olton and Pappas, 1979; White and McDonald, 2002). There are a variety of mazes that vary in working and/or reference memory load and in which different neural regions mediate performance on these tasks. Working memory is disrupted by lesions to the hippocampus (Bannerman et al., 2004; Kubik et al., 2007; Yoon et al., 2008), basal forebrain, and prefrontal cortex (Hammond et al., 2009; Yoon et al., 2008); while reference memory is disrupted by lesions to the hippocampus (Bannerman et al., 2004) and the caudate (Kolbe and Cioe, 1996; White and McDonald, 2002). Estrogens, and in particular estradiol, affect performance on a variety of tasks in which performance depends on different brain regions (i.e. hippocampus, prefrontal cortex (e.g., Sinopoli et al., 2006), amygdala, striatum (e.g., Uban et al., 2012)). However in this review we will focus on tasks that involve the hippocampus.

Adult neurogenesis

Adult neurogenesis in the dentate gyrus consists of at least four processes: cell proliferation (production of new cells), migration (migration of new cells to the appropriate place), differentiation (into a neuron or glia cell or the phenotype of new cells) and cell survival

(cells surviving to maturity). The amount of neurogenesis is determined by changes in any one of these components independently or in concert. For instance, chronic antidepressants increase neurogenesis via increases in cell proliferation independent of any changes in cell survival (Malberg et al., 2000), while testosterone increases neurogenesis via increases in cell survival independent of any changes in cell proliferation (Hamson et al., 2013; Spritzer and Galea., 2007). On the other hand, prenatal alcohol exposure decreases neurogenesis in female rats due to reduction in the percentage of new cells differentiating into neurons (Uban et al., 2010).

Stages of neurogenesis can be studied along with stage-specific endogenous cell markers such as Ki67 (proliferating cells) or doublecortin (immature neurons), or exogenous markers such as bromodeoxyuridine (BrdU). BrdU incorporates into the DNA of mitotic cells during S-phase of the cell cycle, over a period of 2 h (Nowakowski et al., 1989). Thus, BrdU is often used for birthdating and monitoring the fate of divided cells (for discussion on markers see Taupin, 2007). Because BrdU does not exclusively label neurons but also other cell types such as glial cells (Taupin, 2007), it is necessary to co-label BrdU with endogenous markers, such as doublecortin (immature neurons expressed 1-21 days after production) or NeuN (expressed in mature neurons beginning approximately 14 days after production in a rat), depending on your timeline, to determine neuronal phenotype (Figure 2; Brown et al., 2003; Snyder et al., 2009). Modification during any of the four stages may lead to changes in levels of neurogenesis and perhaps function of the hippocampus.

Adult neurogenesis in the dentate gyrus plays a role in spatial memory (Winocur et al., 2006). Ablation of neurogenesis demonstrated a role of adult hippocampal neurogenesis for long-term spatial memory (Snyder et al., 2005) and pattern separation (Clelland et al., 2009), but no significant role for acquisition in the Morris water maze (Shors et al., 2002). However, the

amount of neurogenesis does not always correlate with spatial performance (reviewed in Leuner et al., 2006a). Once the new neuron is produced, it likely undergoes modifications to spines and pruning of dendritic branching that may impact hippocampal function (Zhao et al., 2006). A complete review of this literature is beyond the scope of this review but the reader is directed towards comprehensive reviews on these topics (e.g., Aimone et al., 2014; Christian et al., 2014). A way to determine whether new neurons are active is to examine immediate early gene (IEG) expression in these new neurons. IEGs are activated rapidly after neuronal stimulation and encode transcription factors that regulate the expression of genes that modify the phenotype of the neuron in response to the neuronal stimulation (Sheng and Greenberg, 1990). IEG products such as c-Fos, Arc or zif268 are induced in response to neural stimulation and play an important role in neural plasticity and memory consolidation (Guzowski et al., 2001; Jones et al., 2001). New neurons in the dentate gyrus can be preferentially recruited during spatial memory retrieval depending on the task and age of new neurons (Epp et al., 2011; Kee et al., 2007; Leuner et al., 2006b). The functional implications of the estradiol-dependent modulation of hippocampal neurogenesis are addressed below.

Sex differences in learning, memory, and neurogenesis

Sex differences in learning and memory: spatial navigation and strategy choices

Sex differences in spatial learning, favoring males, exist across a variety of species including humans and rodents (Voyer et al., 1995). For example, men on average outperform women on tasks such as route learning (Galea and Kimura, 1993; Holding and Holding, 1988; Postma et al., 2004), maze navigation (Moffat et al., 1998; Woolley et al., 2010) and spatial rotation tasks (Kaufman, 2007; Parsons, 2004). In rodents, a meta-analysis indicated that male

rats outperform female rats in both water maze and radial arm maze protocols, both generally spatial tasks (Jonasson, 2005). Males also have a greater retention of contextual fear than females (Maren et al., 1994) and this sex difference is regulated by estradiol as sex differences are eliminated when female rats are ovariectomized and re-established when females receive a high dose of estradiol (10 µg; Gupta et al., 2001 ; see also section below).

In addition to spatial performance, there is evidence that males and females use different strategies to solve spatial navigation tasks, which is seen in both humans and rodents (Anderson et al., 2012; Dabbs et al., 1998; Galea and Kimura, 1993; Grissom et al., 2013; Hawley et al., 2012; Lawton, 1994; Williams et al., 1990; Silverman and Choi, 2006). Females preferentially use egocentric cues while males preferentially use allocentric cues (Cherney et al., 2008; Grissom et al., 2013; Galea and Kimura, 1993, Williams et al. 1990). For instance, more men used geometric cues and more women used landmark cues to reach a destination (Andersen et al., 2012; Galea and Kimura, 1993). Interestingly, Chamizo et al. (2011) found sex differences favouring men, in a 3-D virtual environment Morris Water maze task but only when the task required higher cognitive demands, indicating that sex differences are only detected when the task is more difficult.

In agreement with human studies, there are sex differences in strategy choice in rodents performing cue competition tasks (Grissom et al., 2013, Hawley et al., 2012; Williams et al., 1990). For example, Grissom et al. (2013) showed that in a cue competition task male rats used a hippocampus-dependent place strategy that preferentially relies on extra-maze spatial cues, while female rats were more likely to use a striatum-dependent response strategy that relies on visible landmark cues, though this latter finding did not reach statistical significance. These data

indicate that not only are there sex differences in performance but also in strategy choice to solve spatial tasks.

Although this review centers on estrogen's effects on hippocampus dependent learning and plasticity, adult androgen levels have been linked to both spatial performance and strategy choice in males (Spritzer et al., 2013). For example, castration decreased the accuracy in spatial working memory while testosterone administration improved spatial working memory (Gibbs and Johnson, 2008; Spritzer et al., 2008). Hawley et al. (2012) found that male rats showed a bias toward a place strategy, and castration reduced the preference for this learning strategy. Furthermore, low testosterone increased the use of a motor-response strategy or a cued-response strategy while high testosterone led to a preference for a place strategy in dual-solution water maze (Spritzer et al., 2013). Perhaps not surprisingly, ovarian hormones influence spatial performance and strategy choices in females and we review below the effects of estrogens both naturally and by manipulation on cognition and neurogenesis in adult females.

Sex differences in adult hippocampal neurogenesis

There are sex differences in hippocampal neurogenesis in adult rats and voles. In both rodents, females have higher levels of cell proliferation compared to males in the dentate gyrus (Galea and McEwen, 1999; Tanapat et al., 1999). These sex differences are dependent on natural fluctuations of gonadal hormones (see below). However, male rats can have higher levels of immature neurons than female rats in adulthood (Hillner et al., 2013; Lee et al., 2014; but see Chow et al., 2013). Interestingly, differences in cell proliferation between the sexes or during the reproductive cycle are not always seen across mammalian species. For example, no sex or seasonal differences in hippocampal neurogenesis is observed in squirrels (Lavenex et al., 2000)

but there was more doublecortin expression in the hippocampus of opposite-paired versus same-sex paired eusocial rodents, such as naked mole rats (Peragine et al., 2014). In laboratory mice, research is equivocal as some studies have found no significant differences in neurogenesis between males and females (C57BL/6J strain; Ben Abdallah et al., 2010; Klaus et al., 2012; Lagace et al., 2007; and B6SJL strain; Ma et al., 2012) while other studies have found sex differences in cell proliferation and survival, favouring females, in the hippocampus of adult C57BL/6J mice (Roughton et al., 2012). Some of these discrepancies may be due to the fact that not all studies compensate for sex differences in hippocampal volume (larger in males than females) by using a density measure for BrdU-labelled cells or examine estrous cycle phase in females. The research community is encouraged to examine these important factors when investigating sex differences in neurogenesis in the future.

Functional implications: are the effects on neurogenesis related to changes in cognition?

IEG expression in adult-born neurons is increased after spatial learning (Jessberger and Kempermann, 2003; Kee et al., 2007; Snyder et al., 2009) and spatial memory retrieval (Snyder et al., 2005; Epp et al., 2011). Thus far, the majority of studies examining IEG expression after spatial learning have been conducted in male animals. One study examined sex differences in activation of 20 days old adult-born cells in the dentate gyrus in response to spatial memory retrieval in the Morris water maze (Chow et al., 2013). Greater activation of new neurons in the dentate gyrus was strongly associated with better performance in female, but not in male, rats. The greater activation in females in response to spatial memory retrieval (Chow et al., 2013) may be due to differences in excitability of new neurons or sex differences in the timing of when males and females recruit young neurons during spatial learning. Méndez-López et al. (2009)

reported that there is a greater increase in c-Fos expression in female relative to male rats in the hippocampus during spatial training. These sex differences in excitability may be caused by ovarian hormones (reviewed below). Further research is needed to determine whether there are sex differences in how and when adult-born neurons are recruited during spatial learning and memory.

Effects of natural variations in estrogens on learning, memory, and neurogenesis

Most studies have examined the effect of estradiol but there are three forms of estrogens, estradiol, estrone, and estriol, with estradiol being the most potent. Estradiol exerts its physiological effects by binding to the classical estrogen receptors (ER α and ER β), localized in nuclear and extranuclear sites, including the membrane (McEwen et al., 2012; McEwen and Milner, 2007; Vasudevan and Pfaff, 2008), and to the membrane estrogen receptor GPER, localized in the plasma membrane and endoplasmic reticulum (Prossnitz et al., 2008). Both ERs and GPER are expressed in the hippocampus (Brailoiu et al., 2007; Hazell et al., 2009; Perez et al., 2003; Towart et al., 2003), making it an important target for estrogens.

Natural variations in estrogens and cognition

Estrous cycle in rodents and cognition

Females in proestrus (high estradiol and progesterone) perform worse in spatial learning tasks such as the Morris water maze compared to females in estrus (low estradiol; Frye, 1995; Galea et al., 1995; Warren and Juraska, 1997). However, a study that pre-exposed rats to the water maze prior to training found no significant effects of estrous cycle on spatial performance (Berry et al., 1996), indicating that novelty stress may impair performance during proestrus.

Furthermore stress reactivity is higher during proestrus compared to estrus in rats (Viau and Meaney, 1991). Other studies have found that acute stress negatively affects spatial performance in females (Beiko et al., 2004) and impaired learning of the eye-blink conditioning task in proestrous, but not estrous, females (Shors et al., 1998). Another study however found that acute restraint stress facilitated spatial memory in the Y-maze in both proestrous and estrous females (Conrad et al., 2004). Taken together, the role of stress in mediating learning across the estrous cycle may be specific to the task and/or type of stressor.

Task-specific impairments in performance during proestrus likely depend on the brain region recruited during the procedure. Proestrous rats show less freezing in the hippocampus-amygdala-dependent contextual fear conditioning task (Markus and Zecevic, 1997), but acquire the hippocampus-cerebellum-dependent classical eye-blink conditioning task much faster than non-proestrous rats (Shors et al., 1998). However, Chang et al. (2009) found no effect of estrous cycle on freezing in the contextual fear conditioning task in female rats, which may be partly attributed to strain differences (Sprague-Dawley vs Fischer 344) and differences in time intervals between fear conditioning and testing (2 weeks vs 24 h).

As both estradiol and progesterone fluctuate across the estrous cycle, several studies have attempted to tease out the relative contribution of each hormone on learning and memory in female rats. Chesler and Juraska (2000) found that short-term injections of estradiol and progesterone 4 h prior to training had no significant effect on acquisition in the water maze task, but both hormones together impaired learning. Frye and colleagues (2007) found that acute injections of estradiol, progesterone, or a combination of both improved object placement memory when administered immediately after training. Finally, Milad et al. (2009) blocked either estradiol or progesterone (using receptor antagonists) in proestrous animals 30 min prior to

extinction training in a fear conditioning task and found that while the rate of extinction did not differ between groups, blocking progesterone receptors increased the percentage of freezing during training. However, blocking either estradiol or progesterone receptors alone prior to training impaired extinction recall when animals were tested the next day, suggesting that hormone conditions during training can affect memory retrieval at a later time (Milad et al., 2009). Taken together, these studies show that both estradiol and progesterone can affect learning and memory across the estrous cycle, but the relative role of either hormone depends largely on the timing of training and type of task.

Menstrual cycle in women and cognition

In women, contextual memory, spatial ability, and recognition memory for negative images are better during the early follicular phase (low estradiol) than during the mid-follicular phase and the luteal phase (high estradiol) of the menstrual cycle (Hampson, 1990; Milad et al., 2006; Bayer et al., 2014). Verbal fluency is positively correlated with estradiol levels, while spatial performance is negatively correlated with estradiol levels (Hampson, 1990; Maki et al., 2002). These studies collectively suggest that hippocampus-based tasks are negatively affected during reproductive cycles characterized by high estradiol, but tasks requiring recruitment of the prefrontal cortex or other areas may show opposite effects.

Although there are seemingly inconsistent results on cycle and learning, this may be attributable to a number of factors that are not always accounted for in the literature. 1) In both the estrous cycle and menstrual cycle, both estradiol and progesterone fluctuate rapidly and a difference of a few hours can matter dramatically for the levels of estradiol. For example, levels of estradiol peak in the afternoon of proestrus in female rodents (Smith et al., 1975). 2) Age and

parity modulate levels of estradiol and progesterone seen across the estrous/menstrual cycle. Briefly, parity results in reduced levels of estradiol across the menstrual (Bernstein et al., 1985; Dorgan et al., 1995) and estrous (Bridges and Byrnes, 2006) cycle in both women and rodents, respectively. These rapidly changing effects, coupled with age and parity differences in absolute values across reproductive cycles, suggest that these are important factors to keep in mind when researching the effects of cycling in females (Box 1).

Any changes in cognition or neurogenesis during the estrous or menstrual cycles could be due to changes in ovarian hormone levels including estrogens and progestins. In this review, we focus on the effects of estrogens but it is important to keep in mind that progesterone or estradiol/progesterone ratio could regulate cognition and neurogenesis (as noted in the preceding section).

Pregnancy, reproductive experience and cognition in rodents

In a rodent pregnancy, estradiol levels are lower (but progesterone levels are higher) during the first two weeks of pregnancy, which is followed by higher levels of estradiol in the final days of pregnancy (while progesterone levels decline), and finally, a rapid drop upon parturition with the expulsion of the placenta, and both estradiol and progesterone levels remain low during the early postpartum period (Rosenblatt et al, 1988; Hapon et al., 2003; Paris and Frye, 2008; Barha and Galea, 2010).

In general, early- and mid-pregnancy (lower levels of estradiol) are associated with enhanced spatial performance, but the opposite is true during late pregnancy (higher levels of estradiol; Galea et al., 2000; Macbeth et al., 2008). Additionally, the early postpartum period is characterized by impaired hippocampus-dependent learning, while the late postpartum period

and after weaning are characterized by enhanced hippocampus-dependent learning compared to non-parous rats (Darnaudéry et al., 2007; Kinsley et al., 1999; Gatewood et al., 2005; Kinsley et al., 2011). These changes do not completely mirror changing levels in estradiol concentration and it is important to note that there is a curvilinear relationship between estradiol level and spatial performance, as discussed below. In addition, other hormones such as oxytocin, prolactin, corticosterone or changes in the estradiol/progesterone ratio may also regulate cognition across gestation, lactation, and the post-weaning period, but are beyond the scope of this review.

The effects of parity to modulate cognition are modified partly by age and amount of parity, as multiparous rats outperformed primiparous rats on the object placement task throughout the majority of the gestational period (Paris and Frye, 2008). Well beyond the early postpartum period, reproductive experience is associated with better spatial ability. Studies show that primiparous rats, after weaning, had enhanced spatial working and reference memory compared to nulliparous females (Kinsley et al., 1999, Pawluski et al., 2006a). The parity-related differences may be due to alterations in endogenous hormone levels. Primiparous rats have lower levels of estrogen during proestrus compared to nulliparous rats when measured two weeks after weaning, and were also less responsive to stress, suggesting alterations to the hypothalamic–pituitary–adrenal axis (HPA; Bridges and Byrnes, 2006). However, this is beyond the scope of this review and the reviewers are directed to other reviews on the topic (Roes and Galea, in press).

Pregnancy and postpartum effects on cognition in women

The hormonal profile during human pregnancy is different than during rodent gestation, with estradiol (as well as progesterone and cortisol) rising throughout gestation, then dropping upon parturition (Abou-Samra et al., 1984 Magiakou et al., 1997; Pawluski et al., 2009). A meta-

analysis showed that during pregnancy, cognitive impairments are observed in tasks that require more mental effort, such as free recall and working memory tasks (Henry and Rendell, 2007), although other studies failed to find differences in working memory and spatial performance in pregnant women (Glynn, 2010; Henry and Sherwin, 2012). Fetal sex might partially account for these inconsistencies, as Vanston and Watson (2005) found that women carrying male fetuses performed better on working memory and mental rotation tasks compared to women carrying a female fetus throughout pregnancy, an effect that persisted up to 19 months postpartum. Alternatively, differences in environment might also account for inconsistencies between studies. Cuttler et al. (2011) found comparable performances between pregnant and non-pregnant women when prospective memory tasks were performed in laboratory settings, but found impairments in pregnant women when tasks were performed in the context of their everyday lives.

Studies show that impairments in verbal recall memory occur during the second and third trimester of pregnancy (de Groot et al., 2006; Glynn, 2010), but return to control levels 6 to 12 months postpartum (Glynn, 2010; Silber et al., 1990), although de Groot et al. (2006) did observe impairments at 32 weeks postpartum. Additionally, Glynn (2010) found that higher estradiol levels across gestation were associated with poorer performance in verbal recall memory, while Henry and Sherwin (2012) found no association between estradiol levels and verbal memory in the third trimester. These studies suggest that estradiol levels during early pregnancy may be more predictive of verbal recall memory than in later time points. Studies in women have found that during pregnancy and early postpartum, biparous or multiparous women have poorer verbal recall memory compared to primiparous women who in turn are worse than nulliparous controls (Sharp et al., 1993; Glynn, 2012). Taken together, it is possible that working memory and spatial performance vary during pregnancy depending on fetal sex and environmental factors, while

verbal recall memory is more dependent on steroid hormone levels and previous reproductive experience.

Natural variations in estrogens and neurogenesis

Estrous cycle and neurogenesis

In proestrous rats and mice, cell proliferation is increased in the hippocampus compared to estrous and diestrous rats (Rummel et al., 2010; Tanapat et al., 1999; Tzeng et al., 2014 but see Lagace et al., 2007). Cell survival is also increased for up to 14 days after BrdU injection during proestrus, but normalizes by day 21 as BrdU-labelled cell survival was similar to the level of estrous rats (Tanapat et al., 1999). Female meadow voles during the breeding season have higher levels of estradiol (but also higher levels of corticosterone) and less cell proliferation compared to females during non-breeding seasons (Galea and McEwen, 1999; Ormerod and Galea, 2001). It is important to note that studies so far have only observed the effects of estrous cycle on neurogenesis in adult females with no prior reproductive experience. Given the evidence that primiparous rats had lower estradiol levels during proestrus compared to nulliparous rats (Bridges and Byrnes, 2006), it is unclear how neurogenesis would be affected once cycling resumes after pregnancy, and whether or not an estrous cycle effect would persist in reproductively experienced animals. Finally, given that estradiol levels begin to decline with estropause in middle-aged rats (Chakraborty and Gore, 2004), it would be interesting to examine how estrous cycle affects neurogenesis across adulthood and into old age.

Reproductive experience and neurogenesis

The brain changes drastically across pregnancy and the postpartum period. Hippocampal volume is significantly reduced in pregnant rats (Galea et al., 2000) and overall brain volume is reduced in humans but return to pre-pregnancy level after parturition (Oatridge et al., 2002). In mice, BrdU-labelled cell survival is decreased during the second week of gestation but return to nulliparous levels 21 days after parturition (Rolls et al., 2008). In wild meadow voles, pregnant females during breeding season show a reduction in cell proliferation compared to non-pregnant females during the non-breeding seasons (Galea and McEwen, 1999). In rats, neither cell proliferation nor survival varies during early or late pregnancy, as no significant differences were seen in the early (gestational day 1 or 7) or late (gestational day 21) gestational periods compared to nulliparous rats (Furuta and Briges, 2005; Pawluski et al., 2009). However, late pregnancy (gestational day 18) is associated with increased expression of polysialylated form of the neural cell adhesion molecule (PSA-NCAM, marker of synaptic plasticity (Seki, 2002)) in rats (Banasr et al., 2001). Therefore, pregnancy itself does not alter neurogenesis levels across pregnancy in rats, but neurogenesis may be enhanced at mid-gestation.

In contrast to pregnancy, the postpartum is associated with significant changes to neurogenesis in the hippocampus (Darnaudéry et al., 2007; Pawluski and Galea 2007; Leuner et al., 2007). In the early postpartum, cell proliferation is decreased 1 day after parturition for up to 14 days postpartum in primiparous rats (Darnaudéry et al., 2007; Leuner et al., 2007; Pawluski and Galea, 2007; Hillerer et al., 2014) but returns to nulliparous levels by postpartum day 28 (Leuner et al., 2007). In support of the idea that changes in cell proliferation are hormone-related, a hormone-simulated pregnancy in female rats followed by a cessation of estradiol in the 'postpartum' period replicated the reduction in cell proliferation seen in the natural postpartum

period (Green and Galea, 2008). Other studies show that cell survival is reduced at postpartum day 21 (Pawluski and Galea, 2007; Hillerer et al., 2014; Workman et al., 2015). Additionally, primiparous rats, but not biparous rats, show reduced hippocampal neurogenesis across the postpartum relative to nulliparous rats (Pawluski and Galea, 2007; Darnaudéry et al., 2007). Interestingly, pup exposure to nulliparous rats increased both cell proliferation and cell survival compared to nulliparous, primiparous, and biparous rats (Pawluski and Galea, 2007). Therefore, pup exposure alone increased neurogenesis, possibly as a result of enhanced enrichment from the pups, but pregnancy (with or without pup exposure) decreased cell proliferation and neurogenesis across the postpartum (Pawluski and Galea, 2007). Taken together, pregnancy and the postpartum produce changes that have long-lasting negative effects on neurogenesis during the postpartum period, which is possibly related to their poorer performance on spatial tasks during the early postpartum period (Darnaudéry et al., 2007).

Natural variations in estrogens and neurogenesis: Functional implications

Estrous cycle and neurogenesis: Relation to learning

Changes in learning performance over the estrous cycle may be related to changes in neurogenesis. During proestrus, there is an increase in cell proliferation in the dentate gyrus (Tanapat et al., 1999; Rummel et al., 2010). Proestrous females prefer the use of hippocampus-dependent place strategies and show an increase in cell proliferation compared to rats not in proestrus (Korol et al., 2004; Rummel et al., 2010). In young adult female rats, performance during spatial training was positively correlated with activation of newborn cells (measured by co-labeling BrdU with the immediate early gene product zif268) in the dorsal, but not ventral, dentate gyrus (Chow et al., 2013). As the dorsal hippocampus is associated with spatial learning

and memory, while the ventral hippocampus is associated with emotional responses (Moser et al., 1993; Kjelstrup et al., 2002), cell activation was likely in response to spatial memory retrieval rather than stress. Furthermore functional magnetic resonance imaging (fMRI) studies show that women in the menses phase (low estradiol) and men have lower cortical activation and better performance during a mental rotation task compared to women in the high estradiol phase (Moody, 1997; Hausmann et al., 2000; Dietrich et al., 2001). Collectively, these data indicate that less activation is associated with better spatial performance (perhaps due to greater signal to noise ratio). Additionally, as mentioned above, cell proliferation was increased in females during proestrus, but this is not associated with better spatial performance despite the use of a more effective strategy (Rummel et al., 2009; Warren and Juraska, 1997). This emphasizes the fact that greater neurogenesis and greater cell activation does not necessarily lead to better cognitive performance (discussed in detail in section “Manipulation of estrogens and neurogenesis: functional implications”). Further study is needed to establish the relationship between activation of different brain circuits and cell types and cognition across the menstrual or estrous cycle.

Reproductive experience and neurogenesis: Relation to learning

Although cell proliferation and cell survival are not significantly different throughout pregnancy in rats (Banasr et al., 2001; Furuta and Bridges, 2005), there are differences in spatial performance across gestation. Differences in cognitive abilities across pregnancy may be due to regulation of other forms of neuroplasticity rather than through neurogenesis (e.g., changes in PSA-NCAM (Banasr et al., 2001) and dendritic spine density (Kinsley et al., 2006)).

In primiparous rats, spatial ability is impaired during the early postpartum period (Galea et al., 2000; Darnaudéry et al., 2007) and this time point is also associated with less cell

proliferation (during postpartum days 1-8; Leuner et al., 2007; Darnaudéry et al., 2007). The reduction in neurogenesis during the early postpartum is regulated by corticosterone (Leuner et al., 2007), therefore, corticosterone may be the mechanism underlying impairments in cognition via neurogenesis during the early postpartum period in primiparous rats.

Later in the postpartum period, spatial memory is improved in primiparous compared to nulliparous rats (Darnaudéry et al., 2007) and cell proliferation returns to nulliparous levels by postpartum day 28 (Leuner et al., 2007). However, this same time point is accompanied by a reduction in cell survival compared to nulliparous rats when observed on postpartum day 21 (Pawluski and Galea, 2007; Hillerer et al., 2014). Furthermore primiparous rats, at the point of weaning, have decreased dendritic spine density, fewer basal branch points, and shorter basal dendritic processes in the CA1 and CA3 regions (Pawluski and Galea, 2006). However, to our knowledge the link between parity, neurogenesis, and learning has not been directly examined during the postpartum period.

Studies examining learning and memory in the postweaning period found that motherhood enhanced spatial learning and memory compared to nulliparous rats (Kinsley et al., 1999; Gatewood et al., 2005; Pawluski et al., 2006b; Lemaire et al., 2006). But as in the late postpartum period, cell proliferation is not significantly different in primiparous rats from nulliparous rats 2 weeks after weaning (Leuner et al., 2007). Other studies suggest that the memory-enhancing effect of motherhood is seen into middle age (Gatewood et al., 2005, Lemaire et al., 2006) and may be related to increased brain derived neurotrophic factor (BDNF) levels in the CA1 region (Macbeth et al., 2008) and enhanced neurogenesis (Barha et al., in press; Barha and Galea, 2011). Taken together, parity enhances spatial performance long after weaning and this may be mediated through neural plasticity including neurogenesis and dendritic

remodeling in the hippocampus in primiparous and multiparous rats (for a detailed review see Roes and Galea, in press).

Effects of estrogens on learning, memory and neurogenesis and its functional implications

Manipulating the levels of estrogens and the effects on learning and memory

Ovariectomy is associated with impaired cognitive function in young adult humans and rodents using a variety of tasks (e.g., reviewed in Gibbs, 2010; Hogervorst et al., 2000; Sherwin and Henry, 2008). Estradiol treatment can reverse the ovariectomy-induced impairments on learning and memory although the effects depend on the species, strain, type of estrogen, duration and dose of treatment, timing of treatment (before/after training) and type of learning (Figure 1). Perhaps not surprisingly, estrogens regulate hippocampus-dependent memory in tasks in which sex differences in performance are seen, such as place memory (novel object placement), context memory (contextual fear conditioning), and spatial memory (water maze, radial arm maze). Intriguingly, while ovariectomy impairs spatial cognition in young adult rats, ovariectomy enhances spatial cognition in middle-aged and older adult rats (Barha et al., in press; Bimonte-Nelson et al., 2003). We review here the literature on the effects of different doses of estrogens on cognition and neurogenesis. It is important to be aware that studies do not always verify their levels of estradiol and this may be problematic when using silastic pellets as they can result in variable levels of estradiol (Ingberg et al. 2012, Ström et al., 2008, Ström and Ingberg, 2014; Theodorsson et al., 2005). We refer to high estradiol as being levels approximate to afternoon of proestrus, while low as being diestrous levels. Where appropriate, we have indicated when the dose is ‘supraphysiological’ and include these as they may be meaningful, as

dramatically high levels of estradiol are seen in pregnancy (human), and disease (e.g., cancer; Key et al., 2002) and can be the result of diet (Wu et al., 1999).

Effects of acute exposure to estrogens on cognition

Acute treatment with high physiological or supraphysiological estradiol enhances place memory (using a novel object placement task) in adult ovariectomized rats when administered before or after training which indicates an effect on memory encoding and consolidation (Luine et al., 2003 [15µg/kg]; Frye et al., 2007 [0.9mg/kg]; Jacome et al., 2010 [estradiol benzoate: 50 µg/kg]). Both ER α and ER β agonists (systemic and intra-hippocampal) immediately after training enhanced place memory compared to controls, suggesting that the effects of estradiol on place memory consolidation are mediated by both ER α and ER β in mice and rats (Boulware et al., 2013, Frye et al., 2007; Jacome et al., 2010, but see Jacome et al., 2010 for no effect of ER α agonist using systemic dose and see Frye et al., 2007 for no effect of ER β agonist using systemic dose). Using a Y-maze task that also relies upon the detection of spatial novelty, Hawley et al. (2014) found that GPER activation (using the agonist G1) before training improved spatial recognition similar to estradiol in ovariectomized female rats. These studies suggest that both ERs and GPER might be involved in spatial recognition in rats and mice.

High estradiol also enhances memory consolidation in the spatial Morris water maze in both young and aged female rats (Packard, 1998) and mice (Gresack and Frick, 2006; Harburger et al., 2007) via an ER β mechanism (Rhodes and Frye, 2006). In a delayed non-match to place, post-trial phase injection of supraphysiological estradiol (20 µg) or an ER β agonist (WAY-200070; 10 mg/kg) but not an ER α agonist (PPT; 10 mg/kg) improved memory performance in adult female rats (radial arm maze; Liu et al., 2008). This research suggests a critical role for

ER β on hippocampus-dependent spatial reference and possibly working memory but for both ER α and β for novel place recognition in adult female rats. This may be due to different brain circuits recruited during the different tasks. The Morris water maze relies on the hippocampus and to some extent the prefrontal cortex (Fantie and Kolb, 1990) while the novel object placement task relies on the hippocampus and to some extent the cingulate and fornix (Ennaceur et al., 1997). Future studies would benefit from a circuit-based approach to better understand how different brain circuits are recruited during different tasks.

In ovariectomized adult female rats, medium and high doses (1 and 10 μ g) of estradiol and 17 α -estradiol (the optical isomer of 17 β -estradiol) impair, while low doses (0.3 μ g) facilitate, contextual fear memory compared to controls (Barha et al., 2010). Estrone impaired contextual fear memory at a medium dose (1 μ g; Barha et al., 2010). In contrast, Chang et al. (2009) found no significant effect of estradiol (high and supraphysiological doses, 10 or 20 μ g) or ER agonists (PPT and DPN) on contextual fear conditioning in female rats. Differences between the studies could be due to timing and dose of treatments (multiple vs single injection) as Chang et al. (2009) rats received two injections of the hormone and agonists (24 h apart) during training and were tested 24 h after the last injection. Interestingly, estradiol promotes contextual fear extinction through activation of ER β (Chang et al., 2009). Contextual fear conditioning is dependent on the integrity of the hippocampus and amygdala. Future research should investigate how estrogens affect each of these brain regions and how it is affecting performance (e.g., which receptors and pathways are involved).

Effects of chronic exposure to estrogens on cognition

When reviewing literature on estrogens' effects on cognition close attention needs to be directed to the type of estrogen, dose, treatment regimen (e.g., acquisition vs consolidation) and memory assessed (e.g., working vs reference memory). Low doses of estradiol improve spatial working memory while high doses of estradiol impair spatial working and reference memory (using a radial arm maze task) in female rats compared to ovariectomized controls (Bimonte and Denenberg, 1999; Daniel et al., 1997; Galea et al., 2001; Holmes et al., 2002) suggesting a dose-dependent relationship between estradiol and spatial working performance (Figure 1).

Ovariectomy impairs spatial working and reference memory in a radial arm maze task (Gibbs and Johnson, 2008) and low levels of estradiol (serum levels ~28 pg/ml) restored accuracy and working memory performance but not reference memory in young adult female rats (Gibbs and Johnson, 2008; Holmes et al., 2002). On the other hand, chronic high estradiol has no significant effect on spatial working memory in a radial arm maze in adult female rats (5-10 µg/day of estradiol benzoate or Silastic capsule producing estradiol levels ~90 pg/ml; Galea et al., 2001; Holmes et al., 2002; Luine and Rodriguez, 1994). Chronic low or medium estradiol (producing serum levels ~15-25 pg/ml or 50-90 pg/ml) enhances acquisition of the delayed matching-to-position (DMP) T-maze task, which assesses spatial working memory, in ovariectomized adult and middle-aged female rats (Gibbs, 1999; Gibbs, 2000). Performance in the DMP T-maze task depends on cholinergic innervation of the hippocampus (reviewed in Gibbs, 2010). Estradiol enhances basal forebrain cholinergic function and performance on the DMP task is mediated by both ERs and GPER (Hammond et al., 2009; Hammond et al., 2012). Chronic treatment with ER α , ER β , and GPER agonists increases the rate of acquisition of the DMP task similar to estradiol (Hammond et al., 2009). In addition, the GPER antagonist (G15) impairs acquisition in ovariectomized female rats treated with estradiol in this task (Hammond et

al., 2012). Taken together these studies suggest that low to medium levels of estradiol facilitate spatial working memory, possibly via all known ERs and GPER, while higher levels of estradiol do not affect working memory.

In terms of reference memory, high to supraphysiological estradiol benzoate (10 $\mu\text{g}/300\text{g}$) impaired spatial reference memory performance in adult female voles and rats (Galea et al., 2001; Galea et al., 2002). However, when using less reference memory load tasks and administering estradiol well before the training began, medium levels of estradiol enhanced, while high levels did not affect, reference memory in ovariectomized adult female rats (Davis et al., 2005: radial arm maze and estradiol pellets releasing 45–80 pg/ml; McClure et al., 2013: Morris water maze and 10 $\mu\text{g}/300\text{mg}$ of estradiol).

Chronic treatment with high estradiol (33 $\mu\text{g}/\text{kg}$ for 15 days) did not affect contextual fear conditioning in ovariectomized rats (Barker and Galea, 2010), while, chronic high estradiol (Silastic capsules producing estradiol levels similar to proestrous) facilitates contextual fear memory relative to ovariectomized mice (Jasnow et al., 2006). Unlike acute exposure (see above), chronic estradiol does not affect contextual memory in female rats, while it has a positive effect in female mice. Future research is needed to understand these species differences and determine the mechanisms of estrogens in contextual memory.

Of course estradiol interacts with other hormone and neurotransmitters to regulate cognition. For example, as mentioned previously estradiol enhances basal forebrain cholinergic function which is necessary to regulate cognitive performance in the DMP task (reviewed in Gibbs, 2010). Estradiol also interacts with dopamine and serotonin to regulate mood and cognition, but a complete review of estradiol's effects on neurotransmitters or neurotrophic

factors to mediate effects on cognition is beyond the scope of this review (see: Fink et al., 1998; Jacobs and D'Esposito, 2011; Joffe and Cohen, 1998).

Finally, other estrogens also affect hippocampal-dependent memory, although there are fewer studies. Chronic high (8 µg/day), but not low (2.6 and 4 µg), estrone impairs spatial working memory in middle-aged ovariectomized rats (Engler-Chiurazzi et al., 2012) but chronic high estrone (10 µg) had no significant effect on spatial reference memory in ovariectomized young adult female rats (McClure et al., 2013). Chronic treatment with Premarin (which is comprised of 50% estrone sulphate; 1 and 2 µg) impaired both spatial working and reference learning in a radial arm maze in ovariectomized young adult rats (Barha and Galea, 2013). While in middle-aged ovariectomized rats, chronic high Premarin (20-30 µg) enhanced spatial working memory when given cyclically (Acosta et al., 2009) or via osmotic minipumps (Engler-Chiurazzi et al., 2011). Thus estrone or Premarin can have deleterious effects in younger adult females, while estrone or Premarin may be beneficial in middle-aged adults. There are similar age-dependent effects of Premarin to affect cognition in women (see below and reviewed in Hogervorst et al., 2000; Sherwin and Henry, 2008).

Aging, estrogens, and cognition

Estrogens and cognition in aging rodents

Contrary to what is seen in young adults (reviewed in Gibbs, 2010; Hogervorst et al., 2000; Sherwin and Henry, 2008), long-term ovariectomy improves spatial working and reference memory in nulliparous aged females (Barha et al., in press; Bimonte-Nelson et al., 2003). Progesterone (pellets producing ~30 ng/ml) reverses the improvements in spatial working and reference memory seen in older female rats after ovariectomy (Bimonte-Nelson et al., 2004),

suggesting that progesterone can have detrimental effects on spatial memory in aged rats. Chronic medium levels of estradiol (30 day silastic capsule, producing mean serum levels ~40-50pg/ml) improved spatial reference memory in middle-aged (12-16 months old) rats in the Morris water maze (Kiss et al., 2002; Talboom et al., 2008) and increased hippocampal BDNF levels (Kiss et al., 2012). In aged ovariectomized rats (24 months old), chronic high estradiol did not improve spatial reference memory (capsule producing serum levels ~100pg/ml; Talboom et al., 2008), while in intact aged mice (27–28 month), medium estradiol (5 µg) enhanced spatial reference memory and increased hippocampal synaptic plasticity (using the presynaptic protein synaptophysin; Frick et al., 2002). Differences between aging studies could be due to species and strain differences, the dose of estradiol and type of administration (e.g., injections vs pellets, cyclic vs tonic), the effect of other gonadal hormones (e.g., ovariectomized vs intact animals), and training parameters. Chronic estradiol treatment (serum levels ~26–47 pg/ml) during middle-age enhances spatial working memory in a radial arm maze and increases expression of ER α in the hippocampus in multiparous female rats (Rodgers et al., 2010). Further research by the same group showed that increasing ER α in the hippocampus enhances memory in aging female rats in the absence of estradiol (Witty et al., 2012). In contrast, in nulliparous aged female rats, chronic high estradiol (producing estradiol levels ~ 90 pg/ml) has no effect on spatial working memory using a radial arm maze (Luine and Rodriguez, 1994). Thus, in addition to differences in experimental design discussed above, it is important to consider reproductive experience when comparing aging studies as number of aging studies have utilized retired breeders.

Aging, estrogens and cognition in women

In women, there is evidence supporting the role of hormone therapy in preventing cognitive decline (reviewed in Sherwin, 2003) but the timing of initiation and type of hormone therapy are important factors. The critical period hypothesis proposes that hormone therapy will only have cognitive benefits if given close to the onset of menopause (reviewed in Brinton, 2008; Daniel and Bohacek, 2010; Gibbs, 2010; meta-analysis by Hogervorst et al., 2000). Indeed research shows that if treatment is initiated in early menopause or just prior to menopause, 80% of randomly controlled trials (RCTs) using estradiol-based therapies while 20% of RCTs using conjugated equine estrogens therapies (estrone-based) show improved cognition. However, if hormone treatment is initiated significantly after menopause the percentage of improvement drops to 40% for estradiol-based therapies and none of the RCTs using estrone-based therapies showed a positive effect while 40% had a detrimental effect on cognition (Hogervorst et al., 2000; Ryan et al., 2008). This research suggests that for hormone replacement to be beneficial, treatment needs to start as soon as gonadal hormones begin to decline. Indeed, preclinical studies agree with these findings in women as estradiol enhances acquisition of a spatial task in older rats when treatment is initiated 3 months but not 5 or 10 months after ovariectomy (Gibbs, 2000; Daniel et al., 2006). The efficacy of estradiol treatment begins to decline 3-5 months following hormone deprivation in female rodents, possibly due to reduced ER α expression (Zhang et al., 2009) and thus sensitivity to estrogens in the hippocampus (reviewed in Daniel, 2013). In addition, the type of hormone replacement is important, whether it is estradiol or estrone based, as different estrogens have different effects on cognition (Hogervorst et al., 2000; Ryan et al., 2008; Ryan et al., 2009). Finally, reproductive experience (pregnancy and motherhood) can influence how estrogens modulate cognition (reviewed in Roes and Galea, in press) and neurogenesis (reviewed next).

The effects of estrogens on neurogenesis

Estrogens and cell proliferation

Estradiol influences neurogenesis in the dentate gyrus by modulating both cell proliferation (Figure 3) and survival (Figure 4) of young neurons but these effects are time-, dose-, age-, and sex-dependent (reviewed in Galea et al., 2013). In adult female rats, short-term ovariectomy reduces cell proliferation, while high estradiol (10 µg) restores levels of proliferation to those seen in sham controls (Tanapat et al., 1999). A single injection of estradiol (10 µg) rapidly increases cell proliferation after 30 min and 2 h, but not after 4 h in ovariectomized adult female rats (Barha et al., 2009; Tanapat et al., 2005; Tanapat et al., 1999). Interestingly, estradiol's ability to modulate cell proliferation is rapid and dose-dependent, with both a lower (0.3 µg; diestrous range) and higher dose (10 µg; proestrous range) increasing cell proliferation 30 min after exposure while medium or supraphysiological doses (1 µg, 50 µg) having no significant effect on cell proliferation (Barha et al., 2009; Tanapat et al., 2005). Different types of estrogens also increase cell proliferation. Acute estrone (0.3 and 10 µg) and 17α-estradiol (1 µg) rapidly increase cell proliferation after 30 min in young adult and middle-aged multiparous ovariectomized female rats (Barha and Galea, 2011; Barha et al., 2009). Estradiol benzoate (more slowly metabolized than estradiol) increases cell proliferation after 4 h but decreases cell proliferation after 48 h of exposure in female rats and voles (Ormerod and Galea, 2001; Ormerod et al., 2003). The fast acting effect of estradiol to increase cell proliferation has been attributed in part to estradiol's effects on serotonin (Banasr et al., 2001) while the slower effect of estradiol to decrease cell proliferation has been attributed to estradiol's effects on adrenal hormones (Ormerod et al., 2003).

The ovariectomy-induced decrease in cell proliferation is also affected by the time elapsed since ovariectomy. Short-term (6-7 days) but not long-term (21 or 27 days) ovariectomy decreases cell proliferation in young adult rats (Banasr et al., 2001; Green and Galea, 2008; Tanapat et al., 2005). In addition, estradiol given 28 d after ovariectomy does not increase cell proliferation unlike estradiol given 7 d after ovariectomy in rats (Tanapat et al., 2005). This research indicates that progenitor cells in the dentate gyrus are no longer sensitive to acute estradiol after long-term deprivation, reminiscent of the critical period hypothesis. As discussed above, estradiol fails to modulate spatial memory (see section “Aging, estrogens and cognition in women”) and to increase ER expression after long-term ovariectomy compared to what is observed when estradiol replacement starts immediately after ovariectomy (Bohacek and Daniel, 2009). Together this research indicates that the hippocampus becomes resistant to estradiol treatment after long-term gonadectomy. This could be due to compensatory mechanisms that increase, with time after ovariectomy, the production of extragonadal sources of estradiol (Zhao et al., 2005). Synthesis of *de novo* steroids can also occur locally in the brain as enzymes required for steroid production from cholesterol are expressed in the brain and in particular, aromatase, is expressed in the hippocampus of rats, mice, and monkeys (Fester et al., 2011). There is evidence that local estradiol affects cell proliferation in hippocampal slices of 5-day old rats (Fester et al., 2006) but it is less clear that there is considerable neurosteroid production after long-term ovariectomy in adult female rats in the hippocampus (Barker and Galea, 2009). The role of neuroestrogens in the adult hippocampus is a relatively new area of research and will be important in determining the potential role, if any, that neurohormones play in regulating learning and memory and neuroplasticity.

Some of the effects of estradiol on hippocampal cell proliferation are mediated by ERs.

ERs are found in progenitor cells in the subgranular zone of the dentate gyrus in rats (Mazzucco et al., 2006; Perez-Martin et al., 2003) and voles (Fowler et al., 2005). Administration of an ER α (PPT) and ER β (DPN) agonist increases cell proliferation (Mazzucco et al., 2006), while the effects of estradiol are partially blocked with an ER antagonist (ICI 182,780) in ovariectomized rats (Nagy et al., 2006). Interestingly, the ER agonists (alone or in combination) did not increase proliferation to the levels seen with estradiol (Mazzucco et al., 2006) suggesting that the estradiol induced increase in cell proliferation cannot be completely explained by the actions of ERs. GPER is also expressed in the dentate gyrus, and in the CA1, CA2, and CA3 regions of the hippocampus in adult female rats (Brailoiu et al., 2007; Duarte-Guterman et al., submitted). Surprisingly, activation of GPER with an agonist (G1) decreases, while estradiol increases, cell proliferation in ovariectomized female rats (Duarte-Guterman et al., submitted). This research suggests that GPER has a role in hippocampal neurogenesis but this role is independent of estradiol.

Estrogens and cell survival

Chronic estradiol administration to ovariectomized female rats produces different results depending on when new neurons are examined relative to estradiol exposure (Figure 4). New neurons produced and surviving after estradiol treatment has begun, have an increased cell survival (McClure et al., 2013), while new neurons produced just prior to estradiol treatment, have a decreased cell survival (Barker et al., 2008; Chan et al., 2014). Giving BrdU prior to treatment will ascertain the effects of estradiol on cell survival independent of cell proliferation, while giving BrdU after estradiol treatment will include the effects of estradiol on both cell proliferation and cell survival (Figure 4). Thus given that estradiol increases cell proliferation in

the short term, we believe these differing effects of estradiol on the survival of these BrdU-labelled cells are due to the effects of estradiol to increase cell proliferation. Conversely, high estrone treatment (10 µg) for 20 days decreased the survival of new neurons produced and surviving under an estrone environment in young adult female rats (McClure et al., 2013). This is particularly interesting as the same dose of estrone increases cell proliferation (Barha et al., 2009) and this suggests that most of these cells that proliferate under estrone do not survive in an estrone-enriched environment. In contrast, a longer period (33 days) of treatment with Premarin, an estrone-based hormone replacement therapy, increased cell survival when BrdU was given prior to treatment, however this was only true in spatially-trained rats as there was no significant effect on cell survival in cage controls (Barha and Galea 2013) suggesting that the effect of estrogens on neuroplasticity can be modified by experience such as spatial training. Further, the ability of estradiol to promote dendritic spine density in the CA1 field of the hippocampus is attenuated when female rats performed a hippocampus-dependent task (Frick et al., 2004).

Estradiol has a less prominent role in regulating cell survival in males. Chronic (15-30 days) administration of estradiol benzoate in adult male rats had no significant effects on cell survival or proliferation (Barker et al., 2008; Spritzer et al., 2007). Similarly, three days of injection with estradiol did not increase cell survival in male mice when examined 28 days later (Zhang et al., 2010), but it is unclear whether estradiol at later time points may produce an effect. In male meadow voles, estradiol increased cell survival only if given days 6-10 after BrdU (Ormerod et al., 2004) which is roughly during the axon extension phase of cell development (Hastings and Gould, 1999). Therefore, in male rodents, estradiol either does not affect cell survival or only does so when immature neurons extending their axons are exposed to estradiol.

Aging, estrogens, and neurogenesis

Neurogenesis levels decrease with age in the hippocampus of both rodents and humans (Kuhn et al., 1996; Gould et al., 1999; Eriksson et al., 1998; Epp et al., 2013). In female rats, cell proliferation begins to decrease in middle age (10-12 months; Kuhn et al., 1996) but does not continue into old age, as 27 month old rats show similar rates of cell proliferation as middle-aged rats in both male and female rats (Kuhn et al., 1996; Rao et al., 2005; Rao et al., 2006). Decreased neurogenesis with age appears to be due largely to a decreased number of precursor cells dividing, resulting in less cell proliferation (Olariu et al., 2007).

Interestingly, estrogens seem to regulate neurogenesis differentially in young versus aged female rats. Acute high dose (10 μ g) of 17 β -estradiol or estradiol benzoate increases cell proliferation in young adult, but not middle-aged, nulliparous Sprague Dawley and Wistar-Imamichi female rats (Tanapat et al., 1999; Barha et al., 2009; Chiba et al., 2007; Barha and Galea, 2011). However, another study showed that chronic injections of estradiol (150 μ g given weekly) increases cell proliferation in older (22 month) female Wistar albino rats (Perez-Martin et al., 2005). These studies suggest that the hippocampus loses its ability to respond to acute peripheral estradiol administration in nulliparous aging female rats.

Reproductive experience also affects brain responsivity to estrogens in later life, as acute 17 β -estradiol, 17 α -estradiol, or estrone increased cell proliferation in middle-aged multiparous, but not nulliparous, rats (Barha and Galea, 2011). This may be related to the fact that BDNF is modulated by estradiol (reviewed in Scharfman and MacLusky, 2006) and parity at least in the CA1 region (Macbeth et al., 2008) and also regulates neurogenesis (Scharfman et al., 2005; Zhou et al., 2005). Therefore, the neurogenic effects of acute estradiol may be more potent in older female rats with prior reproductive experience.

Manipulation of estrogens and neurogenesis: functional implications

Some studies have tried to link changes in learning and memory with changes in neurogenesis and a complete review of this literature is beyond the scope of the review but the reader is directed elsewhere (Cameron and Glover, 2015; Epp et al., 2013; Galea et al., 2013; Leuner et al., 2006a). Indeed the learning experience itself can alter neurogenesis levels (reviewed in Epp et al., 2013; Glasper et al., 2012; Opendak and Gould, 2015) as well as the effects of estrogens on neural plasticity (Barha and Galea, 2013; Frick et al., 2004) and these are important factors to keep in mind. Perhaps counterintuitively, merely increasing the number of new neurons is not always related to better hippocampus-dependent memory. For example, seizures and traumatic brain injury increase neurogenesis, conditions that are not linked to better memory (Parent, 2007; Richardson, et al., 2007). A study implicated the increased neurogenesis to impaired memory after seizures (Jessberger et al., 2007) and more recently increases in neurogenesis has been found to enhance forgetting (Akers et al., 2014). Computer modeling has found that there is an optimal level for neurogenesis to be helpful for memory (Aimone et al., 2009; Butz et al., 2006). Indeed, increased levels of neurogenesis under pathological conditions (e.g., seizures, traumatic brain injury) may be detrimental. In earlier stages of Alzheimer's Disease (AD) there is an increase (Jin et al., 2004) and at later stages a decrease in neurogenesis (Ekonomou et al., 2015) and the initial increase may be seen as a compensatory mechanism to replace neurons. While new neurons are created, these neurons may not be making appropriate connections. For example, new neurons created under seizures conditions are ectopic (Jessberger et al., 2007; Scharfman et al., 2007) and have different electrophysiological properties than those new neurons created under running (Jakubs et al., 2006), perhaps due to their inappropriate synaptic contacts. Thus, it is not the number of new neurons that may be important, and not even

how many new neurons are activated (via IEG) in response to appropriate stimuli but how those new neurons may be related to the memory itself. For example, Premarin increased neurogenesis but impaired spatial reference and working memory in the radial arm maze in adult female rats (Barha and Galea, 2013). Furthermore, low but not high Premarin reduced BrdU/zif268 activation, while both doses of Premarin impaired spatial acquisition. When the authors examined the relationships between activation of new neurons in response to spatial memory performance, total errors was positively correlated with activation of new neurons in the high Premarin group, suggesting that new neurons were not contributing to better memory in this group (Barha and Galea, 2013). In adult female rats, high estradiol (10 µg) increased the survival and activation of new neurons after water maze training and was positively related to spatial reference memory performance but this was not true of high estrone (McClure et al., 2013). Very few studies have examined the impact of new neurons activated in response to spatial memory, especially in female rodents, and the percentage of activated new neurons is quite low. Still, much could be gained from determining the extent to which new neurons are involved in spatial memory and researchers are encouraged to implement these analyses in their studies. Future studies are needed to determine the direct consequences of the effects of estrogens on neurogenesis, and other forms of neural plasticity in the hippocampus.

Conclusions and future directions

There is considerable research demonstrating that sex and estrogens interact with reproductive experience and aging to modulate hippocampal learning, memory, and neurogenesis. Research is ongoing to determine whether and how sex and sex hormone alterations in neuroplasticity are related to changes in cognition (see Box 1 for recommendations

for future studies). The integrity of the hippocampus is affected in neurodegenerative and neuropsychiatric diseases and thus understanding how sex and sex hormones influence hippocampal function can have wide-ranging implications for the development of better treatment options for these devastating diseases.

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Box 1. Implications for clinical research and recommendations for future studies

There is considerable research demonstrating that sex and estrogens modulate hippocampal learning, memory, and neurogenesis. As outlined in this review, the effects of estrogens on cognition can be varied and depend on many factors including age, sex, reproductive experience, and type of estrogen. Meta-analyses suggest that estradiol formulations have more cognitive beneficial effects than conjugated equine estrogens when given during the menopausal transition (Hogervorst et al., 2000; Ryan et al., 2008). New research indicates that other factors such as surgical menopause and reproductive factors may all play a role in the effects of hormone therapy on cognition later in life (for review see Roes and Galea, in press). The research community is encouraged to consider the many reproductive factors as well as hormone therapy formulations when disseminating results of studies. Only in this way, will this research lead to improved treatment options to combat cognitive decline in women (and men). Here, we propose a summary of what we believe should be taken into account in future studies both in preclinical and clinical research.

- 1) Estrogen type: estrone- vs estradiol-based therapy or selective estrogen receptor modulators
- 2) Dose
- 3) Timing of hormone therapy – studies point to early in menopause and not later (critical period hypothesis).
- 4) Interactions between estrogens and progestins: what type and dose of progestin to use
- 5) Route of administration: transdermal patch vs oral
- 6) Reproductive experience

FIGURES

Figure 1. Theoretical dose-dependent curvilinear effects of estradiol (E2) on (A) spatial working memory and (B) spatial reference memory in young adult ovariectomized (OVX) female rats and voles. Working memory (performance relies on the ventral hippocampus and the prefrontal cortex) can be defined as manipulation and retrieval of trial unique information to guide prospective action (Baddeley, 2003) while reference memory (performance relies on the dorsal hippocampus and caudate nucleus) can be defined as a long-term memory for events or stimuli (Olton and Pappas, 1979; White and McDonald, 2002). Low physiological levels of estradiol (diestrus levels) improve while high levels of estradiol (afternoon of proestrus) impair working memory compared to ovariectomized controls. Low levels of estradiol (diestrus) have no effect while high levels of estradiol (afternoon of proestrus, supraphysiological) impair spatial reference memory performance compared to ovariectomized controls. See text for details and discussion.

Figure 2. (A) Timeline of adult neurogenesis in the rat hippocampus and (B) photomicrograph of a new neuron expressed at the border between the granule cell layer and the hilus in the dentate gyrus. Dividing cells will form two daughter cells and express endogenous markers of cell proliferation such as Ki67. Doublecortin (DCX) is expressed in new immature neurons from approximately day 1 until day 21 (Brown et al., 2003). Bromodeoxyuridine (BrdU) is a DNA synthesis marker that is incorporated into any cell synthesizing DNA within 2 h of injection. If BrdU is given at day 0, BrdU-labelled cells will also co-express markers for mature neuronal proteins (such as NeuN) as early as 2 weeks after production. It is important to note that timeline of maturation of new neurones differs between adult rats and mice (Snyder et al., 2009). B)

BrdU-labelled cell in the granule cell layer (BI; red), cells in the granular cell layer labelled with the neuronal marker NeuN (BII; green), and merged image showing co-labelled cell with BrdU (red) and NeuN (green) (BIII).

Figure 3. Effects of acute 17β -estradiol (E2) or estradiol benzoate (EB) treatment on cell proliferation in the dentate gyrus of adult female rats and voles. Timeline of BrdU injection and hormone administration. Females were ovariectomized and allowed to recover for approximately 7 or 28 days at which point they received a single injection of E2 or EB followed by a BrdU injection. Rodents are perfused within 24-48 h of BrdU injection therefore BrdU-labelled cells are newly divided daughter cells and this paradigm measures cell proliferation. High dose of E2 increases cell proliferation within 30 min or 2 h after BrdU injection but if BrdU is given 4 h after E2 exposure, there is no significant change in cell proliferation. EB (more slowly metabolized than E2) increases cell proliferation 4 h after BrdU injection but decreases cell proliferation if BrdU is injected 48 h after EB injection. E2 does not increase cell proliferation after long-term ovariectomy (28 days) in young adult female rats. See text for details and discussion.

Figure 4. Effects of chronic 17β -estradiol (E2) or estradiol benzoate (EB) treatment on survival of new neurons in the dentate gyrus of adult (A) female and (B) male rats. Timeline of BrdU injection and hormone administration. BrdU-labelled cells after 16-30 days express mature neuronal markers (e.g., NeuN) and this paradigm examines the effects of E2 or EB on the survival of BrdU-labelled cells. If BrdU is given 24 h before hormone treatment, the experiment examines the effects of hormone exposure on the survival of new cells independent of any initial

cell proliferation effects of the hormone. If BrdU is given 24 h after hormone exposure, the experiment examines the effects of hormone exposure on both cell proliferation and survival. Chronic EB exposure decreases cell survival in adult female rats when BrdU is given prior to EB but chronic E2 increases cell survival if BrdU is given after E2 treatment has initiated. In young adult male meadow voles, EB treatment 6–10 after BrdU injection (during the cell maturation stage of axon extension) increases cell survival. However, chronic EB treatment does not affect cell survival in young adult male rats. See text for details and discussion.

Figure 1

(A) Spatial working memory

(B) Spatial reference memory

Figure 2

(A)

(B)

Figure 3

Paradigm	Changes in cell proliferation	Study
↑	Tanapat et al. (1999)
↑	Ormerod et al. (2003) Barha et al. (2009) Mazzucco et al. (2006)
↓	Ormerod et al. (2003)
↑	Tanapat et al. (2005)
■
■
↑	Barha et al. (2009)

Figure 4

(A) Female rodents

(B) Male rodents