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The endocrine-brain-aging triad where many paths meet: female reproductive hormone changes at midlife and their influence on circuits important for learning and memory⋆

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Abstract

Female mammals undergo natural fluctuations in sex steroid hormone levels throughout life. These fluctuations span from early development, to cyclic changes associated with the menstrual or estrous cycle and pregnancy, to marked hormone flux during perimenopause, and a final decline at reproductive senescence. While the transition to reproductive senescence is not yet fully understood, the vast majority of mammals experience this spontaneous, natural phenomenon with age, which has broad implications for long-lived species. Indeed, this post-reproductive life stage, and its transition, involves significant and enduring physiological changes, including considerably altered sex steroid hormone and gonadotropin profiles that impact multiple body systems, including the brain. The endocrine-brain-aging triad is especially noteworthy, as many paths meet and interact. Many of the brain regions affected by aging are also sensitive to changes in ovarian hormone levels, and aging and reproductive senescence are both associated with changes in memory performance. This review explores how menopause is related to cognitive aging, and discusses some of the key neural systems and molecular factors altered with age and reproductive hormone level changes, with an emphasis on brain regions important for learning and memory.

Keywords

Estrogen; Androgen; Progesterone; Cholinergic; GABAergic; ERK; Aging; Learning; Memory; Cognition; Gonadotropins; Brain; Ovarian; Hormone; Steroid

1. Introduction to menopause and the aging brain: The endocrine-brain-aging triad

During aging, humans and other animals commonly experience cognitive changes. Neurobiological alterations concomitant with aging can impact brain regions that are critical for the regulation of attention, learning, and memory processes, such as the frontal cortex,

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basal forebrain, and hippocampal formation. In females, changes in cognitive function during midlife are often associated with reproductive senescence. Reproductive senescence occurs in women when the finite ovarian follicle pool is depleted. Women are born with all of the immature ovarian follicles they will ever have; it has been estimated that human females have over half a million immature ova at birth (Gougeon, 2010; Wallace and Kelsey, 2010). Approximately 400 of these immature follicles fully mature and are ovulated throughout the reproductive life stage between puberty and menopause, and as such, over 99% of follicles undergo atresia, or programmed cell death. This normal apoptotic process begins at birth and continues until the follicle pool is exhausted around the fifth decade of life (Hsueh et al., 1994).

During the reproductive life stage, the ovary is the main synthesis site of circulating sex steroid hormones, including estrogens, progesterone, and androgens. Estrogens are primarily synthesized by growing ovarian follicles, progesterone predominantly by the corpus luteum after ovulation and in small amounts by growing follicles, and androgens by both ovarian interstitial tissue and the adrenal glands. The production and regulation of these hormones are mediated by the hypothalamic-pituitary-gonadal (HPG) axis. The feedback loop includes the hypothalamus, which produces and releases gonadotropin releasing hormone (GnRH) into the anterior pituitary gland, initiating the synthesis and secretion of the gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH are two key hormone regulators of ovarian follicle development and the ovarian cycle. Once the follicle pool is depleted through natural atresia and ovulation, the ovaries do not generate a sufficient amount of estrogens and progesterone to sustain the normal uterine cycle. Although the natural transition to reproductive senescence is not completely understood, it is clear that it is not an abrupt event; rather, it is thought that when a critical threshold of remaining ovarian follicles is reached, women begin to experience the transitional phase to menopause, which involves intermittent ovulatory cycles, significant fluctuations in ovarian hormone levels, and variable but rising FSH and LH levels. This process culminates with an eventual cessation of menses and infertility, referred to as menopause, and occurs at the average age of 51–52 in women (Hoffman et al., 2012; NAMS, 2014). This gradual menopause transition may last up to ten years prior to the final menstrual period, signaling the end of the reproductive life stage (Harlow et al., 2013). In addition to these physiological changes, women often report undesirable symptoms including hot flashes, genitourinary symptoms, and changes in sleep, mood, and memory during the menopause transition (Sullivan Mitchell and Fugate Woods, 2001; Weber et al., 2013; Weber and Mapstone, 2009).

How, then, do these factors associated with menopause relate to the brain and behavior? Basic science research on the role of hormones and behavior began as early as the mid 19th century with Arnold Berthold’s classic experiments evaluating the role of the testes in sex behaviors in roosters, which showed that castration decreased aggression and male sex behaviors. He found that when he surgically implanted testes back into the rooster’s abdominal cavity, they reestablished a blood supply, and both aggressive and sexual behavior were also reinstated (Berthold, 1849). From these experiments, Berthold hypothesized that a substance was secreted from the testes into the bloodstreamto trigger these behaviors. It was not until some fifty years later that the term “hormone” was coined and defined by William Bayliss and Ernest Starling. By the 1930’s and 40’s, Dr. Frank Beach began his influential

work on early life hormone manipulations and rodent sexual behavior. Beach and colleagues systematically manipulated sex hormone exposure by surgically removing the gonads of male and female rats and observing altered sex behaviors. He also found that phenotypic sex behaviors (e.g. mounting male sex behavior, lordosis female sex behavior) could be altered or induced by the manipulation of sex hormones in males and females (Beach, 1942, 1941). In the decades following these landmark studies, researchers went on to recognize the role of sex hormones and their influence on the brain with regard to sexual differentiation and neuroendocrine modulation of reproduction, including the discovery of the sexually dimorphic nucleus of the preoptic area, and how the size of this brain region can be altered depending on early life gonadal hormone exposures (Gorski, 1972; Gorski et al., 1978). Additionally, the role of estrogen as an activator of female-typical sexual behavior early in life was noted in Dr. Christina Williams' research, showing that administering a supraphysiological dose of estradiol benzoate to rat pups as young as 4–6 days old facilitated the expression of lordosis (a receptive behavior) and of ear wiggling (a proceptive behavior) (Williams, 1987). The McEwen laboratory found that male pups that were administered an aromatase inhibitor, which prevents the metabolic conversion of androgens to estrogens, exhibited lordosis behavior in addition to phenotypic male sex behaviors in adulthood. When these aromatase inhibitor-treated males were gonadectomized as adults and subsequently administered estradiol benzoate and progesterone, they also exhibited lordosis and proceptive behaviors, suggesting the mechanisms driving phenotypic female sex behaviors can develop independently of male sex behaviors (Davis et al., 1979). Through the decades, research has shown that just as the male brain is actively masculinized by sex steroids, the female brain is actively feminized by sex steroids (Fitch and Denenberg, 1998); this has been exemplified for gross brain structure (Bimonte et al., 2000a, 2000b), cortical ultrastructure (Kim and Juraska, 1997), as well as behavior (Stewart and Cygan, 1980; Zimmerberg and Farley, 1993). These findings moved the field forward by challenging the traditional tenet that estrogens have a passive role in sexual differentiation of the brain. Since this time, the field of neuroendocrinology and aging has learned that the role of circulating ovarian hormones is not limited to reproductive functions and behaviors, and the brain areas that mediate them, but also extends to modulating memory and other cognitive processes, as well as their related brain regions. In fact, we have learned that in addition to impacts on brain health, the effects of estrogens, progestogens, and androgens on the body are diverse and manifold, including, but not limited to, influences on cardiovascular and bone health (Engler-Chiurazzi et al., 2016; Turgeon et al., 2006; Wise et al., 2009).

While acknowledging that sex steroid hormones have an impact on a myriad of systems with important functional and health outcomes, this review will focus on the brain and cognition. That sex hormones and gonadotropins could impact non-reproductive domains of the brain and behavior is not unexpected given the discovery of mechanisms which could mediate such effects. Indeed, there are steroid hormone and gonadotropin receptors in many areas of the brain, including the hippocampus and frontal cortex, two brain regions that are critical for effective memory functioning in everyday life. These memory functions include spatial, working, and reference memory. Spatial memory is hippocampal dependent and involves the use of distal cues to navigate through an environment. Working memory depends on both the frontal cortex and hippocampus, and is a type of short-term memory that involves updating

information. For example, working memory requires manipulating information, such as mental arithmetic. Reference memory is a form of long-term memory that remains consistent across time; for example, one would utilize reference memory to navigate the route driven from home to work each day (Bimonte-Nelson et al., 2015). A substantial amount of research, from which key findings are highlighted and explored below, has been dedicated to elucidating the cognitive effects of ovarian hormones, particularly estrogens, and how changes in circulating hormones can impact the brain and behavior across the lifespan (See Fig. 1).

2. On the role of midlife changes in ovarian hormones, gonadotropins, and cognitive function

2.1. Estrogen and progesterone as the traditional key ovarian hormone players

As aging ensues, female mammals typically experience a cessation of reproductive capacity in mid- to end- of life. Most animals are not long-lived following reproductive senescence; humans are one of the unique exceptions to this rule. People are living longer than ever before, with the average female lifespan surpassing 81 years in developed countries such as the United States (Murray et al., 2015). However, the age at menopause does not seem to be changing with increased longevity (Sherwin, 2003). This means that women are now living in a postmenopausal state, with significantly reduced circulating ovarian hormone levels, for a substantial part of their lives. This underscores the need to understand the effects of aging and related hormone loss on the body, including on the brain and its function.

There has been ample research, both in basic science and human realms, suggesting that the loss of ovarian hormones has a negative impact on a variety of body systems. These adverse effects are especially robust when an abrupt hormone loss occurs, such as that associated with ovariectomy (Ovx; the surgical removal of the ovaries). Ovarian hormone loss is associated with a decline in cognitive function both in humans (Nappi et al., 1999; Rocca et al., 2007; Sherwin, 2003) as well as in animal models (Daniel, 2013; Frick, 2015; Koebele and Bimonte-Nelson, 2015; Korol and Pisani, 2015; Luine, 2014). Animal models have provided an excellent framework to elucidate the effects of ovarian hormones on the brain and behavior. For example, seminal preclinical work in the field of neuroendocrinology, aging, and cognition has shown that Ovx impairs spatial memory performance, and that subsequent estrogen treatment can improve memory performance following Ovx, at least for a period of time (Bimonte and Denenberg, 1999; Bohacek et al., 2008; Savonenko and Markowska, 2003; Talboom et al., 2008; Wallace et al., 2006). Interestingly, transient 17β-estradiol treatment after Ovx can enhance memory performance, as well as increase hippocampal choline acetyltransferase (ChAT; the synthesizing enzyme for acetylcholine) and estrogen receptor alpha (ERα) levels, even after the estrogen treatment has been terminated (Rodgers et al., 2010). However, timing of 17β-estradiol replacement is critical; spatial memory performance was improved only when hormone treatment was initiated immediately after Ovx, but not after five months of hormone deprivation (Daniel et al., 2006). It is also notable that estrogen replacement following Ovx is more efficacious in young and middle-aged animals than in aged animals (Diz-Chaves et al., 2012; Savonenko and Markowska, 2003), and that chronic estrogen treatment can improve cognitive

performance, but only after priming with a cyclic regimen of 17β-estradiol injections (Markowska and Savonenko, 2002). Moreover, animals' responsiveness to the enhancing effects of 17β-estradiol or estradiol benzoate changes with age (Foster et al., 2003; Talboom et al., 2008),which may be related to estrogen receptor expression in the hippocampus (Foster, 2012). ERα and ERβ are associated with a range of intracellular signaling molecules that are rapidly activated in the presence of estrogens. Remarkably, Ovx animals receiving hippocampal lentivirus injection of ERα, which increases ERα expression, displayed enhanced spatial working memory, even in the absence of high circulating estrogen levels (Foster et al., 2008; Witty et al., 2012). Lentiviral delivery of ERα to the hippocampus also increased phosphorylated extracellular regulated kinases (ERK1/2; discussed in more detail below) in rats, suggesting that signal transduction pathways important for learning and memory are, in part, moderated by estrogen receptor expression and activity (Witty et al., 2012). Taken together, these novel findings indicate that serum estrogen levels alone cannot necessarily dictate or predict cognitive outcomes; they are part of a complex and interactive system involving many cellular and molecular mechanisms that impact memory performance in a collaborative fashion.

Further elaborating on this tenet, estrogens do not operate on the brain and body in isolation. Progestogens are a class of steroid hormones that include endogenous progesterone and synthetic progestins that bind to the progesterone receptor. Progesterone is an important component of the reproductive cycle, and is especially critical for the maintenance of pregnancy. In a non-pregnant female, the main release of progesterone occurs during the endogenous female reproductive cycle from the corpus luteum after ovulation. With follicular depletion and ensuing menopause, corpora lutea formation is attenuated, and therefore there is a lack of elevated progesterone. In a broad context, the scientific study regarding the impact of the shifts in progesterone across the female lifespan is important because of the systematic and rapid alterations in progesterone levels across the regular reproductive cycle in adulthood, markedly elevated levels with pregnancy, as well as the decreased levels that occur into old age. Determining the impact of progestogens on the brain and other systems is crucial, given the wide use of bioidentical and synthetic progestogens in hormone therapies and contraceptives. The effects of progestogens specifically on the brain and its functions is a growing area of research; in fact, the work is yielding strong evidence that progestogens have marked impacts on brain areas integral to many reproductive and non-reproductive behaviors, including translating effects to cognition.

Interestingly, our laboratory has found that the beneficial effects of estrogen treatment on spatial memory can be reversed by concomitant progesterone administration (Bimonte-Nelson et al., 2006), and that administering the synthetic progestin medroxyprogesterone acetate (MPA) to Ovx rats impaired performance on a spatial working memory task (Braden et al., 2011, 2010). However, it seems that progestins are not unequivocally harmful to cognition. Our laboratory and others have recently demonstrated that different classes of synthetic progestins commonly used in HT formulations, including levonorgestrel and norethindrone acetate, can have differential effects on spatial memory performance compared to MPA (Braden et al., 2016; Gambacciani et al., 2003; Simone et al., 2015; Tierney et al., 2009).While MPA administration has been shown to produce detrimental,

long-lasting cognitive impairments (Braden et al., 2011, 2010), and norethindrone acetate dose-dependently impaired spatial memory, levonorgestrel has been shown to have a null or even enhancing effect on spatial memory performance (Braden et al., 2016). In the context of translational research, finding a null effect of a progestin is a better outcome than the generally detrimental effects of MPA; that is, it is preferable for women to use a progestin that will have no effect or a beneficial effect on memory, rather than utilize a known cognitively impairing option like MPA. Thus, these novel findings regarding the differential effects of progestogens on cognition warrant further investigation into progestin type, dose, and timing of treatment to produce an optimal brain aging profile while maintaining the important protective effects that progestogens provide for other body systems.

Notably, the age at which ovarian hormone changes occur is also an important factor in cognitive outcomes. Some research shows that women who experience surgical menopause prior to natural, transitional menopause onset have poorer verbal memory scores and may have a greater risk for developing cognitive impairments, as well as dementia, later in life (Nappi et al., 1999; Rocca et al., 2007). Our laboratory recently extended these findings using the 4-vinylcyclohexene diepoxide transitional menopause rodent model, which acts by chemically inducing ovarian follicular depletion to produce an ovarian and hormone profile similar to women undergoing the transition to menopause (Koebele and Bimonte-Nelson, 2016).We found that animals that underwent the transition to menopause in young adulthood exhibited working memory impairments compared to normally aging adult rats, whereas transitionally menopausal middle-aged rats performed similarly to middle-aged control rats. These memory impairments were evident early in the menopause transition, particularly when working memory load was taxed (Koebele et al., 2017). These cumulative findings suggest that it is not only essential to consider hormone type, timing, and dosing regimen, but also an individual's reproductive history and status, as well as age, as important factors for understanding the potential of hormone therapy to have neuroprotective effects.

2.2. Androstenedione: long ignored but not unimportant

Androstenedione is an androgen synthesized by the adrenal glands and interstitial ovarian tissue, as well as by the thecal cells of maturing follicles. The aromatase enzyme converts androstenedione to estrone and 17β-estradiol; androstenedione can also be converted to testosterone via the enzyme17β-HSD; both of these androgens and their metabolites can impact the brain and cognition (Bimonte-Nelson et al., 2003; Camp et al., 2012; Mennenga et al., 2015b). In the context of menopause, research shows that, while estrogen and progesterone production declines substantially with reproductive senescence, the postmenopausal ovary continues to produce androgens in rodents (Mayer, 2004) and in humans (Fogle et al., 2007). In fact, it has been estimated that in the postmenopausal state, the ovaries continue to produce about 30% of the circulating androstenedione levels and 50% of total testosterone levels (Vermeulen, 1976). Recent research in postmenopausal women shows that exogenous testosterone can enhance memory (Davis et al., 2014; Davison et al., 2011), but endogenous testosterone levels may differentially impact cognition, such that a lower testosterone to estrogen ratio is better for memory performance (Ryan et al., 2012). Thus, the cognitive effects of androgens likely depend on a woman's background

hormone profile in the postmenopausal state, and should be taken into consideration when interpreting the effects of ovarian hormones on cognitive outcomes.

Although the effects of endogenous and exogenous estrogens and progestogens have been the focus of research related to cognitive function, the role of androgens (particularly androstenedione) in learning and memory remains somewhat elusive, and not as well defined as estrogens and progestogens. Our laboratory has shown that in a transitional menopause rat model, animals with higher naturally circulating androstenedione levels tended to make more working memory errors on the water radial-arm maze (Acosta et al., 2009). Given that androgens can convert to estrogens, we recognized this novel finding could inform important innovations in the realm of hormone therapy options for women. Thus, intrigued by this correlation, we continued to explore the role of androstenedione on memory in the middle-aged female rat. We found that in middle-aged Ovx rats, a high dose of androstenedione impaired spatial working memory and reference memory (Camp et al., 2012). Androstenedione can be aromatized to estrone, an estrogen that we have shown to impair memory in the Ovx rat model (Engler-Chiurazzi et al., 2012). Consequently, our laboratory systematically evaluated whether the apparent detrimental effects of androstenedione on memory were due to binding to the androgen receptor or androstenedione's conversion to estrone via the aromatase enzyme. Results indicated that blocking aromatase enzymatic activity via anastrozole reversed androstenedione-induced spatial memory impairments in young Ovx rats, but blocking the androgen receptor did not prevent detrimental effects on memory, suggesting that the conversion of androstenedione to estrone influences cognitive performance (Mennenga et al., 2015b). Collectively, these findings point to a crucial role of androgens, a long ignored yet ostensibly critical factor in understanding the role of the hormone milieu on cognition in the menopausal woman. Future research should continue to focus on understanding how circulating androgen levels impact the brain and body of aging women, and how maintaining a "golden ratio" of androgens to estrogens may be key to preserving cognition in the postmenopausal life stage.

2.3. What about those gonadotropins? Cognitive effects of LH and FSH during menopause and aging

The reproductive system in females is regulated by communication and interactions with numerous hormones from the hypothalamus, pituitary, and ovaries. Thus, ovarian-derived steroids, such as estrogens, progesterone, and androgens, are not the only hormones to become dysregulated with age and reproductive senescence. Research in recent years has indicated that changes in gonadotropins, namely FSH and LH, have a major role in cognitive changes and risk factors for developing age-related neurodegenerative disorders. FSH and LH are glycoprotein hormones released from the anterior pituitary, and they each have critical effects on body growth and maturation, as well as reproductive functions. FSH is released to stimulate the growth of immature ovarian follicles, resulting in a gradual rise in circulating estrogen levels during the first half of the cycle. Once estrogen levels reach a certain threshold, an LH surge occurs, which triggers ovulation and concurrently initiates corpus luteum development from the remaining ovarian follicle, which produces progesterone in preparation for egg fertilization. Once the follicle pool falls below a critical threshold, typically in midlife, the normal feedback from the ovaries to the hypothalamus

and pituitary becomes disrupted. Thought to be a compensatory mechanism, increased FSH and LH levels are released in the system's attempt to stimulate normal follicular growth and ovulation. Seminal work from the laboratories of Dr. Andrea Gore and Dr. Phyllis Wise has provided evidence that perturbations in the cyclic release of GnRH and subsequent release of gonadotropins occur before alterations in regular estrous or menstrual cyclicity becomes apparent, and that changes in N-methyl-D-aspartate (NMDA) receptor function may play a key role in disrupted GnRH release and feedback (Gore et al., 2000a; Gore et al., 2000b; Scarborough and Wise, 1990; Wise, 1982).

Some human research suggests that it is the alterations in gonadotropin levels, over and above declines in circulating ovarian hormone levels, that result in the cognitive changes observed during aging (Webber et al., 2005). In fact, higher circulating FSH and LH levels have been associated with neurodegenerative disease and pathologies in clinical populations (Bowen et al., 2002, 2000; Short et al., 2001). Basic science research using rodent models has further substantiated this tenet. For example, Dr. Gemma Casadesus and colleagues found that transgenic mice that overexpress LH receptors performed poorly on a hippocampal-dependent Y-maze task, while LH receptor knock out mice were not impaired, despite increased circulating LH levels (Casadesus et al., 2007). Furthermore, this group has shown that pharmacologically down-regulating serum LH improves cognitive performance after Ovx in wildtype and a triple transgenic mouse model of Alzheimer's disease (Blair et al., 2016; Palm et al., 2014); in wild type animals, decreasing LH serum levels benefitted memory performance, even after exogenous 17β-estradiol treatment was no longer effective in enhancing memory following Ovx (Blair et al., 2016). In addition, our laboratory has shown an inverted-U association between LH levels and cognitive performance in middle-aged female rats. Specifically, for animals with their ovaries (sham and follicle-deplete via experimental induction), higher LH levels were associated with poorer memory performance. Conversely, for Ovx animals, higher LH levels tended to be associated with better memory performance (Acosta et al., 2009). It is clear that in addition to circulating steroid ovarian hormone levels, gonadotropins also play a part in mediating cognitive performance, and these effects likely depend on background hormone milieu. Overall, these exciting findings point to novel pathways to explore to fully understand the impact of a dysregulated hypothalamic-pituitary-ovarian feedback loop, especially regarding the transition to reproductive senescence as related to the trajectory of cognitive aging.

3. Aging, ovarian hormones, and altered neural systems

The brain is a highly plastic organ. It adapts and changes throughout the lifespan, constantly revising and redacting information in order to adjust to an organism's ever changing environment. Neural systems and biochemical mediators are affected by many factors that are modified with age and interactions with the environment. A fundamental factor influencing the brain beginning early in life is sex steroid hormones. It is well established that androgens and estrogens play a key role in organizing the developing brain, and set it up to respond in a particular way following sexual maturity of an organism. Many of these neural systems and molecular pathways that are impacted by age and reproductive hormones are also associated with learning and memory processes. Here, we focus upon the cholinergic and GABAergic systems, which are two of the most well-studied neural systems

critical for learning and memory processes that are concomitantly impacted by age and ovarian hormones. The effects of age and altered ovarian hormone levels on dendritic morphology, as well as ERK1/2 signaling, a ERα-linked signaling pathway, are also discussed.

3.1. The cholinergic system

Age and ovarian hormones, both endogenously circulating and exogenously administered, impact a myriad of factors in the brain, including, but not limited to, growth factors (e.g., neurotropins), the inflammatory response, the immune response, mitochondrial function, and the cholinergic system. The latter involves the neurotransmitter acetylcholine, which also has diverse functions on the brain. One of acetylcholine's significant functions is to act as a key regulator of learning and memory consolidation. The basal forebrain is a primary synthesis site for acetylcholine in the mammalian forebrain. It is known that there are long-range projections from the basal forebrain to the frontal cortex as well as the hippocampus, crucial brain structures for learning and memory consolidation. Beginning in the 1980's, landmark research has indicated that age impacts morphology and functionality of the brain's cholinergic system. For example, aged animals showed a decline in ChAT and acetylcholinesterase (AChE; the enzyme that breaks down acetylcholine) activity in the basal forebrain and hippocampus (Springer et al., 1987). Recently, the Bizon laboratory reported a decreased number of ChAT-immunoreactive (ChAT+) basal forebrain neurons in aged males rats compared to young adult male rats (Bañuelos et al., 2013). By examining $p75^{NTR}$ expression, a growth factor receptor that often co-localizes with cholinergic neurons, Veng and colleagues reported a reduction in density and presence of healthy cholinergic neurons in both aged male and female rats compared to younger animals (Veng et al., 2003). Aged males exhibited smaller cholinergic neuron somas compared to younger males, while aged females did not show a reduction in mean soma size (Veng et al., 2003). In addition to age-related alterations in cholinergic neurons, Ovx has been associated with a decline in ChAT activity, while subsequent 17β-estradiol administration restored ChAT activity in the female rat basal forebrain and projection sites into the frontal cortex and CA1 region of the hippocampus (Gibbs, 1994; Luine, 1985; Singh et al., 1994). Further, lesions to the medial septum and vertical/diagonal bands of the basal forebrain resulted in impaired spatial memory performance and prevented the memory enhancing effects of 17β-estradiol (Gibbs, 2002; Hagan et al., 1988). It is important to note that age and ovarian hormone loss do not necessarily affect the number of ChAT-producing neurons in the basal forebrain, but do impact the functional integrity of the cholinergic system (Gibbs, 2003). Recently, it has been shown that GPR30, a membrane bound G-protein coupled estrogen receptor distinct from ERα and ERβ, exhibits co-localization with basal forebrain cholinergic neurons and likely mediates some of estrogens' effects on both basal forebrain cholinergic integrity and resulting cognitive outcomes (Hammond et al., 2011; Hammond and Gibbs, 2011; Ping et al., 2008). Thus, ovarian-derived hormones likely play a significant role in the neuroendocrine modulation of the cholinergic-hippocampal pathway. Most research on this subject has been evaluated in the Ovx model, where the ovaries, which are the endogenous source of circulating gonadal hormones, are surgically removed, and subsequent exogenous hormone therapy is administered. The effects of estrogens on cholinergic neurons in the basal forebrain are not always consistent, however. For example, many studies have shown

that after Ovx, exogenous administration of 17β-estradiol can increase ChAT+ neurons in the basal forebrain (Engler-Chiurazzi et al., 2012; Gibbs, 1997); it is of note that other estrogen types initiate varied effects on this measurement. Indeed, tonic administration of estrone, a weaker metabolite of 17β-estradiol, failed to impact ChAT+ neurons in the rat basal forebrain (Engler-Chiurazzi et al., 2012), and the synthetic estrogen used in oral contraceptives, ethinyl estradiol, decreased the number of ChAT+ neurons in the basal forebrain following chronic administration in an Ovx rat model (Mennenga et al., 2015a). These diverse effects of estrogens on one system highlight the complexity of estrogens' actions in the brain, and underscore the importance of taking multiple factors into account when assessing estrogens' effects on the brain and other body systems, such as type of estrogen, dose, route of administration, and timing of administration (for review, see Koebele and Bimonte-Nelson, 2015).

3.2. The GABAergic system

Adding complexity to understanding the system, many cholinergic projections from the basal forebrain synapse onto γ-aminobutyric acid (GABA) ergic cortical neurons; GABA is the primary inhibitory neurotransmitter in the brain and an important neuromodulator for normal cognitive processes, including hippocampal and cortical function. Acetylcholine release onto these GABAergic neurons in the hippocampus may modulate hippocampal theta wave oscillations through both direct and indirect pathways; these hippocampal theta rhythms play a role in regulating memory consolidation and synaptic plasticity (Dannenberg et al., 2015). The basal forebrain also has long-range GABAergic projections to the frontal cortex and hippocampus, both of which are thought to play a regulatory role in normal neural activity. Among its many roles, GABA signaling in the brain is a key regulatory factor in normal memory formation and maintenance (Kalueff and Nutt, 1997; Katz and Liebler, 1978). Inhibitory GABAergic neurons and signaling appear to become dysregulated with aging (Shetty and Turner, 1998; Stanley and Shetty, 2004). Animal models with altered GABA signaling, both systematically and with normal aging, show altered cognition with changes to the system, both in relation to cognitive aging and other psychiatric disorders (Bañuelos et al., 2013; McQuail et al., 2015). For example, the Bizon laboratory found that younger animals had better performance on the probe trial of the spatial reference memory Morris water maze compared to aged rats. The basal forebrain was immunohistochemically processed for ChAT and glutamate decarboxylase 67 (GAD67; the synthesizing enzyme for GABA). For GAD67-immunoreactive (GAD67+) neurons, there was no overall difference between young and aged rats. However, when aged rats were sub-classified into spatially-unimpaired and impaired groups, aged-spatially-impaired rats were found to have significantly more GAD67 + neurons compared to both young and aged-spatially-unimpaired animals. Further, this group showed a negative correlation with spatial memory performance in aged rats, such that a greater number of GAD67 + neurons was associated with poorer memory performance (Bañuelos et al., 2013). This laboratory also recently found that aged male rats have impaired performance on a set-shifting task, and that poorer performance on this task was associated with fewer GABA(B) receptors in the medial prefrontal cortex of the aged animals, but not the young animals. Directly infusing a GABA(B) receptor agonist into this brain region enhanced performance on the set shifting

task for the aged animals (Beas et al., 2016), suggesting that cognitive changes with age are in part modulated by GABAergic signaling.

In addition to age-related changes in the GABAergic system and subsequent memory performance, ovarian hormones influence the GABAergic system. While some research suggests that 17β-estradiol can influence GABAergic signaling in the hippocampus (Wójtowicz and Mozrzymas, 2010), the majority of studies thus far have focused on the role of progesterone and GABAergic functioning. For example, our laboratory and others have shown that progesterone decreased GAD65 + 67 protein levels in the hippocampus and increased GAD65 + 67 protein levels in the entorhinal cortex (Braden et al., 2010) as well as decreased GAD activity in several brain regions, including the dorsal hippocampus, as measured by kinetic studies (Wallis and Luttge, 1980). Furthermore, an in situ hybridization study revealed that 12 h after treatment, progesterone, but not MPA, reduced hippocampal mRNA expression of the α4 subunit of the GABA(A) receptor (Pazol et al., 2009), suggesting that different progestogens can have variable impacts on the GABAergic system. We recently showed that in a middle-age Ovx rat model, progesterone administration resulted in transient working memory impairments on a spatial memory task, but concomitant delivery of bicuculline, a GABA(A) receptor antagonist, obviated these memory impairments (Braden et al., 2015). Finally, the recent finding that cholinergic neurons may also co-release GABA adds an additional level of complexity wherein the full impact on cognitive function has yet to be determined (Tritsch et al., 2016). Nonetheless, whether there are sex differences in how GABAergic circuitry and signaling are affected by aging, as well as how endogenous alterations and exogenous administrations of other sex steroid hormone levels impact this system, remains somewhat elusive and warrants further investigation.

3.3. MAPK/ERK1/2 signaling pathway

A wide range of intracellular pathways and kinases are known to be important for normal learning and memory processes (Giese and Mizuno, 2013), many of which are recruited downstream of estrogen receptor activation. One pathway in particular, the extracellular signal-regulated kinases, known as ERK1/2, p44/42, and classical mitogen-activated protein kinases (MAPKs; in humans, ERK1 = *MAPK3*), has diverse functions in regulating learning and memory (Atkins et al., 1998; Bozon et al., 2003; Fasano and Brambilla, 2011). Estrogen receptors are thought to activate ERK1/2 via production of cyclic adenosine monophosphate (cAMP) and/or interactions with growth factor receptors. Age-related brain changes in ERK1/2 signal transduction have not yet been extensively studied; however, one experiment found that aged male rats had decreased ERK1/2 activity in the cortex compared to younger rats (Zhen et al., 1999). Further investigations into how aging alters ERK1/2 signaling are necessary to elucidate whether aberrant signal transduction has functional consequences on cognitive outcomes across the lifespan. Given that ERK1/2 is ubiquitously expressed throughout the brain and other organs, it is important to evaluate potential age-related changes in multiple cognitive brain regions, as well as consider sex, age, and hormone status as factors influencing ERK1/2 expression and signaling. Indeed, in recent years, 17β-estradiol has been proposed to regulate ERK1/2 activity in both in vitro and in vivo studies. Dr. Karyn Frick's laboratory has demonstrated that intraperitoneal injections and

intracerebroventricular or hippocampal infusions of 17β-estradiol activated ERK2 and enhanced object recognition memory (Fernandez et al., 2008; Frick, 2015; Lewis et al., 2008). Temporal parameters may impact the outcome of estrogen effects on this system; in aged Ovx rats, the amount of time since Ovx (and therefore ovarian hormone deprivation) impacted subsequent effects of 17β-estradiol on ERK1/2 phosphorylation, which were dependent upon brain region (Pinceti et al., 2016). Additionally, findings from Dr. Thomas Foster's laboratory revealed that ERα lentivirus injection directly into the hippocampus of middle-aged Ovx rats (i.e. animals with low endogenous estrogen levels) increased ERK1/2 phosphorylation and enhanced memory performance (Witty et al., 2012), suggesting that estrogen receptor activity, and possibly brain-derived estrogens, can activate and alter signal transduction pathways critical for learning and memory formation. These novel findings point to ERK1/2 signaling as another important biochemical mediator to investigate in the context of aging-and menopause- related brain changes. The interactions between ERK1/2 and the multitude of other hormone-linked pathways on learning and memory processes are only beginning to be explored. Further investigations in this newer field of how ovarian hormone fluctuations and aging impact these biochemical signaling pathways are currently underway.

3.4. Age- and ovarian hormone- influenced structural brain changes

In addition to the age- and menopause- related alterations observed in many neural systems and signaling pathways, the field is beginning to understand how aging and the ovarian hormone milieu impact the brain and other systems at the structural level. While neuron number does not necessarily decline in a healthy aging brain, age-related alterations in dendritic length (Pyapali and Turner, 1996), branching (Markham et al., 2005), and spine density and synapses (Adams et al., 2010; Geinisman et al., 1992) occur in several species, including rodents and non-human primates; these changes are seen in many brain regions, including those that regulate cognitive processes (Dickstein et al., 2013). Ovarian hormones can also affect dendritic morphology, and these alterations are sex-specific. For example, Miranda and colleagues demonstrated that aged Ovx rats showed decreased dendritic spine density in the dentate gyrus of the hippocampus compared to younger Ovx females deprived of hormones short-term; however, short-term estrogen administration, even in old age, increased spine density to the level of an adult female, and long-term estrogen replacement did not affect spines. Males did not show the same pattern of responsiveness to hormone deprivation and subsequent estradiol benzoate administration, suggesting that estrogen effects on dendritic spines are both sex- and time- dependent (Miranda et al., 1999). Furthermore, 17β-estradiol administration immediately after Ovx increased CA1 apical spines and enhanced memory performance, but if 17β-estradiol was given after 10 weeks of hormone deprivation, these morphological and behavioral changes were not as pronounced, again suggesting that temporal dynamics of estrogen administration matter for memory effects (McLaughlin et al., 2008). Middle-aged, ovary intact female rats showed impaired object recognition memory and a significant decrease in apical dendritic spines in pyramidal neurons within the CA1 regions of the hippocampus compared to young rats, but no differences in basal dendritic spines or pyramidal neurons in the CA3 region were apparent (Luine et al., 2011). Estrogen and progesterone likely regulate dendritic spines through NMDA receptors (Woolley and McEwen, 1994), and it is noted that there is natural variation

in dendritic spines across the estrous cycle (Woolley and McEwen, 1993). These studies collectively indicate that both aging and ovarian hormone fluctuations have the capacity to trigger structural brain changes at multiple levels, and that there is marked plasticity both during aging and across the reproductive cycle; this is true even though we note that the extent and efficacy of this plasticity likely waxes and wanes across the lifespan. It is possible that organizational effects not only occur during early development, but that reorganizational events also exist across the lifespan as natural, significant fluctuations in ovarian hormones occur, such as with puberty, pregnancy, and menopause.

4. Conclusions and future directions for understanding the complex interactions among female reproductive hormones, age, and neurobiological alterations underlying cognitive processes

Accumulating evidence points to roles for both age and reproductive hormones on the brain and behavior throughout life. Knowledge about the complex interactions within this endocrine-brain-aging triad is growing in breadth and depth as scientific discoveries are made, and continuing this work will yield new insights into how these paths meet and influence each other. Given the continuously increasing average human lifespan, it is more important than ever for the field of neuroendocrinology and aging to better understand how aging and the long-lasting changes in gonadal hormones and gonadotropins that occur in midlife affect the neural circuits and molecular mechanisms related to learning and memory. Thus far, discoveries have included multiple neural systems, domains of function, and biochemical mediators, such as the basal forebrain-hippocampal cholinergic pathway, GABAergic transmission, ERK1/2 signal transduction, and structural brain changes. It is of particular interest to understand how the neurobiological and neurochemical changes associated with menopause and aging alter the underlying circuitry of cognitive pathways, and if these systems compensate by using alternative mechanisms or undergo a rewiring to return to homeostasis as aging occurs. Elucidating the changes in these molecular mechanisms with age and ovarian hormone milieu in a systematic and demonstrable fashion will yield insight into how and when the brain responds to endogenous hormone changes as well as to potential exogenous hormone treatment. This will, in turn, drive progress forward toward development and optimization of opportunities and choices for women undergoing the transition to menopause that not only addresses the undesirable symptoms associated with menopause, but also that potentially prevents, attenuates, or postpones the onset of cognitive or affective changes for at-risk women during aging. In order to move toward this realm of discovery, it should be recognized that female reproductive hormones, including sex steroids and gonadotropins, have a powerful impact on many complex and interactive neural systems that influence cognitive outcomes throughout life. Indeed, it seems that exposure to these hormones, whether transient or long-lasting, can change the course of future responses and brain health.

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Abbreviations

ChAT	choline acetyltransferase
AChE	acetylcholinesterase
BF	basal forebrain
Ovx	ovariectomy
GAD	glutamate decarboxylase
GABA	γ-aminobutyric acid
ERK	extracellular regulated kinases
MPA	medroxyprogesterone acetate
ER	estrogen receptor
LH	luteinizing hormone
FSH	follicle stimulating hormone
HSD	hydroxysteroid dehydrogenase
HPG	hypothalamic-pituitary-gonadal
GnRH	gonadotropin releasing hormone

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Examples showing that many structural and molecular modulators of cognition become dysregulated with age and are sensitive to changes in circulating ovarian hormone levels

BRAIN CHANGE	AGING	OVARIAN HORMONES
Basal Forebrain-Hippocampal Cholinergic System	• Aged male rats showed decreased ChAT and AChE activity in the BF and hippocampus *(Springer et al., 1987)* • Aged male rats had decreased BF ChAT+ cell counts *(Bañuelos et al., 2013)* • In aged male rats, $p75^{NTR}$+ neurons (a growth factor receptor that often co-localizes with cholinergic neurons) in the BF decreased in size and density, and showed atrophy *(Veng et al., 2003)*: • Aged female rats did not exhibit a change in BF $p75^{NTR}$+ cell size, but atrophy and decreased density were observed, suggesting a sex difference in age-related BF cholinergic neuron changes	• Ovx decreased ChAT activity in the frontal cortex and hippocampus *(Singh et al., 1994; Gibbs, 2003)* • Ovx + 17β-estradiol increased BF ChAT activity *(Luine, 1985; Singh et al., 1994)* • Loss of BF cholinergic neurons prevented memory enhancing effects of 17β-estradiol *(Gibbs, 2002)* • 17β-estradiol or estradiol benzoate dose, treatment duration, and timing differentially impacted the number of ChAT+ cells in the BF *(Gibbs, 1997, 2003; review: Gibbs, 2010)* • Ovx + ethinyl estradiol, a synthetic estrogen used in oral contraceptives, decreased BF ChAT+ neuron counts *(Mennenga et al., 2015a)*

GABAergic System	• Aged, spatial memory-impaired rats had increased GAD67-immunoreactive cell counts in the BF *(Bañuelos et al., 2013)*
	• Aged male rats had and fewer GABA(B) receptors in the medial prefrontal cortex *(Beas et al., 2016)*:
	  • Fewer GABA(B) receptors correlated with poorer performance on a set-shifting task
		• Ovx + progesterone and Ovx + MPA decreased GAD65+67 protein levels in the hippocampus and increased GAD65+67 protein levels in the entorhinal cortex *(Braden et al., 2010)*
		• Ovx + progesterone impaired spatial working memory *(Braden et al., 2015)*:
		  • Concomitant administration of a GABA(A) receptor antagonist obviated this impairment

ERK1/2 Signaling Pathway	• Aged male rats have decreased ERK1/2 activity in the striatum and hippocampus *(Zhen et al., 1999)*: • Distribution of ERK changed with age, such that older males show clustered, rather than distributed, immunoreactive ERK1/2	• Intracerebroventricular or hippocampal infusions of estradiol activated ERK2 and enhanced memory performance *(review: Frick et al., 2015)* • Time between Ovx and acute, subcutaneous estrogen treatment impacted ERK1/2 activation profiles *(Pinceti et al., 2016)* • ERα lentiviral injection, which increases ERα expression, increased hippocampal ERK1/2 activation in the absence of increased circulating estrogen levels *(Witty et al., 2012)*

Dendritic Complexity	• Dendritic length in hippocampal neurons increased in aged rats *(Pyapali & Turner, 1996)*
	• Dendritic spine density and synapses can decrease *(Adams et al., 2010; Geinisman et al., 1992)* but may also not show differences with age *(Markham et al., 2005)*
	• Age-related altered dendritic complexity may depend on sex, where females show less decline in neuronal complexity than males *(Markham et al., 2005; review: Dickstein et al., 2013)*
		• Long-term Ovx decreased dendritic spine complexity in the hippocampus in females; males did not show the same pattern dendritic spine loss following gonadectomy *(Miranda et al., 1999)*:
		  • Ovx + long term estradiol benzoate administration did not alter spine density
		  • Estradiol benzoate treatment after short-term Ovx rescued spine density; in males, estradiol decreased spine density
		• Ovx + estradiol increased hippocampal dendritic spine density; effects were more pronounced when hormone treatment began immediately *(McLaughlin et al., 2008)*

Fig. 1.

Literature demonstrating the discoveries of brain changes in regions that regulate cognitive processes, and that are sensitive to both aging and ovarian hormones. ChAT=choline acetyltransferase; AChE=acetylcholinesterase; BF=basal forebrain; Ovx=ovariectomy; GAD=glutamate decarboxylase; GABA=ɣ-aminobutyric acid; ERK=extracellular regulated kinases; MPA = medroxyprogesterone acetate; ER = estrogen receptor.