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Trajectories and phenotypes with estrogen exposures across the lifespan: What does Goldilocks have to do with it?

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Abstract

Estrogens impact the organization and activation of the mammalian brain in both sexes, with sex-specific critical windows. Throughout the female lifespan estrogens activate brain substrates previously organized by estrogens, and estrogens can induce non-transient brain and behavior changes into adulthood. Therefore, from early life through the transition to reproductive senescence and beyond, estrogens are potent modulators of the brain and behavior. Organizational, reorganizational, and activational hormone events likely impact the trajectory of brain profiles during aging. A “brain profile,” or quantitative brain measurement for research purposes, is typically a snapshot in time, but in life a brain profile is anything but static – it is in flux, variable, and dynamic. Akin to this, the only thing continuous and consistent about hormone exposures across a female's lifespan is that they are noncontinuous and inconsistent, building and rebuilding on past exposures to create a present brain and behavioral landscape. Thus, hormone variation is especially rich in females, and is likely the destiny for maximal responsiveness in the female brain. The magnitude and direction of estrogenic effects on the brain and its functions depend on a myriad of factors; a “Goldilocks” phenomenon exists for estrogens, whereby if the timing, dose, and regimen for an individual are just right, markedly efficacious effects present. Data indicate that exogenously-administered estrogens *can* bestow beneficial cognitive effects in some circumstances, especially when initiated in a window of opportunity such as the menopause transition. Could it be that the age-related reduction in efficacy of estrogens reflects the closure of a late-in-life critical window occurring around the menopause transition? Information from classic and contemporary works studying organizational/activational estrogen actions, in combination with acknowledging the tendency for maximal responsiveness to cyclicity, will elucidate ways to extend sensitivity and efficacy into post-menopause.

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Keywords

Organizational; Activational; Development; Brain; Aging; Estrogen; Hormones; Memory; Menopause; Female

Introduction

The phenotype. As scientists, the word ‘phenotype’ means something different to each of us, depending upon our scientific query at hand. The traditional definition of a phenotype is the outward observable traits or characteristics of an organism that can be measured. Any phenotype to mention is a composite resulting from multiple factors. But, *which* factors? This is the question that helps drive scientific discovery forward. What factors impact observable, and potentially quantifiable, composites in our world? If we start with the basic description of a mammal, for example, an initial question would certainly be, “Is it a male or female?” Why ask this question? The answer is that males and females have markedly different phenotypes; sex and hormones matter.

A brief primer on mammalian sexual differentiation

For a behavioral endocrinologist, it all begins with sex. According to the long-standing model based on a plethora of research, for mammals, sexual differentiation of the gonads results from a cascade of events initiated by the chromosomal constitution of the animal (Figure 1a). This model is traditionally linear: chromosomal constitution leads to gonadal constitution which leads to phenotypic constitution. If the Y chromosome is present, testes develop, testosterone and other hormones are secreted, and male internal and external genitalia develop. If no Y chromosome is present, ovaries develop, no testosterone is secreted, and female internal and external genitalia develop. Thus, under this tenet female genital development is thought to develop by “default” – in the absence of gonadal hormone stimulation. This widely accepted model of default genital development for the female has been updated as the field garners new information. A series of gene transcription factors have been implicated in a more active process of ovarian development. For example, the *FOXL2* gene is a notable contender to be an ovary-determining gene. As the earliest known marker, *FOXL2* is necessary to differentiate testes development from ovary formation, and it plays a key role in actively suppressing *SOX9*, a downstream target through which the sex-determining-region Y (*SRY*) gene induces testes formation (Georges et al., 2014; Kalfa et al., 2008; Schmidt et al., 2004; Uhlenhaut et al., 2009). Furthermore, female mice without *Foxl2* (*Foxl2*^{-/-} mutants) do not undergo normal ovarian follicle development, experience pervasive neonatal follicular atresia, and are sterile (Ottolenghi et al., 2005; Schmidt et al., 2003; Uda et al., 2004; Uhlenhaut et al., 2009). Depleting *Foxl2* can trigger a cascade of events including upregulation of genes that produce male phenotypic gonad development (Garcia-Ortiz et al., 2009; Ottolenghi et al., 2005; Uhlenhaut et al., 2009). *Foxl2* may not only be critical during prenatal development, but also across the lifespan (Uhlenhaut et al., 2009). In fact, experimentally-induced *Foxl2* loss in eight-week-old mice resulted in ovarian granulosa cells morphing into Sertoli-like cells, and thecal cells beginning to upregulate an enzyme controlling testosterone biosynthesis (Uhlenhaut et al., 2009). There are likely other genomic processes acting in concert with *Foxl2*, but this recent evidence provides novel

pathways to explore with regard to sexual differentiation and active development of the female phenotype. In addition, there is fascinating ongoing work in the field of sexual differentiation to better elucidate the role of gonadal hormones, as well as the newly recognized unique impact of genetic sex, as direct factors in the phenotypic outcome of an individual (Arnold et al., 2004; Bakker and Baum, 2008). Indeed, non-linearity may exist in the genetics-determines-gonads-determines-phenotype model, such that the resulting phenotype is *directly* influenced by genomic mechanisms, which likely work in synergy with gonadal hormones and even epigenetic effects (McCarthy et al., 2012; McCarthy and Arnold, 2011). These intriguing new discoveries continue to modify more traditionally-accepted models and will aid in elucidating the complex nature of sex differences and steroid hormone effects on multiple systems spanning early development to reproductive senescence and beyond, impacting the phenotype throughout life.

Brain sexual differentiation in mammals

In mammals, the traditional model of sexual differentiation of the brain parallels that of the genitalia, maintaining that if testosterone exposure occurs early in life during a defined critical window, it exerts permanent effects resulting in a male phenotype. This traditional model also holds the tenet that if the brain is not exposed to gonadal steroids during this critical window, this will result in a female phenotype. Therefore, in this model the “normal” female phenotype has been considered to be organized by default. The updated model builds upon abundant research and the traditional framework, incorporating new evidence and views (Figure 1b). Accumulating behavioral and brain data have demonstrated that in fact, normal female brain development and organization depends on estrogen exposure, and that female brain organization is an active process that is *not* by default.

A flow diagram depicting sexual differentiation of the mammalian brain and phenotype is presented in Figure 2. For the purposes of discussion here, masculinization is defined as the induction of the male phenotype, feminization as the induction of the female phenotype, demasculinization as removing the potential for the development of male traits, and defeminization as removing the potential for the development of female traits. A phenotypically “normal” female is both feminized and demasculinized, while a phenotypically “normal” male is masculinized and defeminized. Much of the research leading to our understanding and defining of these processes has been done in rodents; hence, the work discussed in this section is largely based on rodent experimental evaluations. Regarding the process of sexual differentiation of the mammalian brain, testosterone is released from the gonads, and is thought to lead to masculinization in the male via direct and indirect routes. Indeed, testosterone readily crosses the blood brain barrier and is converted in the brain to 17 β -estradiol via the aromatase enzyme. Numerous rodent studies have shown that high doses of 17 β -estradiol can be masculinizing to the brain and behavior, including reproductive and non-reproductive brain areas and behaviors. Why then do female rodents not experience the same masculinization from estrogen exposure during gestation? Alpha-fetoprotein (AFP), a transient plasma protein circulating during fetal development, has a high binding affinity and capacity for the estrogens rodents are exposed to during gestation (likely maternal in origin) (Figure 3). Once bound to AFP, the estrogen compound is sufficiently large such that it can no longer cross the blood brain

barrier, effectively preventing estrogen-induced brain masculinization and defeminization in females during the early perinatal timeframe (Figure 3; Attaradi and Ruoslahti, 1976; Benno and Williams, 1978; McEwen et al., 1975). Bakker et al. assessed the principle that AFP prevents estrogens from exerting masculinizing and defeminizing effects in the brain by testing female mice without AFP (*Afp*^{-/-} mutant); consequently estrogen could enter the brain in the *Afp*^{-/-} mutants (Bakker et al., 2006). These *Afp*^{-/-} mutant animals did not exhibit female-typical sex behavior and had increased male-typical sex behavior compared to wild-type female mice, supporting the tenet that when AFP is not present, estrogens enter the brain and subsequently masculinize and defeminize (Bakker et al., 2006). Moreover, females lacking AFP had male-like quantities of tyrosine hydroxylase expression in the anteroventricular nucleus of the preoptic region, an area important for female reproductive function, and administering treatment of an aromatase inhibitor to the mother during pregnancy (effectively attenuating estrogen production and exposure in the fetus) produced a normal female phenotype for the measured variables in the female *Afp*^{-/-} mice that was similar to that of wild-type females (Bakker et al., 2006). This research provides further support that estrogens exert brain masculinizing and defeminizing properties during development, and that the presence of AFP can prevent these actions in the female. The system is really quite remarkable. Indeed, in rodents, AFP levels become undetectable in the brain after post-natal day (P) 7 (Ali et al., 1981a), around the same time that the ovaries increase in activity and produce detectable levels of gonadal hormones (Mannan and O'Shaughnessy, 1991; Picut et al., 2014; Sokka and Huhtaniemi, 1995; Weniger et al., 1993). Because the female brain seems to have an extended period of sensitivity to gonadal hormones beyond the neonatal period (discussed in more detail in the section: "On the framework of organizational and activational effects of ovarian hormones on the brain"), these estrogens of ovarian origin are likely necessary for normal female brain development, and they can now access the brain during this time since AFP no longer attenuates brain access (Toran-Allerand, 1984; for review, see Bakker and Baum, 2008).

We would be remiss if we did not note that the questions of sexual differentiation of the brain in humans are difficult to address due to the obvious ethical considerations of systematic experimental manipulation. However, some work, including in human brain extracts and cord serum, has questioned whether AFP binds to estrogens in humans in a manner similar to rodents; thus, the role of AFP in human sexual differentiation is still highly controversial (Ali et al., 1981b; Nunez et al., 1974; Swartz and Soloff, 1974). Nonetheless, sexual differentiation and the role of AFP has been extensively studied and characterized in the rodent model, providing a solid foundation and framework for understanding the role of gonadal hormones in sexual differentiation of the brain.

Estrogens, the brain, and cognition

Estrogens are potent modulators of the brain, including in regions known to affect learning and memory. Many of these cognitive brain regions are sensitive to changes as aging ensues. Thus, it makes sense that estrogens impact the brain and its functions across the entire lifespan in various contexts. When we think about the scientific literature, in this realm where we control the environment as much as possible, we can initiate measurable and impactful effects of estrogens on the phenotype. Are these effects potent enough to be

meaningful in the real world, such that they would be impactful to a woman who has estrogen levels in flux across a short period of time (e.g. the menstrual cycle), as well as with greater marked alterations such as during pregnancy and as aging and menopause ensue? The 1998 special issue on estrogen and cognition in *Hormones and Behavior* discussed estrogen exposure and its influence on the brain across the lifespan, including estrogen's impact on brain morphology and learning and memory, as well as the mechanisms and sites of action through which estrogens exert their effects (Williams, 1998). In 2013, *Brain Research* published a special issue on the “window of opportunity” for hormone therapy interventions in the context of cognitive brain aging, reflecting the extension and direction of this research area over the years. Basic science research on estrogen exposure, as well as related cascades of action and life-long influences on the female brain, is especially important and relevant for translational impact to the clinic. Indeed, in the United States alone, the number of people aged 65 and over is projected to increase to encompass 20% of the general population by 2020 (U.S. Census Bureau, 2010, 2007), and the National Institute on Aging reports that by 2050, the world population of individuals 65 and over is expected to reach 1.5 billion, triple the population estimate of the same age group in 2010 (National Institute on Aging, 2011). Each of the subfields within basic science and clinical investigations of estrogens, the brain, and cognition continues to pique the curiosity of neuroscientists. In the 17 years since the last special issue on estrogen and cognition in *Hormones and Behavior*, we have gleaned much insight into the field, opening doors to even more, seemingly infinite, inquiries about estrogen's impact on cognitive processing throughout the lifespan and the fascinating enigma that is the brain.

On the framework of organizational and activational effects of ovarian hormones on the brain

The turn of the 20th century was abound with empirical evaluations in the field of sex differences and behavior. In the early years of this research, Parkes described “internal secretions of the ovary” (Parkes, 1927, p. 1663) as critical to development, the menstrual and estrous cycles, and pregnancy maintenance, and that injections of “extracts of corpus luteum” (Parkes, 1927, p. 1665) disrupt the normal estrous cycle (Parkes, 1927). Young and Beach began investigating these internal secretions, later known as gonadal hormones, and their relationship to sex behaviors in the 1930's and 1940's. Beach found that gonadal hormones had sex-specific effects on the presence or absence of phenotypic mating behaviors, and that these behaviors could be manipulated by altering hormone levels. For example, his laboratory found that testosterone propionate induced male-typical sex behaviors, in addition to female receptivity sex behaviors, in female rats that underwent ovariectomy (surgical removal of the ovaries; Ovx) prior to the onset of puberty (Beach, 1942), and that gonadally intact males demonstrated female-typical sex behaviors, in addition to male-typical sex behaviors, when given large doses of androgen in adulthood (Beach, 1941).

In 1959, Phoenix, Goy, Gerall, and Young posited the theory of organizational and activational effects of gonadal hormones (Phoenix et al., 1959; Young, et al., 1964). This dichotomy is based primarily on the parameters of gonadal differentiation and on androgenic

actions in the male. A myriad of research has ensued in the area of sex differences and sexual differentiation of the body and brain. Many of these investigations are based on the classic concept of critical periods in which gonadal hormone exposure must occur at just the right time during development to effectively and permanently organize an organism to respond normally upon sexual maturity. In the 1970's, Jost and others studied embryonic development of the gonads, postulating that testes develop earlier in gestation and by an active process, while ovaries were considered to develop in the absence of testes formation (for discussion, see Jost et al., 1973). These classic and landmark studies were critical to the field, driving further research and contributing to the establishment of the long-held dogma that many female systems develop by “default.”

Organizational effects of hormones have varied definitions depending on the discussant, but they are typically operationally defined as permanent and occurring early in development. *Activational effects* of hormones are typically operationally defined as transitional, depending on the immediate presence of the hormone for the effect, and occurring later in development. Under the tenet of this traditional model, sex differences reflect the organizing and permanent actions of sex steroids present during an early development critical period, while activational effects “activate” underlying previously-organized substrates specifically in adulthood. In the landscape of traditional as well as newer models, organizational and activational effects of sex steroids work in concert. In fact, although early sex hormone exposure plays a significant role in organizing brain substrates driving sexually differentiated behaviors, many of these effects are realized only with exposure to the activating sex hormones; in many cases, the “male” or “female” phenotype is expressed normally only with the activational hormone present (Beatty, 1992). As a result of this synergy of organizational and activational effects, “male” and “female” brains are not likely to respond to the same circulating/activating hormones in an equivalent fashion because the structures being activated have had different prior organizational hormone exposures and organizational consequences (Beatty and Beatty, 1970).

Evidence accumulating over the last few decades has blurred the traditional operational definitions of the organizational and activational dichotomy, especially regarding the temporal distinction wrapped into the definitions. More recent views of the organizational/activational tenet suggest that the temporal parameters should not be so strictly defined, and that the distinction between effects is not necessarily linearly related in time. Rather, whether “the induced changes represent permanent or transient effects, whenever in life they occur” (Fitch and Denenberg, p. 312, 1998) should provide the distinction. Examples of findings blurring the traditional dichotomy include activation of ear wiggling sexual behavior in female rat pups by estrogen, when pups were as young as six days of age (Williams, 1987), and non-transient alterations in brain morphology following post-pubertal sex hormone manipulations (Bimonte et al., 2000a, 2000b, 2000c; Bloch and Gorski, 1988; Pappas et al., 1979; Rodriguez-Sierra, 1986).

Nearly 30 years ago, researchers were already rethinking the idea that organizational effects occur only early in development. A variety of brain regions and behaviors are differentially impacted by exposure to estrogens and/or androgens, with females showing extended sensitivity to hormonal manipulations and changes in anatomical and behavioral outcomes

(for review see: Fitch and Denenberg, 1998). For example, Rodriguez-Sierra showed that estradiol benzoate administration on P25 (prior to puberty but after neonatal brain organization) modified synaptogenesis in several brain regions within days after estrogen treatment, and that these estrogen effects may be sex-specific to females (Rodriguez-Sierra, 1986). In my (HBN) doctoral research in the Denenberg laboratory, we found that ovarian hormones actively feminized the corpus callosum, and that this effect was not reversible within the extended timeframes into adulthood that were assessed. These data indicate that the permanent, potentially “organizational” effects of ovarian hormones can extend into adulthood for females (Bimonte et al., 2000a). Moreover, in females there is flexibility in the window of sensitivity for normal feminized brain organization. There has been extensive research on the corpus callosum that nicely illustrates this idea. It is known that in the normally developing rat the organizing effects of androgen on the male corpus callosum end by P1 (Bimonte et al., 2000c; Fitch et al., 1991, 1990; Mack et al., 1996), but the female corpus callosum is sensitive to ovarian hormone input at least up to P70 (adulthood). Pre-pubertal Ovx enlarges, or defeminizes, the corpus callosum; ovary transfer post-puberty on P70 can counteract this effect, resulting in a smaller (feminized) corpus callosum in adulthood (Bimonte et al., 2000b). In addition, there is evidence that neonatal estrogen exposure is necessary for brain feminization later in life. Exposure to the estrogen receptor blocker tamoxifen from birth until Ovx on P25 resulted in a larger corpus callosum size even after estradiol was given starting at P70, whereas animals that had normal ovarian hormone exposure until P25 Ovx responded as would be expected to exogenous estradiol administration beginning on P70 — the size of the corpus callosum was smaller in a feminized fashion. One could interpret these findings as meaning that the presence of ovarian hormones through the first 25 postnatal days of life allowed estradiol to later feminize the corpus callosum. If estrogens were not impacting the brain during this early timeframe, attenuating estrogen receptor stimulation would have had no impact on the response to the activating effects of estrogens when given later. Collectively, these studies implicate an active role of ovarian hormones in organizing the female brain, and an overall extended period of sensitivity in females relative to the period of sensitivity in males.

Hormone induction of sex differences in the brain

Since the landmark work of Gorski and colleagues in the late 1970's and early 1980's studying the sexually dimorphic nucleus of the pre-optic area (SDN-POA; Gorski et al., 1978), there has been a plethora of research exploring sexually differentiated brain structures grossly and at the microscopic level. An aim driving this work is to discover modifications in brain morphology, which may help explain some sexually dimorphic patterns of behavior. Many studies have manipulated parameters in early life. Intact male rats show asymmetric cortical thickness, which appears to be dependent on androgen exposure during the neonatal period since P1 gonadectomy prevents this asymmetry (Diamond et al., 1981; Stewart and Kolb, 1988). This cortical asymmetry is not noted in ovary intact females, and there is no impact of P1 Ovx (Diamond et al., 1981; Stewart and Kolb, 1988). Ovarian hormone effects on cortical thickness have been studied by others as well. Rats Ovx on P1 that received ethinyl estradiol between days P40-90 have thinner cerebral cortices than ovary-intact vehicle injected animals, but rats Ovx on P1 that receive progesterone injections exhibit thicker cortices compared to sham animals, suggesting that treatment with estrogens beyond

the perinatal period can alter cortical thickness, and that different ovarian hormones can exert opposing effects on brain development (Pappas et al., 1979). Differences in dendritic spine morphology can be found in a variety of brain structures that are affected by gonadal hormone exposure. This includes the natural cyclic changes observed in spine density across the estrous cycle (Gould et al., 1990; Luine, 2014; McEwen, 2002; Shors et al., 2001; Woolley et al., 1990), and alterations that occur as a result of some experimental manipulations (e.g. stress; for discussion, see McLaughlin et al., 2009). As well, sex-specific responses in the spines of dentate granule cells in the hippocampus of aging rats after estradiol benzoate administration have been demonstrated (Miranda et al., 1999).

Using *in vivo* micro dialysis in freely behaving rats, Mitsushima and colleagues have presented data indicating that there are sex-specific effects of gonadal hormones on acetylcholine release in the hippocampus, and that these effects rely on prior brain organization by hormones. In this study, to evaluate activational effects, male and female rats were gonadectomized at eight weeks of age, and rats received a silastic implant containing either 17 β -estradiol, testosterone, or oil vehicle (Mitsushima et al. 2009a). Testosterone treatment restored acetylcholine levels in gonadectomized males to the level of intact males, but testosterone treatment did not restore acetylcholine levels in Ovx females to the level of ovary-intact counterparts. In Ovx females, 17 β -estradiol administration restored acetylcholine levels to those of intact females, but 17 β -estradiol administration did not restore acetylcholine levels in gonadectomized males to those of intact males (Mitsushima et al. 2009a). Mitsushima and colleagues also examined organizational effects of gonadal steroids on acetylcholine release in females. On P0 and P1, female rats were given an injection of testosterone, 17 β -estradiol, dihydrotestosterone (DHT), or oil vehicle. At eight weeks of age, rats were then Ovx and given a silastic implant with testosterone. *In vivo* microdialysis to assess acetylcholine release in the hippocampus showed that early life exposure to testosterone and 17 β -estradiol increased acetylcholine levels when given testosterone in adulthood, but early life exposure to DHT did not increase acetylcholine levels when testosterone was administered. The collected findings indicate that priming females with testosterone or 17 β -estradiol immediately after birth masculinizes acetylcholine release patterns and levels, and that the cholinergic system can be organized by perinatal gonadal hormone exposure (Mitsushima et al. 2009a). The authors suggest that, as noted in many studies in the literature, Ovx animals may not perform well on some learning and memory tasks because they produce inadequate amounts of hippocampal acetylcholine (Mitsushima et al., 2009a, 2009b).

(Re)organizational events across the lifespan

It is possible that non-transient organizational hormone events in the brain can continue across the lifespan, challenging the long-held dogma that organizational effects are limited to the neonatal period of development, and supporting the updated distinction of the organizational and activational dichotomy depending on whether the effects are permanent or transient, irrespective of the life timeframe during which they occur (e.g., see Arnold and Breedlove, 1985; Rodriguez-Sierra, 1986; Fitch and Denenberg, 1998; Stewart and Kolb, 1988). For example, Bloch and Gorski found that in gonadectomized male rats, sexually dimorphic structures within the preoptic area are impacted by exogenous estrogen and

progesterone administration when given in adulthood, with results indicating some feminization since the response was in the female direction (Bloch and Gorski, 1988). Moreover, while it is clear that gonadal hormones play a significant role in organizing the brain during early development, there are other hormonal exposures that can result in seemingly permanent effects on the brain, even if they occur later in life. An extended and systematic series of studies from the Juraska laboratory has beautifully shown that puberty marks another period of architectural remodeling in the brain, including alterations in neurons and glia, and that ovarian hormones play active roles in these events (see Juraska et al., 2013, for review), which could be considered reorganizational effects. Evidence from our laboratory extends the idea of reorganizational effects to exogenous and synthetic hormone exposures, testing the permanence of effects of hormones that are taken by women. We have recent data suggesting that exposure to the synthetic progestin medroxyprogesterone acetate (MPA) in adulthood — even if that administration is discontinued — negatively impacts female rats' working memory performance with detrimental effects persisting up to four months after transient exposure is terminated (Braden et al., 2011). While the impact of MPA on cognition could be considered activational because the brain was organized early on to respond to hormones, even a transient exposure appears to have a long-lasting, and potentially permanent, impact on the trajectory of cognitive performance with aging. Hence, this pattern of effects resulting from the adult transient exposure to this synthetic hormone might be considered a reorganizational event.

Pregnancy is another life event marked by potent steroid hormone changes; some of these changes appear to result in long-term brain and behavioral effects in females such that they respond differently to other hormonal exposures later in life compared to females who have never experienced pregnancy-related hormone changes. Might this be considered a reorganizational hormone event? Parity not only impacts maternal behaviors in rats, but it also induces changes in spatial learning and memory performance, stress behaviors, and alterations in related brain measurements (Gatewood et al., 2005; Kinsley et al., 1999; Macbeth and Luine, 2010; Macbeth et al., 2008; Pawluski et al., 2005). For example, there was a reduction in number of amyloid precursor protein immunoreactive cells in the hippocampi of parous rats compared to nulliparous rats (Gatewood et al., 2005), and a noted increase in monoaminergic activity in the olfactory bulb, and BDNF in the hippocampal CA1 region and medial septum, in multiparous versus nulliparous animals (Macbeth et al., 2008). Additionally, an acute (10 μ g) injection of 17 β -estradiol, 17 α -estradiol, or estrone increased the number of BrdU-immunoreactive cells in the dentate gyrus of middle-aged Ovx multiparous rats compared to age-matched virgin rats, indicating that previous sexual or reproductive experience impacts responsiveness to estrogens later in life (Barha and Galea, 2011). Of note, parity (i.e. pregnancy, lactation, and pup experience [Kinsley et al., 1999]) involves other hormone fluctuations, including a significant and extended increase in progesterone levels accompanying the increase in estrogen levels, and these gonadal hormones likely act in concert to result in the observed long-term changes in the brain and behavior associated with motherhood. Less is known about the long-term effects of pregnancy experience in humans, but research suggests that there are changes associated with hormonal fluctuations during and after pregnancy that affect future brain morphology and behavior (Glynn, 2010; for discussion, see Macbeth and Luine, 2010).

Clearly, gonadal hormones act upon a variety of systems and brain structures that work together to result in the observable outcomes of hormone actions. Many of these observations have been carried out at adolescent or young adult time points, and we note that these hormone exposures likely influence the trajectory of aging such that the brain has “activational” responses dependent not only on the organization that occurs very early in life, but also to reorganizational events that occur across the whole lifespan, including very late in life. These long lasting (potentially permanent) effects of estrogen exposures, such as from pregnancy, exogenous hormone administration (e.g., contraceptives, hormone therapy), and menopause, may be considered “reorganizational” rather than “activational” as their effects appear to persist long after the initial exposure is terminated, and they have been found to change the trajectory of responsiveness later in life. This is not mutually exclusive of the waxing and waning in sensitivity to estrogens across the lifespan; indeed, a decrease in sensitivity does not mean activational or reorganizational effects cannot occur. McCarthy notes that the developing and the reproductively senescent time periods may have more in common than previously recognized (McCarthy, 2011). Research elucidating how steroid hormones organize the brain throughout life can yield insight into optimizing the hormone milieu during menopause, including providing perspectives and considerations for menopause trajectories and hormone therapy options.

Parameters for understanding the impact of hormones on the brain and cognition: evaluating learning and memory in the rodent model

Mazes and memory

Since the early 20th century, a variety of innovative tasks have been implemented to study learning and memory in the rodent (Bimonte-Nelson, 2015), and these maze tasks have been utilized to gain understanding of hormone effects on the brain and cognition. The radial-arm maze and Morris maze are two apparatuses often used to study estrogen effects on cognition; therefore, their brief histories and methods will be highlighted here to set the backdrop for later discussion in this review.

In the 1940's, Edward Tolman and colleagues created a “sunburst” maze, which was comprised of several arms radiating from a circular arena, to evaluate rodent navigation and formation of a cognitive map (Tolman, 1948; Tolman et al. 1946). These groundbreaking experiments sparked a revolution in the use of mazes to study learning and memory for spatial navigation in laboratory rodents. Spatial navigation is defined as utilizing distal or extra-maze cues to navigate in an environment. In the 1970's, David Olton pioneered an eight-armed radial arm maze, based on Tolman's sunburst design, to test rodent cognition. Each arm was baited with a food reward, and once a food reward was obtained it was not replaced within a day, requiring the animals to remember and update which spatial locations they had already visited. Olton and colleagues noted that animals most often relied on spatial cues to efficiently solve the radial-arm maze (Olton and Samuelson, 1976). The radial-arm maze continues to be one of the most utilized maze paradigms in animal models of learning and memory to evaluate *reference memory*, a form of long-term memory wherein information stays constant, and *working memory*, a form of short-term memory that is updated within a testing session (Bimonte-Nelson et al., 2015). Many studies using the land

version of this task have contributed significantly to our understanding of ovarian hormone effects on cognition (e.g. Daniel et al., 1999; 1997; Fader et al., 1999; Gibbs and Johnson, 2008; Holmes et al., 2002; Witty et al., 2013), and the land version has also been used for notably translational working and reference memory assessments using Premarin, a widely used hormone therapy in women (Barha and Galea, 2013). One caveat to the land version of the radial-arm maze is that animals are typically food deprived, and food reward is given upon successful choices, to motivate rodents to perform in the maze. Using appetitive motivation can add complexity to interpretations of hormone research, as longer-term food restriction can impact cyclicity and alter circulating levels of LH, FSH, and estradiol (Ahmed et al., 2012) in the female rodent. As a solution to this concern, a water version of the radial-arm maze was developed in the Denenberg laboratory in the late 1990's; of note, others were utilizing water escape in other maze tasks as well (for review see: Bimonte-Nelson et al., 2015). The water version of this task relieves the need for food deprivation by using hidden escape platforms (thereby removing the animal from the water, which is the negative reinforcer) and returning the animal to a warm, heated home cage as a positive reinforcer across all trials (Bimonte and Denenberg, 1999). Working memory load is taxed in the water version of the radial-arm maze by placing platforms in the end of each arm, and removing each platform without replacement once the animal locates it within a day. A reference memory component can be added to this paradigm by placing escape platforms in a subset of the arms. In this maze testing protocol, the animal not only has to utilize working memory to remember where it has been within a day, but it also must use reference memory to remember where it should never go (i.e. arms that never contain a platform) (Bimonte-Nelson, 2015). Figure 4a provides a schematic of a common radial-arm maze setup.

Another invaluable task for studying spatial navigation and cognition in the rodent model is the Morris water maze, developed by Richard Morris in the early 1980's. This simple, yet cleverly designed, water escape task involves a circular tub of water in which a hidden platform is submerged just beneath the surface of the water; rodents are tasked with navigating to the hidden escape platform, typically using spatial cues placed around the room (Figure 4b; Morris, 1981; 1984; Morris et al., 1982; for review and discussion from a personal perspective, see: Morris, 2015). This task often (and optimally, should) include a probe trial, where the platform is removed from the maze, and animals are allowed to swim freely for 60 seconds. The probe trial is critical to evaluate the use of spatial localization to the hidden platform. The Morris water maze is traditionally a measure of spatial reference memory, but many laboratories have altered the paradigm to evaluate working memory, the use of distal and proximal cues, and room geometry and cue learning (Pearce et al., 1998; Clark et al., 2007). Other tasks including, but not limited to, the T maze, Y maze, delayed-match-to-sample, novel object recognition, and place recognition have also been used to quantify hormone effects on learning and memory performance in rodents (Bimonte-Nelson et al., 2015; Chisholm and Juraska, 2012; Daniel et al., 2005; Foster et al., 2003; Frick and Gresack, 2003; Gibbs, 2002; Gibbs et al., 2009; Johnson et al., 2002; Nelson et al., 2012; Orr et al., 2009). Many of the experiments discussed in this review utilize a battery of these tasks to evaluate the effects of gonadal hormones on rodent cognition.

Cyclic and tonic hormone exposures can differentially impact maze learning and memory

The female brain is designed to respond in a “permanently transient” nature (see: Fitch and Denenberg, 1998). In fact, as the decades have progressed, evidence has accumulated that the organized hormonal cyclicity in females results in responses that are the most potent, and potentially optimal, from a cyclic pattern of hormone exposure as opposed to a tonic pattern of hormone exposure. Cyclic patterns of hormone exposure via periodic injections that result in a repeated rise and fall of circulating hormone levels more closely model natural endogenous rhythms inherent to the female, while a steady tonic exposure may be suboptimal because there is a lack of cyclic pulsatile exposures (Figure 5). This could be especially meaningful in the context of hormone therapy effects on cognition. Several rodent studies from our and other laboratories indicate that tonic hormone exposure (such as via silastic implants, hormone pellets, or osmotic pumps) may not be an optimal route of administration for estrogens, at least in relation to performance on cognitive measures. This is not to say that tonic estrogen treatments can not exert positive effects on cognition. They can. However, effects with tonic estrogen exposure seem to be quite particular to get an efficacious response, especially with regard to dose (Bimonte and Denenberg, 1999; Bimonte-Nelson et al., 2006; Engler-Chiurazzi et al., 2011; see also Engler-Chiurazzi et al., 2012). Cyclic exposure (such as daily or weekly injections) may provide a solution to a stunted response with tonic administration since it capitalizes on the female's tendency to have naturally cyclic physiological rhythms. Markowska and Savonenko performed a longitudinal, repeated-measures study in female rats over a nine-month span, beginning in middle age, to elucidate the cognitive impact of gonadal hormone deprivation followed by tonic and/or cyclic 17 β -estradiol administration. Results indicated that 17 β -estradiol administration was most effective when tonic treatment was primed first with cyclic injections, compared to tonic treatment alone; the authors conclude that “this paradigm appears to be highly beneficial for cognitive improvement and may produce fewer side effects than continuous daily administration” (Markowska and Savonenko, p. 10994, 2002). Subsequent research supports the tenet that cyclic 17 β -estradiol administration is beneficial to cognition; assessments to test these effects have included the traditional spatial reference memory Morris water maze and the delayed-match-to-sample spatial working memory version of the Morris water maze (Bimonte-Nelson et al., 2006; McLaughlin et al., 2008; Sandstrom and Williams, 2001, 2004). There has also been work expanding applications to other types of estrogen formulations used in women. For example, our laboratory has shown that a cyclic regimen of conjugated equine estrogens (CEE, Premarin) improved learning and memory in middle-aged Ovx rats and increased choline acetyltransferase (ChAT, the synthesizing enzyme for acetylcholine) immunoreactive cell counts in the basal forebrain compared to controls given vehicle (Acosta et al., 2009a). However, when we extended this work to systematically test whether etiology of menopause impacted outcomes, we found that cyclic CEE was only beneficial to learning and memory when the rats had their ovaries removed via Ovx surgery. Animals that were ovary intact but treated with 4-vinylcyclohexene-diepoxy (VCD), a drug that selectively depletes ovarian follicles in rodents and provides a model of transitional menopause when follicle-deplete ovaries are retained, were impaired by the same CEE treatment regimen on both working and reference memory as tested in the water radial-arm maze (Acosta et al., 2010). It is noteworthy that

hormone effects were most pronounced at the latter trials, when the working memory load was highest.

Progestogens and combination hormone therapy

If a woman has her uterus, a progestogen component of hormone therapy is necessary to protect against unopposed estrogen-induced endometrial hyperplasia and cancer (NAMS, 2012). Because over 80% of women maintain their ovaries and uterus throughout the natural menopause transition (NAMS, 2015), progestogens are a widely used component of hormone therapy, and an important factor to consider in a preclinical research setting to provide a translational approach. Preclinical evaluations should include clinically-used progestogens, as well as novel options that may be promising therapeutic candidates. Careful consideration should be given to systematically evaluate progestogen effects when given alone, as well as potential interactions when given in concert with estrogens. We will only discuss natural progesterone and synthetic progestins (collectively called progestogens) in brief here only to aid discussion of estrogen effects, the topic of this review paper.

A commonly prescribed progestin component of hormone therapy, MPA, has been shown to induce long lasting spatial memory impairments on the water radial-arm maze and the Morris water maze in the rodent (Braden et al., 2011, 2010), as has natural progesterone (Bimonte-Nelson et al., 2004; Braden et al., 2010; Sun et al., 2010). It is also noteworthy that administration of allopregnanolone, a progesterone metabolite, was detrimental to episodic memory, but not semantic or working memory, in healthy women (Kask et al., 2008), and it impaired spatial reference memory in young rats (Frye and Sturgis, 1995; Johansson et al., 2002). However, there are many studies showing that progesterone can enhance some cognitive measures in rodents, mostly in the realm of non-spatial memory (Frye et al., 2007; Frye and Walf, 2008; Harburger et al., 2008; Lewis et al., 2008; Orr et al., 2009). The mechanisms of both the detrimental and enhancing effects of progestogens are currently being deciphered in the field, and the work in this area is noteworthy and convincing (Braden et al., 2011, 2010; Fortress et al., 2014; Harburger et al., 2009; Orr et al., 2012; for review see: Singh and Su, 2013a, 2013b). The disconnect between the efficacious and detrimental effects of progestogens on the brain and cognition have yet to be unraveled; this will be a critical factor to consider when designing effective hormone therapies since estrogens and progestogens will need to be combined in many clinical cases. Of course, the brain is not the only consideration when searching for effective hormone therapies. A multiple systems approach must be taken.

Combination estrogen/progestogen treatments have been tested in rodent models. Using middle-aged Ovx rats, the Juraska laboratory has shown that four weeks of tonic 17 β -estradiol plus progesterone treatment, tonic 17 β -estradiol treatment, or acute 17 β -estradiol treatment prevented overnight forgetting in the Morris water maze compared to vehicle controls (Markham et al., 2002). As well, work from this laboratory has demonstrated that six months of chronic treatment with MPA in combination with 17 β -estradiol enhanced learning on a delayed alternation task (Chisholm and Juraska, 2012), but impaired learning on the spatial reference memory Morris water maze task (Lowry et al., 2010), as compared to treatment with 17 β -estradiol alone. The latter results correspond to findings in our

laboratory. We have assessed the cognitive impact of cyclic versus tonic 17β -estradiol administration, alone or combined with cyclic progesterone, in middle-aged Ovx rats using the spatial reference memory Morris water maze as well (Bimonte-Nelson et al., 2006). Results indicated that animals receiving the cyclic biweekly (i.e. every other week) 17β -estradiol treatment performed better on the spatial reference memory Morris maze task compared to Ovx controls, and biweekly treatment was as effective as a low dose of tonic 17β -estradiol treatment. The enhanced performance from a biweekly injection indicates that middle-aged females *can* respond to an intermittent exposure to 17β -estradiol and still benefit from hormone treatment, but with overall less exposure than a tonic treatment; this administration method may additionally mitigate negative effects associated with long-term, chronic estrogen administration (Bimonte-Nelson et al., 2006). Of note, progesterone administration diminished the benefit for both types of estrogen exposure. Clearly, the net cognitive outcome of estrogens is impacted by many factors, and the presence of progestogens is a key player of the result.

Menopause: Etiology, evolution, and estrogens

Etiology: On the physiology of reproductive senescence

Both males and females experience a variety of changes associated with aging. The phenotype as aging ensues differs by sex. In most species it is common for males to retain reproductive capacity well into old age, while females usually experience reproductive senescence beginning in middle age. Different profiles of reproductive senescence in females are noted across species. In humans, it is widely accepted that women are born with a finite pool of immature eggs; only a small proportion of these follicles will mature to end-stage ovulation. Ovarian follicles develop from germ cells during gestation, and these immature eggs exist at various stages of follicular growth at any time point up to reproductive senescence. Beginning in gestation, organizational processes within the ovary, including follicular development and degeneration, take place to set up ovarian function later in life. Throughout a woman's lifetime, the majority of these immature follicles will undergo atresia, and once the follicles are gone, women can no longer reproduce (Hsueh et al., 1994). Although the precise mechanism by which follicles are selected to mature to ovulation is unknown, most immature ova degenerate, and the rate of this attrition likely increases in a woman's 30's and 40's (Richardson et al., 1987); however, others argue that follicle attrition does not accelerate with age (Leidy et al., 1998; Wu et al., 2005). Among the complex cellular and molecular mechanisms involved in follicle development, maintenance, and degeneration, studies using animal models implicate estrogens, insulin-like growth factor 1, follicle stimulating hormone (FSH), and human chorionic gonadotropin in protecting ovarian follicles from undergoing apoptotic atresia; androgens, gonadotropin-releasing hormone (GnRH) (or a GnRH-like substance in the ovary), inhibin proteins, and interleukin 6, likely play a role in regulating follicular atresia (Hsueh et al., 1994). Pinpointing regulatory factors of the maturation and degeneration of ovarian follicles will provide insight into mechanisms of menopause, and could potentially lead to markers of menopause and reproductive senescence for use in clinical diagnosis and practice. Work in this area thus far has afforded our understanding that a drastic drop in estrogen and progesterone levels accompanies menopause as ovarian follicle reserves become deplete and ovulation halts. In

fact, degenerating follicles tend to have an increased androgen to estrogen ratio within the follicular fluid, and that ratio may be key to understanding the role of these steroid hormones in follicular maintenance and attrition (Carson et al., 1981; Hsueh et al., 1994; Maxson et al., 1985; McNatty et al., 1979a, 1979b). Indeed, the androgen androstenedione becomes the primary hormone released from the follicle-deplete ovary after menopause (Timiras et al., 1995), marking the end of an estrogen-dominant hormonal milieu of the female.

The mechanism and pattern of change with age-related reproductive senescence differs between female rats and women. While in women ovarian hormone loss during natural, transitional menopause is ultimately due to follicular depletion, the rat undergoes estropause with aging rendering a persistent estrus state characterized by chronic anovulation and typically resulting in moderate estrogen levels, or a pseudopregnant/persistent diestrus state showing relatively high progesterone levels due to increased ovulation and corpora lutea (Meites and Lu, 1994). While the ovarian follicle pool in rodents also diminishes with aging, hypothalamic-pituitary-gonadal (HPG) axis dysregulation plays the chief role in rodent reproductive senescence, during which time normal and regular cyclic ovulation ceases to occur (Finch, 2014). Importantly, irrespective of the mechanism, at rodent reproductive senescence there are changes in circulating gonadal hormone levels, as well as changes in ratios of these steroid hormones, relative to those in adults of reproductive capacity, and the cyclic nature of the female rodent eventually comes to a halt.

Evolution: conservation of reproductive senescence across time and species

A variety of hypotheses regarding the evolution of menopause have been proposed. The prevalent though debated “Grandmother Hypothesis,” popularized by G.C. Williams in 1957, states that menopause — which was thought to be uniquely human at that time — might be a result of natural selection because it is an adaptive advantage for non-reproducing females to continue to care for, and put resources into, the survival of their genealogical lineage (Kuhle, 2007; Williams, 1957). A grandmother's contribution of resources, particularly food resources, to mothers and children could increase the children's potential to survive to reproductive age as well as maintain the health of the mother (Hawkes et al., 1998). This is in line with the closely related “Mother Hypothesis,” which postulates that births are riskier for the mother at a later age, so it is more advantageous to cease reproductive ability early and invest in surviving children due to the extended period of infancy in humans which necessitates a large parental investment (Hawkes and Coxworth, 2013; Pavard et al., 2008; Wu et al., 2005). As a social species, devoting food resources and continued care to offspring that have a greater chance of survival is more advantageous than continued reproduction of children that may not survive infancy; likewise, mother mortality can compromise survival rates of immature children so maternal maintenance is also an important factor (Hawkes et al., 1998; Kuhle, 2007; Pavard et al., 2008). In sum, the “Grandmother” and “Mother” hypotheses are based on the idea that menopause is an evolutionarily adaptive trait that prevents maternal mortality and supports surviving children to maturity and reproductive age.

But, is menopause really adaptive? Other theories suggest that menopause is simply a result of physiological deterioration with age (Ward et al., 2009) or an artifact of an increased

lifespan (Wu et al., 2005). However, this seems unlikely considering that women tend to live longer than men and that the average lifespan is increasing, yet the age of menopause onset has remained relatively stable (Amundsen and Diers, 1973, 1970; Mennenga and Bimonte-Nelson, 2013; Regan and Partridge, 2013; Seifarth et al., 2012; Sherwin, 2003), indicating that the number of reproductive-age years is not proportionally increasing along with the rising average lifespan. Additionally, the rate at which chimpanzees (the closest living relatives to humans) and human females' ovarian follicles deplete is remarkably similar, yet a chimpanzee's lifespan is much shorter than modern humans and they lack an extended post-menopausal life stage, indicating that humans' unique capacity for longevity evolved long ago (Gems, 2014). Despite the fact that the average life expectancy started to increase in the twentieth century (Hawkes and Coxworth, 2013), anthropological evidence suggests that "menopause and post-reproductive life are not new phenomena" (Sievert, p. 1153, 2014) even in early civilizations and other hominid ancestors (Kuhle, 2007; Sievert, 2014).

Information obtained from female ancient Egyptian mummies indicates that women lived into their forties (Allam et al., 2011), and estimates in early hunter-gatherer populations suggest that some women lived beyond 45 years of age (Hawkes, 2004). If reproductive senescence were simply an epiphenomenon of physical deterioration, it would be plausible to expect males to also go through a complete cessation of reproductive ability (perhaps even earlier due to their shorter average lifespan). Recent evidence suggests that males do experience a decrease in gonadal hormone production and reproductive capabilities, aptly termed "andropause." With andropause, a gradual but progressive decline in androgen levels occurs during aging, albeit less extreme than the drastic drop in gonadal hormone levels women experience associated with menopause (for discussion see Matsumoto, 2002).

Although some research indicates that the quality and quantity of sperm appears to remain relatively stable at least into the sixth decade of life (Gallardo et al., 1996; Matsumoto, 2002), the caliber of sperm vitality into old age remains controversial (for review, see Plas et al., 2001). A recent meta-analysis of human semen quality suggests that sperm viability decreases with age, including decreased motility and increased DNA fragmentation (Johnson et al., 2015). There is some evidence that advanced paternal age is associated with decreased semen quality, reproductive success, and healthy offspring (Kovac et al., 2013). Although the impact of aging on male fertility is less understood, an absolute cessation of reproductive capacities is not a predictable event in the male reproductive lifespan as it is in women. Thinking about menopause in terms of cellular- and brain- driven processes can provide understanding of an inevitable deterioration of a finite follicle pool, but does not necessarily explain the post-reproductive lifespan seen in women and a few other species.

Perhaps the more interesting question is not how or why menopause evolved, but *why* humans have the capacity for a long post-menopausal stage of life, and *why* females remain at all responsive to estrogen in a post-menopausal state. Though whales and elephants may have a comparable post-reproductive lifespan to humans (Kuhle, 2007; Lahdenperä et al., 2014; Ward et al., 2009), they are not close relatives to our species, and extended longevity after reproductive senescence has not been observed in non-human primates (Gems, 2014; Sievert, 2014). Cooperative social dynamics and relatedness to other conspecifics within each species can also be a tool to understand longevity beyond reproductive senescence (Johnstone and Cant, 2010). The "Patriarch Hypothesis" postulates that older men continue

to reproduce late in life with younger women, and this selection for older male fathers increases the probability for offspring to also have a long lifespan. According to this hypothesis, longevity in women is a byproduct of selective mating with older males (Gems, 2014; Kuhle, 2007). This theory also leads to evidence for antagonistic pleiotropy, or the effect that a particular genetic trait may have benefits early in life (e.g. evolutionary selection for high reproductive success), but may involve trade offs resulting in disadvantageous health effects related to androgens and decreased longevity. In fact, some studies suggest that eunuchs, especially when castrated pre-pubertally, tend to have longer lifespans than gonadally-intact men (for discussion see Gems, 2014; Regan and Partridge, 2013; Seifarth et al., 2012), implicating a role for androgens in a shorter lifespan for the phenotypically normal male. Yet another theory involves the evolution of menopause as a side effect of reduced paternal investment, whether by premature death or preference for younger mates (Kuhle, 2007). Clearly, a variety of anthropological and evolutionary theories, often based on social structure, have been put forth to understand the development of reproductive senescence and the post-menopausal life stage. Despite controversial origins, it is clear that some mothers and grandmothers exhibit exceptional longevity. It is possible that their particular aging profile provides them with an evolutionary advantage to support the survival of reproductively viable offspring, and therefore the propagation of their progeny.

Estrogens: Longevity, cellular aging, and menopause status

Telomere length, and more importantly the rate at which they are shortened, is considered to be a marker of cellular aging that impacts longevity (Aviv et al., 2005; Hawkes and Coxsworth, 2013; Pines, 2013). Telomeres are repetitive nucleotide sequences of non-coding DNA that exist at the ends of chromosomes to prevent the loss of critical information located on the chromosome during cell replication (Blackburn and Szostak, 1984). Telomerase, a reverse transcriptase enzyme, maintains these nucleotide sequences and as a result, conserves telomere length and cell functioning. Over time, repeated cell division leads to the gradual shortening of telomeres and eventual cell death (Pines, 2013; Misiti et al., 2000). Recent evidence suggests that estrogens impact telomere length (Aviv et al., 2005, Bayne et al., 2011; Hanna et al., 2009; Lee et al., 2005; Pines, 2013), and play a regulatory role in telomerase activity (Gopalakrishnan et al., 2013; Misiti et al., 2000). Due to this apparent regulatory function, telomeres have been an exciting new avenue to explore in the context of female longevity and menopause status. Females have exposure to estrogens across the lifespan, which is in accordance with the tenet that women tend to have longer telomeres than men, and also tend to outlive men by several years (Aviv et al., 2005; Regan and Partridge, 2013).

What happens to telomere length during reproductive senescence? A study utilizing young aromatase knock out (ArKO) mice indicates that the ArKO animals have less telomerase activity than wild-type mice in granulosa cells in ovarian follicles; further, three weeks of chronic treatment with 17 β -estradiol increased telomerase activity and lengthened telomeres in the ArKO mice (Bayne et al., 2011). Likewise, Gopalakrishnan and colleagues found a correlation between plasma 17 β -estradiol levels and telomerase activity during aging in the Japanese medaka fish, which has an established sex difference in lifespan with female fish living longer than males (Gopalakrishnan et al., 2013). Circulating 17 β -estradiol levels in

female fish were positively correlated with telomerase activity and telomere length, indicating a relationship between estrogens and telomeres (Gopalakrishnan et al., 2013). In accordance with animal studies, post-menopausal women aged 55 to 69 who were on a long-term hormone therapy regimen (more than five years of 0.625 mg of CEE or 2 mg of 17 β -estradiol with a progestin component) had longer telomeres than post-menopausal women within the same age range who had never been exposed to exogenous hormone therapy after menopause (Lee et al., 2005), and correlations have been reported between maternal estriol levels during pregnancy and their infant's telomere length (Entringer et al., 2014). Other research has shown that women with longer telomeres were older at the time of natural menopause, while women who underwent surgical menopause showed no associations between age at menopause and telomere length (Gray et al., 2014), and that women who experienced premature ovarian failure tended to have longer telomeres compared to control participants (Hanna et al., 2009). Of note, especially lending biological support to the previously discussed idea that older fathers positively impact the lifespan of children, a positive correlation between paternal age when an infant is born and telomere length in that child has been noted (Kuhle, 2007; Prescott et al., 2012). Collectively, this growing research area indicates that steroid hormones may have organizational effects on children's telomeres that can be influenced by endogenous estrogens later in life and that exogenous hormone treatment impacts telomerase activity. Further research is necessary to understand the impact of estrogen exposure across the lifetime, cellular aging processes as a marker of reproductive senescence, as well as a potential link to longevity in a post-menopausal state. These cellular aging-based theories and the evolutionary perspectives discussed here act in concert to help piece together a history of longevity in humans, as well as factors contributing to menopause and its transition.

The human menopause transition

Although the cellular aging processes linked to menopause and estrogen exposures are not yet well understood, the field has straightforward knowledge that in women, follicle reserve is correlated with menstrual status (Richardson et al., 1987), and can mathematically predict the age at which menopause occurs (Hansen et al., 2008; Wallace and Kelsey, 2009). What comes first in humans, HPG axis dysfunction or ovarian follicle failure? This has been a “chicken-or-the-egg” question in research for many years, with data supporting evidence for both phenomena contributing to the reproductive senescent state in women. O'Connor and colleagues studied 108 women between the ages of 25-58 over the course of five years who were not users of exogenous hormone treatment, and analyzed menstrual cycle data and daily urine specimens for pregnanediol-3-glucuronide, which is an inactive metabolite of progesterone, as well as FSH, luteinizing hormone (LH), and an estrone metabolite (O'Connor et al., 2009). Women were classified into reproductive stages ranging from regular menstrual through irregular/extended cycles to post-menopause. Results showed that advancing reproductive stage and age were predictors of more anovulatory cycles, with reproductive stage being a stronger predictor than age for the number of anovulatory cycles; only after age 45 did the likelihood of anovulatory cycles increase (O'Connor et al., 2009). Accurately classifying the onset of anovulatory cycles may be a tool that can aid in determining the most beneficial time to administer hormone therapy. Finch notes that

alterations in hypothalamic function are more extreme in aging rodents than in women undergoing the transition to menopause (Finch, 2014), although changes in HPG feedback and sensitivity occur in some women during the menopause transition (Weiss et al., 2004) and estrogen levels in women actually increase during the transitional phase before the final decline in the post-menopausal state (Santoro et al., 1996). In women and in rodent models, changes in FSH and LH secretion patterns precede ovulatory irregularity; these alterations likely induce the variability in menstrual cycles during the menopause transition (Downs and Wise, 2009; Wise et al., 1999). Overall, evidence exists for both neuroendocrine changes and alterations in the ovaries' responsiveness to gonadotropins during mid-life that lead to eventual ovarian failure. Although the question of "what comes first" remains to be conclusively answered, Phyllis Wise's work has suggested that reproductive senescence in the female is ultimately a consequence of a breakdown in communication between the brain and the ovaries that results in the end of the reproductive stage in the female lifespan (Downs and Wise, 2009; Wise et al., 2002; 1999).

Understanding the mechanisms driving menopause is as important as understanding the consequences of menopause if we want to optimize the trajectory of the menopause transition and beyond, with the ultimate goal to obtain excellence in women's health. The same template can be used for estrogen effects: understanding the mechanisms driving estrogen effects is as important as understanding the consequences of estrogenic hormone therapy so that we can optimize quality of life during the menopause transition and beyond.

Natural menopause is, in fact, a transition, not a sudden life event. This provides a multitude of opportunities to optimize and individualize estrogen exposures and timeframes in the context of hormone therapies. In 2001, the Stages of Reproductive Aging Workshop (STRAW) described a staging system ranging from -5 to +2 that encompasses typical stage length, menstrual cycle activity, and endocrine status: -5 to -3 signify the reproductive years, -2 to -1 are considered the menopausal transition, 0 marks the final menstrual period, and +1 and +2 are the early and late post-menopausal life stages, respectively (Harlow et al., 2012; Soules et al., 2001). STRAW resolved that "the menopause transition" is a more appropriate term to use in scientific literature, and that vague and/or inconsistent nomenclature such as "perimenopause" and "climacteric" are generally only appropriate in the clinic and lay press (Soules et al., 2001). The menopause transition usually begins in a woman's 40's and typically lasts six or more years. It is characterized by menstrual cycle irregularity and erratic hormone levels, and is accompanied by an array of physiological indicators including vasomotor symptoms, vaginal dryness and atrophy, sleep and affect disturbances, and memory complaints (Al-Safi and Santoro, 2014; NAMS, 2015; Richardson et al., 1987; Timiras et al., 1995; Weber et al., 2013). The transitional stage extends through menopause (i.e. the last menstrual period). Menopause is retrospectively confirmed after one year of a complete cessation of menses (NAMS, 2015; Hoffman et al., 2012).

Estrogen responsiveness, menopause, and the organizational/activational framework

What does Goldilocks have to do with it?

That menopause is transitional provides opportunities abound for therapeutic windows for estrogen-containing hormone therapy. Preclinical scientists have been systematically testing window of opportunities of estrogen effects on the brain and cognition. The collected rodent research supports the tenet that there is a sensitive window in middle age wherein lasting changes can ensue from a transient exposure to estrogens, even when not present at the exact time of assessment (Figure 6). Perhaps this window of sensitivity is another organizational period wherein estrogen exposure can alter the future aging trajectory in a beneficial way, and this temporary exposure is not as “transient” as once thought – even when the hormone exposure ends, it could initiate a cascade of effects that result in a different brain profile than one that has never been exposed to additional hormone treatment. When we consider the various parameters necessary to obtain beneficial impacts of hormone therapy on cognition, we recognize a Goldilocks phenomenon wherein conditions including, but not limited to, timing, dose, and hormone combination must be *just right* — not too late or too early, not too low or too high, with or without specific other hormones—to achieve efficacious effects. It is probable that organizational, reorganizational, and activational hormone events impact the trajectory of brain profiles during aging. Of note, a “brain profile” or quantitative brain measurement for research purposes, is typically just a snapshot in time, but in life a brain profile is anything but static – it is in flux and variable and dynamic – waxing and waning with repeated and noncontinuous exposures to hormones, other chemicals, and situational life events. The only thing continuous and consistent about hormone exposures across a female's life is that they are variable and changing, building and rebuilding on past exposures to create a current “present” brain and behavioral landscape. Variation and plasticity are especially rich in females, and are likely the destiny for maximal responsiveness in the female brain. Hormone exposures across the lifespan, reproductive history and current state, and the innate tendency for maximal responsiveness to cyclicity should be taken into consideration when researching an effective hormone therapy. Preclinical data suggest that cognitive responsiveness to estrogens can be reinstated, even in old age and after a period of hormone deprivation, given the ideal parameters. What these “ideal” parameters are... that is the query that needs to be answered next, and that is the query many hormone researchers are fervently trying to address. Part of the challenge in addressing this, however, is that what is ideal for one woman may not be ideal for another (see the section: “Estrogen replacement during aging as a viable option for cognitive maintenance or enhancement in women: Translating basic science research to the clinic” for further discussion). Research to understand factors impacting which type of therapy could be efficacious for a woman's profile can be driven forward by continuing methodical and systematic manipulations using preclinical models.

Estrogens and the aging brain

Adding further complexity to the picture, there is evidence that the brain's response to estrogens decreases in old age, and which patterns of hormone administration initiate

optimal responsiveness changes in old age as well. The aging brain can still be responsive to estrogens given the “proper” parameters... if they are just right. Tom Foster's laboratory showed that cognitive benefits of chronic estradiol benzoate treatment on memory consolidation rely on complex age and dose interactions; aged animals required a higher dose of estradiol to produce the same effects seen in young and middle-aged animals with lower doses (Foster et al., 2003). Our laboratory has shown that there is less responsiveness to Ovx and estrogen replacement in old age in rodents. For example, tonic 17 β -estradiol replacement improved reference memory performance on the Morris water maze for young and middle-aged, but not aged, rats (Talboom et al., 2008). In a repeated measures design, Markowska and Savonenko showed that the working memory benefits of long-term tonic 17 β -estradiol treatment were significantly enhanced by priming with estradiol injections (given every four days) in middle-aged Ovx animals (Markowska and Savonenko, 2002). Rodgers and colleagues also found that a transient exposure to 17 β -estradiol in middle-age had a lasting beneficial effect for spatial memory similar to that observed with continuous estrogen treatment in middle-aged Ovx rats, even when the treatment had been discontinued for several months (Rodgers et al., 2010). Thus, the beneficial effects of estrogens were seen even when the “activating” hormone, estrogen, was not present at the time of testing, further supporting the revised operational definitions of the organizational/activational dichotomy. In fact, one may consider these effects to be reorganizational.

Of note, benefits of estrogens on cognition in old age have been seen in non-human primates. Aged Ovx monkeys given cyclic estradiol cypionate (one injection every three weeks) showed spatial working memory enhancements compared to the aged Ovx group; cyclic 17 β -estradiol-induced enhancements were so substantial that performance of this group did not differ from the young group (Rapp et al., 2003). There has been some work testing estrogen effects on cognition in old age using mice as well. Neither daily nor intermittent (every four days) 17 β -estradiol injections for three months aided spatial reference or working memory in Ovx mice aged 21 months at test; in fact, the intermittent treatment impaired both memory types (Gresack and Frick, 2006). These null and negative cognitive impacts of estrogen could be due to different parameters for effectiveness in mice, or to missing a critical window of efficacy since treatment started in late middle age but testing occurred in old age. Further assessment to understand the mechanisms of why variations in responsiveness might be occurring across rodent species could inform parameters for successful and efficacious hormone treatments.

Research in women points to marked variations in the cognitive response to hormone therapy, with both age and timing relative to menopause acting as key players in this variation in responsiveness to exogenous treatment. The Women's Health Initiative Memory Study (WHIMS) indicated that the most commonly used (at that time) estrogen component of hormone therapy, CEE, might be detrimental to memory performance, and that the addition of the progestin MPA increases risk for mild cognitive impairment and dementia (Espeland et al., 2004; Shumaker et al., 2004, 2003; for review see: Coker et al., 2010). These negative findings were not expected and left the field whirling – many were perplexed by the outcome. As scientists and clinicians reflected upon these findings and dug deeper into factors that could have influenced the outcome, much discussion centered on the older age of the women and a potential missed window of opportunity for sensitivity and

responsiveness. Indeed, many women who participated in that study had been deprived of endogenous estrogens for an extended period of time before the therapy began, and they were 65 years of age or over (Shumaker et al., 1998). Recently published preliminary results from the WHIMS-Young cohort, which included women who were 50-55 years of age at the time of CEE hormone therapy with or without MPA, indicate that seven years after experimental trials there were no long-term beneficial or detrimental cognitive effects of hormone therapy when initiated at an earlier age (Espeland et al., 2013); it is possible that hormone administration even earlier, during the early- to mid- menopause transition, would have yielded positive effects (see section: “The human menopause transition”). In fact, other research in women has shown cognitive benefits with hormone therapy being initiated early- to mid- menopause transition (Maki et al., 2011). This cumulative evidence points to temporal factors, including both age and timing of administration, as significant to attaining maximum benefits from estrogen-based hormone therapy that extend into the post-menopausal stage of life.

It is biologically plausible that estrogens can impact the brain in middle-to-old age. For example, Miranda, Williams, and Einstein provide evidence for hippocampal responsiveness to estrogen during an older timeframe; estrogen-induced dendritic spine increases in the granule cells of the dentate gyrus were demonstrated in middle-to-old age (16-20 months old) Ovx rats after estradiol benzoate administration, but not in young (5 months old) Ovx rats (Miranda et al., 1999). Interestingly, these spine increases were seen with a short-term (two consecutive daily injections) cyclic dose, but not with a long-term chronic dose, suggesting that females respond optimally to a cyclic, rather than tonic, exposure to estrogen at a neuronal level. Relevant to sex differences as discussed herein, in this study estradiol benzoate treatment also had divergent effects depending on sex (Miranda et al., 1999). In contrast to the estradiol benzoate-induced increase in spines in older Ovx female rats, there was an estradiol benzoate-induced decrease in older gonadectomized male rats. These divergent responses to estrogen depending on sex and age lend support to the tenet that male and female brains are not only organized to have distinct responses to gonadal hormones, but also may have dynamic responses to the same hormone throughout the lifespan.

Understanding estrogen receptors can help us get hormone therapy just right

In addition to many peripheral tissues, the brain contains ample ERs, including both α and β subtypes. Of the three estrogens, 17 β -estradiol, estrone, and estriol, it is noteworthy that 17 β -estradiol binds to estrogen receptor (ER) ER α and ER β with the strongest affinity as compared to estrone and estriol. Estrone binds these receptors with the next strongest affinity, and estriol binds these receptors with the weakest affinity of the three estrogens (Kuhl, 2005; Kuiper et al., 1997). Moreover, 17 β -estradiol and estrone bind ER α and ER β with about equal preference, while estriol, though a very weak estrogen in comparison, has a slight preference for ER β compared to ER α (Kuiper et al., 1997). The hippocampus, a key brain structure for learning and memory, contains both α and β ERs, with greater levels of ER β than ER α in humans and rats (Foster, 2012). Because ER α transcription processes are more active than ER β , ER β may, in part, serve as a regulator for ER α , at least in the hippocampus (Bean et al., 2014; Han et al., 2013). Indeed, the ratio of ER α to ER β may play a chief role in how estrogens ultimately impact behavior. Estrogens may act through

traditional signaling pathways, as well as exert rapid non-genomic changes associated with a third receptor type referred to as GPR30, a membrane coupled receptor.

Estrogen receptors have been shown to decrease in response to tonic 17 β -estradiol treatment (Brown et al., 1996). It is well documented that cyclic 17 β -estradiol treatment (repeated intermittent injections of estradiol) results in a *temporary* decrease in estrogen receptors beginning within an hour of injection while receptors translocate to the cell nucleus; within about 12 hours, the total number of estrogen receptors again become elevated as they return to the cytoplasm of the cell. This process has been termed receptor recycling, and occurs in brain as well as peripheral tissues (Blaustein, 1993; Kassis and Gorski, 1981; Rosser et al., 1993). Further, there is evidence that 17 β -estradiol injection results in synthesis of new estrogen receptors after about 12 hours post-injection, thereby increasing total receptor levels (Rosser et al., 1993; Sarff and Gorski, 1971). Aged female rats exhibit fewer estrogen receptors compared to young rats in certain brain regions, including cognitive regions such as the cortex (Wilson et al., 2002), and most research suggests that aged female rats further decrease estrogen receptors in response to tonic 17 β -estradiol stimulation (Chakraborty and Gore, 2004 but see Funabashi et al., 2000). Hence, it makes sense that there might be particular estrogen administration regimens that are more beneficial for cognitive function, and that which of these regimens is most efficacious depends on age. Cyclic estrogen treatment may provide more benefits than tonic treatment due to the intermittent interruption of treatment inherent to the cyclic regimen. In fact, there is evidence that intermittent exposure to 17 β -estradiol sensitizes neural tissues to subsequent 17 β -estradiol stimulation, resulting in increased 17 β -estradiol-induced behavioral responsiveness (Clark and Roy, 1983). Moreover, Rodgers and colleagues found a lasting effect of transient 17 β -estradiol exposure in the hippocampus; specifically, short-term exposure increased estrogen receptor-alpha (ER α), but not estrogen receptor-beta (ER β), and effects persisted after treatment had been terminated (Rodgers et al., 2010). These data indicate that intermittent exposure to 17 β -estradiol in middle age could permit lasting benefits on the brain and its functions, including enhancing the ability to respond to estrogens.

Estrogen receptors, estrogen synthesis, and the aging brain

It is important for the field to gain understanding of how exogenous hormone treatments impact receptor function and gene transcription that results in both rapid and long-term effects. Some studies have shown beneficial spatial memory effects when young and middle-aged Ovx females were chronically administered the ER α agonist PPT, the ER β agonist DPN, or the GPR30/GPER1 agonist G-1 (Frye et al., 2007; Hammond et al, 2009), although, as is characteristic of estrogen treatments, an inverted U-shape dose response is likely whereby too low or too high of a dose could cause impairments (Bean et al., 2014). Relative binding affinities to ER α versus ER β , dose, and administration type (i.e. cyclic or tonic) should be considered when assessing current and future hormone therapy options. It is also important to acknowledge recent work regarding the potential for *de novo* neurogenesis of steroids in the hippocampus. In addition to the ovaries, 17 β -estradiol is likely locally synthesized in the brain, including in the hippocampal region (McCarthy, 2011, 2008; Nunez and McCarthy, 2009; Prange-Kiel et al., 2003). There is evidence that aromatase, key to the biosynthesis of estrogens, has neuroprotective actions for a variety of conditions including

brain injury and neurodegeneration. In fact, aromatization of androgens to estrogens may be a key factor in its protective effects, and aromatase activity could be used as a novel target for cognitive maintenance (Azcoitia et al., 2001; Garcia-Segura et al., 1999a, 1999b). Barker and Galea found that local synthesis in the brain could be hindered by a long period of hormone deprivation (Barker and Galea, 2009; also discussed in: Bean et al., 2014), as occurs in the post-menopausal state. Collectively, this work indicates that if hormone therapy is to be utilized, initiating the therapy before the brain and body have experienced an extended period of time with very low estrogen levels would likely be most beneficial to cognition.

An upregulation of ER α in the hippocampus, even in the absence of ovaries or estrogen treatment, may be sufficient to benefit spatial learning and memory. Tom Foster's group used a lentivirus to deliver the gene that encodes ER α directly to the hippocampus of adult Ovx ER α knock out (KO) mice. They found better spatial memory performance in ER α KO mice given the lentivirus compared to ER α KO controls without the virus, suggesting that increasing hippocampal ER α in the absence of giving exogenous estrogens can enhance performance (Foster et al., 2008). Interestingly, female ER α KO mice showed spatial memory impairments compared to wild-type mice, while male ER α KO mice did not show this detriment (Foster et al., 2008; Fugger et al., 2000, 1998), indicating that ER α expression in the hippocampus specifically impacts female performance on spatial memory tasks. Recently, these findings were extended to an Ovx middle-aged rat paradigm. Viral vector delivery of the gene that encodes ER α directly to the hippocampus resulted in cognitive benefits as well as increased ERK/MAPK levels, again without any additional exogenous estrogen treatment (Witty et al., 2012). Further research using viral vector techniques also suggests that decreasing ER β levels in the hippocampus improves spatial memory performance without exogenous 17 β -estradiol treatment, lending support to the idea that the ratio of ER α to ER β is a principal factor in the regulation of estrogens' impact on cognition (Han et al., 2013). The assimilation of this exciting line of research suggests that estrogen receptors in the female brain can remain active even in the absence of endogenous gonadal hormones, and can help researchers illuminate novel treatment options during the menopause transition as well as sustained cognitive performance in a post-menopausal state.

Estrogen treatment during aging as a viable option for cognitive maintenance or enhancement in women: Translating basic science research to the clinic

When, which, and how?

A multitude of studies emerged following the WHIMS assessing the effects of common hormone therapies on memory. Conflicting evidence from the pre-clinical and clinical studies indicates beneficial, detrimental, or null impacts of estrogens on memory. Researchers have set out to elucidate the impact of estrogen therapy on cognition around the time of menopause, and we have quickly learned that there are many factors that contribute to determining the optimal parameters. This is reminiscent of a classic Goldilocks phenomenon. For example, the dose cannot be too low or too high, the duration cannot be too short or too long, the timing cannot be too early or too late, this list could go on.

Conditions need to be *just right*. And, what equals “just right” is not the same for all women. That is, one dose and duration might be optimal for one woman, but suboptimal or even detrimental to another woman. In addition, the animal work indicates that there are a considerable number of potentially interactive factors to bear in mind, including, but certainly not limited to, the type of estrogen given, route of hormone administration, status of reproductive senescence/follicular depletion, age at which hormone is administered, presence or absence of a progestogen, and the presence or absence of ovaries (Acosta et al., 2013; Chisholm and Juraska, 2013; Luine, 2014; Mennenga and Bimonte-Nelson, 2013), as well as the appropriate battery of assessments to tap into estrogen-modulated systems.

A critical period hypothesis regarding the timing of hormone therapy administration and its cognitive outcome has gained substantial support in the rodent and human literature, garnering much discussion and consideration (Acosta et al., 2013; Daniel and Bohacek, 2010; Maki, 2013; Rocca et al., 2011, 2010; Singh et al., 2013; Singh and Su, 2013b). One of the first reports indicating the importance of the timing of hormone therapy in an animal model comes from the laboratory of Robert Gibbs, stemming in part from data showing that weekly injections of 17 β -estradiol and progesterone administered shortly after Ovx improved spatial memory task acquisition, but the same treatment administered several months after Ovx did not produce this benefit (Gibbs, 2000a).

The window of opportunity for exogenous hormone exposure likely has to do with neuroendocrine changes associated with erratic endogenous hormone levels concomitant with the menopause transition. One system of particular relevance and interest is the cholinergic system. The cholinergic system is intimately linked to cognition and shows age-related alterations; moreover, as discussed earlier, it is impacted by estrogen exposure (e.g., Daniel and Bohacek, 2010; Gibbs, 2000b, 2000c; Luine et al., 1985). It is therefore not surprising that growing evidence points to the cholinergic system as a common denominator in the relationships among aging, memory, and estrogens. Gibbs showed that long-term, but not short-term, deprivation of gonadal hormones decreased cellular ChAT mRNA levels in the basal forebrain of middle-aged Ovx rats (Gibbs, 1998), and that injection of estradiol benzoate with or without progesterone, or continuous administration of 17 β -estradiol alone, increased ChAT activity after two weeks compared to control animals; however, these effects were not apparent after four weeks (Gibbs, 2000c). Moreover, middle-aged rats receiving tonic 17 β -estradiol treatment immediately after Ovx showed increased hippocampal ChAT protein levels compared to Ovx rats that received the same hormone treatment five months after Ovx (Bohacek et al., 2008). Increased ChAT protein levels remained elevated in the hippocampus for at least two months after 17 β -estradiol treatment was terminated (Rodgers et al., 2010), indicating a role for estrogens promoting long-lasting neuroprotective effects if they are administered during a window of opportunity in middle-age, potentially during the menopause transition.

Further evidence supporting a critical window of opportunity for exogenous estrogen treatment on the brain and cognition comes from a systematic series of elegantly performed studies from the Daniel laboratory. In 2009, work from this laboratory lent support to the tenet that timing of estrogen administration matters for brain regions subserving learning and memory (Bohacek and Daniel, 2009). Middle-aged rats that were given tonic 17 β -

estradiol immediately after Ovx showed increased ER α levels in the hippocampus, and there was no change in the prefrontal cortex; however, when tonic 17 β -estradiol treatment was started five months after Ovx, 17 β -estradiol increased ER α in the prefrontal cortex, and there was no change in the hippocampus (Bohacek and Daniel, 2009). Because the prefrontal cortex is a brain region important for attentional processes, subsequent evaluations focused on whether attention was impacted by estrogens, and whether the impact was variable depending on timing of administration relative to Ovx, as well as duration (length) of treatment (Bohacek and Daniel, 2010). Results showed that both timing of hormone treatment relative to Ovx, and duration of hormone treatment, did indeed impact attention. Specifically, rats receiving tonic 17 β -estradiol treatment immediately after Ovx at 17 months old showed enhanced attention compared to Ovx rats receiving vehicle; yet, 17 month old rats receiving tonic 17 β -estradiol treatment initiated immediately after Ovx at 12 months of age (i.e. five months of continuous 17 β -estradiol exposure) did not show enhancements. These effects suggest that in 17-month old animals, a history of short-term tonic exposure benefits attention, but long-term tonic exposure does not. Moreover, supporting the tenet that timing relative to Ovx impacts performance, Ovx rats receiving 17 β -estradiol treatment at 17 months old, after a five-month ovarian hormone deprivation window, did not show improvements in attention (Bohacek and Daniel, 2010). Similar 17 β -estradiol treatment, parameters, and ages were used to test spatial working memory, and again, enhancements were seen after immediate, but not delayed, treatment (Daniel et al., 2006). Of note, in these studies the middle-aged rats were all retired breeders, so responsiveness to these particular parameters of estrogen treatment may be impacted by prior reproductive experience, as discussed earlier; nulliparous rats could have a different response to the same treatment.

Several animal models exist as translational tools to help us better understand aging and reproductive senescence (Acosta et al., 2013; Brinton, 2012; Mennenga and Bimonte-Nelson, 2013). Much of our understanding of the impact of estrogens on cognition comes from the “blank slate” Ovx rat model, in which the primary source of endogenous gonadal hormones, the ovaries, is removed before exogenous hormone therapies are administered¹. This Ovx model has given us invaluable insight into the specific effects of ovarian hormone loss and exogenous titrated hormone treatments, both individual and combined. Yet, the reality is that most women retain their ovaries and any type of hormone therapy will be acting in concert with numerous other steroid hormones, as well as other body systems, to produce its net behavioral effect. Of note, the VCD model has been, and will continue to be, tremendously advantageous in answering questions about such interactions in that it allows systematic study via induced depletion of ovarian follicles in rodents to more closely model transitionally menopausal women who maintain ovaries (Acosta et al., 2010, 2009b; Mayer et al., 2004, 2002). Animal models could help decipher specifics regarding the impact of follicular depletion on multiple domains and systems (brain and otherwise), noting that predicting menopause status in women could be an immensely clinically beneficial tool. For example, Hansen and colleagues correlated biomarkers of ovarian reserve — including antral follicle count via transvaginal ultrasound; inhibin B, 17 β -estradiol, FSH, and anti-Müllerian hormone (AMH) assays; and quantification of primordial and non-growing

¹It is of note that estrogen can still be locally produced from non-ovarian sources, such as in adipose and breast tissues.

follicles after benign oophorectomy — with the STRAW staging system using menstrual cycle status in premenopausal women. They found a progressive decrease in ovarian reserve of non-growing follicles as the STRAW stages advanced into the menopause transition, and concluded that menstrual cycle classification using the STRAW staging system can inform us about ovarian reserve over and above age alone to better predict the age of menopause onset (Hansen et al., 2012). AMH levels and antral follicle counts appeared to be particularly promising biomarkers of ovarian reserve, as they strongly correlated with ovarian reserve (Hansen et al., 2011). The ability to accurately predict menopause, especially some time before the onset of the menopause transition, has important implications for fertility and overall women's health (for discussion see: Hansen, 2013; Hansen et al., 2012, 2011). Using clinical data as well as translational animal models to predict ovarian failure and reproductive senescence may also shed light on a critical window of opportunity for hormone therapy during the menopause transition.

Hormone therapy options: past, present and future

Since the 1940's when CEE was first on the market, there has been excellent research studying innovative options for hormone therapy. However, at the present time there are still limited alternatives for hormone therapy, and few novel options have come to fruition to use clinically. Those non-CEE options that are widely available utilize 17 β -estradiol administered in various ways (Hoffman et al., 2012), such as:

- ◆ transdermal patches
 - ❖ Alora, Climara, Menostar, Vivelle-dot; in combination with levonorgestrel (Climara Pro) or norethindrone acetate (CombiPatch)
- ◆ gels
 - ❖ *Estrogel, Estrasorb, Divigel, Elestrin, Evamist*
- ◆ oral formulations
 - ❖ Estrace; in acetate form as Femtrace; in combination with the progestin norethindrone acetate (Activella) or drospirenone (Angeliq); or via the synthetic estradiol, ethinyl estradiol, given orally in combination with the progestin norethindrone acetate (Femhrt)

Selective estrogen receptor modulators may be promising options that need further optimization and research (for discussion see Luine, 2014; Pinkerton and Thomas, 2014). Additionally, some hormone therapy options, albeit less common, include the addition of an androgen, often testosterone, to the estrogenic and/or progestogen component. Although controversial, hormone therapy including androgens in menopause has been reported to improve libido, sexual function, and overall well-being, particularly in women with hypoactive sexual desire disorder and after surgical menopause (Hoffman et al., 2012). However, more research is needed to corroborate the efficacy of androgen therapy during the menopause transition and beyond. Moreover, given that another androgen, androstenedione — a precursor to the biochemical synthesis of testosterone and estrone — has been associated with spatial memory impairments in middle-aged female rodents (Acosta et al.,

2010; Mennenga et al., 2014), the use of androgens in hormone therapies for women should be further evaluated for its long-term impact on cognitive function.

In recent years, there has been a trend toward popularizing “bioidentical” hormone therapy and personalized therapy options, as well as non-estrogenic therapy alternatives. The Endocrine Society defines bioidentical hormones as compounds with an identical chemical and molecular structure to endogenously, or naturally, produced hormones (The Endocrine Society, 2006). Bioidentical hormone therapy options could include compounding hormones, creating ratios of 17 β -estradiol, estrone, and estriol, and/or other hormones such as progesterone and androgens into a therapy. The Endocrine Society and the North American Menopause Society recommend women continue to use FDA-approved therapies, whether it be CEE or a 17 β -estradiol-based therapy, and avoid personalized bioidentical compounds until more research evaluates the efficacy, reliability, and safety of these compounds (The Endocrine Society, 2006; Pinkerton, 2014). Focusing on efficacy, reliability, and safety of potential components used in bioidentical hormone therapies is an important endeavor, and will help in the creation of a personalized approach that is standard for care.

The ongoing longitudinal, multi-site, Study of Women's Health Across the Nation, also known as SWAN, is a tremendously informative and multi-dimensional research project aiding in understanding of the menopause transition and other mid-life experiences in women. Recent publications from SWAN have discussed adrenal steroids as a meaningful factor in hormone profiles and symptomology during the menopause transition, and indicate that the variations seen in the increase in weaker androgens during the transition could account for some of the large variation in phenotypes observed in the transition to a post-menopausal state, especially because androstenediol has slight estrogenic properties (Lasley et al., 2012, 2011). Since such variability exists within the population we can only optimize parameters once we better understand the factors that contribute to the multitude of outcomes that impact women's overall health from a multiple systems perspective. Revisiting the traditional organizational and activational framework in the context of possible reorganizational periods throughout the lifespan can give us insight into the variability we see among women, as well as ideas for novel approaches in both basic science and the clinic. Although the Goldilocks phenomenon is a real occurrence when considering the efficacy of estrogen-based therapy for women, personalized treatment options are not a fairy tale – they are a real possibility in the near future to remedy symptomology associated with the menopause transition and optimal aging trajectories in the post-menopausal life stage.

Conclusions: Reflections on reproductive senescence and menopause as the end of an additional critical period

As research has progressed, evidence has accumulated that makes it clear that the parameters of masculinization and gonadal hormone effects on the male brain do not also define the parameters of feminization and gonadal hormone effects on the female brain. In the female, there are likely multiple critical periods of estrogen exposure that impact and organize the brain (Figure 6). There are data demonstrating that the female brain can be organized into

adulthood, therefore with a later critical window as compared to males, and beneficial effects of estrogens given for a short timeframe in young adulthood are maintained even after estrogens are no longer present (e.g., Bimonte et al., 2000a; Rodgers et al., 2010). Moreover, data indicating long-term brain and behavioral changes associated with pregnancy (Barha and Galea, 2011; Gatewood et al., 2005; Macbeth et al., 2008; Macbeth and Luine, 2010; Pawluski et al., 2005) and some progestins used in hormone therapy (Braden et al., 2011, 2010), support the tenet of non-transient effects of steroid hormone exposures across the lifespan. These data lead the mind of the scientist to consider the principle that estrogens could organize and reorganize the female brain even later in life. In fact, this could explain the attenuated responsiveness of estrogens in old age. It is possible that decreased responsiveness to estrogens in old age might in fact reflect the end of an additional critical period in the female. This could explain the age-related decreased sensitivity to estrogen exposure. Whether the mechanisms underlying this change in sensitivity in old age are indeed the same mechanisms driving the end of the critical period in early development remains to be determined. Understanding these mechanisms and identifying factors that allow manipulation of the window of estrogen sensitivity is an exciting direction in the field of translational behavioral endocrinology. Findings could ultimately provide the potential for methodical alterations of responsiveness to estrogens in women, allowing a host of estrogen-sensitive systems to attenuate, shut down, or enrich responses, in turn yielding an optimal aging profile using a personalized individualized approach. Many will continue to search for the key to understanding estrogens' intricate relationships with cognitive processes; while we have unlocked many discoveries and have learned much, we still have much to learn and unexplored routes to travel. Indeed, "still round the corner there may wait, a new road or a secret gate" (J.R.R. Tolkien, 1954).

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Highlights

- ❖ Estrogens actively organize, reorganize, and activate the female brain across the lifespan
- ❖ A Goldilocks phenomenon exists for estrogens; efficacy might present if exposure is just right
- ❖ Exogenous estrogens may benefit cognition when given in a window of opportunity such as the menopause transition
- ❖ Estrogen sensitivity changes with age, perhaps reflecting a sensitive window closure after the menopause transition
- ❖ Research on organizational/activational hormone actions may inform ways to extend brain sensitivity into post-menopause

Figure 1.
a: The standard model of mammalian sexual differentiation holds that male genitalia actively develop in the presence of androgens, while female genitalia develop in the absence of gonadal hormone exposure. 1b: The traditional model of sexual differentiation of the brain holds the same “default” concept for females as the model for genitalia development; the updated model builds on these concepts using new research, and suggests that sexual differentiation of the brain involves active processes of gonadal hormone exposure for both males and females. E = estrogen, T = testosterone

Figure 2.

A flow diagram of sexual differentiation of the mammalian brain and phenotype. The classic model holds that genetic sex determines gonadal sex, and that gonadal sex determines phenotypic sex. The phenotype is directly impacted by hormone secretions from the gonads, occurring during early life as organizational effects, and later in life as activational effects. An updated model incorporates more flexibility in these definitions, and acknowledges newer data showing that females can be sensitive to non-transient steroid hormone effects later in life. Further, phenotypic sex is indirectly impacted by genetic sex via gonadal sex and subsequent hormone release, but accumulating evidence demonstrates that genes can directly impact phenotypic sex as well.

Figure 3.

In rodents, testosterone of testes origin indirectly masculinizes the male brain. Testosterone readily crosses the blood brain barrier and is converted in the brain to 17β -estradiol via the aromatase enzyme, and this 17β -estradiol exposure results in masculinization. The plasma protein alpha-fetoprotein has a high binding affinity and capacity for estrogens in rodents. Once bound to alpha-fetoprotein, estrogen can no longer cross the blood brain barrier. This scenario prevents estrogen-induced brain masculinization in females during the early perinatal timeframe, when extra-ovarian estrogens are present. Remarkably, alpha-fetoprotein levels become undetectable in the brain after P7, around the same time that the ovaries increase in activity and produce detectable levels of gonadal hormones. The extended temporal period of brain sensitivity to gonadal hormones in females suggested in many rodent studies corresponds with the work showing that estrogens of ovarian origin are likely necessary for normal female brain development, and that they can access the brain when alpha-fetoprotein declines after the early perinatal period. Of note, temporally, this is after the window for normal brain masculinization has closed. E = estrogen, T = testosterone

Figure 4.

a) A schematic of an eight-arm radial-arm maze with a subset of arms baited to represent a working and reference memory task. The arms are baited with a food reward in the land version of the maze, and a hidden platform just beneath the surface of the water in the water escape version of the maze. b) A schematic of the Morris water maze, a reference memory task, with the hidden platform positioned in the northeast quadrant of the maze.

Serum Estrogen Levels With Cyclic Versus Tonic Administration Regimens **Figure 5.**

A graphical representation of the way route of administration for estrogens impacts serum levels across time. A cyclic regimen, such as a daily injection in rodents or a daily pill in women, generally results in a rise and fall in circulating hormone levels each day. On the other hand, a tonic regimen, such as a subcutaneous implant in rodents or a transdermal patch in women, generally results in a relatively steady state of circulating hormone levels across time. Research suggests that a cyclic regimen of hormone administration may impart the most beneficial effects for a positive cognitive profile in females.

Figure 6.

Several critical windows of particular sensitivity to estrogen exposure occur across the female lifespan. There is a non-transient impact of estrogen exposures beyond the initial critical organizing period during development which have long-lasting, and potentially permanent, effects on the brain. We hypothesize that these effects, which could be considered reorganizational, impact the way the brain behaves later when estrogen exposures again occur. Activational, organizational, and reorganizational hormone effects are dynamic and multidimensional, and build upon each other to create the phenotype across the lifespan. It is noted that the majority of research from which this model derives is in the rodent; across species the temporal aspects of brain development vary, and this could further impact interpretation of when organizational and activational actions occur.