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ESTROGENS AND AGE-RELATED MEMORY DECLINE IN RODENTS: WHAT HAVE WE LEARNED AND WHERE DO WE GO FROM HERE?

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Abstract

The question of whether ovarian hormone therapy can prevent or reduce age-related memory decline in menopausal women has been the subject of much recent debate. Although numerous studies have demonstrated a beneficial effect of estrogen and/or progestin therapy for certain types of memory in menopausal women, recent clinical trials suggest that such therapy actually increases the risk of cognitive decline and dementia. Because rodent models have been frequently used to examine the effects of age and/or ovarian hormone deficiency on mnemonic function, rodent models of age-related hormone and memory decline may be useful in helping to resolve this issue. This review will focus on evidence suggesting that estradiol modulates memory, particularly hippocampal-dependent memory, in young and aging female rats and mice. Various factors affecting the mnemonic response to estradiol in aging females will be highlighted to illustrate the complications inherent to studies of estrogen therapy in aging females. Avenues for future development of estradiol-based therapies will also be discussed, and it is argued that an approach to drug development based on identifying the molecular mechanisms underlying estrogenic modulation of memory may lead to promising future treatments for reducing age-related mnemonic decline.

Keywords

Estradiol; aging; hippocampus; rat; mouse; menopause; hormone therapy

Introduction

Can estrogen therapy reduce cognitive decline in menopausal women? This seemingly simple question has sparked considerable debate in recent years due to reports from the Women's Health Initiative Memory Study (WHIMS) indicating that treatment with estrogens, either alone or in combination with progestin, failed to prevent age-related memory decline in menopausal women and increased the risk of cognitive decline and dementia (Espeland et al., 2004; Rapp et al., 2003b; Shumaker et al., 2004; Shumaker et al., 2003). Although a follow-up study from the WHI Study of Cognitive Aging (WHISCA) revealed a trend for a positive effect of estrogen plus progestin treatment on figural memory, it also reported that treatment

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impaired verbal memory and had no effect on tests of attention, working memory, spatial ability, fine motor speed, affect, and depression (Resnick et al., 2006). The findings of the Women's Health Initiative (WHI) stand in sharp contrast to previous studies linking ovarian hormone loss to an increased risk of Alzheimer's disease (Launer et al., 1999; Sherwin, 1999; Wolf and Kirschbaum, 2002; Yaffe et al., 2000b; Yaffe et al., 1998; Zandi et al., 2002a), and estrogen therapy to a decreased risk of Alzheimer's disease (Hogervorst et al., 2000; Tang et al., 1996; Yaffe et al., 1998; Zandi et al., 2002b). The WHI reports also conflict with reports from studies enrolling fewer subjects indicating that estrogen therapy in some menopausal women with (Asthana et al., 2001; Asthana et al., 1999; Yaffe et al., 2000a) and without (Caldwell and Watson, 1952; Duff and Hampson, 2000; Duka et al., 2000; Hogervorst et al., 2000; Maki et al., 2001; Sherwin, 1999; Smith et al., 2001; Yaffe et al., 1998) Alzheimer's disease can reduce multiple types of memory decline (although see (Henderson, 2006; Mulnard et al., 2000; Resnick and Henderson, 2002; Wang et al., 2000)). Indeed, the negative findings from WHIMS and WHISCA, combined with the increased risks of breast cancer, stroke, and heart disease reported by the larger WHI studies (Chlebowski et al., 2003; Rossouw et al., 2002; Wassertheil-Smoller et al., 2003), were a surprise to many scientists, physicians, and patients. The WHI trial, the largest of its kind to date, was designed to examine effects of the commonly prescribed Premarin® and PremPro® hormone treatments on many aspects of women's health (e.g., cancer, vascular function, osteoporosis, cognitive function), and its outcome precipitated both a rapid reduction in the number of women taking hormone therapy and substantially altered recommendations for hormone doses and duration of treatment.

Upon reflection, however, the WHI findings are not terribly surprising for numerous reasons, many of which have been articulated elsewhere (Craig et al., 2005; Maki, 2004; Sherwin and Henry, 2008). Among the criticisms leveled against the WHI study design are the fact that subject were too old to benefit from treatment and were not healthy prior to study enrollment. Further, the conjugated equine estrogen formulation of Premarin® is not as potent as estrogen treatments used in other studies (Sherwin and Henry, 2008), and the progestin in PremPro® (medroxyprogesterone acetate) is less neuroprotective than natural progesterone (Nilsen and Brinton, 2003). Premarin®, prescribed to relieve symptoms of menopause, first entered the market in 1942, well before the publication of data supporting an effect of sex-steroid hormones on cognitive function (Caldwell and Watson, 1952). Research using rodents and non-human primates (Hao et al., 2003; Hao et al., 2006; Rapp et al., 2003a; Tang et al., 2004; Tinkler et al., 2004) has since revealed that estrogens and progestins can significantly alter the physiology of "cognitive" regions of the brain, such as the hippocampus and prefrontal cortex, but because this basic research is in its relative infancy, it cannot yet provide the critical information necessary for the design of hormone-based therapies that maximize cognitive benefit. As such, many important questions remain to be addressed. This review aims to identify the issues most crucial to understanding the importance of ovarian hormones to modulating memory in aging females and to provide an overview of data from animal models of cognitive aging which may help shed light on these issues. Because research on the effects of ovarian hormones on memory in aging female rodents (rats and mice) has not previously been reviewed, rodents will be the focus of this discussion. In addition, because the vast majority of this work to date has examined effects of estrogens on types of memory that involve the hippocampus, hippocampal-dependent memory will be discussed most extensively. However, other brain regions and ovarian hormones (e.g., progesterone) will be discussed as appropriate. Finally, directions for future research will be discussed.

Estrogens and the brain

Understanding how estrogens modulate memory can be challenging for numerous reasons, not the least of which is that many brain regions subserve memory formation. With regard to estrogenic modulation of memory, types of memory involving the hippocampus have been

most extensively studied due to the numerous effects of estrogens on this structure (see (Spencer et al., 2008; Woolley, 2007) for recent reviews) and the importance of this brain region in multiple types of memory. The hippocampus, a bilateral medial temporal lobe structure, is critical for various types of memories involving spatial, relational, and contextual information, and is necessary only for consolidation of such memories, not their long-term storage (Eichenbaum, 1997; Eichenbaum, 2002; Squire, 1992). Further, the vulnerability of the hippocampus to aging and Alzheimer's disease (deToledo-Morrell et al., 2007; Driscoll and Sutherland, 2005) makes this brain region of particular interest to the study of estrogens and age-related cognitive function. The basal forebrain, through which the hippocampus receives subcortical information, and temporal cortices adjacent to the hippocampus (e.g., entorhinal and perirhinal cortices), through which the hippocampus receives cortical information, are also particularly vulnerable to the detrimental effects of aging and Alzheimer's disease (Hof and Morrison, 2004). Other brain regions play important roles in different types of learning and memory, for example, the amygdala in emotional memory, the striatum in response learning, and the prefrontal cortex in working memory and executive function (Eichenbaum, 2002; Squire, 2004). Although these brain regions form intricate networks that include the hippocampus, each has distinct memory functions separate from the hippocampus (Eichenbaum, 2002; Squire, 2004).

Estrogen receptors are located throughout the brain, including most of the aforementioned brain regions. The two nuclear estrogen receptors, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ), can be found throughout the cerebral cortex, hippocampus, basal forebrain, and amygdala of the mouse, rat, primate, and human (Milner et al., 2005; Milner et al., 2001; Osterlund et al., 2000; Shughrue et al., 1997a; Shughrue et al., 1997b; Shughrue and Merchenthaler, 2000; Shughrue et al., 2000). In the neocortex, ERα mRNA expression has been weakly detected in laminae IV–V, whereas ERβ mRNA was strongly detected throughout the cortex, particularly in the frontal, parietal, and entorhinal cortices (Osterlund et al., 2000; Shughrue et al., 1997b). ERα and ERβ are expressed in the amygdala in the medial, cortical, and amygdalohippocampal subdivisions (Osterlund et al., 2000; Shughrue et al., 1997b), and ERα expression has also been reported in the central nucleus of the rat (Shughrue et al., 1997b). In the basal forebrain, both receptors colocalize with cholinergic neurons (most of which project to the hippocampus and neocortex), although this is true more for ERα than ERβ (Shughrue et al., 2000). Both receptors are also expressed throughout the dorsal and ventral extent of the hippocampus, particularly in pyramidal neurons of the CA1 and CA3 regions (Shughrue and Merchenthaler, 2000). More recent evidence suggests that these receptors are not limited to the cell nucleus and are, in fact, located throughout neurons in the hippocampus. Ultrastructural evidence demonstrates that ERα is present in the nuclei and cytoplasm of GABAergic interneurons, and in the cytoplasm of pyramidal and granule cells (Milner et al., 2001). In pyramidal neurons, both receptors are also located in dendritic spines, axons, and axon terminals, where ERβ is more widely expressed at extranuclear sites (Milner et al., 2005; Milner et al., 2001). Collectively, the available data demonstrate that both ERs are expressed in brain regions that are critical for learning and memory, thereby providing an opportunity for estrogens to modulate the functioning of these brain regions and the memory processes they subserve. As detailed later in this review, the fact that ERα and ERβ are located at extranuclear sites within hippocampal neurons provides a multitude of potential mechanisms through which these receptors can modulate hippocampal function and hippocampal memory.

Estrogens comprise a class of steroid hormones that includes three biologically significant members: estradiol, estrone, and estriol. All three estrogens are synthesized from the androgens testosterone and androstenedione by the enzyme aromatase. Because androgens are synthesized from progestins, such as progesterone, progestins are obligatory precursors to both androgens and estrogens. In females, the primary sources of estrogens and progestins are the ovaries, although members of both classes of hormones can also be synthesized in the brain

(Hojo et al., 2004b; Kretz et al., 2004; Robel et al., 1995). At puberty, the ovaries begin to produce these hormones in a cyclic fashion in response to hormone signals from the brain. Of most importance to the present discussion is the timing of hormone peaks and troughs. At the beginning of the menstrual cycle, estrogen and progestin levels are low. As ovarian follicles mature, levels of estrogens increase and reach peak levels just prior to ovulation, after which levels decrease to baseline just before the next cycle. Progesterone levels begin to rise just after ovulation, remain elevated through the second half of the cycle, and then decrease to baseline just prior to the next cycle (unless fertilization and implantation occur). In laboratory rodents, such as rats (*Rattus norvegicus*) and mice (*Mus musculus*), this cycle is termed an "estrous cycle", which differs from the menstrual cycle in several ways, including the lack of a true luteal phase and the absence of uterine wall sloughing (Wise, 2000). However, cyclic hormone fluctuations are similar in many respects among rats, mice, and humans, including the surges of estradiol and progesterone just prior to ovulation (McCarthy and Becker, 2002). The rodent estrous cycle is just 4–5 days long, each day corresponding roughly to one of four phases. Of these phases, the adjacent proestrus and estrus phases are particularly noteworthy, where proestrus is characterized by peak estradiol and progesterone levels, and estrus is characterized by trough estradiol and progesterone levels (Allen, 1922; Long and Evans, 1922; McCarthy and Becker, 2002).

Incredibly, the drop in estradiol and progesterone levels that occurs within the approximately 24 hours between proestrus and estrus gives rise to extraordinary alterations in the morphology and physiology of the hippocampus. Indeed, the current study of estrogenic modulation of memory can primarily trace its origins to the seminal discovery that dendritic spine density in the CA1 subregion of the hippocampus is approximately 30% higher during proestrus than during estrus (Woolley et al., 1990; Woolley and McEwen, 1992). Subsequent studies have demonstrated that other aspects of hippocampal physiology fluctuate in a cyclic manner; for example, both CA1 long-term potentiation (Warren et al., 1995) and dentate gyrus neurogenesis (Tanapat et al., 1999) are enhanced during proestrus relative to estrus. Bilateral removal of the ovaries (ovariectomy) also significantly decreases CA1 dendritic spine density, and treatment with the potent estrogen 17β-estradiol (E_2; two injections spaced 24 hours apart) prevents this decrease (Gould et al., 1990; Woolley and McEwen, 1992). Progesterone injection 48 hours after the last E_2 injection initially increases CA1 dendritic spine density, but then sharply decreases spine density more than is observed with E_2 alone (Gould et al., 1990; Woolley and McEwen, 1993). Similar increases in CA1 dendritic spine density have been observed in young and aged rhesus monkeys after cyclic estradiol cypionate treatment (Hao et al., 2003). Despite the fact that both hormones so profoundly affect spine density, the vast majority of subsequent research into hormonal modulation of the hippocampus (and of hippocampal-dependent memory) has focused on E_2. Among the numerous effects of exogenous E_2 on hippocampal function (reviewed in (Woolley, 2007)) are enhancements in baseline synaptic excitability and the magnitude of long-term potentiation (Foy et al., 1999; Woolley, 2007), and suppression of long-term depression (Vouimba et al., 2000). This increased plasticity may result from the activation of N-methyl-D-aspartate (NMDA) receptors on CA1 pyramidal neurons (Woolley and McEwen, 1994; Woolley et al., 1997), made possible, in part, by E_2-induced inhibition of GABA synthesis in the inhibitory interneurons that regulate pyramidal neuron function (Hart et al., 2001; Murphy et al., 1998).

Further, E_2 can influence hippocampal and neocortical plasticity indirectly by enhancing cholinergic input from hippocampal- and cortically-projecting cholinergic basal forebrain neurons (e.g., Gibbs and Aggarwal, 1998; Wu et al., 1999). Among the many effects of E_2 on these neurons, basal forebrain mRNA levels of the cholinergic synthetic enzyme choline acetyltransferase (ChAT) fluctuate during the estrous cycle and are increased in response to E_2 and progesterone after ovariectomy (Gibbs, 1996; Gibbs et al., 1994; Luine, 1985). Neocortical, hippocampal, and basal forebrain ChAT activity and acetylcholine release are also

enhanced by E_2 (Frick et al., 2002a; Gibbs, 2000a; Gibbs et al., 1997), as is high affinity choline uptake (O'Malley et al., 1987; Singh et al., 1994). This modulation of hippocampal and neocortical function by basal forebrain cholinergic neurons is critical with respect to aging, given that pathological changes in these neurons are associated with memory dysfunction in Alzheimer's disease (Auld et al., 2002; Pappas et al., 2000; Perry et al., 1978; Whitehouse et al., 1982).

In addition to the basal forebrain, E_2 influences levels of neurotransmitter systems in other mnemonic brain regions in rodents. For example, in the amygdala, E_2 reduces levels of monoamine oxidase and ChAT (Luine et al., 1975), but increases levels of dopamine and metabolites for norepinephrine and serotonin (Bowman et al., 2002). In the hippocampus, E_2 decreases levels of the serotonin metabolite 5-HIAA, but increases norepinephrine levels (Bowman et al., 2002; Renner and Luine, 1986). In the prefrontal cortex, levels of dopamine, norepinephrine, and serotonin are reportedly decreased after chronic E_2 treatment in ovariectomized rats (Luine et al., 1998). Cyclic E_2 treatment also increases spine density in the dorsolateral prefrontal cortex, but not primary visual cortex, of young rhesus monkeys (Tang et al., 2004), indicating that E_2 influences synaptic plasticity in specifically in cortical regions critical for mnemonic functioning.

Accumulating evidence suggests that many E_2-induced alterations in neural plasticity may be mediated by rapid signal transduction mechanisms. Estrogens have traditionally been thought to act via a "genomic" mechanism, by binding to ERα and ERβ, which act as nuclear transcription factors when the hormone-receptor complex binds to an estrogen response element on DNA. Although many effects of E_2 on the brain are likely the result of genomic action on estrogen response elements, many "non-genomic" mechanisms of estradiol action have recently been identified, including activation of various intracellular signaling cascades. For example, the fact that E_2's effects on baseline hippocampal synaptic transmission and LTP are blocked by protein kinase inhibitors (Gu et al., 1999) suggests that activation of intracellular signaling cascades is necessary for E_2-induced enhancements of hippocampal excitability. Subsequent work has indicated that E_2 can activate signaling cascades that are critical for memory (Adams and Sweatt, 2002), such as the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) (Fitzpatrick et al., 2002; Kuroki et al., 2000; Wade and Dorsa, 2003) and phosphatidylinositol 3-kinase (PI3K) cascades (Mannella and Brinton, 2006; Yokomaku et al., 2003). Further, E_2-induced enhancement of basal forebrain cholinergic function (Pongrac et al., 2004) and CA1 spines (Ogiue-Ikeda et al., 2008) has been shown to depend on ERK activation. These findings are supported by evidence placing ERα and ERβ at extra-nuclear sites throughout hippocampal neurons, including dendritic spines and presynaptic terminals (Milner et al., 2005; Milner et al., 2001). Traditional ERα and ERβ have even been shown to promote gene expression by binding directly to the Fos/Jun complex, thereby entirely bypassing estrogen response elements (Webb et al., 1995). The presence of estrogen receptors in the cell membrane has also been reported, and although the nature of these receptors remains unclear (Toran-Allerand, 2004), it appears as if E_2 can activate signaling cascades by binding to these putative receptors (Fernandez et al., 2008; Kuroki et al., 2000). Interestingly, the enzymes necessary to synthesize E_2 are expressed in the hippocampus, and hippocampal slices can produce E_2 when stimulated by NMDA (Hojo et al., 2004a), suggesting that locally synthesized E_2 may mediate the rapid effects of E_2 on hippocampal physiology. The implications of such rapid changes in hippocampal signaling for future hormone therapy development will be discussed later in this review.

Estradiol and memory in young females

The extant literature generally supports the conclusion that E_2 promotes hippocampal function, which leads to the obvious hypothesis that E_2 should facilitate hippocampal-dependent

memory. Although this is a reasonable hypothesis, directly linking estradiol-induced hippocampal alterations to memory modulation has proven to be a challenge. For example, the increases in spine synapses, LTP, and neurogenesis observed during proestrus relative to estrus during the estrous cycle should lead to enhanced memory during proestrus relative to estrus. However, only one study testing spatial reference memory, a type of long-term memory that is critically dependent on the hippocampus (Morris et al., 1982; Moser et al., 1993) has found this to be so. In this study, young female mice in proestrus learned to find a hidden escape platform in the Morris water maze faster and more accurately than those in estrus (Frick and Berger-Sweeney, 2001). This finding is supported by other work showing that rats in proestrus are more likely than those in estrus to use a spatial learning strategy in dry-land mazes (Korol et al., 2004) and that infusions of E_2 directly into the dorsal hippocampus increase spatial strategy use in ovariectomized rats (Zurkovsky et al., 2007). Nevertheless, the superior spatial performance of proestrus mice in the water maze is inconsistent with studies of female rats using other water maze protocols that reported enhanced spatial reference memory in estrus relative to proestrus (Frye, 1995; Warren and Juraska, 1997) or no effect of cyclic ovarian hormone fluctuations on task performance (Berry et al., 1997). Further, conflicting effects of the cycle have been reported in tests of spatial memory using an object recognition task (Frye et al., 2007; Sutcliffe et al., 2007) and from studies using novel object or social recognition tasks in which rodents must detect the presence of a new object, conspecific, or food (Markham and Juraska, 2007; Sanchez-Andrade et al., 2005; Sutcliffe et al., 2007; Walf et al., 2006). Although the estrous cycle literature, on the whole, is inconclusive, the inconsistencies among estrous cycle studies should not necessarily be interpreted as a lack of effect of circulating estrogens and progestins on memory. Rather, the lack of agreement among these studies may be more indicative of how difficult it can be to pinpoint the behavioral effects of hormones that are in a constant state of flux. Indeed, the lack of consensus about the effects of the estrous cycle on memory in young females complicates the issue of how estrous cycle cessation should affect memory in aging females. If estrous cycling, particularly high E_2 levels, are beneficial for memory, then age-related reductions in E_2 levels should be detrimental to memory. If, however, low circulating E_2 levels are more beneficial for memory, then memory may be only minimally affected by age-related reductions in this hormone. In aging females, the influence of acyclicity on memory may best be understood by examining interactions among age, ovarian deterioration, and duration of hormone deprivation prior to E_2 treatment.

In both young and aging females, one way to isolate the effects of a single hormone on memory is to administer exogenous hormones to ovariectomized rodents. In young ovariectomized female rodents, exogenous E_2 generally improves a short-term form of spatial memory called spatial working memory (Bimonte and Denenberg, 1999; Bohacek and Daniel, 2007; Bowman et al., 2002; Daniel and Dohanich, 2001; Daniel et al., 1997; Fader et al., 1998; Fader et al., 1999; Garza-Meilandt et al., 2006; Gibbs, 1999; Holmes et al., 2002; Luine et al., 1998; O'Neal et al., 1996; Sandstrom and Williams, 2001; Sandstrom and Williams, 2004; Wide et al., 2004), non-spatial working memory (Wide et al., 2004), memory for both the location (Frye et al., 2007; Luine et al., 2003) and identity (Vaucher et al., 2002) of objects, inhibitory avoidance (Frye and Rhodes, 2002; Singh et al., 1994)(but see (Foster et al., 2003)), and trace eyeblink conditioning (Leuner et al., 2004). However, as is true for exogenous E_2 administration in aging females, improvements in young females can depend on numerous methodological variables such as dose (Holmes et al., 2002; Wide et al., 2004), duration of treatment (Luine et al., 1998), route of administration (Garza-Meilandt et al., 2006), extent of daily handling (Bohacek and Daniel, 2007), cognitive demand of the task (Bimonte and Denenberg, 1999), and whether was E_2 was administered prior to training (Daniel et al., 1997; Gresack and Frick, 2004). Additional variables related to the aging process further complicate the design of E_2 treatment studies in aging females, as will be discussed extensively later in this review.

Aging and ovarian hormones

In women, menopause is an inevitable consequence of aging, and this transition occurs, on average, at about age 51. Menopause is a gradual process of change (typically over the course of 2–7 years) resulting in the cessation of menses, profound reductions in ovarian hormone levels, and irreversible ovarian failure (Bellantoni and Blackman, 1996). The impact of menopause, and the consequent ovarian hormone loss, on memory has been the subject of considerable recent study. Numerous reports have linked menopause with memory loss, particularly those studies of surgically menopausal women, most of whom experience significant verbal memory decline after removal of the ovaries (reviewed in (Sherwin, 2006; Sherwin and Henry, 2008)). Among naturally menopausal women, those with low endogenous estrogen levels display worse verbal memory and an increased risk of cognitive decline relative to those with high estrogen levels (Wolf and Kirschbaum, 2002; Yaffe et al., 2000b). Women are also reportedly at increased risk for developing Alzheimer's disease relative to men (Launer et al., 1999; Yaffe et al., 1998; Zandi et al., 2002a), which suggests that estrogen and/or progestin deficiency during middle age may be a critical factor in the development of dementia. Indeed, some studies have shown that hormone therapy can decrease the risk of developing Alzheimer's by nearly one third (Yaffe et al., 1998) and delay the onset of the disease (Tang et al., 1996). Because the female hippocampus relies on hormones such as estrogens as trophic factors during adulthood (Brinton, 2001), estrogen deficiency during menopause may render these neurons more vulnerable to deterioration and exacerbate emerging age-related memory deficits. Studies in rodents lend support to this hypothesis.

Rodents are typically considered "aged" when they are approximately 2 years old. "Middle-aged" rodents average about 16–18 months of age, whereas "young" rodents used for memory experiments are typically 3–4 months of age. With regard to reproductive aging, there are several key differences between rodents and humans. For example, unlike in women, where menopause leads to a total loss of primordial follicles (Richardson et al., 1987), rats and mice do not experience complete follicle loss (Lu et al., 1979; Wise, 2000). In addition, whereas the negative feedback effects of E_2 on gonadotrophins are decreased in menopausal women, which leads to elevated gonadotrophin levels (Crowley et al., 1985; Yen, 1999), such feedback remains intact in aged acyclic pseudopregnant rats, leading to relatively normal gonadotrophin levels (Lu, 1983; Wise, 2000). Nevertheless, reproductive senescence in rodents is similar to menopause in several critical respects, including similar alterations in pulsatile LH release and the LH surge, variability of cycle length prior to acyclicity, and ultimate cessation of hormone cycling (LeFevre and McClintock, 1988; Nelson et al., 1995). In addition, impending reproductive decline in both middle-aged rodents and humans is characterized by increases in FSH and circulating E_2 levels (Downs and Wise, in press; Lu, 1983). Circulating E_2 levels ultimately decline in women and rodents (Lu et al., 1979; Nelson et al., 1995), although they tend to remain elevated in middle-aged rats for quite some time (Morrison et al., 2006). In rats, reproductive decline begins at 9–12 months of age (Finch et al., 1984); by 12 months, approximately 70% of female rats cycle irregularly or are acyclic, and nearly 75% of females become acyclic by 24 months (Markowska, 1999). In mice, reproductive alterations begin at 13–14 months of age (Nelson et al., 1995); by 17 months, approximately 80% of female mice cycle irregularly or are acyclic, and all females mice become acyclic by 25 months (Frick et al., 2000). Although reproductive senescence in non-human primates is more similar to menopause than that in rodents (Morrison et al., 2006), practical considerations, including small size and short lifespan, make rodents an important model system in which to test the effects of E_2 loss and exogenous E_2 on age-related memory decline.

However, a few important caveats are important to keep in mind when extrapolating from rodents to humans. First, differences in the types of estrogens used in many human and rodent studies may limit the applicability of the rodent data to menopausal women. Whereas many

clinical studies, including the WHIMS and WHISCA studies, have administered conjugated equine estrogens (a cocktail of estrogens containing mainly estrone sulfate), rodent studies have typically administered some form of estradiol (either E_2 or estradiol benzoate). In randomized clinical trials of postmenopausal women, E_2 administered intramuscularly or transdermally improved verbal and working memory, whereas oral conjugated equine estrogens did not (see (Sherwin and Henry, 2008) for recent review), suggesting superior efficacy of E_2 over conjugated equine estrogens. Indeed, estrone has a considerably lower binding affinity for ERα and ERβ than E_2 (Kuiper et al., 1997). As such, findings from rodent or human studies using E_2 may not generalize well to conjugated equine estrogens. Second, tests used to measure cognitive function, including memory, differ considerably in rodents and humans, which may also limit application of data from rodents to menopausal women. For example, many clinical studies report an effect of estrogens on verbal memory (whether an improvement as reviewed in (Sherwin and Henry, 2008) or an impairment as shown by (Resnick et al., 2006)), whereas rodents do not have a verbal memory to test, per se. Further, many clinical studies of hormone therapy employ general tests of cognitive function (e.g., the 3MSE) (Rapp et al., 2003b; Shumaker et al., 2003) for which there is no rodent equivalent. When more specific neuropsychological test batteries are used in clinical studies (e.g., digit span, card rotations, California Verbal Learning Test) (Resnick et al., 2006), the investigator can typically tap into more aspects of cognitive function than is possible in a rodent. In addition, most rodent studies utilize tasks based on navigating through the environment (e.g., Morris water maze, radial arm maze, T-maze), or otherwise interacting with stimuli in the environment in a physical way (e.g., investigating an object, moving to avoid a shock), whereas tests used in humans generally involve no physical movement throughout the environment. Although these differences may call into question the applicability of rodent data to humans, the remarkable parallels between the effects of brain lesions and aging on tests designed in rodents and humans to measure the same type of memory suggest a considerable degree of commonality among tests meant to measure similar mnemonic processes in rodents and humans (Rosenzweig and Barnes, 2003; Squire, 1992). In addition, the recent development of virtual computer mazes that simulate movement through mazes such as the Morris water maze allow for better parallels between humans and rodents. Although these virtual mazes are sensitive to sex differences in performance (males outperform females), age (young subjects outperform older subjects), and testosterone levels (high levels correlate with better performance) (Astur et al., 1998; Burkitt et al., 2007; Driscoll et al., 2005), such mazes have not yet been used to study effects of hormone therapy on cognitive function in menopausal women. Adoption of such virtual tools for studies of menopausal women would greatly aid in bridging the methodological gaps between rodents and humans.

The aging brain and estradiol

Certain brain regions, such as the hippocampus, basal forebrain, entorhinal cortex, and prefrontal cortex, are exceptionally vulnerable to the detrimental effects of aging. Age-related deterioration of these brain regions has been extensively documented in several mammalian species, including humans, non-human primates, rats, and mice (e.g. (Burke and Barnes, 2006; Decker, 1987; Erickson and Barnes, 2003; Hof and Morrison, 2004; Morrison and Hof, 2002; Rosenzweig and Barnes, 2003)). Numerous age-related alterations in the rodent hippocampus have been associated with impaired spatial memory including place cell rigidity (Wilson et al., 2003), elevated neurotrophin and protein kinase levels (Bimonte et al., 2003; Columbo et al., 1997), reduced postsynaptic density area (Nicholson et al., 2004) and synaptic proteins (Frick and Fernandez, 2003; Smith et al., 2000), and impaired long-term potentiation (Bach et al., 1999). Elevated protein kinase levels in the prefrontal cortex have also been associated with impaired working memory in aged rats and monkeys (Ramos et al., 2003), and decreased prefrontal dendritic spine density has been related to impaired object recognition in memory in aged female rats (Wallace et al., 2007). Spatial memory deficits in aged gonadally

intact female rats have also been correlated with deterioration of basal forebrain cholinergic neurons (Fischer et al., 1992; Fischer et al., 1989).

Of these brain regions, the hippocampus has been the primary focus of rodent studies examining the mnemonic response to E_2 in aging females due to the extensive literature on estrogenic effects in the hippocampus among young females. In general, the hippocampus of aging female rodents remains responsive to E_2. E_2 treatment in the hippocampus of middle-aged and/or aged females increases levels of synaptophysin and nerve growth factor, augments dentate gyrus dendritic spine density, activates protein kinases, normalizes intracellular calcium homeostasis, and phosphorylates NMDA receptors (Bi et al., 2003; Fernandez and Frick, 2004; Foster, 2005; Frick et al., 2002b; Miranda et al., 1999). The E_2-induced increase in synaptophysin protein levels in aged females has been associated with improved spatial reference memory in the Morris water maze (Frick et al., 2002b). The hippocampus of aging rodents is also susceptible to long-term depression, and the fact that E_2 treatment can block induction of this phenomenon in middle-aged female rats (Foster et al., 2003), may suggest a potential synaptic mechanism through which E_2 improves memory in aging females.

Nevertheless, it is important to remember that the effects of E_2 treatment in the aging hippocampus are dictated by the myriad of age-related alterations to this structure. Among these alterations are reductions in ERα and ERβ immunoreactivity, mRNA levels, and protein levels in the aged female hippocampus (Adams et al., 2002; Mehra et al., 2005; Yamaguchi-Shima and Yuri, 2007), which may alter responsiveness to E_2 in aging females relative to young females. Indeed, several studies have found differing effects of E_2 treatment on the hippocampus in young and aged (22–24 months) females. For example, CA1 pyramidal neurons in ovariectomized aged rats do not respond to E_2 with an increase in dendritic spine density as do those in young females (Adams et al., 2001). Rather, dendritic spine density is increased by E_2 in the dentate gyrus of ovariectomized aged (16–20 months) rats (Miranda et al., 1999). Also, whereas E_2 reduces the amount of ERα immunoreactivity per CA1 synapse in young females, it has no such effect in aged females (Adams et al., 2002). Interestingly, although CA1 spine density is not affected by E_2 in aged females, E_2 does increase the numbers of NMDA receptors per aged CA1 synapse (Adams et al., 2001). E_2 also restores the synaptic distribution of NR2B NMDA receptor subunits in the aged CA1 region to that seen in young females (Adams et al., 2004). Collectively, these studies indicate that the CA1 region in aged females does not respond to E_2 by increasing spine synapses, but rather by modifying the number and distribution of NMDA receptors in existing synapses. Although it is tempting to speculate that alterations in hippocampal NMDA receptors underlie the beneficial effects of E_2 treatment on memory in aging females, E_2-induced changes in glutamatergic plasticity have not been directly linked to E_2-induced memory modulation in young or aging females. In fact, only one study has measured both the neural and mnemonic response to E_2 in the same aged animals. In this study, an E_2-induced increase in synaptophysin protein levels in 27–28 month-old females was associated with improved spatial reference memory in the Morris water maze (Frick et al., 2002b), which provides support for a link between enhancement of synaptic plasticity and memory in aging females. Nevertheless, dearth of studies in aging rodents directly associating E_2-induced changes in the brain and memory underscore the fact that the specific neural mechanisms underlying estrogenic modulation of memory in aging females are very poorly understood. This important issue deserves much more attention in future studies.

Effects of estradiol on memory in aging female rodents

Memory decline has been associated with the loss of estrous cycling in both rats and mice. This relationship has been particularly well described for spatial reference memory tested in the Morris water maze, which declines at an earlier age in females than in males. Significant deficits in females are observed by 12 months in rats and 17 months in mice, whereas such

deficits are not apparent in male rats until 18 months and in male mice until 25 months (Frick et al., 2000; Markowska, 1999). Moreover, the onset of this premature spatial memory decline in females coincides with the cessation of ovarian hormone cycling, as illustrated by the fact that the age at which spatial memory deficits first appear in both species is marked by a sharp decline in regular estrous cycling (Frick et al., 2000; Markowska, 1999) (Fig. 1). In both the Markowska, 1999 and Frick et al., 2000 studies, the low numbers of rodents in each cycling category (regular, irregular, or acyclic) precluded statistically meaningful correlations between cycling status and spatial memory. However, the study by Markowska, 1999 did observe an interesting trend among 12 month-old female rats, whereby performance in a daily probe trial was best in regularly cycling females, intermediate in irregularly cycling females, and worst in acyclic females. Similar trends were reported in females at 18 and 24 months of age (Markowska, 1999), suggesting that disruption of estrous cycling is detrimental to spatial memory throughout the aging process.

Interestingly, mRNA for the nerve growth factor receptor trkA decreases significantly in the medial septal nucleus of the basal forebrain between 13 and 25 months of age in gonadally intact female, but not male, rats (Gibbs, 1998). Nerve growth factor is an important trophic factor for cholinergic neuron survival, and over 90% of medial septal cholinergic neurons express trkA (Gibbs, 1998). A relationship between basal forebrain cholinergic dysfunction and spatial memory loss in aging is supported by studies in 24–29 month-old male rats demonstrating significant correlations between poor spatial reference memory in the water maze and reduced ChAT activity in the hippocampus (Dunbar et al., 1993), basal forebrain, and frontal cortex (Gallagher et al., 1990). Because the majority of trkA-expressing medial septal cholinergic neurons project to the hippocampus, the loss of trkA expression in aging females may suggest a disruption of subcortical cholinergic inputs to the hippocampus, which could contribute to sex differences in spatial memory decline.

One way to test the hypothesis that reproductive aging contributes to memory decline in females is to determine if replacement of estrogens and/or progestins can reverse the observed memory dysfunction. On the face of it, this seems like a relatively simple proposition. However, truly restoring the cycle at any age is exceedingly difficult, given the complex timing of hormone fluctuations during the estrous cycle. Further, the added dimension of aging raises complicated issues with respect to experimental design. Some issues are characteristic of nearly any pharmacological experiment. For example, effects of E_2 on memory may differ based on dose and type of memory tested. For instance, Bimonte-Nelson and colleagues (2006) found that a low dose of E_2 time-release pellets (0.25 mg, 60–day release) administered to 14 month-old ovariectomized rats for four weeks improved spatial reference memory in the water maze, whereas high dose pellets (0.5 mg, 60-day release) had no effect. Among slightly older (17–18 months old) ovariectomized rats, silastic capsules containing a low or high dose of estradiol benzoate had no effect on spatial water maze acquisition, but the high dose improved task retention (Foster et al., 2003). Also among middle-aged females, one of three doses of E_2 administered to 18 month-old ovariectomized mice in the drinking water for 5 weeks impaired spatial reference memory tested in a water-escape motivated verision of the radial arm maze, but robustly improved novel object recognition (Fig. 2). In aged females, daily injections of 5 μg, but not 1 μg, estradiol benzoate to 27–28 month-old intact mice prior to training improved spatial water maze acquisition and increased hippocampal synaptophysin levels (Frick et al., 2002b). Although there is unlikely to be a single dose of E_2 that improves memory on all tasks at all ages, this sampling of studies that have utilized multiple doses of E_2 in aging females illustrates how dose, task, and/or type of memory tested can influence the outcome of E_2 treatment. Unfortunately, many studies to date have been limited in the scope of doses, ages, and tasks used, and therefore, more studies must include multiple doses and multiple tasks to better understand how various types of memory are affected by E_2 treatment. Such information

would be relevant to understanding the specific aspects of cognitive function likely to be affected by estrogen therapy in menopausal women.

In addition to the more obvious issues of dose, task, and type of memory tested, other important factors must be taken into consideration in studies of hormones and aging, including the age of the subjects, presence or absence of the ovaries during treatment, duration of hormone deprivation prior to treatment, timing of treatment relative to testing, cyclic or continuous nature of the treatment, and the influence of progestin co-administration and environmental factors on the mnemonic response to E_2. Most studies manipulate more than one of these factors simultaneously, which provides a challenge to understanding how each factor contributes to the mnemonic effects of E_2. Nevertheless, the sections below will provide a synthesis of this literature to date and suggest avenues for future research. A table detailing most of these studies has been previously published; please see Table 2 in Gresack and Frick, 2006a for more specific methodological information on many of the studies described below.

Age at treatment

A common approach used in many aging studies is to examine effects of E_2 on memory at a single age (i.e. either middle-aged or aged), with treatment effects measured relative to an age-matched vehicle group and/or a young control group. Among middle-aged (14–18 months) female rats and mice tested in such studies, chronic (1–5 weeks) treatment with E_2 via silastic capsules, pellets, or the drinking water improves spatial reference memory in the water maze (Bimonte-Nelson et al., 2006; Markham et al., 2002), spatial working memory in the radial arm maze (Daniel et al., 2006), and novel object recognition (Fernandez and Frick, 2004) (Fig. 2). It is important to note for the Daniel et al., 2006 study that improvements in spatial working memory were observed only in 17 month-old rats receiving treatment immediately after ovariectomy, and not in those whose treatment commenced 5 months after ovariectomy (the issue of duration of ovariectomy prior to treatment will be discussed in detail below). Inconsistent with the positive results of E_2 treatment in middle-aged females are the aforementioned data from 18 month-old ovariectomized mice showing that spatial reference memory tested in the radial arm maze can be impaired by 5 weeks of E_2 administered in the drinking water prior to training (Fernandez and Frick, 2004) (Fig. 2).

Many studies also report beneficial effects of estradiol in aged female rodents. Among aged (22–28 months) female mice, spatial reference memory in the water maze is improved by daily injections (for 9 days) of estradiol benzoate (Frick et al., 2002b) and spatial reference memory consolidation is enhanced by a single injection of E_2 given immediately after training (post-training) (Frye et al., 2005; Harburger et al., 2007). Spatial reference memory, but not spatial working memory, in a win-stay radial arm maze task is also improved by forty days of E_2 treatment via silastic capsules given to 24 month-old female mice 18 months after ovariectomy (Heikkinen et al., 2004). Improvement in other tasks has also been observed; silastic E_2 implants improved spontaneous alternation in a T-maze (Miller et al., 1999) and novel object recognition (Vaucher et al., 2002) in 24 month-old female mice. In contrast to these positive effects of E_2 treatment, negative or null effects of treatment have been reported in other aged females treated with E_2 post-training. In one study, a single E_2 injection given immediately after training to 22 month-old female mice had no effect on novel object recognition (Gresack et al., 2007b), and in another study, chronic E_2 treatment administered via injections of E_2 daily or every 4 days (vehicle injected all other days) to female mice from 18–21 months of age had no effect on novel object recognition and working memory errors in the radial arm maze and a detrimental effect on reference memory errors in the radial arm maze (Gresack and Frick, 2006a). Although these data could indicate that E_2 must be in the circulation during training to improve memory in aged females, the fact that post-training E_2 enhances spatial reference memory in the water maze (Harburger et al., 2007) argues against this as an explanation for

all types of memory. Rather, it may be that E_2 must be in the circulation during testing to improve certain types of memory, like object recognition or spatial memory tested in the radial arm maze, in aged females. This possibility is supported for object recognition by the fact that performance in this task is improved in 22–24 month-old mice by 21 days of E_2 silastics prior to training and testing (Vaucher et al., 2002).

Although the aforementioned studies provide valuable information about the effects of E_2 on memory in aging females, with many findings suggesting that treatment is beneficial, they do not allow the effectiveness of E_2 to be directly compared between middle-aged and aged females. Such comparisons can only be made if females of different ages are given the same E_2 treatment and tested on the same behavioral tasks. Although only a handful of such studies have been conducted, studies of this kind can reveal key insights about how age influences the mnemonic response to E_2. Studies of avoidance learning in female rats indicate that memory at any age is not improved by E_2; treatment either impaired or has no effect on such learning in ovariectomized female rats at 12–13 months, 17–18 months, or 20 months of age (Foster et al., 2003). However, E_2 did protect against the detrimental effects of the cholinergic antagonist scopolamine on T-maze active avoidance in ovariectomized 12–13 month-old, but not 20 month-old, female rats, suggesting protective effects of E_2 in middle-aged, but not aged, females (Savonenko and Markowska, 2003). Benefits limited to middle-aged females have also been observed for other types of memory. For example, E_2 enhanced spatial water maze acquisition in 4 and 16 month-old ovariectomized rats, but not in 24 month-old rats (although minor benefits were observed at this age in a spatial probe trial) (Talboom et al., 2008). More strikingly, our lab recently reported widely discrepant effects of post-training E_2 treatment on spatial reference memory in the Morris water maze and novel object recognition tasks in ovariectomized young, middle-aged, and aged female mice; in 5 month-old females, E_2 enhanced object recognition, but impaired spatial memory, in 17 month-old females, E_2 enhanced both types of memory, and in 22 month-old females, E_2 had no beneficial effect on either type of memory (see Std-Veh and Std-E_2 groups in Fig. 3) (Gresack et al., 2007a).

Collectively, the studies by Savonenko and Markowska, 2003, Talboom et al., 2008 and Gresack et al., 2007a suggest beneficial effects of E_2 in middle-aged, but not aged, females. As such, these findings support the increasingly popular notion that there is a critical period during early menopause in which hormone replacement may effectively improve memory (Maki, 2006; Sherwin, 2006; Zandi et al., 2002b). This so-called “critical period hypothesis” suggests that hormone therapy will only benefit cognitive function if initiated when menopausal symptoms are present during early menopause (i.e., during middle-age) (Maki, 2006). Indeed, meta-analyses of clinical studies in menopausal women report that hormone therapy is more effective in recently menopausal women experiencing physiological symptoms of menopause at the time of treatment than in those who are many years beyond the onset of menopause (Yaffe et al., 1998). The fact that the subjects in the Women’s Health Initiative (WHIMS and WHISCA) studies were all age 65 or older, and were asymptomatic, helps to support the contention that estrogen treatment after the critical period is not an effective means of preventing age-related cognitive decline.

Influence of the ovaries and duration of hormone loss prior to treatment

In addition to age, duration of hormone deprivation prior to E_2 treatment is also relevant to consideration of the critical period hypothesis. In women, age and duration of hormone deprivation are typically linked (except in the case of surgical menopause for younger women), so differentiating between effects of age and length of hormone deprivation in women can be challenging. In rodents, bilateral ovariectomy can be more easily used to distinguish between age and hormone deprivation. Regardless of age, ovariectomy is standard practice in rodent hormone replacement studies, allowing investigators more control over E_2 levels in circulation.

Most investigators ovariectomize their females in close proximity (a month or less) to the start of E_2 treatment, with the assumption that allowing the ovaries to age naturally provides the most useful model of natural brain aging. However, some investigators report interesting differences in the mnemonic response to E_2 between short- and long-term ovariectomy. For example, Daniel, Hulst, and Berberling (2006) tested three groups of ovariectomized rats in the radial arm maze at 17 months of age; one group was ovariectomized at 12 months and treated with E_2-secreting silastic capsules for 5 months prior to testing, and other groups were ovariectomized at 12 or 17 months and treated with E_2 silastics for 1 week prior to testing. Rats in which silastics were implanted at the time of ovariectomy, regardless of whether treatment started at 12 or 17 months, exhibited improved spatial working memory in the radial arm maze when tested at 17 months. In contrast, spatial working memory in rats ovariectomized at 12 months and treated at 17 months was not affected by E_2, suggesting that E_2 treatment is not effective when initiated after 5 months of hormone deprivation. In support of this notion, two other studies reported no effect of E_2 on spatial working memory among rats ovariectomized for 7 or 18 months. In one study, rats ovariectomized at 13 months of age and treated with E_2 silastics for 5 days at 21 months of age showed no improvement in a T-maze delayed non-matching to position task (although spatial working memory was improved in a water maze task) (Markowska and Savonenko, 2002), and in another, rats ovariectomized at 5 months of age and treated for 40 days with E_2 pellets at 23 months of age showed no improvement in radial arm maze and T-maze tasks (Heikkinen et al., 2004). Further, Gibbs, 2000b showed that 10 months of hormone deprivation followed by 6–8 weeks of weekly E_2 injections had no effect on spatial working memory in a delayed non-match to position task among 23 month-old rats. However, a shorter period of hormone deprivation (3 months) could be offset by 5–9 months of E_2 silastic treatment, as spatial working memory was improved at 22–25 months of age (Gibbs, 2000b). Together, these studies indicate that long-term (> 3 months) hormone deprivation reduces the beneficial effect of E_2, and fit well with the critical period hypothesis suggesting that hormone therapy may be most effective when given soon after hormone loss.

Knowing about this critical period may help women decide when to initiate hormone therapy, but this information does not address the question of whether hormone therapy is necessary in the first place. That is, does long-term ovarian hormone loss itself impair memory to the extent that treatment would be required? A handful of studies have examined the effects of long-term ovariectomy on memory, and the results do not provide any clear answers. Bimonte-Nelson and colleagues have demonstrated that 1.5–6 months of ovariectomy starting after 14 months of age *improves* spatial working and reference memory in aged rats tested in a water escape-motivated radial arm maze, whereas 21 days of ovariectomy impairs both spatial working and reference memory in the same task (Bimonte-Nelson et al., 2003; Bimonte-Nelson et al., 2004). This seemingly unlikely benefit of long-term ovariectomy has been linked with elevated progesterone in aged intact rats, a hypothesis supported by detrimental effects of progesterone treatment in ovariectomized aged female rats on spatial working memory in this task (Bimonte-Nelson et al., 2003; Bimonte-Nelson et al., 2004). The improvements induced by long-term ovariectomy are also consistent with the beneficial effects of long-term ovariectomy on delayed recognition learning in monkeys (Lacreuse et al., 2000). However, the beneficial effects of long-term ovariectomy in rats observed by Bimonte-Nelson and colleagues have not been found by other investigators. For example, Sato et al., 2003 reported that 15 month-old rats tested in a dry-land radial arm maze 3 months after ovariectomy were impaired in numerous measures of spatial working memory (Sato et al., 2003). Further, a recent longitudinal study of rats ovariectomized at 13 months found no effect on spatial working memory tested in a T-maze delayed non-match to position task until 7 months after surgery or until 4 months after surgery when delays of 5, 15, or 30 minutes were inserted between trials (Markowska and Savonenko, 2002). This study also found that rats tested 6 months after ovariectomy surgery

were more sensitive to the disruptive effects of scopolamine than sham-operated controls (Markowska and Savonenko, 2002).

In the hippocampus and prefrontal cortex, long-term ovariectomy alters cholinergic function, although these changes have not been directly linked to behavior. In one recent study, ChAT protein levels were increased in the hippocampus, but not prefrontal cortex, of 2 month-old and 15 month-old rats treated for 10 days with E_2 silastics implanted immediately after ovariectomy (Bohacek et al., in press). However, among rats ovariectomized at 10 months of age and treated at 15 months of age, ChAT protein levels were increased in the prefrontal cortex, but not hippocampus (Bohacek et al., in press). Another study of cholinergic function in rats found that ChAT and trkA mRNA in specific basal forebrain nuclei were significantly decreased 6 months, but not 3 months after ovariectomy (conducted at 13 months of age) relative to age-matched gonadally intact controls (Gibbs, 1998). The results of these two studies may indicate that long-term ovarian hormone loss alters the functioning of the septohippocampal and basalocortical cholinergic systems, which could lead to impaired spatial working memory in tasks, like the radial arm maze, in which hippocampal and cortical cholinergic involvement has been demonstrated (Olton et al., 1992; Sengstock et al., 1992). However, the work by Bimonte-Nelson and colleagues is inconsistent with the notion that long-term ovariectomy is detrimental to neural and mnemonic functioning. Given that Bimonte-Nelson and colleagues have replicated the beneficial effects of long-term ovariectomy on spatial working and reference memory (Bimonte-Nelson et al., 2003; Bimonte-Nelson et al., 2004), the discrepancy between their data and those of others may be related to the stress of water maze testing, which has been shown to interfere with the hippocampal response to E_2 treatment (Frick et al., 2004) (Fig. 4). This, and other, possibilities must be addressed before conclusions can be made in rodents about the effects of long-term ovariectomy on memory. Additional data concerning effects of long-term ovariectomy on other types of memory and in mice should be collected to determine the extent to which any of these findings generalize to other cognitive domains and other rodent species.

Another critical issue related to the ovaries is whether they are present during treatment and testing. Because most women retain their ovaries at menopause, this issue is clearly relevant to clinical use of hormone therapies, and it is surprising that more studies have not been conducted using gonadally intact female rodents. To date, only three studies have tested the effects of E_2 on gonadally intact aging females, so it is too premature to judge whether the presence of ovarian tissue affects the mnemonic response to E_2. However, the results, at least for spatial reference memory, suggest a positive effect of E_2 in intact aging females. One study from my lab reported that 5 μg estradiol benzoate given to gonadally intact 27–28 month-old female mice for 5 days prior to Morris water maze testing, and then each day 4 hours prior to testing, significantly improved spatial task acquisition (Fig. 5A) and increased hippocampal levels of the presynaptic protein synaptophysin (Fig. 5B) (Frick et al., 2002b). This effect was dose-dependent, as a 1 μg dose had no effect on spatial memory or synaptophysin levels (Frick et al., 2002b). Interestingly, the 5 μg dose had no effect on spatial water maze acquisition in ovariectomized 17 month-old mice (unpublished observations), although it is unclear whether this difference was due to age or ovariectomy surgery. Another study of gonadally intact 20 month-old female mice found that a single post-training injection improved spatial water maze retention and inhibitory avoidance (Frye et al., 2005). However, neither the Frick et al., 2002b or Frye et al., 2005 studies included comparisons to ovariectomized rats, so neither could address whether the presence of ovaries afforded an advantage over the absence of ovaries in response to E_2.

A comparison between intact and ovariectomized females was provided by a study of 20 month-old female rats that were untreated or were treated with E_2 silastics for 6 days after sham or ovariectomy surgery. In this study, total number of errors in a T-maze active avoidance task

was not affected by ovariectomy or E_2 treatment, however among untreated rats, ovariectomized rats were more sensitive than intact rats to the disruptive effects of the cholinergic antagonist scopolamine (Savonenko and Markowska, 2003). In another measure of performance, E_2 treatment increased the number of trials to criterion performance in intact rats relative to ovariectomized rats, indicating a detrimental effect of E_2 on the performance of intact rats (Savonenko and Markowska, 2003). These results seem to contrast with the beneficial effects of E_2 in intact aged female mice described above, and resolving this discrepancy will require considerably more data on this subject. Task, dose, and type of memory tested could all contribute to the differences between studies, as could species. In intact middle-aged mice, levels of E_2 and progesterone are reduced relative to intact young mice (Nelson et al., 1992), whereas in intact aged rats, E_2 levels are similar to and progesterone levels are more than 4-fold higher than intact young rats (Bimonte-Nelson et al., 2003). Given that the hormonal milieu of the intact aging rat and mouse differs considerably, baseline E_2 and progesterone levels may influence the extent to which ovariectomy influences memory on its own and in combination with E_2. As mentioned previously, elevated endogenous progesterone levels in aged rats have been hypothesized to underlie observed impairments in spatial working and reference memory in a water escape-motivated radial arm maze (Bimonte-Nelson et al., 2003; Bimonte-Nelson et al., 2004). Because most women taking hormone therapy retain their ovaries, addressing the discrepancies between the mouse and rat data, as well as understanding whether the ovaries influence the mnemonic response to E_2, is imperative to the application of rodent data to menopausal women.

Timing of treatment relative to training

Although the aging studies discussed thus far suggest that E_2 can influence certain types of memory, the vast majority are confounded by the fact that E_2 administration prior to training can influence numerous non-mnemonic performance factors such as motivation, attention, and sensorimotor function (McGaughy and Sarter, 1999; Morgan and Pfaff, 2001; Pfaff et al., 2002), in addition to memory. Therefore, this work does not address the issue of whether E_2 specifically modulates memory in tasks designed to test memory. This shortcoming can be addressed if E_2 is given immediately after training (post-training) in tasks where rodents are trained in a single day and then treated with E_2 immediately after training. Most studies conducted in both young and aging rodents utilize a form of E_2 encapsulated in 2-hydroxypropyl-β-cyclodextrin which can successfully cross the blood-brain barrier and is metabolized within 24 hours (Pitha et al., 1986; Taylor et al., 1989). Retention is then tested 24 or more hours later. Because E_2 is not in the circulation during training or testing, its specific effects on memory consolidation can be examined in the absence of non-mnemonic confounds, which may confound interpretation of the data (McGaugh, 1989).

All post-training studies conducted to date report beneficial effects of E_2 on memory consolidation. In the Morris water maze, young ovariectomized rats (Packard and Teather, 1997b) and mice (Gresack and Frick, 2006b) receiving a single intraperitoneal (i.p.) injection of 0.2 mg/kg cyclodextrin-encapsulated E_2 immediately after eight consecutive spatial training trials remembered the location of the hidden escape platform 24 hours later significantly better than those receiving vehicle, 0.1 mg/kg E_2, or 0.4 mg/kg E_2 (Fig. 6A). The memory facilitation produced by E_2 is time-dependent, as administration 2 hours post-training does not enhance memory in this task (Packard and Teather, 1997b). Using this same protocol, post-training injection of 0.2 mg/kg E_2 also significantly enhanced spatial reference memory consolidation in ovariectomized 22 month-old mice (Harburger et al., 2007) (Fig. 7). This beneficial effect in aged females is supported by another study in intact 24 month-old female and male mice in which post-training injections of 10 μg E_2 dissolved in oil enhanced spatial memory in the water maze using a different 2-day testing protocol (Frye et al., 2005). However, when given immediately after training each day in a 5-day Morris water maze protocol, post-training i.p.

injections of 0.2 mg/kg E_2 improved spatial reference memory consolidation in 17 month-old, but not 22 month-old, ovariectomized mice (Gresack et al., 2007a) (Fig. 3). These findings raise two interesting points. The first is the discrepancy between the effects of post-training E_2 on spatial memory in aged females in the 2-day and 5-day Morris water maze protocols. Although the reasons behind this discrepancy are currently unclear, we have previously hypothesized that the stress due to repeated daily injections may contribute to the lack of effect of E_2 in the 5-day protocol (Gresack et al., 2007a). The second point, which is consistent with the critical period hypothesis, is the effectiveness of the same E_2 treatment in middle-aged, but not aged, females, again illustrating the fact that age can play a pivotal role in the ability of E_2 to enhance memory.

In addition to spatial reference memory, other types of memory are also improved by post-training E_2. In young rodents, a single post-training systemic injection of cyclodextrin-encapsulated or oil-dissolved E_2 also enhances non-spatial reference memory consolidation (Farr et al., 2000), spatial working memory consolidation (Gresack and Frick, 2004), and spatial and non-spatial object memory consolidation (Frye et al., 2007; Gresack and Frick, 2004; Gresack and Frick, 2006b; Gresack et al., 2007a; Gresack et al., 2007b; Luine et al., 2003; Walf et al., 2006) (Fig. 6B). Like spatial reference memory consolidation, the beneficial effects of E_2 on object memory consolidation occur rapidly, as injections given 2–3 hours post-training to young ovariectomized females do not object recognition memory (Fernandez et al., 2008; Luine et al., 2003; Walf et al., 2006). Further, post-training infusions of 5 μg cyclodextrin-encapsulated E_2 directly into the dorsal hippocampus of young female rats and mice enhance both spatial reference memory (Packard and Teather, 1997a) and object recognition memory (Fernandez et al., 2008) consolidation (Fig. 6C). Also like spatial reference memory, the effects of acute post-training E_2 depend on age, such that an improvement is seen during a 48-hour retention test in ovariectomized 17 month-old females treated with a single 0.2 mg/kg E_2 injection, but not during either 24- or 48-hour retention tests in 21–22 month-old females (Gresack et al., 2007a; Gresack et al., 2007b) (see Std-Veh and Std-E_2 groups in Fig. 3). Again, these data fit well with the critical hypothesis. Moreover, comparisons with other studies invite some speculative conclusions. For example, the fact that spatial reference memory consolidation was enhanced in aged females in the 2-day Morris water maze task by a single E_2 injection (Harburger et al., 2007) (Fig. 7), but was not enhanced in the radial arm maze by chronic E_2 injections (Gresack and Frick, 2006a), may suggest that post-training E_2 in aged females can improve memories that are simple, like the location of a single platform overnight, but not those that are significantly more complex, like the location of multiple platforms over the course of a two-week testing period. Further, the fact that a single post-training injection of E_2 in aged females could enhance 24-hour retention in the 2-day spatial water maze task, but not 24- or 48-hour object memory consolidation in the object recognition task, may imply that spatial reference memory is more sensitive than object recognition memory to the effects of acute E_2 treatment in aged females. Finally, the fact that object recognition was improved in 22–24 month-old ovariectomized mice by pre-training E_2 administered via silastic capsules (Vaucher et al., 2002), but not by post-training E_2 injections (Gresack and Frick, 2006a; Gresack et al., 2007a; Gresack et al., 2007b), may indicate that E_2 must be in the circulation for object memory consolidation to be enhanced in aged females.

No study has directly compared the effects of pre- and post-training E_2 treatment on memory in young or aging rodents, so the importance of circulating E_2 to enhancing memory in aging females is unknown. Nevertheless, the data thus far indicate that E_2 can specifically enhance certain types of memory in middle-aged and aged female rodents in the absence of performance-related confounds, which is important for the development of future hormone-based treatments for reducing age-related memory decline in humans. Although effects of a single post-training E_2 injection may not seem relevant to issues of age-related memory decline, the distinction between E_2 effects on memory and other psychological processes is important

for the use of hormone therapy in menopausal women; if motivational or affective changes alone are responsible for hormone therapy-induced improvements in memory tasks, then treatments that directly target these processes, rather than memory, could be used instead of hormones. Although it is unlikely that the effects of E_2 in memory tasks are due entirely to non-mnemonic factors, understanding exactly how E_2 modulates memory may lead to the development of novel treatments that produce the beneficial effects of E_2 without having to administer the hormone itself. Such development may provide a useful and effective strategy for reducing age-related memory decline, and would require the elucidation of the molecular mechanisms underlying estrogenic modulation of memory. As will be discussed later this review, the rapid time frame in which post-training E_2 enhances memory consolidation may provide a unique opportunity to understand the molecular mechanisms through which E_2 modulates memory.

Type of treatment

The nature of the E_2 treatment itself also requires careful consideration. The duration of treatment (e.g., acute or chronic) may not substantially affect the ability of E_2 to improve memory in aging females, given that acute post-training and longer-term pre-training treatments can both improve the same type of memory at a given age. For example, spatial memory in the water maze is improved in aged female mice by a single post-training injection of E_2 (Frye et al., 2005; Harburger et al., 2007) and by estradiol benzoate given 5 days prior to and then throughout testing (Frick et al., 2002b). One study of middle-aged females directly compared the effects of acute (2 days of injections) and chronic (28 days) E_2 treatments on spatial water maze acquisition and found that both treatments improved performance (Markham et al., 2002). Thus, other factors, such as age at treatment, duration of hormone deprivation, and inclusion of a progestin, may be more important determinants of how a given E_2 treatment will affect a specific type of memory. Nevertheless, too few studies comparing the effects of acute and chronic treatments have been conducted in aging females to conclude that duration of treatment is not an important factor influencing the mnemonic response to E_2.

Perhaps more important than duration of treatment may be whether the treatment reproduces hormone fluctuations similar to the natural cycle. Hormone therapies prescribed for women, including those used by the WHI studies, do not simulate the cyclic nature of estrogen and progestin fluctuations characteristic of the menstrual cycle. Women in the WHI received daily doses of 0.625 mg conjugated equine estrogens (CEE; Premarin®) or 0.625 mg CEE plus 0.25 mg medroxyprogesterone acetate (MPA; PremPro®). The “continuous” nature of these treatments, where the same dose of hormone is administered each day, may contribute to their failure to improve memory, given that female brains are exposed to cycling levels of estrogens and progestins for much of their lives. At this time, however, it is unclear if cyclic hormone therapy is any more effective in enhancing cognitive function than continuous treatment. Because truly replicating the cycle is very difficult, the studies published thus far have attempted to simulate only certain aspects of the cycle (typically, the preovulatory estrogen surge). As such, these treatments may be considered “intermittent” rather than “cyclic”.

Only three studies thus far have directly compared the effects of continuous and intermittent E_2 treatments in aging female rodents, so very little data is available on the subject. One study, in which rats were ovariectomized at 13 months of age, found that continuous and intermittent E_2 treatments were similarly beneficial. Starting at 14 months of age, rats were treated with E_2-secreting silastic pellets (in low and high doses) or with one injection of 10 μg E_2 every other week for four weeks prior to spatial Morris water maze testing (Bimonte-Nelson et al., 2006). Although the high dose E_2 pellet had no effect on performance, the low dose E_2 pellet and intermittent E_2 injection treatments significantly improved task acquisition (Bimonte-

Nelson et al., 2006). Another study in rats ovariectomized at 13 months at treated at 18–20 months found that the effects of continuous E_2 treatment (via pellets) on spatial working memory were greatly enhanced by priming with E_2 injections (given 3 of 4 days for 4 cycles) (Markowska and Savonenko, 2002). The results of these studies contrast with other data collected in mice ovariectomized at 18 months of age and treated with vehicle every day, 0.2 mg/kg cyclodextrin-encapsulated E_2 every day, or 0.2 mg/kg E_2 every 4 days from 18 to 21 months of age (Gresack and Frick, 2006a). In this study, continuous E_2 treatment had no effect on spatial working or reference memory tested in the radial arm maze or on object recognition, whereas intermittent E_2 treatment impaired spatial reference memory and tended to impair spatial working memory and object recognition (Gresack and Frick, 2006a). A primary factor in the discrepancy between the Bimonte-Nelson et al., 2006 and Gresack and Frick, 2006a studies could be age at treatment, as subjects in the two studies differed greatly in age (benefits were seen by Bimonte-Nelson and colleagues at 15 months of age, but not by Gresack and Frick at 21 months of age). Further, differences in route of administration (pellets vs. injection) and type of E_2 (cyclodextrin-encapsulated or not) may also contribute to the discrepant results. However, although the Bimonte-Nelson et al., 2006 study found a beneficial effect of intermittent treatment, whereas Gresack and Frick, 2006a found a detrimental effect of such treatment, neither study found that cyclic E_2 treatment was more effective than intermittent E_2 treatment, which is very useful information. Nevertheless, too little data have been published comparing continuous and intermittent E_2 treatment regimens to judge the relative efficacy of these treatments on memory in females of any age. This critically important issue deserves much more future study aimed at determining: 1) the cognitive domains in which continuous and intermittent E_2 treatment differ in effectiveness, 2) whether age at treatment or duration of hormone loss influences the effectiveness of comparable continuous and intermittent E_2 treatments, and 3) whether treatments that more accurately mimic the natural cycle (including progesterone fluctuations) are any more effective in modulating memory in aging females than continuous or intermittent treatments with E_2 alone.

Co-administration of a progestin

The effects of progestins, such as progesterone, on memory at any age are not well understood. Of particular relevance to this review are effects of progesterone on memory when combined with estrogens. Data from the WHI indicate that treatment with CEE plus the synthetic progestin MPA increased the risk of global cognitive decline and dementia (Rapp et al., 2003b; Shumaker et al., 2003), and impaired verbal memory (Resnick et al., 2006). It is easy to suggest that MPA was responsible for the detrimental effects of the WHI treatment, since MPA has been shown to reduce CEE's neuroprotective effects on hippocampal neurons (Nilsen and Brinton, 2002) and to act similar to glucocorticoids (Poulin et al., 1989), which promote neurodegeneration and cognitive impairment during aging (McEwen et al., 1999). However, the arm of the WHI in which only CEE was administered also found an increased risk of global cognitive decline and dementia (Espeland et al., 2004; Shumaker et al., 2004), which may suggest little effect of progestins, like MPA, on cognitive function in older women.

The current rodent literature adds little to help resolve this issue. Two studies report beneficial effects of treatment with chronic E_2 plus progesterone on memory in ovariectomized rats, one testing spatial working memory and the other testing spatial reference memory. In one study, rats ovariectomized at 13 months of age were implanted with E_2-secreting silastic capsules either immediately or 3 months after ovariectomy, or received weekly injections of 10 μg E_2 followed 48 hours later by 500 μg progesterone starting 3 months after ovariectomy (Gibbs, 2000b). Spatial working memory in a delayed non-match to position task was tested in all rats at 22–25 months of age. Although rats receiving injections of E_2 plus progesterone, but not E_2 alone, reached criterion performance faster than controls, all treatments reduced the mean number of errors per testing block (Gibbs, 2000b), suggesting a benefit to both types of

treatment. In another study, rats were ovariectomized at 14 months of age and treated at 15–16 months of age with 2 days of E_2 (16.67 μg/day) or 28 days of E_2 or E_2 plus progesterone (via silastic capsules); all treatments improved spatial Morris water maze task acquisition (Markham et al., 2002). Although these studies might suggest that E_2 plus progesterone treatment is as effective, if not slightly more effective, at improving spatial memory in aging females, other studies report diametrically contrasting results. For example, in another study using the spatial Morris water maze task, progesterone given continuously (via pellets) completely reversed the beneficial effects of continuous or intermittent E_2 on spatial task acquisition in 15 month-old ovariectomized rats (Bimonte-Nelson et al., 2006). Similarly, immediate post-training i.p. injection of 20 mg/kg cyclodextrin-encapsulated progesterone completely reversed the memory enhancing effects of 0.2 mg/kg cyclodextrin-encapsulated E_2 in 22 month-old ovariectomized mice tested in a 2-day spatial Morris water maze task (Harburger et al., 2007) (Fig. 7). The reasons for the striking inconsistencies between these reversals and the beneficial effects of E_2 and progesterone (Gibbs, 2000b; Markham et al., 2002) are not obvious. As such, the data regarding the influence of combined hormone therapy on memory in aging females are inconclusive. However, understanding how both estrogens and progestins affect cognitive function is of tremendous clinical importance, given that co-administration is recommended for women with a uterus because of the protection from uterine cancer afforded by a progestin (Persson et al., 1996). Future work will need to assess how combined treatment influences different types of memory at different ages, and to determine, if applicable, the most effective methods of administering combined treatment for reducing age-related memory decline.

Environmental factors

Finally, environmental factors, such as education, appear to alter the mnemonic response to E_2. Clinical trials of hormone therapies are susceptible to a “healthy user bias” due to the fact that women who initiate hormone therapy are generally more educated (Keating et al., 1999; Launer et al., 1999; Tang et al., 1996) and healthier (Matthews et al., 1996) than women who do not elect treatment. This selection bias may skew perceptions about the effectiveness of hormone therapy because women who are well educated (> 11 yrs) are less likely to develop dementia than poorly educated (< 8 yrs) women (Launer et al., 1999). One recent longitudinal study reports that high childhood cognition levels are associated with an older age of menopause onset and better cognitive performance at menopause (Kok et al., 2006). Furthermore, some evidence suggests that estrogens may more effectively improve cognition in women with less education than in those with greater education (Matthews et al., 1999), a situation that could result if differences in baseline neural and cognitive function allow more room for improvement in women with less education. The over-representation of well-educated women in studies of hormone therapy may lead to a false perception that such treatment is ineffective for all women, when, in fact, certain populations (e.g., those with less education) may benefit from treatment.

We sought to examine this issue in an aging mouse model using a paradigm called environmental enrichment. Environmental enrichment treatments involve housing rodents together in cages with cognitively and physically stimulating objects. Controls are group (social) or singly (isolated) housed and not exposed to enriching objects. Enrichment improves hippocampal-dependent memory and enhances hippocampal morphology and plasticity in young-adult (Davis et al., 2004; Duffy et al., 2001; Kempermann et al., 1997; Rampon et al., 2000), middle-aged (Frick et al., 2003; Kempermann et al., 1998), and aged (Frick et al., 2002b; Nakamura et al., 1999; Soffié et al., 1999; Winocur, 1998) rats and mice. A link between enrichment and endogenous ovarian hormone levels was provided by a 1999 study in which spatial reference memory in the Morris water maze was impaired in intact female rats relative to ovariectomized rats; this impairment was eliminated by exposure to environmental

enrichment (Daniel et al., 1999). Our laboratory subsequently showed that exposing female mice to an enriched environment from weaning to adulthood influences their mnemonic response to exogenous E_2 (Gresack and Frick, 2004). Mice were exposed for 3 hrs/day to enriched or control environments from 3 weeks to 7 months of age, at which point they were given i.p. injections of 0.2 mg/kg E_2 immediately after training in the radial arm maze and novel object recognition tasks. E_2 enhanced spatial working memory and object recognition in mice raised in standard environments, but had no effect on those raised in enriched environments (Gresack and Frick, 2004), suggesting that enrichment prevents E_2 from improving memory. We subsequently replicated the effect on object memory consolidation in young mice exposed to 24 hour/day enrichment from 3 weeks to 5 months of age (Gresack et al., 2007a) (Fig. 3D), and in those exposed to 4 weeks of enrichment starting at 5 months of age (Gresack et al., 2007b). Interestingly, different relationships between enrichment and post-training E_2 treatment were observed in middle-aged and aged female mice raised in standard or enriched housing conditions. Among middle-aged females, object memory was enhanced by E_2 regardless of housing condition (Fig. 3E), whereas spatial reference memory in the Morris water maze was enhanced by E_2 in standard housed, but not enriched, mice (Fig. 3B) (Gresack et al., 2007a). In aged females, E_2 alone had no effect on object or spatial memory (Fig. 3C and 3F), and interfered with the beneficial effects of enrichment on object memory (Fig. 3F) (Gresack et al., 2007a; Gresack et al., 2007b). As mentioned earlier, these data support the critical period hypothesis, and are consistent with clinical data suggesting that estrogen treatment may be most effective in women with less education (Matthews et al., 1999). As such, the rodent data suggest that estrogen treatment may be most beneficial for cognition in women who have experienced recent ovarian hormone loss and less cognitive and/or physical stimulation. Although it is uncertain how well these findings will generalize to humans, they clearly indicate that environmental factors should be considered in assessing the ability of E_2 to reduce age-related memory decline.

What's next for hormone therapy?

The studies conducted thus far have provided invaluable insights into how hormones such as E_2 and progesterone modulate memory in aging females. Among the most firm conclusions to be drawn from the available data are that middle-aged female rodents seem to benefit more than aged rodents from several types of E_2 treatments, and that environmental factors influence the mnemonic response to E_2. Further, post-training studies have revealed that E_2 can specifically modulate memory consolidation in middle-aged and aged females. However, many issues remain unresolved as described above. Specifically, questions of how duration of ovarian hormone deprivation, presence of the ovaries, addition of a progestin, and cyclicity of treatment influence the mnemonic response to E_2 need to be more fully addressed. Of greatest importance to women considering hormone therapy may be understanding the influence of ovarian hormone deprivation on the mnemonic response to E_2, given that such information could weigh heavily in their decision to initiate hormone therapy. Further, determining the dose and duration of treatment necessary to benefit memory, the length of time any benefits may last post-treatment, and the relative effectiveness of estrogen and estrogen plus progestin treatments for modulating various types of memory should help individual women decide on a treatment that will work best for them. Ideally, these issues should be addressed systematically using common methods and hormone formulations, with results replicated within and between labs to establish external validity and generalizability among rodent species and to menopausal women.

Because many women will take hormone therapy to alleviate menopausal symptoms, it is incumbent upon the research community to determine how treatment may affect cognitive function in these women, both during and after treatment. However, estrogens and progestins are complicated compounds that affect tissues throughout the body. Thus, it is reasonable to question the desirability of working towards the goal of optimizing ovarian hormone treatments

to modulate memory, as it may not be possible to develop treatments that have no effects in peripheral tissues such as the breast, uterus, or heart.

SERMs

One way in which to reap the benefits of ovarian hormone treatment, while mitigating its side effects, is through the use of selective estrogen receptor modulators (SERMs). Historically, SERMs have been non-steroidal compounds that act as estrogen agonists in some tissues and antagonists in others. A comprehensive review of the SERM literature is beyond the scope of this review (see (Zhao et al., 2005) for a recent review), but some of the most common of these mixed agonist/antagonist SERMs are discussed below, including tamoxifen, raloxifene, phytoestrogens, and ICI 182,780. In general, these SERMs have been disappointing in terms of their ability to improve memory in female rodents and postmenopausal women. Tamoxifen, a SERM used for the prevention and treatment of breast cancer in postmenopausal women, acts as an ER antagonist in tissues such as breast and an ER agonist in several tissues including bone, liver, and uterus (Mitlak and Cohen, 1997; Shang and Brown, 2002). In cultured hippocampal neurons, tamoxifen has modest effects as an ER agonist, but acts as a competitive antagonist in the presence of E_2 (Zhao et al., 2005). In postmenopausal women, tamoxifen use has been associated with impaired verbal memory (Jenkins et al., 2004) and an increase in reported memory problems (Paganini-Hill and Clark, 2000). These data are consistent with studies in mice showing that tamoxifen impairs spatial memory consolidation and retrieval (Chen et al., 2002a; Chen et al., 2002b). Raloxifene, which is currently approved for the treatment of osteoporosis, also acts a both an ER agonist and antagonist depending on the tissue (Wijayarante et al., 1999). In hippocampal cultures, raloxifene's neuroprotective effects suggest action as a partial to full ER agonist (Zhao et al., 2005). However, treatment of postmenopausal women with raloxifene for 3 years had no effect on verbal memory or attention (Yaffe et al., 2001). Similarly, raloxifene did not mimic the beneficial effects of E_2 on spatial working memory in young female rats (Gibbs et al., 2004) or aged rhesus monkeys (Lacreuse et al., 2002). Phytoestrogens are plant-derived estrogens that are structurally similar to endogenous estrogens, and include such compounds as genistein, genistin, diadzein, and formononetin (Zhao et al., 2005). Phytoestrogens exhibit neuroprotective abilities in hippocampal cultures, but do not promote neurite outgrowth (Zhao et al., 2005). One study in rats found that soy phytoestrogens significantly improved working memory in the radial arm maze (Pan et al., 2000). However, studies of women eating soy diets suggest no beneficial effect of these compounds; in women already using estrogen therapy, a high soy diet actually blocked the beneficial effects of the therapy (Kreijkamp-Kaspers et al., 2004; Rice et al., 1995). Perhaps the most promising of this group of SERMs may be ICI 182,780, which is typically considered a pure anti-estrogen. Recent work suggests that this compound may actually act as an estrogen agonist in the hippocampus, as it increases kinase activation, attenuates glutamate excitotoxicity, and protects against β-amyloid induced neurotoxicity similar to E_2 (Zhao et al., 2005). One recent study in young female rats found that ICI 182,780 alone enhanced spatial learning, despite the fact that it also reversed the beneficial effects of E_2 on spatial learning when the two compounds were given in combination (Zurkovsky et al., 2006). However, ICI 182,780 may only have agonist-like properties under certain circumstances. For example, my lab recently found that, unlike E_2 alone, ICI 182,780 alone did not enhance novel object recognition in young female mice, although it blocked the beneficial effects of E_2 when the compounds were given in combination (Fernandez et al., 2008). Thus, in order for ICI 182,780 to be considered a potential SERM for reducing age-related memory decline, the conditions under which it may act as an agonist must be delineated, and its effectiveness in aging females must be established. An additional complication with the potential clinical use of ICI 182,780 and similar compounds is that they cannot cross the blood brain barrier. However, studies are currently underway to design ICI-like compounds that can do so (Zhao et al., 2005).

One of the primary hurdles to the development of compounds that selectively bind to one of the cloned ERs is that the ligand binding domains of ERα and ERβ share over 50% homology and differ in only two amino acids within the binding site (Mosselman et al., 1996). Despite this challenge, some relatively selective SERMs that act only as ER agonists (not antagonists) have recently been developed and the results, thus far, seem promising. The most selective of these compounds are the ER-specific agonist propyl pyrazole triol (PPT), which has 410-fold selectivity for ERα over ERβ (Stauffer et al., 2000), and the ER-specific agonist diarylpropionitrile (DPN), which has 70-fold selectivity for ERβ over ERα (Meyers et al., 2001). Thus far, these compounds appear to improve certain types of memory in young ovariectomized rodents. In young female rats, DPN modestly improved spatial Morris water maze acquisition (Rhodes and Frye, 2006), whereas PPT enhanced spatial learning in an object placement task (Frye et al., 2007). Although both compounds have been reported to enhance novel object recognition in rats (Walf et al., 2006), my laboratory has found a beneficial effect of only DPN on novel object recognition in young female mice (Fernandez et al., 2006). Together, these data seem to suggest an important role of ERα in mediating memory for object locations and for ERβ in spatial reference memory and object recognition. However, it is important to note that both DPN and PPT can still bind to both ERs, which complicates the interpretation of their effects. Furthermore, these drugs also presumably bind putative membrane estrogen receptors, so even if they were completely selective for one of the cloned receptors, it would still be difficult to elucidate the specific receptor mechanisms through which they work. Regardless of receptor specificity, it remains possible that these compounds could be sufficiently selective to benefit cognitive function without causing clinically significant side effects in other tissues.

Molecular-based therapies

An alternative to further refining SERMs would be to elucidate the molecular mechanisms underlying memory-enhancing effects of hormones and develop drugs that target those mechanisms. As mentioned earlier in this review, E_2 can rapidly affect cellular function in a way that cannot be attributed to traditional genomic action of ERα and ERβ. For example, E_2 potentiates kainate-induced currents in cultured hippocampal CA1 neurons within 3 minutes of application, and this effect is independent of ERα and ERβ activation (Gu et al., 1999). In CA1, E_2 also rapidly enhances field excitatory postsynaptic potentials (EPSPs) (Bi et al., 2000; Foy et al., 1999; Teyler et al., 1980), increases the amplitude of intracellular EPSPs (Wong and Moss, 1992), and potentiates excitatory postsynaptic currents (Rudick and Woolley, 2003). Accordingly, long-term potentiation in CA1 is enhanced by E_2 (Bi et al., 2000; Foy et al., 1999), an effect that is blocked by the tyrosine kinase inhibitor, PP2 (Bi et al., 2000). Numerous other studies have demonstrated that exogenous E_2 activates several signaling cascades hippocampal neurons, including the ERK/MAPK (Fitzpatrick et al., 2002; Kuroki et al., 2000; Wade and Dorsa, 2003), PI3K/Akt (Mannella and Brinton, 2006; Yokomaku et al., 2003), tyrosine kinase (Bi et al., 2000), and protein kinase A (PKA) (Shingo and Kito, 2005) pathways.

The ERK/MAPK pathway is of particular interest for hormonal modulation of memory given recent data showing that this pathway is critical for many kinds of learning and memory, including hippocampal-dependent learning and memory (see (Adams and Sweatt, 2002; Sweatt, 2004) for reviews). ERK is one of a family of MAP kinases that is phosphorylated (i.e., activated) as part of a G-protein initiated signal transduction cascade. Ligand (e.g., a growth factor or hormone) binding to a G-protein activates the molecules (in order) Ras, Raf, MAPK/ERK Kinase (MEK), and ERK (Adams and Sweatt, 2002). Phosphorylated ERK (pERK) can then translocate into the cell nucleus where it leads to phosphorylation of cAMP response element binding protein (CREB). Phosphorylated CREB (pCREB) then binds to the DNA response element CRE, which mediates transcription of numerous genes and leads to the

translation of proteins including synaptic proteins like synaptophysin (Adams and Sweatt, 2002). Although other signal transduction cascades (e.g., cAMP/PKA, PKC) are implicated in memory consolidation, particular attention has been focused on ERK because ERK activation is necessary for PKA and PKC to activate CREB (Adams and Sweatt, 2002; Impey et al., 1998a; Murphy and Segal, 1997). Both pERK and pCREB are increased in the hippocampus within 1 hour after training in hippocampal-dependent tasks such as the spatial water maze (Blum et al., 1999), object recognition (Kelly et al., 2003), contextual fear conditioning (Atkins et al., 1998; Impey et al., 1998b), and inhibitory avoidance (Taubenfeld et al., 1999; Taubenfeld et al., 2001). Further, treatment with MEK inhibitors such as PD098059 or an anti-sense CREB oligonucleotide completely blocked training induced increases in pERK and pCREB (Atkins et al., 1998; Guzowski and McGaugh, 1997; Selcher et al., 1999). Importantly, these compounds also block long-term memory consolidation. In the water maze, infusion of PD098059 or an anti-sense CREB oligo into dorsal hippocampus prior to training impaired 48-hour retention (Blum et al., 1999; Guzowski and McGaugh, 1997). PD098059 also impaired 48-hour retention when injected immediately, but not 1 hour, after training, suggesting a 1 hour time window in which ERK regulates memory consolidation (Blum et al., 1999; Guzowski and McGaugh, 1997).

Recent work suggests that E_2 alters hippocampal physiology by activating ERK and CREB. In hippocampal cell lines, E_2 increased pERK, pCREB, and CRE-mediated gene transcription within 10–20 minutes of application (Fitzpatrick et al., 2002; Wade and Dorsa, 2003). MEK inhibitors completely blocked both these effects and also blocked E_2-mediated neuroprotection from β-amyloid and excitotoxicity (Bi et al., 2000; Fitzpatrick et al., 2002; Wade and Dorsa, 2003; Wade et al., 2001). In cultured hippocampal neurons, a MEK inhibitor blocked the E_2-induced increase in synaptic protein levels (Yokomaku et al., 2003) and an anti-sense CREB oligo blocked E_2-induced increases in pCREB and spine density (Murphy and Segal, 1997). In the intact rat, a single intracerebroventricular infusion of E_2 increased pERK in CA1 and CA3 within 5 minutes (Kuroki et al., 2000). In addition, the phosphorylation of the p42 isoform in the hippocampus is significantly reduced by ovariectomy in rats and restored by E_2 replacement (Bi et al., 2001). Although some of these effects may be mediated by classical estrogen receptors, other studies suggest non-genomic mechanisms (Wade et al., 2001; Watters et al., 1997). These studies include data indicating that a bovine serum albumin-conjugated form of E_2 (BSA-E_2) that cannot pass through the cell membrane can induce similar changes in hippocampal ERK activation as free E_2 (Fernandez et al., 2008; Kuroki et al., 2000). Regardless of the specific receptor mechanism, the data clearly indicate an important role for ERK and/or CREB in mediating E_2-induced changes in hippocampal plasticity. The question remains as to whether these molecules are also involved in E_2-induced alterations in memory.

Evidence from my laboratory suggests that ERK activation is necessary for E_2 to modulate memory. Using post-training E_2 treatments, we have found that the 0.2 mg/kg dose of E_2 that enhanced spatial and object memory consolidation (Fig. 3, Fig. 6) also significantly increased activation of the p42 isoform of ERK in the dorsal hippocampus within 60 minutes of a single i.p. injection or 5 minutes of an intracranial infusion (Fernandez et al., 2008;Lewis et al., 2008) (Figs. 8 and 9). Increases in both ERK activation and in object memory were significantly attenuated by dorsal hippocampal infusion of the cAMP inhibitor Rp-cAMPs or the NMDA antagonist APV (Fig. 8), suggesting that the E_2-induced enhancement of object memory involves NMDA receptors and protein kinase A (PKA) activation in the dorsal hippocampus (Lewis et al., 2008). However, neither treatment completely blocked the E_2-induced increase in dorsal hippocampal ERK activation, indicating the involvement of other kinases upstream of ERK (e.g., receptor tyrosine kinases, protein kinase C). Regardless of the upstream activators, the involvement of ERK appears to be necessary for post-training 0.2 mg/kg E_2 to enhance object memory consolidation, as demonstrated by the fact that systemic MEK inhibition by SL327 completely blocked dorsal hippocampal ERK activation (Fig. 9A and 9B)

and that dorsal hippocampal infusion of the MEK inhibitor U0126 completely blocked the E_2-induced enhancement of object memory consolidation (Fig. 9C) (Fernandez et al., 2008). Further, this study also demonstrated that effects of E_2 on object memory and dorsal hippocampal ERK activation could be mediated entirely by membrane-associated estrogen receptors; a membrane-impermeable form of E_2, bovine serum albumin-conjugated E_2 (BSA-E_2), had the same memory-and ERK-enhancing effects as E_2 (Fig. 9D and 9E), and effects on object memory were blocked by intrahippocampal infusion of U0126 (Fig. 9E) (Fernandez et al., 2008).

Downstream from ERK, a recent microarray analysis from my laboratory identified several genes of interest in the dorsal hippocampus altered by 0.2 mg/kg E_2 1 hr after injection, including reduced expression of insulin-like growth factor (IGF) binding protein 2 (IGFBP2) (Pechenino and Frick, 2007). Numerous interactions between IGF and E_2 have been previously documented (Mendez et al., 2006). IGFBP2 typically acts to sequester IGF-1 and prevent this protein from activating elements of the IGF cascade such as PI3K and Akt (Chesik et al., 2007). Thus, reduced expression of IGFBP2 would lead to greater availability of IGF-1, which should lead to increased activation of PI3K and Akt. Because PI3K can lead to ERK activation, an E_2-induced reduction in IGFBP2 should lead to increased activation of ERK, thereby providing another route by which E_2 can modulate ERK. Together, the data from this molecular line of experimentation demonstrate that dorsal hippocampal ERK activation is critical to the beneficial effects of acute E_2 treatment on object memory consolidation, and suggest that the development of treatments that increase ERK activation in the dorsal hippocampus may be one way in which to obtain the beneficial effects of E_2 without incurring the side effects of hormone treatment.

However, as with behavior, it is important to consider how age-related alterations in the hippocampus may affect the molecular response to E_2. Because hippocampal ERK phosphorylation and ERK mRNA levels are reduced in aged rats (Bi et al., 2003; Gooney et al., 2004; Simonyi et al., 2003; Zhen et al., 1999), E_2 may activate ERK to a lesser extent in aged animals. Such a reduction in ERK signaling could diminish the mnemonic response to E_2 in aged females. Indeed, recent work has shown that age-related reductions in the response of basal forebrain neurons to intrahippocampal infusion of another important modulator, nerve growth factor, are due to disruptions in basal forebrain ERK activation (Williams et al., 2007; Williams et al., 2006). Nevertheless, because the aged hippocampus is able to mount robust ERK activation in response to other growth factors (Mo et al., 2005), E_2 may still be able to activate ERK in the aged hippocampus. Because this effect has yet to be tested, it remains to be seen whether any ERK activation produced in the aged hippocampus by E_2 is necessary for, or is even associated with, E_2-induced improvements in learning and memory among middle-aged and aged females. Such work will be pivotal to establishing the validity of this approach for the design of treatments for reducing age-related memory decline.

Although this line of research is in its infancy, an approach focusing on molecules through which E_2 acts to modulate memory may lead to specific targets to which non-steroidal drugs that mimic the beneficial effects of E_2 can be designed. Because these drugs would modulate the downstream effectors of estrogen receptors, rather than the receptors themselves, resulting drugs should not produce the detrimental side effects inherent to hormone- and SERM-therapy. Critical to this approach is the validation of molecular targets in aging females, by not only establishing that E_2 activates these targets in the aging hippocampus, but also that such activation is necessary for E_2 to enhance memory. If such targets can be identified and validated in aging females, then this molecular approach to hormone therapy may ultimately prove useful in providing treatments that can safely and effectively reduce age-related memory decline.

Conclusions

Studies conducted in aging female rodents have begun to shed light on the importance of estrogens and progestins as critical modulators of age-related memory decline. There are numerous practical and scientific advantages of using rodents as models of age-related cognitive decline; of these, the short rodent life span should allow for relatively rapid advances in understanding how factors like age, hormone deprivation, type of treatment, and the environment influence the neural and mnemonic response to E_2. The fact that fewer than two-dozen empirical studies have been published examining effects of E_2 treatment on memory in aging female highlights the need for much more research on this topic. Continuing this work is of great importance to both gerontology and women's health, given how little is currently known about ovarian hormone therapy in aging females. Further, the development of non-steroidal treatments that mimic the beneficial effects of E_2 on memory but lack side effects in peripheral tissue is also paramount to reaping the potential benefits of hormone therapy. As such, interdisciplinary studies that aim to identify the molecular mechanisms through which E_2 modulates memory in young and aging females should set the stage for hormone-based therapies of the future.

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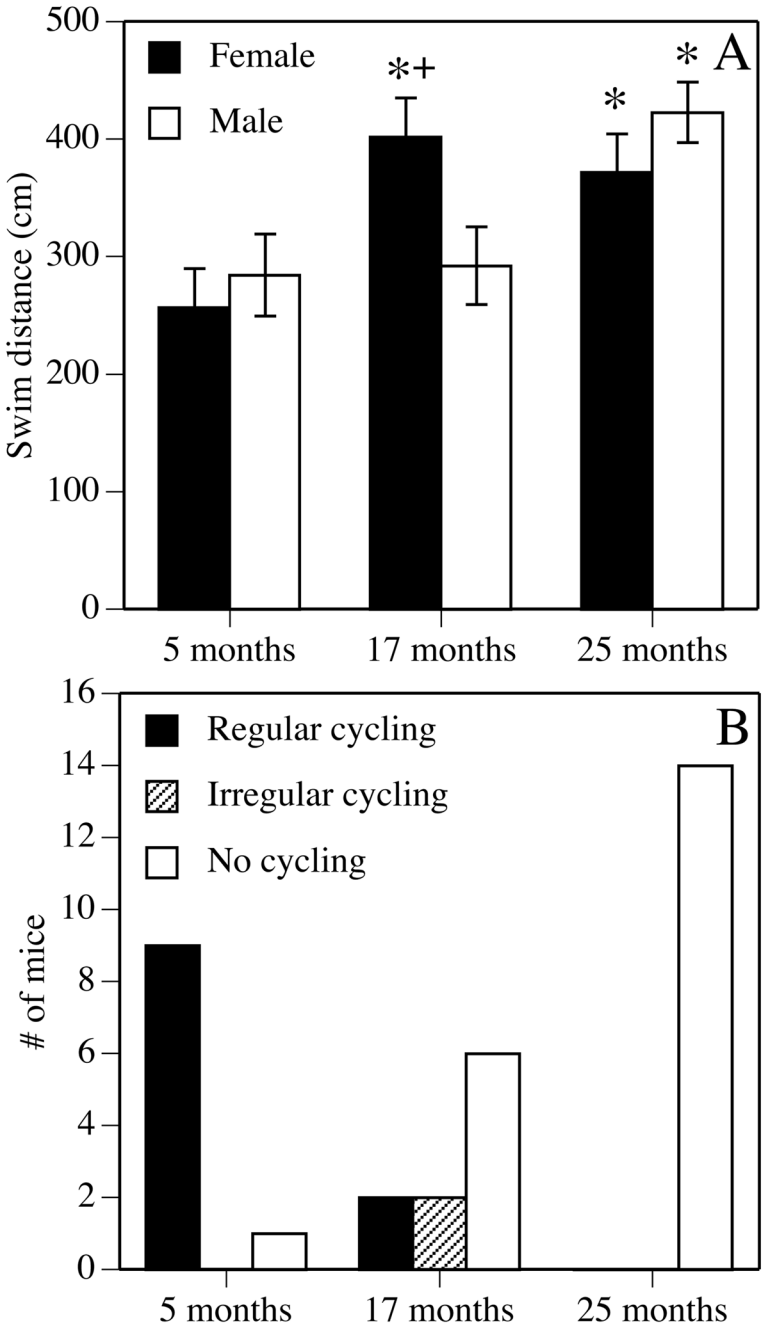

Figure 1.

Effects of age on spatial reference memory in the Morris water maze (A) and estrous cycling (B). (A) Gonadally intact male and female mice were tested for 5 days in a spatial Morris water maze task at 5 (young), 17 (middle-aged), or 25 (aged) months of age. Each bar represents the mean ± standard error of the mean (SEM) swim distance for all 5 days of testing; lower numbers indicate better performance. Middle-aged and aged females were significantly impaired relative to young females, whereas only aged males were impaired relative to young males (*$p < 0.05$). Middle-aged females were also significantly impaired relative to middle-aged males (+$p < 0.05$). This pattern of data indicates that the onset of spatial reference memory decline in females occurs at an earlier age in females than in males. Adapted from (Frick et al.,

2000; Frick et al., 2002a). (B) Estrous cycling was measured using daily vaginal lavage. The incidence of regular 4–5 day estrous cycles declined with age, such that no aged females were observed cycling regularly, whereas the number of mice failing to cycle increased with age. Irregular cycling, consisting of prolonged cycles, was observed among some middle-aged females. Adapted from (Frick et al., 2000).

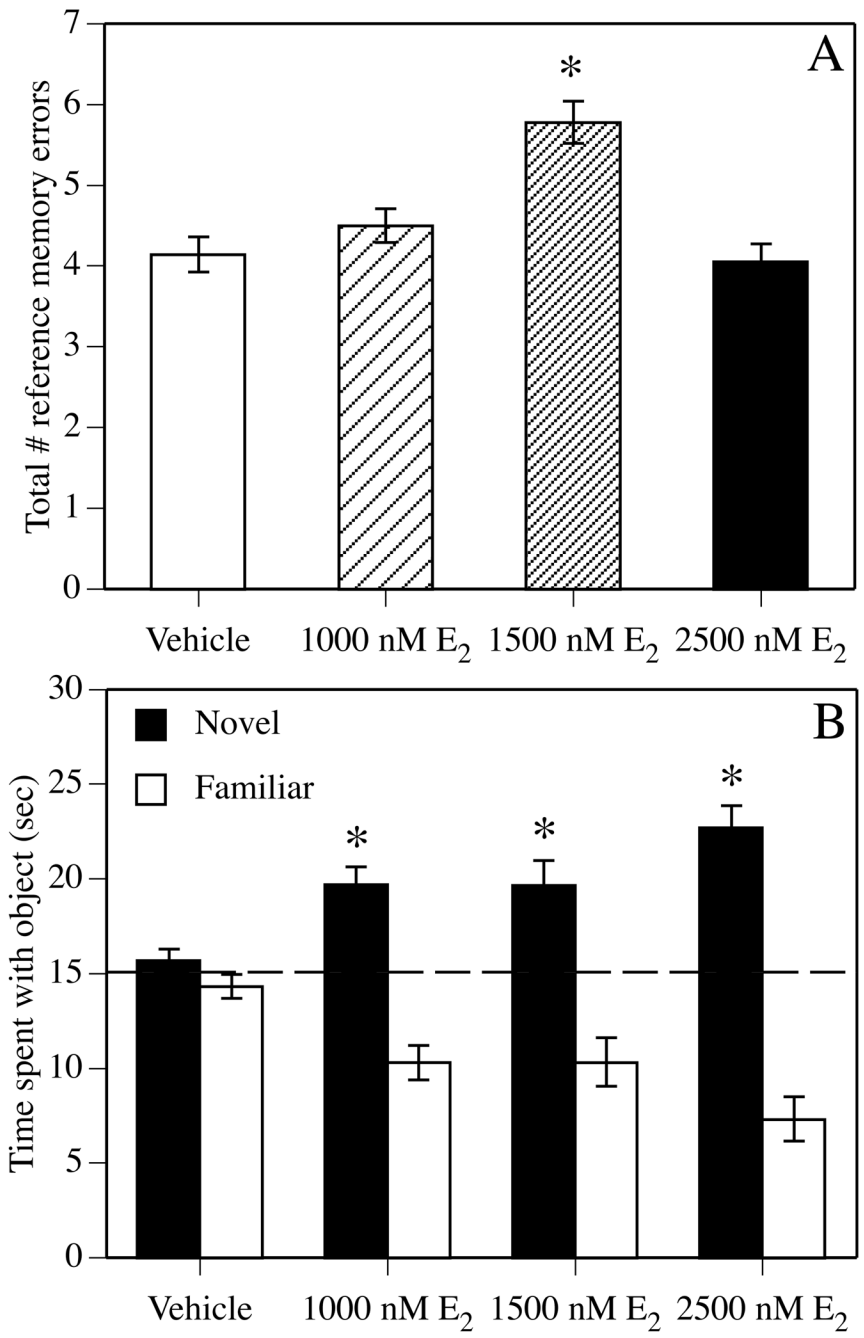

Figure 2.

Vehicle or E_2 were dissolved in ethyl alcohol and delivered via the home cage drinking water for 5 weeks prior to, and then during, testing in water-escape motivated radial arm maze and novel object recognition tasks. (A) In the radial arm maze, 1500 nM E_2 significantly increased the number of spatial reference memory errors made during testing (*$p < 0.05$ relative to vehicle controls). Each bar represents the mean (± SEM) of 15 days of testing. (B) During novel object recognition training, mice accumulated 30 seconds exploring two identical objects and then were immediately injected with vehicle or E_2. Forty-eight hours later, all doses of E_2 significantly increased the time spent with the novel object relative to chance (dashed line at 15 seconds; *$p < 0.05$ relative to chance), indicating intact memory for the familiar object.

Each bar represents the group mean (± SEM) for the retention trial. Adapted from (Fernandez and Frick, 2004).

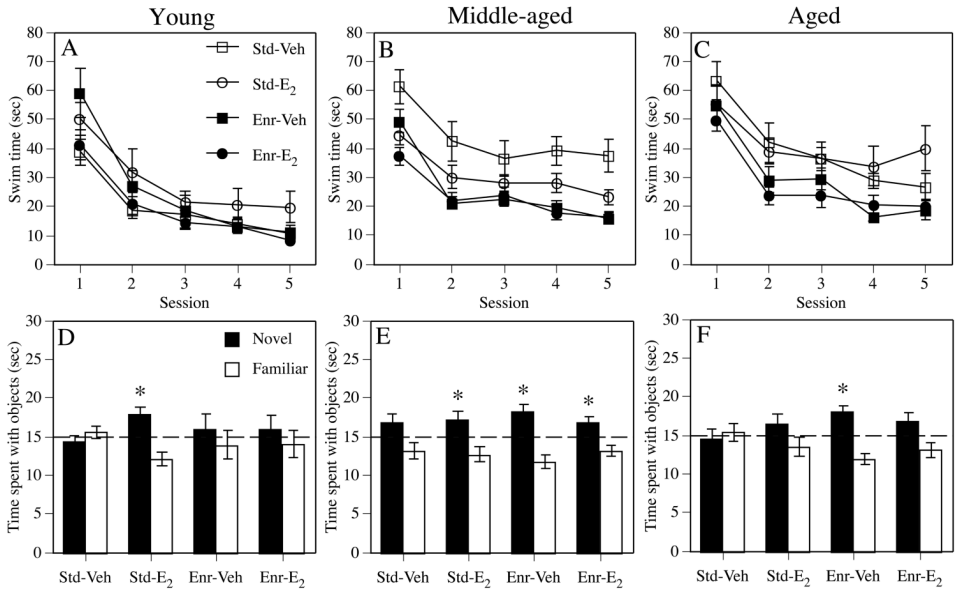

Figure 3.

Female mice were housed from the age of 3 weeks in standard (Std) conditions (5 mice/shoebox cage) or enriched (Enr) conditions (up to 10 mice in a large cage with many objects) up to and through behavioral testing at 5, 17, or 22 months of age. Mice were ovariectomized approximately 2 weeks before behavioral testing and were injected i.p. with vehicle (Veh) or 0.2 mg/kg E_2 immediately after training each day. (A–C) Mice were tested for 5 days in a spatial Morris water maze task. Among young females, spatial memory was impaired by E_2 alone, but improved by the combination of E_2 and enrichment. Among middle-aged females, E_2 improved spatial memory in standard-housed mice only, whereas enrichment improved spatial memory regardless of hormone treatment. Among aged females, only enrichment improved spatial memory. Each point represents the mean (± SEM) for one session. (D–F) Only E_2 alone enhanced 48-hour object recognition relative to chance (dashed line at 15 seconds; $*p < 0.05$) in young females, whereas only enrichment alone enhanced object recognition in aged females. Object recognition in middle-aged females was enhanced relative to chance by E_2 alone, enrichment alone, and the combination of both treatments. Each bar represents the group mean (± SEM) for the retention trial. Adapted from (Gresack et al., 2007a).

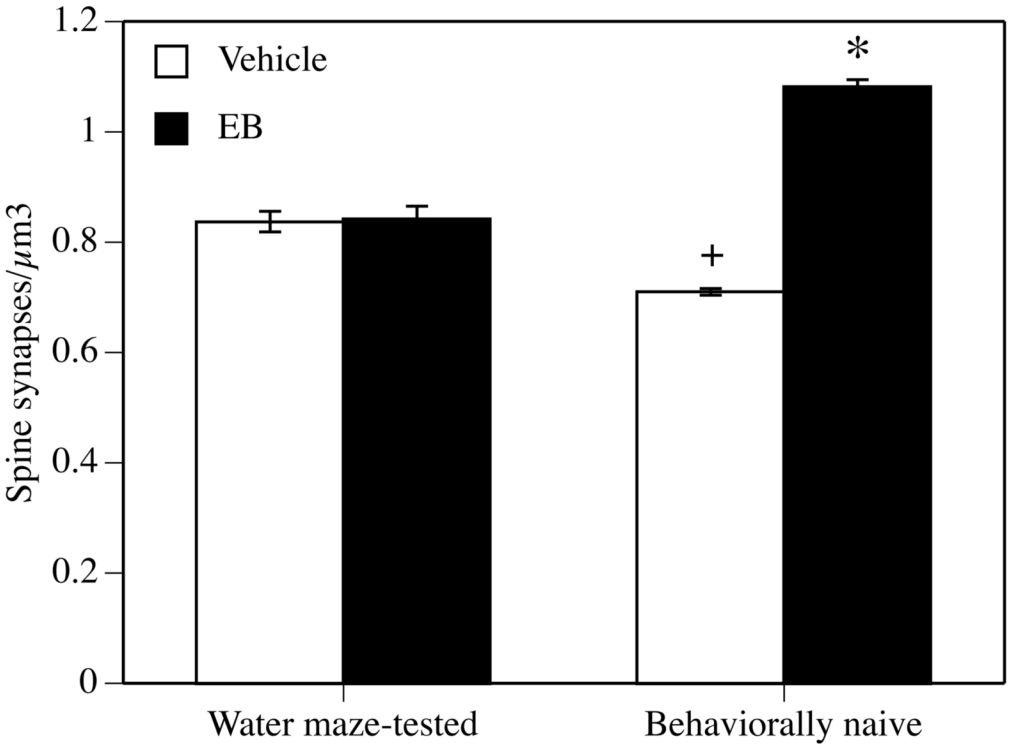

Figure 4.

CA1 spine synapse density in vehicle- and estradiol-treated rats that were behaviorally naïve or tested in the Morris water maze. Young ovariectomized rats were given two injections, 24 hours apart, of sesame oil or 10 μg estradiol benzoate (EB). Forty-eight hours after the second injection, rats were tested in a 1-day spatial Morris water maze task and then immediately perfused for analysis of CA1 spine synapse density. Spine synapse density did not differ between vehicle and EB-treated rats tested in the water maze. In contrast, behaviorally naïve EB-treated rats exhibited a significantly higher density of spine synapses than behaviorally naïve vehicle controls and than both groups tested in the water maze (*$p < 0.05$ relative to all other groups). Behaviorally naïve controls also had fewer spines than both water maze-tested groups (+$p < 0.05$ relative to all other groups). Each bar represents the mean (± SEM). Reprinted from (Frick et al., 2004).

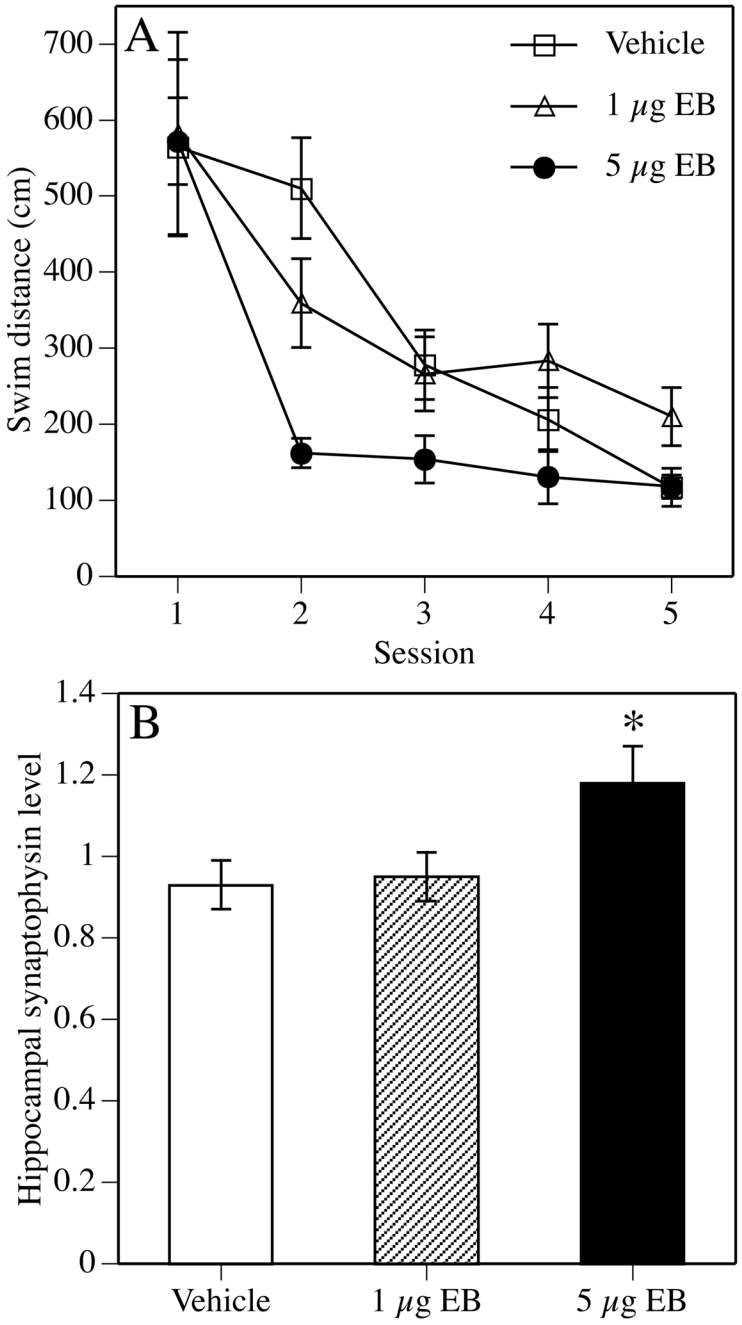

Figure 5.

Gonadally intact 27–28 month-old female mice were injected subcutaneously with sesame oil vehicle or 5 μg estradiol benzoate (EB) for 5 days prior to spatial Morris water maze testing, and then each day 4 hours prior to testing. (A) Although all mice learned to find the platform, mice receiving 5 μg EB learned significantly faster than vehicle controls or mice receiving 1 μg EB. Each point represents the mean (± SEM) for an entire session. (B) At the conclusion of testing, synaptophysin protein levels were measured in whole hippocampus. Synaptophysin levels are expressed as the amount of synaptophysin in each sample relative to the amount of synaptophysin in a homogenate of whole mouse brain. Mice receiving 5 μg EB exhibited

significantly higher hippocampal synaptophysin levels than vehicle controls. Adapted from (Frick et al., 2002b).

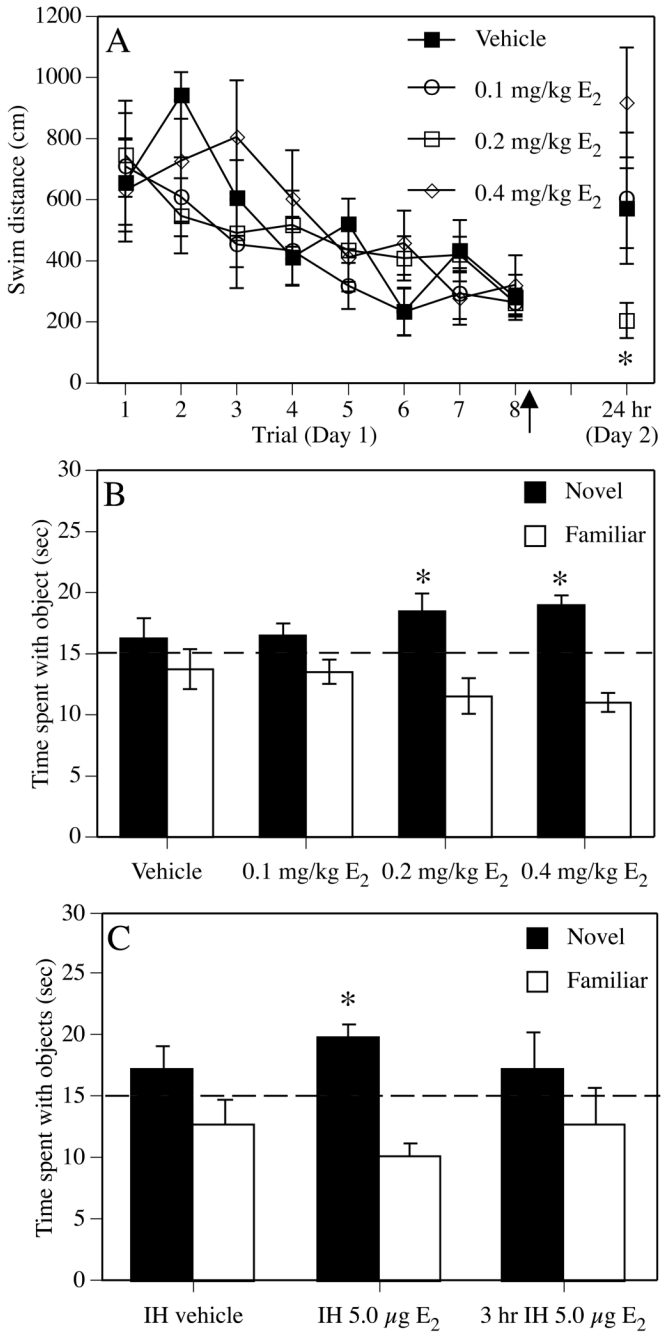

Figure 6.

Effects of post-training estradiol on memory consolidation in young ovariectomized mice. (A) 0.2 mg/kg E_2 significantly improved spatial memory retention in the Morris water maze. All groups learned to find the platform similarly on Day 1 (training trials 1–8). Mice were injected intraperitoneally (i.p.) with vehicle or E_2 immediately following trial 8 (arrow). Twenty-four hours later, only mice receiving 0.2 mg/kg E_2 remembered the platform location, as indicated by shorter swim distances on Day 2 relative to vehicle controls and mice receiving 0.4 mg/kg E_2 (*$p < 0.05$). Each point represents the mean (± SEM) for a single trial. (B) During object recognition testing, groups receiving 0.2 mg/kg or 0.4 mg/kg E_2 spent significantly more time than chance (dashed line at 15 seconds; *$p < 0.05$) with the novel object 48 hours after

injection), suggesting intact memory for the familiar object. (A) and (B) reprinted from (Gresack and Frick, 2006b). (C) Intrahippocampal infusion of E_2 immediately, but not 3 hours later, also enhances novel object memory consolidation. Mice receiving immediate bilateral dorsal hippocampal infusions of 5 μg E_2, but not vehicle, spent significantly more time than chance with the novel object 48 hours after infusion (dashed line at 15 seconds; $*p < 0.05$). For both panels, each bar represents the group mean (± SEM) for the retention trial. Adapted from (Fernandez et al., 2008).

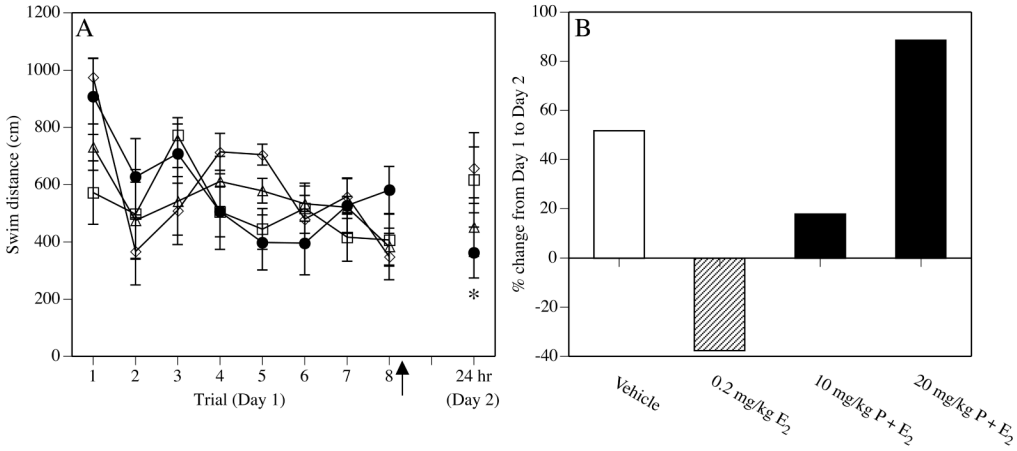

Figure 7.

Aged ovariectomized females were trained in a spatial Morris water maze task and then immediately injected i.p. (arrow) with vehicle, 0.2 mg/kg E_2, 10 mg/kg progesterone + 0.2 mg/kg E_2, or 20 mg/kg progesterone + 0.2 mg/kg E_2 (n = 11 for vehicle, n = 10 for all other groups). (A) All mice learned to find the platform during training on Day 1. On Day 2, the performance of all mice but those receiving 0.2 mg/kg E_2 alone deteriorated relative to that during the last trial of Day 1 (*$p < 0.05$ for the 0.2 mg/kg E_2 group Day 1 vs Day 2), suggesting that only 0.2 mg/kg E_2 alone enhanced memory for the platform location in aged females. The 20 mg/kg dose of progesterone completely blocked this effect. (B) Percent change from trial 8 of Day 1 to trial 1 of Day 2. Positive numbers indicate worse performance on Day 2 relative to Day 1. Only 0.2 mg/kg E_2 alone improved performance from Day 1 to Day 2. Adapted from (Harburger et al., 2007).

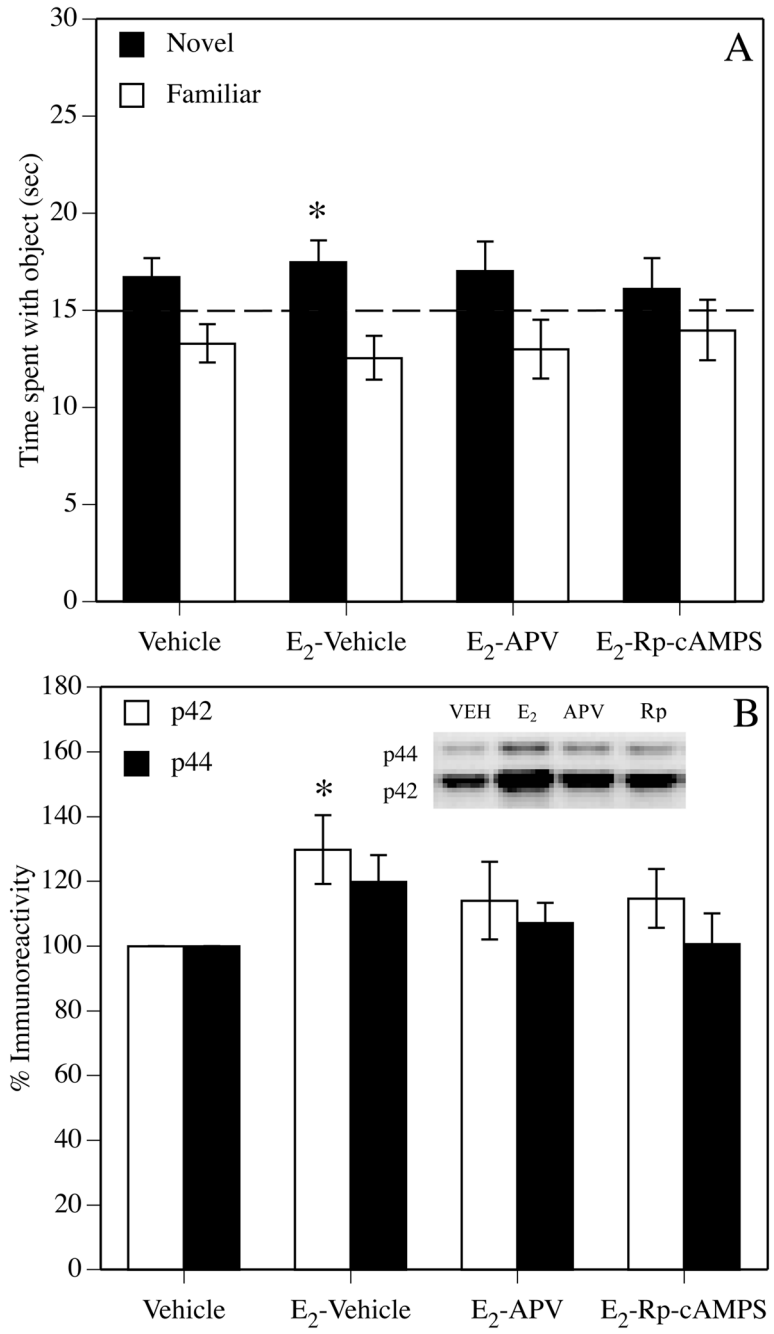

Figure 8.

(A) Mice were trained in the novel object recognition task and then immediately injected i.p. with vehicle or 0.2 mg/kg E_2 and intrahippocampally infused with vehicle, the NMDA receptor antagonist APV (D-2-Amino-5-phosphonovaleric acid; 5.0 mg/ml, 2.5 μg/side), or the cAMP inhibitor Rp-cAMPS (Rp-Cyclic 3′,5′-hydrogen phosphorothioate adenosine triethylammonium salt; 36 mg/ml, 18.0 μg/side). Mice treated with i.p. E_2 and intrahippocampal vehicle (E_2-Vehicle) spent significantly more time with the novel object than mice treated with i.p. and intrahippocampal vehicle (Vehicle; $*p < 0.05$ relative to Vehicle). Neither group treated with i.p. E_2 and intrahippocampal APV or Rp-cAMPS (E_2-APV and E_2-Rp-cAMPS) spent significantly more time with the novel object than vehicle controls,

suggesting that dorsal hippocampal infusions of either drug reduced the E_2-induced enhancement of object recognition. The dashed line at 15 seconds represents chance performance. Each bar represents the group mean (± SEM) for the retention trial. (B) Effects of E_2 on dorsal hippocampal activation of the two isoforms of ERK, p42 and p44. Western blotting was used to measure phospho-ERK levels, which were normalized to total p42/p44 ERK levels. Data are presented as percent increase in immunoreactivity relative to vehicle controls. E_2 alone significantly increased levels of phosphorylated p42 30% above vehicle controls ($*p < 0.05$). Intrahippocampal infusion of APV or Rp-cAMPS attenuated this increase (14% over control). Neither APV nor Rp-cAMP completely blocked the activation of p42. Each bar represents mean (± SEM) immunoreactivity. Inset: Representative Western blots for phosphorylated/total p42 and p44. Reprinted from (Lewis et al., 2008).

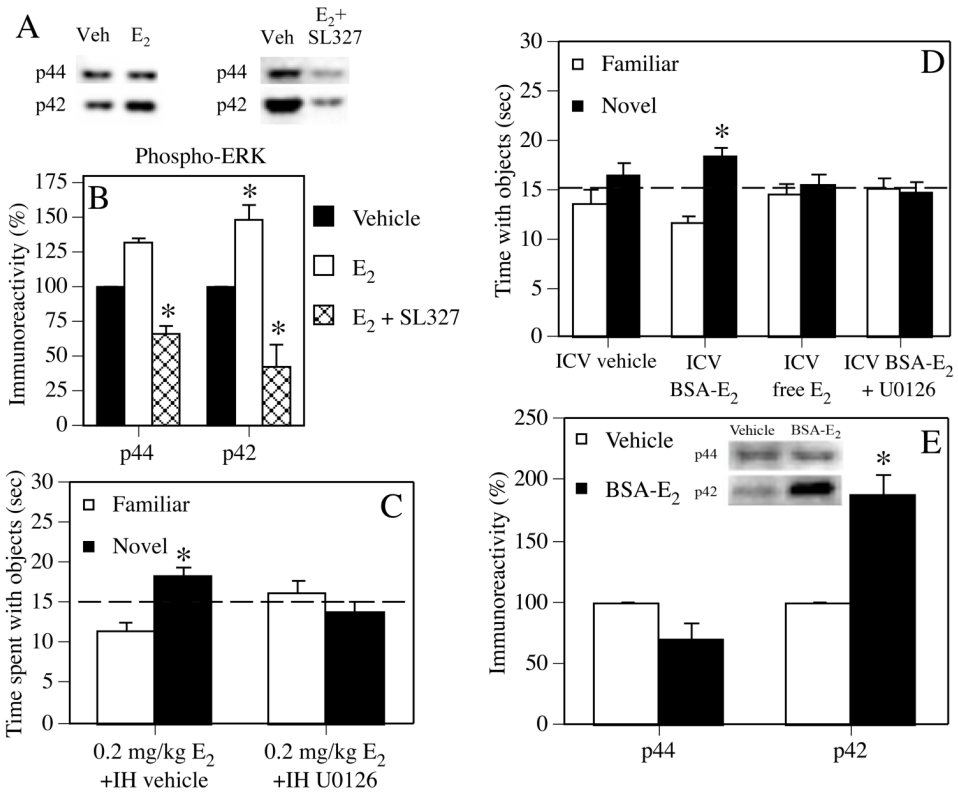

Figure 9.

(A) Representative Western blots showing phosphorylated p42 and p44 ERK protein levels in dorsal hippocampus 1 hour after i.p. 0.2 mg/kg E_2 injection. (B) E_2 significantly increased phospho-p42, but not phospho-p44, ERK levels, and 30 mg/kg SL327 completely blocked this increase. Each bar represents mean (± S.E.M.) percent change from Vehicle (Veh) controls (*$p < 0.05$ relative to vehicle). (C) Mice receiving 0.2 mg/kg E_2 plus intrahippocampal (IH) infusions of vehicle spent significantly (*$p < 0.05$) more time with the novel object than chance (dashed line at 15 seconds) 48 hours after training, thus, demonstrating memory for the familiar object. This beneficial effect of E_2 was blocked by IH infusion of U0126 (0.5 μg/side). (D) Mice receiving intracerebroventricular (ICV) infusions of bovine serum albumin-conjugated E_2 (BSA-E_2, 5 μM) spent significantly more time with the novel object than chance. This effect was blocked by IH infusions of U0126 (0.5 μg/side). Controls receiving ICV infusions of vehicle or 850 pg/μl regular E_2 (free E_2) did not prefer the novel object. Each bar represents the mean (± S.E.M.) time spent with each object (*$p < 0.05$ relative to chance). (E) ICV BSA-E_2 infusions significantly increased phospho-p42, but not phospho-p44, ERK levels in the dorsal hippocampus 5 minutes after infusion. Each bar represents mean (± S.E.M.) percent change from vehicle controls (*$p < 0.05$ relative to vehicle). Inset: Representative Western blots showing phosphorylated p42 and p44 ERK protein levels.