Region-specific Mechanisms of Estrogen and Age on Neuronal Ensemble Activity During Spatial Navigation

by

Kristen Elizabeth Pleil

Department of Psychology & Neuroscience
Duke University

Date:_____

Approved:

_____
Christina L. Williams, Supervisor

_____
Warren H. Meck

_____
Staci D. Bilbo

_____
R. Alison Adcock

Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor of Philosophy in the Department of
Psychology & Neuroscience in the Graduate School
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ABSTRACT

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ABSTRACT

Estradiol modulates the use of spatial navigation strategies in female rats. The presence of circulating estradiol enhances learning on tasks that require the use of a hippocampus-dependent place strategy and impairs learning on tasks that require the use of a dorsal striatum-dependent response strategy. When either strategy may be used successfully, estradiol biases females to use a place strategy. While this behavioral effect has been well-described in the young adult female rat, little is known about the mechanisms in the brain that underlie it or how it changes across age. The experiments in this dissertation examined how age, previous experience, and hormonal condition affect the ability of estradiol to modulate learning during explicit training of place and response tasks, as well as navigation strategy use during ambiguous navigation tasks. Age highly influenced the ability of estradiol to influence strategy use. While female rats could use place and response strategies to navigate by postnatal day (PD) 21, estradiol did not bias them to use a response strategy until PD26, just before puberty. In adulthood, previous navigation experience and estradiol interacted to influence navigation strategy use on a series of experiences to an ambiguous navigation task. And, estradiol impaired learning during explicit response training but did not affect place learning. In middle age, estradiol further impaired response learning but still did not affect place learning. Long-term hormone deprivation, however, was detrimental to acquisition of a place task but did not affect response learning.

These experiments also examined the effects of estradiol on activity, plasticity, and reliability of neuronal ensembles in several subregions of the hippocampus and striatum during spatial navigation using cellular and molecular techniques that take advantage of the kinetics of the immediate-early genes *c-fos* and *Arc*. Increased activation and plasticity during active exploration across several subregions of the hippocampus and striatum reflected similar inputs to these neural systems and similar effects of exploration. However, estradiol modulated the plasticity and reliability of neuronal ensembles in the hippocampus and striatum specifically during goal-directed spatial navigation. Estradiol increased plasticity in CA1 of all behaviorally-trained rats, but only place strategy users displayed high reliability in this plasticity across training and probe trials on a navigation task. Estradiol prevented increase in plasticity and reliability in the dorsolateral striatum displayed by low estradiol response strategy users. These experiments reveal how several factors, including age, influence estradiol's modulation of spatial navigation strategy use and suggest functional mechanisms by which this modulation occurs.

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INTRODUCTION

Overview

Estradiol modulates the functions of the hippocampus (HPC) and dorsal striatum (DS) in the adult female rat, and prior research has used spatial navigation tasks that rely on these brain regions to examine the effects of estradiol on behaviors dependent on these brain regions. Estradiol facilitates HPC-dependent place learning and impairs DS-dependent response learning; and, when either a place or response strategy may be employed successfully, estradiol biases a female to use a place strategy over a response strategy. The effects of estradiol on explicit learning are thought to be caused by the female's bias to use a place strategy, which facilitates learning when a place strategy must be used and impairs learning when a response strategy is used. Thus, both behavioral effects of estradiol on spatial navigation are hypothesized to be a consequence of the same effects of estradiol on hippocampal and/or striatal function that change the interaction between the HPC and DS as they compete for control over navigation behavior. While the effects of estradiol on spatial navigation behavior have been well-described in the young adult female rat, very little is known about the mechanisms by which estradiol influences hippocampal and striatal function, as well as the interaction between the two neural systems. In addition, because research has focused on the young adult female rat, there is currently almost no understanding about what factors, such as age and previous experience, modulate the presence and robustness of this effect.

This dissertation examines the factors that influence estradiol's ability to modulate spatial navigation strategy use, including age and prior navigation experience, as well as the functional mechanisms by which estradiol modulates spatial navigation strategy use. Therefore, this introduction a) describes the parallel memory systems that are capable of supporting spatial navigation via distinct strategies, b) details the hormonal and task parameters that are currently known to influence the robustness of estradiol's behavioral effects, c) explains the possible functional mechanisms by which the HPC and DS may compete for control over navigation behavior, and d) describes how estradiol might influence these mechanisms to modulate navigation behavior.

Parallel Neural Systems for Spatial Navigation

Throughout the past several decades, the plasticity of the mammalian brain has been illustrated in many neural systems required for survival. These systems are highly flexible so that they can adapt their functions to meet the demands of the environment. The HPC and DS are each a crucial part of plastic neural systems that are capable of guiding spatial navigation (White and McDonald, 2002). The HPC guides navigation behavior using the relationship between cues in the external environment to provide the organism a "place" strategy (e.g., Tolman, 1948; O'Keefe and Dostrovsky, 1971; O'Keefe and Burgess, 1996), while the DS provides the organism with a motor "response" strategy by forming a representation based on internal movement cues (e.g., Packard et al., 1989; Packard and McGaugh, 1992). Many researchers believe that having multiple

navigation strategies available is essential for the cognitive flexibility needed for fast and accurate navigation in any context (e.g., Sherry and Schacter, 1987). However, when both strategies can be successfully employed, the HPC and DS may directly compete for control over navigation behavior. Lesions or inactivation of the HPC impair place learning and sometimes even enhance response learning, and lesions or inactivation of the DS impair response learning and sometimes even enhance place learning (Olton and Samuelson, 1976; Olton and Papas, 1979; Mitchell and Hall, 1988; Devan et al., 1999; Chang and Gold, 2003a). When either the HPC or DS may be used for successful navigation, lesioning or inactivating one system does not impair performance on the task and may even improve the rate of acquisition (Packard et al., 1989; McDonald and White, 1993).

When both neural systems are intact, the salience of external and internal cues modulates the activity of the HPC and DS (Mizumori et al., 2000a). For example, when external cues are available and salient, the HPC is likely to be more activated than the DS and consequently guide navigation behavior; when external cues are not available, internal cues are salient and the DS is much more likely to control behavior. When both types of cues are available, drugs and other activating agents that alter the activation levels of the HPC and DS modulate the use of place and response strategies (Matthews et al., 1999; McNay et al., 2001; Korol et al., 2004). For example, on a spatial navigation task that can be solved using either strategy, injecting glucose directly into the HPC causes an increase in the percent of rats that use a hippocampal strategy to solve the task,

while an injection into the DS causes a higher percentage of rats to use a striatal strategy (Canal et al., 2005). And, glucose administration to the DS impairs learning of a place task (Pych et al., 2006), supporting the hypothesis that relative activation in the HPC and DS influences navigation behavior. Ch. 2 tests this hypothesis by assessing the amount of activation in the HPC and DS while rats perform place and response navigation tasks.

Anatomical organization

The HPC and DS receive similar sensory and reward information but in very different ways. While the HPC receives its primary input from the entorhinal cortex (EC) and processes information during spatial navigation via its recurrent circuit, the DS receives extremely complex and overlapping inputs from many brain areas (see Figure 1). Within each system, specific subregions contribute to the function of these processes. Because the organization of processing within the HPC and DS are extremely different, it is not surprising that the styles of processing are very different. The HPC is able to be very flexible, as it tracks many events and stimuli simultaneously and in succession and filters information while the DS is much less flexible, as a particular behavior is triggered in response to specific internal or external stimuli (White and McDonald, 2002). However, both neural systems send output to cortical and motor areas (Wyss, 1981; McGeorge and Faull, 1989) that may converge at the prefrontal cortex (PFC; White and McDonald, 2002). The unique processing styles of the HPC and DS, as well as the intrinsic and extrinsic connections of each system, likely affect how estradiol modulates

the functions of specific hippocampal and striatal subregions during spatial navigation. Therefore, chapters 2, 3, and 4 examine each subregion separately to determine the roles of each one and the functional connections between them.

Hippocampus

The EC integrates and projects environmental sensory information from visual, olfactory, and perirhinal cortices to the HPC via the perforant pathway (Andersen et al., 1966b, 1966a; Hjorth-Simonsen, 1972; Hjorth-Simonsen and Jeune, 1972; Steward and Scoville, 1976). The HPC also receives input from the basal forebrain (medial septum and diagonal band of Broca) via the fimbria-fornix pathway (Lewis et al., 1967; Fonnum, 1970; Lindvall and Bjorklund, 1974; Amaral and Kurz, 1985; Wainer et al., 1985) and the amygdala (Pitkanen et al., 2000) that modulate hippocampal learning and memory (White and McDonald, 2002). The HPC includes the dentate gyrus (DG) and CA1–3 subfields of the HPC, and the subiculum (Swanson et al., 1978; Sharp and Green, 1994). The majority of information from the perforant and fimbria-fornix pathways enters the HPC via the DG, which filters information and sends it to CA3 (Blackstad et al., 1970; Gaarskjaer, 1978b, 1978a). CA3 projects to CA1, which projects to the subiculum (Swanson et al., 1978), and the subiculum projects to the EC (Beckstead, 1978). While this is the primary pathway by which information travels within the HPC, CA3 projects onto itself and contributes feedback information to the DG to filter information (Amaral and Witter, 1989; Scharfman, 2007). In addition, the EC projects to each subregion of the

HPC, including a bidirectional connection with CA1 (Yeckel and Berger, 1990; Witter, 1993; Deller et al., 1996; Naber et al., 2001)

Information is sent from the HPC to other brain regions via two primary pathways, the fimbria-fornix and the retrohippocampal output. The fimbria-fornix serves as both an efferent and afferent pathway between the HPC and subcortical areas including the lateral septum (Siegel et al., 1974), supramammilary bodies of the thalamus (Meibach and Siegel, 1975), ventromedial hypothalamus (Irle and Markowitsch, 1982), and the nucleus accumbens of the ventral striatum (VS; Kelley and Domesick, 1982). In addition to these direct connections to the HPC, the fimbria-fornix may also serve as a direct pathway from the septum to EC (Swanson and Cowan, 1979; Gaykema et al., 1990). The retrohippocampal output connects the subiculum to the entorhinal, prefrontal, and cingulate cortices (Swanson and Kohler, 1986) as well as the medial portion of the DS (Groenewegen et al., 1987).

As implied by the circuitry within the HPC and its efferent and afferent pathways, the HPC integrates environmental information during spatial navigation and distributes this information to higher order cortical areas that modulate ongoing navigation behavior (Rezai et al., 1993; Stuss et al., 1995). Hippocampal neurons known as place cells represent specific spatial locations, and together, the population forms a “cognitive map” of the external environment (e.g., O'Keefe, 1978). The connections between structures within the HPC circuit are plastic and therefore able to change in order to provide a means of memory formation by the system (Malenka and Nicoll, 1993; McNaughton,

1993). The mechanisms likely responsible for this synaptic plasticity are long-term potentiation (LTP) and long-term depression (LTD) that allow for the flexibility of representation within the system (e.g., Bliss and Lomo, 1973). And, the connections between the HPC and subcortical regions, especially the nucleus accumbens, which encodes important information about the reward component during spatial navigation, suggests further involvement in choice behavior and motor planning (White and McDonald, 2002; Adcock et al., 2006).

Dorsal striatum

The lateral (DLS) and medial (DMS) portions of the DS serve slightly different functions but are fused together and share information with one another via intimate connections. The DS receives converging, overlapping, and organized inputs from several subcortical and sensory cortical areas (Beckstead, 1979; Graybiel and Ragsdale, 1979; Battaglini et al., 1982; Gerfen, 1984; Jayaraman, 1985; Donoghue and Herkenham, 1986; McGeorge and Faull, 1989; Groenewegen et al., 1990). When a particular stimulus is presented, a corresponding corticostriatal loop is triggered that represents an action, and reward helps strengthen the association between the stimulus and response (for review, see Packard and Knowlton, 2002). This representation is stored in the DMS (Yin et al., 2005a; Yin et al., 2005b; Yin and Knowlton, 2006). Over time, the repeated presentation of the stimulus triggers the activation of the same loop and produces a

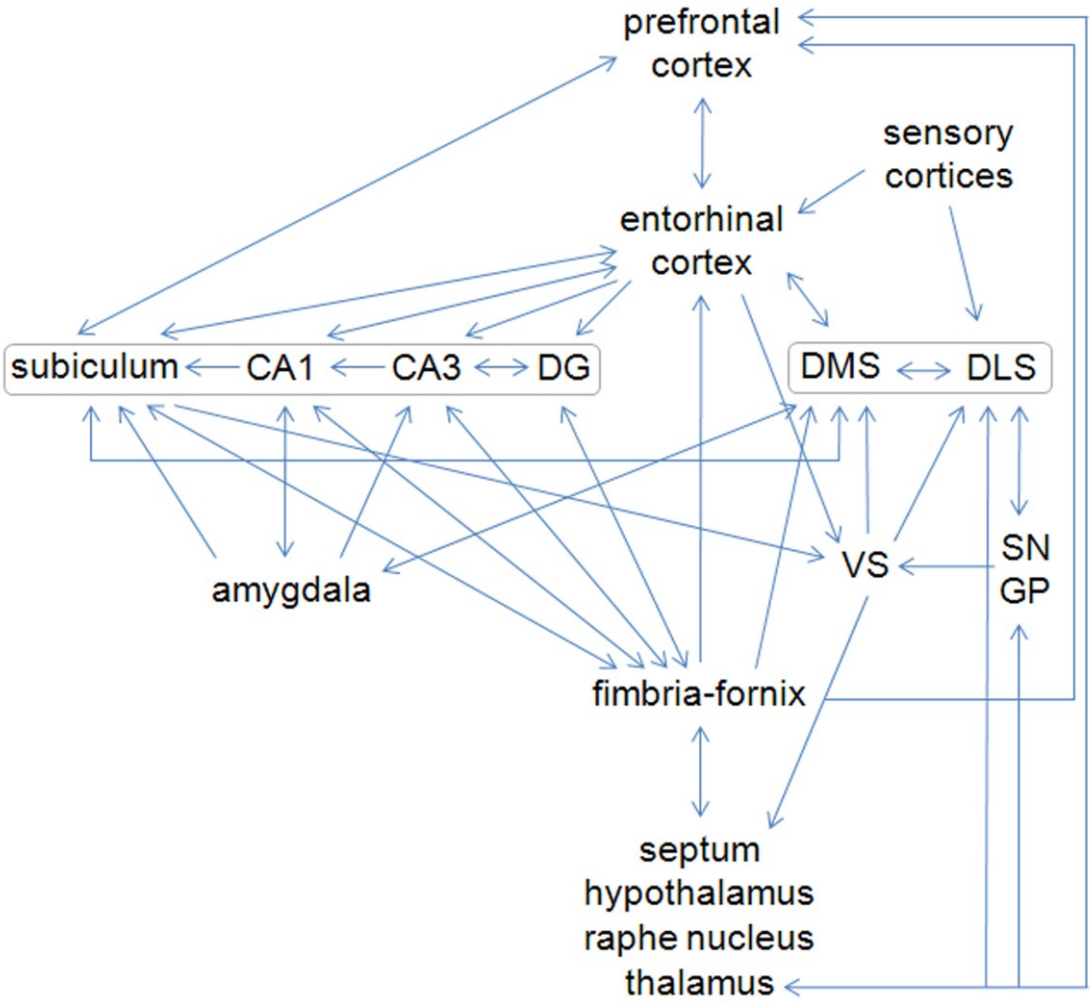

Figure 1: Brain circuitry that contributes to the functions of the HPC and DS.

habitual response. As this occurs, the representation anatomically shifts from the DMS to the DLS (Yin et al., 2004; Yin and Knowlton, 2006; Yin et al., 2006).

The DMS has bidirectional, direct pathways with the amygdala, EC, subiculum, and ventral HPC (Sorensen and Witter, 1983; Sorensen, 1985; Swanson and Kohler, 1986; Groenewegen et al., 1987; McGeorge and Faull, 1989). The DLS receives topographically-mapped inputs from motor and pre-motor cortices, as well as the visual, auditory, olfactory, and somatosensory cortices. In addition to these cortical inputs, the amygdala (Kelley et al., 1982) and thalamus (Kalil, 1978; Royce, 1978; Gerfen, 1985; Gerfen et al., 1987) project to the DS, and topographically-organized dopaminergic inputs from the substantia nigra pars compacta (Gerfen et al., 1987) converge on the DLS. The topographical organization of projections onto the DLS afford it a systematic way to integrate information and learn relationships between stimuli and movements, which are strengthened by reward information from the substantia nigra (White, 1989a, 1989b; Brown, 1992; White and Milner, 1992; Flaherty and Graybiel, 1994; Graybiel et al., 1994; Graybiel, 1995). The DLS projects to the globus pallidus and substantia nigra (Gerfen, 1985), which are involved in motor function, and the substantia nigra projects to the anterior thalamus, which sends signals to the PFC (Berendse and Groenewegen, 1991; Moga et al., 1995). These efferents allow the DLS to convey integrated information to areas that control motor behavior and top-down modulatory brain areas that can further influence motor output. Therefore, the function of the DS is to slowly acquire and

execute motor programs instantiated in cortico-striatal loops (Packard and Knowlton, 2002).

While the HPC receives very few primary inputs and the DS receives complex overlapping and converging inputs onto its interior structures, they both receive similar sensory and reward information. They are able to use this input in very different ways to form representations of the environment. Both the HPC and DS project to cortical and subcortical regions, including the PFC, nucleus accumbens, amygdala, and thalamus. Many researchers posit that the site of comparison between hippocampal and dorsal striatal outputs is at the PFC, because of its role in decision making and other associated processes, as well as its reception of input from both memory systems (White and McDonald, 2002). The differences in inputs and processing styles of these systems likely contribute to the effects of estradiol on the brain and behavioral measures used in the four chapters of this dissertation.

Estradiol Modulates Spatial Navigation Strategy Use

The hormone estradiol appears to modulate spatial navigation strategy use via local action in the HPC and DS. Direct administration of estradiol to the HPC but not overlying cortex enhances the rate of place learning via estrogen receptors, but unlike activating agents like glucose, estradiol administration to the DS but not overlying cortex impairs response learning (Zurkovsky et al., 2006; Zurkovsky et al., 2007). While it is possible that estradiol could also modulate the functions of other brain regions, these data

suggest that the local effects of estradiol within the HPC and DS are sufficient to elicit the observed behavioral effects. Because estradiol appears to enhance hippocampal function and impair dorsal striatal function, the behavioral effects just described also occur when estradiol is present systemically (see Korol, 2004; Daniel, 2006; Galea et al., 2008) and have been demonstrated by comparing females in a variety of hormonal conditions (e.g., Galea et al., 2000; Sandstrom and Williams, 2001, , 2004; Luine et al., 2006). The most typical research design compares the behavior of intact female rats in different estrous cycle phases, or ovariectomized females with those replaced with estradiol. The rat estrous cycle is completed every four to five days, so a rat is in a different estrous cycle phase every day. Estradiol peaks in proestrus when luteinizing hormone, follicle-stimulating hormone, and progesterone are low, and all four hormones are low during a majority of the rest of the cycle. Therefore, it is possible to study the effects of estradiol (along with related ovarian, hypothalamic and pituitary hormones) on the intact female brain by comparing rats in proestrus with those in estrus. However, studying the intact rat does not allow us to isolate the specific effects of estradiol from other ovarian hormones. Several methods of estradiol replacement after ovariectomy have been developed to examine the specific effects of estradiol on spatial navigation, including chronic administration of estradiol via silastic capsules, which keeps estrogen levels constant for up to months, and acute administration of high doses of estradiol, which increase circulating estrogen levels to those at or above what they are at proestrus in the intact female rat and similarly produce behavioral estrous (Rubin and Barfield,

1983a, 1983b; Etgen, 1984). Acute replacement of estradiol to ovariectomized females produces similar effects on spatial navigation strategy use to those found in rats at proestrus (e.g., Galea et al., 2000; Sandstrom and Williams, 2001, , 2004; Luine et al., 2006), suggesting that estradiol and not other ovarian, pituitary, or hypothalamic hormones is mediating these behavioral effects. We have used both designs in the experiments in this dissertation.

The effects of estrogen on spatial navigation strategy have been examined using two basic behavioral paradigms: 1) pitting the two systems directly against each other by using navigation tasks that can be solved using either a place or response strategy and observing which strategy is used, or 2) training on either a hippocampal-dependent *or* striatal-dependent task and assessing the amount of competition (interference) from the other system by observing the rate of learning. For example, a rat biased to use its HPC will use a place strategy when it has a choice and will learn a place task quickly but a response task slowly. When a plus maze task can be solved using either a place or response strategy, intact females in proestrus (high levels of estradiol) and estrus (low estradiol) do not differ in their learning rates; however, those in proestrus are more likely to use a place strategy and those in estrus are more likely to use a response strategy on an immediate probe after learning criterion is reached (Korol et al., 2004). In ovariectomized rats, implantation of a silastic tube containing 5% 17-β estradiol benzoate plus daily subcutaneous injections of 10 μg/kg 17-β estradiol benzoate, which produces a high level of estrogen as that in proestrus (75-90 pg/ml), biases rats toward using a place strategy,

while implantation of the silastic tube alone, which produces a low level of circulating estrogen similar to that in estrus (20 pg/ml), biases them to use a response strategy (Quinlan et al., 2008).

Estradiol enhances place learning on both appetitively (Daniel et al., 1997; Gibbs, 1999; Daniel and Dohanich, 2001; Heikkinen et al., 2002; Liu et al., 2008) and aversively-motivated navigation tasks (Bimonte and Denenberg, 1999; Frick and Berger-Sweeney, 2001; Sandstrom and Williams, 2001; Frye and Rhodes, 2002; Sandstrom and Williams, 2004), as well as on tasks like spontaneous alternation (Walf et al., 2009) and object recognition (Luine et al., 2003; Wallace et al., 2006; Fernandez et al., 2008; Walf et al., 2009) that do not require food deprivation, shock, or cool water for motivation. However, under certain conditions, estradiol has either no effect or impairs performance on many of these same tasks, including radial-arm maze (Luine and Rodriguez, 1994; Luine et al., 1998; Galea et al., 2001; Holmes et al., 2002; Ziegler and Gallagher, 2005), water maze (Frye, 1995; Galea et al., 1995; Berry et al., 1997; Warren and Juraska, 1997; Fugger et al., 1998; Chesler and Juraska, 2000), active avoidance (Diaz-Veliz et al., 1989; Daniel et al., 1999), and working memory (O'Neal et al., 1996). These varying effects of estradiol may be due to differences in the task, paradigm of hormone administration, dependent measures, age, or species used in the studies (for review, see Daniel, 2006). In addition to the effects of estrogens on hippocampal-dependent spatial navigation tasks, estrogens have also been shown to impair learning on a number of tasks that rely on the DS, including win-stay, stimulus-response (Galea et al., 2001), cued

(Daniel and Lee, 2004), and response navigation tasks (Korol and Kolo, 2002; Davis et al., 2005). While the behavioral effects of estradiol on spatial navigation behavior have been examined using a number of tasks and hormonal paradigms, the functional mechanisms by which these behavioral effects occur remain unknown and are therefore examined in chapters 2 and 3.

Circulating Estradiol Levels Vary Across Age

While the examination of the hormonal modulation of spatial navigation strategy use in female rats has uncovered a robust behavioral effect, it has been limited to young adults during a first navigation experience. Whether these effects of estrogen on spatial navigation are evolutionarily adaptive or simply an artifact of estrogen's modulation of other functions, such as reproductive behavior, is unknown. While young adult female rats in the wild are often either pregnant or lactating, it might be adaptive to be biased to use a place strategy during proestrus in order to search for a mate. Therefore, it might be possible that a female's sensitivity to estradiol varies with age.

There is some evidence that age impacts both the ability to successfully navigate through space and the sensitivity of the HPC and DS to estradiol, so estradiol may only modulate navigation strategy use during young adulthood when females are cycling regularly and the HPC and DS are most likely to respond to changes in estradiol levels. While females are able to navigate to a hidden platform that is marked with a proximate cue in a Morris water maze at postnatal day (PD) 17, they are unable to navigate using

the relationship of distal cues in the environment until PD20 (Rudy et al., 1987; Akers and Hamilton, 2007). In addition, females have very little circulating gonadal hormones until puberty, which occurs approximately at PD34-44. Then hormones begin to fluctuate every 4-5 days, as previously described. As females age, their cycles become irregular and either go into constant estrus followed by constant diestrus or directly into constant diestrus, when estrogen levels become low and steady (LeFevre and McClintock, 1988).

Several studies have found that estradiol administration has no effect on spatial memory during place navigation tasks in middle-aged females, suggesting that females may lose sensitivity to the effects of estradiol on hippocampal function by middle-age (Feng et al., 2004; Ziegler and Gallagher, 2005). However, other studies have found that estradiol enhances place memory in middle-aged rats, just as it does in young adult females (Markham et al., 2002; Acosta et al., 2008). These differing results may be partially due to individual differences in age-related variation and/or decrease in circulating estradiol levels and estradiol sensitivity in the HPC (LeFevre and McClintock, 1988). It is also likely that the DS loses sensitivity to estradiol (Roy et al., 1982), but little is known about how this affects navigation behavior. Together, the results of these studies suggest that age greatly modulates the ability of estradiol to influence navigation behavior because of the drastic age-related changes in rats' hormonal profiles. The effects of developmental and adult aging on estradiol's modulation of spatial navigation strategy use are examined in chapters 1 and 2. And, just as experience with cyclic estradiol changes across age, rats likely have many navigation experiences before and during

adulthood, so previous navigation experience may interact with age to influence the ability of estradiol to gain control over navigation behavior during development. This question is examined in Ch. 1.

Activity and Plasticity of Hippocampal and Dorsal Striatal Ensembles are Altered During Spatial Navigation

Studies using inactivation and modulatory substances to examine strategy use support the hypothesis that behavioral control during spatial navigation is determined by the relative amount of activation and/or plasticity within these neural systems and that estradiol modulates navigation behavior by activating the HPC and/or suppressing the DS. Several studies have used immediate-early genes (IEGs) to examine hippocampal and striatal activation and plasticity in intact male rats during navigation, but the effects of estradiol on hippocampal and striatal activation and plasticity in females during spatial navigation have not been examined. Thus, chapters 2, 3, and 4 of this dissertation use IEGs to examine the effects of estradiol on activation and plasticity in several hippocampal and dorsal striatal subregions during spatial navigation.

Activity

One means of examining ongoing neuronal ensemble activity during a behavioral task is to examine the expression of activity-dependent genes. Neural activity dynamically regulates the transcription of specific genes via several distinct transduction

pathways. *c-fos* is a regulatory transcription factor IEG activated via a cAMP response element binding protein (CREB) pathway when a neuron is activated (for review, see Guzowski, 2002). It is called an IEG because it is one of the first genes transcribed after synaptic activity and does not require *de novo* protein synthesis in order to be expressed. While IEGs like *c-fos* do not alter synapses, they serve as markers for cellular activation and synaptic activity. Expression of Fos protein peaks 90-120 min after the gene is activated and declines for the following several hours until the protein is nearly completely degraded 4 hours after induction (Morgan et al., 1987; Sagar et al., 1988; Hoffman et al., 1993; Hoffman and Lyo, 2002).

Fos protein expression has been used to examine the amount and pattern of activation in the HPC and DS while rats perform spatial navigation tasks (Colombo et al., 2003; Teather et al., 2005; Gill et al., 2007), but these studies have examined only gonadally intact male subjects and the results have not been consistent. For example, when male rats were trained to find food-baited arms on a radial-arm maze using either a place or response strategy and then were sacrificed 30 min later, approximately 60 minutes after *c-fos* was induced, Fos protein expression in the HPC was similar regardless of task type, but Fos expression in the DLS was considerably greater in rats that learned the response strategy (Gill et al., 2007). These data suggest that both the HPC and DS are activated and likely encode information during both navigation tasks but that there was only a relationship between DLS activation and which strategy was used. In contrast, another study showed that when rats were trained on either a place or visible

platform version of the water maze and sacrificed 90 min after task acquisition, those that learned the place task had significantly more Fos expression in the CA1 region of the HPC than those that learned the visible platform task or controls that did not learn the task, but rats in all groups had the same amount of *c-fos* activation in the DS (Teather et al., 2005). Similarly, a third study showed that when males were trained on an ambiguous navigation task that could be solved using either a place or response strategy, rats that used a place strategy had significantly greater Fos activation in the DG and CA1 of the HPC than response strategy users when sacrificed 60 min after the task, but there were no differences in Fos expression in the DS (Colombo et al., 2003). In contrast to the first study, the results of these two studies suggest that the amount of hippocampal activation (not dorsal striatal activation) determines which strategy is used. Differing task demands and sacrifice time points may contribute to the conflicting activation patterns observed in the HPC and DS during spatial navigation, because these studies may have captured Fos expression in response to different phases of learning and performance. Ch. 2 examines Fos expression induced in the HPC and DS during the early stage of learning.

Together, the results of the studies just described suggest that the HPC and DS are both engaged in task performance regardless of what strategy is being employed and support evidence that they interact in a complex fashion during spatial navigation (Knierim, 2006; DeCoteau et al., 2007a, 2007b). However, they do not support the hypothesis that the relative amount of hippocampal and striatal activation determines navigation strategy use. Rather, it is likely that the HPC and DS interact in a more

complex way during spatial navigation (Gold, 2004; DeCoteau et al., 2007a, 2007b); (Knierim, 2006). *In vivo* electrophysiology studies have shown that there are neurons in both the HPC and DS that are activated during place and response navigation and code for both external spatial cues such as location and head direction and internal cues like forward motion, velocity, and left and right turns (Ranck, 1973; O'Keefe, 1976; Olton et al., 1978; McNaughton et al., 1983; Taube et al., 1990; Mizumori and Williams, 1993; McNaughton et al., 1994; Skaggs et al., 1995; Taube, 1995; Zhang, 1996; Poucet, 1997; Redish and Touretzky, 1997; Jog et al., 1999; Mizumori et al., 2000b). While both the HPC and DS code for similar types of information, the HPC has a much larger population of neurons that attend to environmental information, and the DS attends more to internal movement cues, and this difference becomes greater as learning progresses (Holscher et al., 2004). In addition, these subpopulations of neurons in the HPC and DS respond differently to changes in the environment and task demands (Mizumori et al., 2000a; Ragozzino et al., 2001; Mizumori et al., 2004; Yeshenko et al., 2004). The complexity of changes that occur in the patterns of activity in the HPC and DS throughout learning suggests that the plasticity occurring at synapses within hippocampal and striatal ensembles may be more important for controlling navigation strategy use than the level of neural activation *per se*. However, while these studies were conducted during explicit place and response tasks, electrophysiological recordings from the HPC and DS during a win-stay cued (striatal-dependent) version of the plus maze task suggest that the DS does not always code for spatial location *per se* (Berke et al., 2009). When the cued goal arm

for a given trial was the same arm that the rat had visited on the previous trial, so the rat could not only go to the cued arm but to the same spatial location, the DS was not shown to have pure place cells. Rather, some striatal neurons coded for the same relative spatial location on all arms of the maze, but firing depended on whether the rat was traveling toward or away from the center platform, suggesting that striatal neurons that code for spatial location actually code for relative position on the maze. Together, these studies suggest that the activity patterns of the HPC and DS during spatial navigation are complex and depend on task demands. As such, measuring activation alone may not allow us to observe the functions of the HPC and DS during navigation. Therefore Ch. 3 examines the plasticity in hippocampal and striatal ensembles during spatial navigation performance.

Plasticity

Activity-dependent IEGs like *c-fos* are useful markers of neural activation, but they are unable to provide specific information about the plasticity of specific neurons throughout learning. However, several studies have used the late effector IEG activity-regulated cytoskeleton-associated protein (*Arc*) as a marker of plasticity in hippocampal and striatal ensembles during learning in males. *Arc* is required for NMDA receptor-dependent synaptic plasticity (Lyford et al., 1995; Lanahan and Worley, 1998; Plath et al., 2006), late-phase long-term potentiation (Link et al., 1995; Guzowski et al., 2000), and long-term memory consolidation (Guzowski et al., 2000; Plath et al., 2006; Miyashita

et al., 2008) that is required for spatial navigation learning and memory (Guzowski et al., 2001a; Daberkow et al., 2007). For example, *Arc* is induced in the HPC during place navigation tasks including the Morris water maze (Guzowski et al., 2001a; Fletcher et al., 2006) and spatial exploration (Guzowski et al., 1999; Vazdarjanova et al., 2002; Ramirez-Amaya et al., 2005; Vazdarjanova et al., 2006), and *Arc* RNA expression in the CA1, CA3 and DG of the HPC is correlated with learning a hippocampal-dependent spatial navigation task but not a cued control task (Guzowski et al., 2001a). Likewise, more DS neurons express *Arc* during response learning than under control conditions (Daberkow et al., 2007). These studies have used male subjects and have not addressed differences in plasticity in the HPC and DS during spatial navigation performance. Therefore Ch. 3 examines *Arc* expression in females that used place and response strategies on an ambiguous spatial navigation task.

There is also evidence that reliability in the expression of plasticity is necessary for successful strategy use. For example, theta rhythm, which is extracellular oscillatory EEG activity between 3 and 12Hz, is important for the neural plasticity needed for learning and memory (Mehta et al., 1997; Mehta et al., 2000; Buzsaki, 2005). In the HPC, theta rhythm becomes synchronized with place cell firing (Maurer and McNaughton, 2007) to orchestrate the timing of spatial coding (O'Keefe and Recce, 1993), and striatal theta rhythm becomes altered over the course of response learning and increases as the rat produces a turn (DeCoteau et al., 2007b). The results from these studies suggest that the reliability of plasticity within the HPC and DS across learning may be important for

successful navigation behavior guided by that system, so that when either strategy may be successfully employed, the system with greater reliability will guide navigation behavior.

Interestingly, the known kinetics of *Arc* have provided a means by which we can directly measure the reliability of neuronal ensembles across multiple behavioral epochs. *Arc* mRNA is expressed within the nucleus of a neuron *by* 2 minutes and *up to* 16 minutes post-stimulus and then migrates to the neuron's cytoplasm where it is detectable 20-45 minutes post-experience (Guzowski et al., 1999, , 2001b; Vazdarjanova et al., 2002). Therefore studies have used a technique called cellular compartment analysis of temporal activity by fluorescence *in situ* hybridization (catFISH) to visualize *Arc* transcription within specific neurons in response to two discrete behavioral epochs (Guzowski et al., 1999, , 2001b; Vazdarjanova et al., 2002). For example, when a rat is exposed to a novel environment for five min and then exposed to the same environment again 20-30 min later and then immediately sacrificed, cytoplasmic *Arc* is detected in CA1 neurons that transcribed *Arc* during the first exposure and intranuclear *Arc* foci are expressed in CA1 neurons that transcribed *Arc* during the second exposure. Thus, neurons that reliably displayed synaptic plasticity during both exposures express both intranuclear foci and cytoplasmic *Arc*. This technique has demonstrated that the induction of *Arc* within individual CA1 pyramidal neurons is highly reliable across two exposures to the same context separated by 20-30 min (Guzowski et al., 1999; Guzowski et al., 2001a; Vazdarjanova and Guzowski, 2004), similar to electrophysiological recordings of place cells in the HPC across exposures to the same location within an environment

(Muller et al., 1987; Thompson and Best, 1990; Wilson and McNaughton, 1993; Leutgeb et al., 2004). While several techniques provide temporal and cellular resolution or spatial resolution, *Arc* catFISH is unique because it provides all three, allowing us to visualize plasticity within individual neurons in large ensembles at multiple time points. This technique is used in chapters 3 and 4 to examine the reliability of neuronal ensembles in hippocampal and striatal subregions across trials of a spatial navigation task.

Estradiol Modulates Activation and Plasticity in the Hippocampus and Dorsal Striatum

No previous empirical research has examined the functional mechanisms by which estradiol influences spatial navigation behavior. However, previous findings in males (discussed above) that the HPC and DS are differentially activated during the use of place and response strategies support the hypothesis that estradiol may influence navigation behavior by altering hippocampal and striatal activation. That is, estradiol may increase hippocampal activation and/or decrease striatal activation at the onset of or during spatial navigation task performance to modulate strategy use and learning rate. Independent of learning, estradiol induces *c-fos* expression in several brain regions including the HPC within 90 min of administration in mice (Dominguez-Salazar et al., 2006). While estradiol's effects on baseline *Arc* expression have not examined, estradiol has been shown to increase hippocampal plasticity via a number of mechanisms on several different time scales, from rapid, transient mechanisms of neuronal excitability to

prolonged mechanisms that alter gene expression (Aenlle and Foster, 2009; Aenlle et al., 2009; Pechenino and Frick, 2009). Similar estradiol administration regimens to those that have demonstrated estradiol's effects on place and response learning (Korol and Kolo, 2002) have been shown to increase hippocampal NMDA receptor expression and binding (Weiland, 1992; Gazzaley et al., 1996; Romeo et al., 2005), as well as NMDA receptor-dependent dendritic spine density (Gould et al., 1990; Woolley et al., 1990; Woolley and McEwen, 1992, , 1993; Murphy and Segal, 1996; Woolley et al., 1997), which have both been correlated with performance on hippocampal-dependent tasks (Daniel and Dohanich, 2001; Leuner et al., 2003). And, while estradiol does not alter the baseline intrinsic properties of CA1 neurons, it increases synaptic excitability in response to stimulation (Wong and Moss, 1992; Kim et al., 2006; Scharfman and MacLusky, 2006) and long-term potentiation (Cordoba Montoya and Carrer, 1997). Estradiol has also been shown to have a negative effect on plasticity in the DS that may contribute to its ability to impair response learning. Estradiol decreases NMDA receptor binding in the DS (Cyr et al., 2000), and administration of dopamine receptor antagonists and estradiol similarly bias rats to use a place strategy (Quinlan et al., 2008), suggesting that estradiol may decrease the activation and plasticity of the DS. Because these effects of estradiol on hippocampal and striatal activation and plasticity appear to occur in the absence of behavior, one way that estradiol may bias rats to use a place strategy is by priming the female HPC to become preferentially activated and used at the onset of spatial navigation behavior. Therefore, chapters 2, 3, and 4 assess the activation and plasticity of

hippocampal and striatal subregions during both behavioral performance and control conditions to determine whether spatial navigation behavior is required to elicit any effects of estradiol on the activation and plasticity of the HPC and DS related to the behavioral effects of estradiol.

Given the current neural and behavioral evidence about how estradiol might modulate navigation strategy use, there are three possible relationships between estradiol, hippocampal and striatal ensemble activity/plasticity, and navigation strategy use that we might find. One possible relationship is that all place strategy users show one pattern of hippocampal and striatal activity, while response strategy users show a different pattern, regardless of estradiol levels. In this case, the presence of estradiol would usually bias the brain to display the "place" pattern. The second possible outcome is that hippocampal and striatal activation and plasticity are always modulated by estradiol in a specific way. For examples, rats with high estradiol levels might always display increased hippocampal plasticity and decreased striatal plasticity compared to rats with low estradiol, and therefore, they would most likely use a place strategy. The third and least likely possibility is that the pattern of activation associated with the use of each strategy is different depending on a rat's estradiol level. For example, rats with high estradiol levels that use a place strategy would display a different pattern of hippocampal and striatal activation/plasticity than low estradiol place strategy users and high estradiol response strategy users.

Summary of Dissertation Research

While the behavioral effects of estradiol on spatial navigation strategy use have been well-characterized in the young adult female rat, surprisingly little is known about how parameters of age and experience affect navigation behavior and modulate the robustness of estradiol's modulation of navigation strategy use. In addition, we have very little understanding about the functional mechanisms by which strategy use is determined, especially in females, and have almost no evidence for how estradiol modulates these mechanisms to influence navigation behavior. These two issues are the major focus of the experiments in this dissertation. Therefore, these experiments characterize estrogen's impact on hippocampal and striatal-dependent spatial navigation as rats in various hormonal conditions develop and age using novel behavioral tasks and measures. They also use what is known about the kinetics of the immediate-early genes *c-fos* and *Arc* to assess ensemble activity and plasticity in the HPC and DS during spatial navigation to determine how estradiol alters patterns of activation and plasticity to influence navigation behavior.

Ch. 1 examines the developmental and experiential conditions under which estradiol can modulate spatial navigation strategy use by a) determining when during development these navigation strategies can be used as well as when estradiol can first modulate their relative use, and b) using a within-subjects design in young adulthood to determine how experience with the task modulates the ability of estradiol to control spatial navigation strategy use. Establishing the behavioral parameters of this effect

provides a strong base for examining the functional mechanisms by which estradiol modulates navigation strategy use. This chapter has previously been published as an empirical journal article (see Pleil and Williams, 2010).

Ch. 2 further examines the effects of aging on estradiol's ability to modulate navigation learning, as well as the effects of duration of hormone deprivation on learning by testing young adult and middle-aged ovariectomized females on place and response tasks. These hormonal manipulations were also used to examine the hypothesis that relative activation in the HPC and DS influences spatial navigation learning, and that this is the means by which estradiol influences place and response learning, by quantifying Fos protein expression in several hippocampal and striatal subregions in response to early learning on these place and response tasks.

Because Ch. 2 showed that estradiol does not to modulate spatial navigation learning via differential activation of the HPC and DS, Ch. 3 examines the hypothesis that the plasticity and reliability of hippocampal and striatal ensembles determines spatial navigation strategy use and that this is the means by which estradiol modulates strategy use, by quantifying *Arc* mRNA expression in young adult intact females during place and response strategy use on an ambiguous navigation task. In addition, Ch. 3 examines the effects of estradiol on hippocampal and striatal plasticity and reliability during spatial exploration to understand whether estradiol alters plasticity under baseline conditions to "prime" the female brain to be place-biased or if explicit learning is required to achieve this bias.

Because active exploration, but not estradiol, was associated with increased plasticity in a number of hippocampal and striatal subregions in Ch. 3, and specific strategy use and estradiol interacted to affect plasticity and reliability in other subregions, Ch. 4 examines the effects of estradiol on plasticity and reliability during passive exploration to determine whether the lack of effect of estradiol on plasticity during exploration was overshadowed by effects of swimming, including the stress associated with swimming without escape, the motor behavior required to swim, or the inattention to external cues. In addition, Ch. 4 examines the effects of landmark cue disturbance within an environmental context on hippocampal and striatal plasticity to further understand how estradiol may prime the female brain to use a place strategy by focusing the female's attention on specific salient external cues.

CHAPTER 1. THE DEVELOPMENT AND STABILITY OF ESTRADIOL-MODULATED SPATIAL NAVIGATION STRATEGIES

In young adult female rats, the presence of circulating estradiol at the proestrus level (~90 pg/ml) significantly increases the probability of using a place strategy over a response strategy when either can be successfully employed. When estradiol is not present or is present at low, estrus levels (~32 pg/ml), a response strategy is much more likely to be used (Korol et al., 2004). While this behavioral effect is well-established in the young adult female, very little is known about how it develops across age. Current findings suggest that a response strategy is the "default" strategy when no estradiol is present, so one might predict that prepubertal females would be biased towards using a response strategy until the rise in estradiol signaling the start of puberty activates neural networks to modulate place and response strategy use. However, this has not been examined, and very little research has even examined the development of the ability to use place and response strategies. Both male and female rats are able to locate a hidden platform in a water maze that is marked with a proximate cue by postnatal day (PD) 17, but they cannot use extra-maze cues to find the hidden platform until PD20 (Rudy et al., 1987; Akers and Hamilton, 2007). This indicates that hippocampal and striatal-dependent navigation strategies develop independently but are available well before puberty, however little research has examined the development of the use of a motor response strategy that requires more than a simple response to a single stimulus. In addition, it is

unclear whether use of a response strategy is the "default" strategy for young rats when both strategies may be used and whether neural networks for navigation are sensitive to estradiol action prior to the onset of estrous cyclicity as are other neural and behavioral systems, such as female mating behaviors including lordosis and ear wiggling (Williams, 1987; Williams and Blaustein, 1988).

In addition to questions about the development of estradiol-modulated spatial navigation, studies in adult females have all used a between subject experimental design (Korol and Kolo, 2002; Korol et al., 2004; Davis et al., 2005; Quinlan et al., 2008), and therefore it is unclear whether navigation strategy use varies across the estrous cycle within individual rats or estradiol only biases rats on a first navigation experience. While there is no evidence that learning and working memory requiring place navigation varies across the estrous cycle in individual rats (Stackman et al., 1997), this issue has been examined very little. Because most mammals encounter specific spatial navigation tasks numerous times throughout life and females' estradiol levels greatly vary from day to day, it is possible that previous experience using one navigation strategy may overshadow estradiol status to control strategy use on subsequent experiences with the task or interact with estradiol to modulate strategy use in a complex fashion. Thus, estradiol may only modulate strategy use in inexperienced females or the robustness of the effect may depend on previous navigation experience.

In order to avoid food deprivation in our juvenile subjects, we used a simplified water-based T-maze task in which the hidden escape platform could be found using either

a place or response strategy to determine the development of estradiol-modulated navigation in rats from 16 to 26 days of age. Additionally, we retested females several times in adulthood at various stages of their estrous cycle to determine a) whether estradiol is able to maintain control over strategy selection over several experiences with the navigation task and b) the extent to which previous experience in the task interacts with the ability of estradiol to influence strategy use.

Materials & Methods

Subjects

Subjects were 80 female offspring from 10 timed-pregnant Sprague-Dawley CD rats purchased from Charles River Laboratories (Kingston, NY). Pregnant dams arrived in our colony at Duke University on their ninth day of gestation and were singly housed in individually-ventilated, transparent shoebox cages with corn cob bedding and ad libitum access to water and a standard diet (Rodent Diet 5001, PMI Nutrition International, Inc., Brentwood, MO). The temperature-controlled colony room was maintained on a 12:12 hr light:dark cycle with lights on at 7 a.m. daily. At birth, pups from all 10 litters were sexed and randomly assigned to foster mothers, and litters were culled to approximately 6 females and 4 males. Pups were weaned on postnatal day 26 (PD26) after the last age group of juveniles was tested. Females that were also behaviorally trained as adults were pair-housed at weaning, with the same food and living conditions described for dams above.

Treatments and experimental time points

Two females from each litter (n = 20 at each age) were behaviorally trained at PD16, PD21, or PD26. Forty-eight and 24 hours prior to the training day, one female from each litter was administered a subcutaneous (s.c.) injection of 5μg 17 β-estradiol (Sigma-Aldrich, St. Louis, MO) dissolved in 0.1ml sesame oil (Sigma-Aldrich, St. Louis, MO) in the nape of the neck, and the other pup from each litter was injected with the oil vehicle alone. Twice the amount of estradiol given on the same schedule to the adult female rat produces circulating estradiol just above those found in the intact rat in proestrus (75-90 pg/ml; Viau and Meaney, 1991), increases hippocampal CA1 spine density (Woolley and McEwen, 1993), and increases learning rate when a place strategy is needed to solve a hippocampal-dependent maze task (Korol and Kolo, 2002).

All oil-treated juveniles were raised to adulthood and tested again three times, at 3.5, 4.5, and 5.5 months of age in a repeated measures design. In order to determine estrous cycle status and confirm normal cycling, vaginal samples were taken daily at 09:30 for 3 weeks prior to each adult test. Cells were collected on a moistened cotton swab, rolled onto a glass slide, and examined at 10X magnification to determine estrous cycle status based on the proportion of leukocytes, epithelial, cornified, and nucleated cells (Matthews and Kenyon, 1984).

In addition to the 60 rats already described, twenty rats were ovariectomized (OVX) either at PD22 or PD44 (n = 10 each) and tested at 3.5 months of age. These ages were chosen because in our vivarium, offspring of timed pregnant Sprague Dawley rats

reach puberty at PD34 as defined by vaginal opening; therefore, rats were ovariectomized either before or after puberty.

Ovariectomies

In order to remove the source of circulating gonadal hormones, rats were anesthetized for with a combination of 80 mg/kg ketamine plus 10 mg/kg xylazine and ovariectomized via bilateral dorsal incisions through the skin and muscle walls of the abdomen. The ovary and ovarian fat on each side of the body were exposed, tied off, and surgically removed with a scalpel. The site of removal was cauterized and carefully placed back into the abdominal cavity. The muscle wall and skin were sutured and antibiotic cream was applied to the wound site. Buprenorphine (0.5 mg/kg, i.p.) was administered at the end of all surgeries and again 12 hr later as analgesic. Rats were kept warm on heating pads until they woke up and then returned to their cagemates. Powdered food was offered in bowls on the cage floor for several days until rats were seen eating from the overhead food bin. All rats recovered without complications and were included in the study.

Apparatus and testing room

All behavioral training took place in a clear Plexiglas plus-maze placed inside a black plastic pool with a diameter of 1.8m. Each arm of the plus maze was 50.8 cm long and 15.2 cm wide, with a 15.2 cm x 15.2 cm center and 30 cm high walls. The maze was

filled with approximately 15.5 cm of water mixed with black water-based paint, which was maintained at 24-25 ºC. A platform with diameter 10.2 cm, hidden just below the water's surface, was placed at the end of the goal arm. Rats could be prevented from entering arms of the maze by inserting a clear piece of Plexiglas at the base of the arm (see Figure 2).

The maze was located in a 6.5 m x 3.8 m rectangular room (1.3 m from the North wall and 0.7 m from the East wall) that was rich with cues including a curtain, table with computer, cart with rat cages, counter, shelves, and walls with posters and objects with high-contrast patterns. We hung three additional cues of different shapes, colors, and contrast patterns 75 cm above the height of the water and outside the radius of the maze to provide additional extramaze cues in order to ensure that any failure of task learning in juveniles was not due to the rats' poor visual acuity at these young ages (as in Figure 2). Hanging cues were quasi-randomly placed in four possible locations and counterbalanced across estradiol-treated rats within each juvenile age group, as well as within each adult test and across adult tests for each rat. No significant differences in latency or strategy were observed across cue configurations, therefore, all groups were combined for further data analysis. Oil-treated littermate controls were always trained in the same apparatus configuration as their estradiol-treated littermates.

Behavioral training and probe testing

Behavioral training and probe testing took place within a single session that consisted of 10 training trials and one probe trial during the lights-on portion of the day. Rats were trained in groups of four, which consisted of two estradiol-treated females from different litters and their two oil-treated littermates. Each rat was removed from its litter and placed in a clean holding cage with its littermate. Holding cages were then transported on a cart to the maze room, where the cart remained throughout behavioral training. During the 10 training trials, rats were always started from the arm closest to the experimenter (South arm) and the goal arm was either the West or East arm (counterbalanced across rats and tests). Clear plexiglass inserts blocked the other two arms, forming an "L" shaped route such that the rat could only enter the correct arm of the maze (see Figure 2). The apparatus remained in the same configuration for all 10 training trials.

To begin the first trial of the session, one rat was taken from its holding cage and placed in the South arm of the maze. A trial was considered complete when the rat climbed onto the escape platform at the end of the goal arm (Figure 2a). If the rat did not find the platform within 60 s, it was guided to the platform by the experimenter. The rat was allowed to stay on the platform for 15 s and then placed on a dry towel in an opaque bucket. All walls of the maze were wiped with ethanol between trials to remove intramaze odor cues. Five consecutive trials were run in this fashion. The rat was then dried with a towel, and returned to its holding cage that was warmed by a space heater.

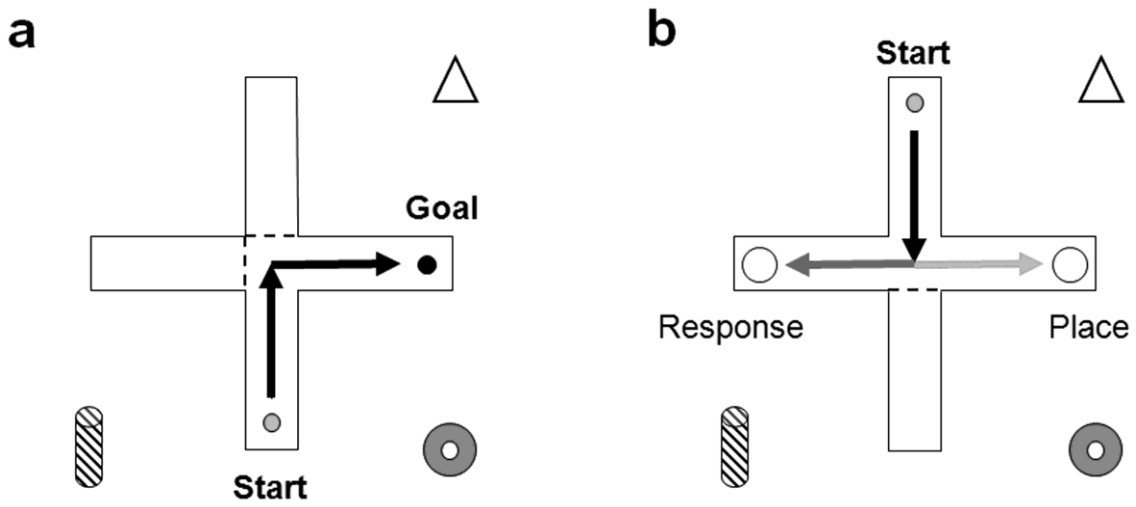

Figure 2: Ambiguous water T-maze taska) During the 10 training trials, rats were started from the South arm of the maze and only the goal arm was available. b) On the probe trial, rats were started from the North arm and both adjacent arms were available.

The cue configuration and/or platform location were changed if necessary and the next rat completed 5 trials. After all 4 rats had completed 5 trials and were resting in holding cages, the first rat received 5 more training trials. Immediately following the last trial, the platform was removed and the Plexiglas inserts were moved so that only the South arm was blocked off, forming a T-shaped maze, in order to conduct a probe trial (see Figure 2b). Cues remained in the same configuration, and the rat was started from the North arm and allowed to swim for 60 s. During all training and probe trials, latencies to reach the end of any arms entered and latency to mount the platform were recorded by the

experimenter and the path of the animal was recorded in real time using HVS Image software (Buckingham, WH, UK).

All rats were trained once as juveniles, at PD16, 21, or 26. Those administered oil as juveniles were raised to adulthood and tested again three times in adulthood as described above. All procedures were approved by the Institutional Animal Care and Use Committee of Duke University.

Time spent in start arm as a measure of strategy use

After 10 training trials, rats completed a probe trial which required them to start the maze from a novel arm 180º from the start arm used during training. Rats that returned to the same spatial location in the room that the platform had been located during training were considered to have used a "place" strategy, and rats that made the same turn (i.e., left or right) that they had taken on the training trials were categorized as using a "response" strategy. In order to confirm that rats were using different strategies to navigate rather than choosing an arm randomly, time spent in each segment of the maze (i.e., start arm, middle, goal arm) for each rat on each trial was examined. Rats trained at PD21 or PD26 that were categorized as place strategy users spent significantly more time in the start arm on the probe trial than during the last three training trials, when performance latency had reached asymptote (PD21: $t(9) = 3.51$, $p = 0.007$; PD26: $t(14) = 2.32$, $p = 0.036$), but rats at PD16 did not ($p > 0.80$; see Figure 3a). This increase in time spent occurred only in the start arm and occurred in a significant proportion of PD21 and

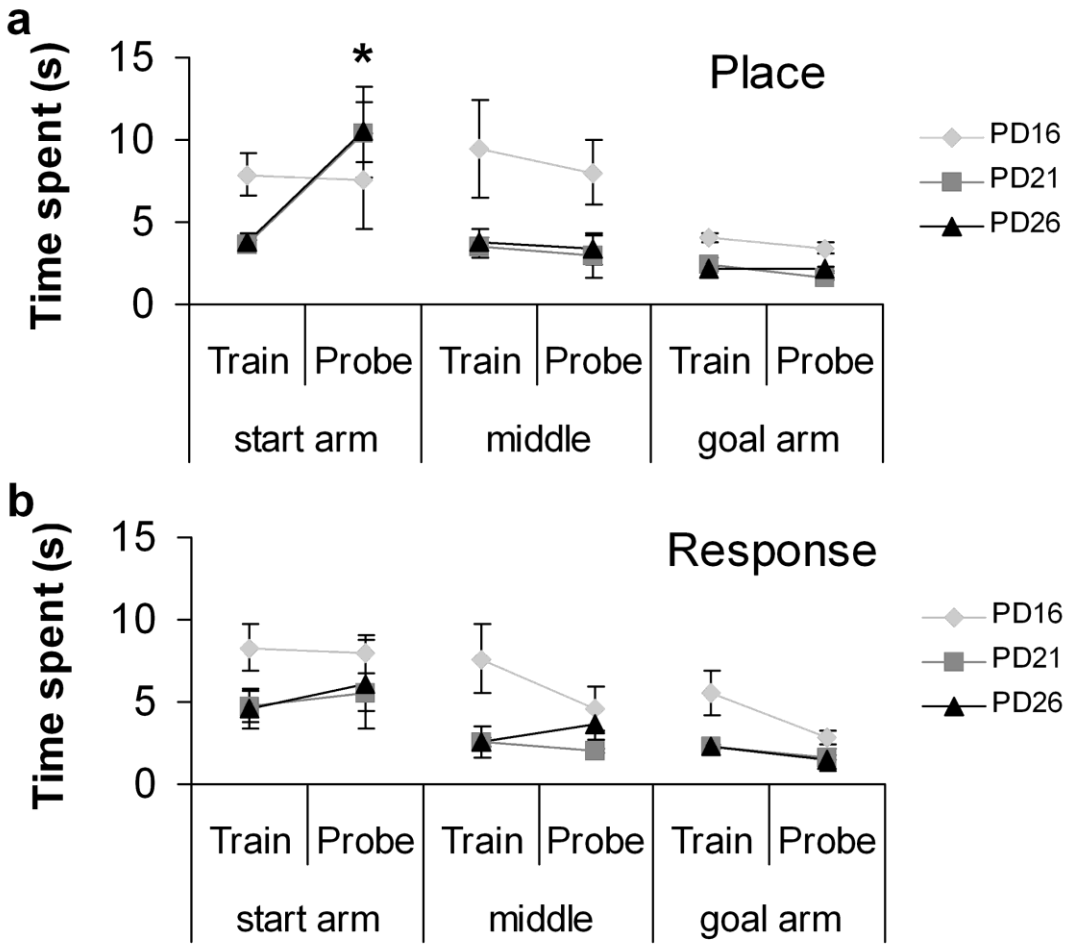

Figure 3: The difference between the time spent on the probe trial and training trials 8-10 for each segment of the maze. a) PD21 and PD26 rats that were categorized as using a place strategy spent significantly more time in the start arm during the probe than the last three training trials, while this was not the case for any other segment for PD21and PD26 rats or any segment for PD16 rats; b) All rats that used a response strategy spent a similar amount of time in each maze segment for the probe and training trials. * indicates $p < 0.05$.

PD26 place strategy users (PD21: $\chi 2 = 11.15$, $p = 0.0008$; PD26: $\chi 2 = 8.89$, $p = 0.002$), but was random in PD16 rats ($\chi 2 = 0.09$, $p > 0.75$). Once PD21 and PD26 place strategy rats reached the center of the maze, they spent no more time in these areas on the probe trial than they did on trials 8-10 ($p > 0.10$; Figure 3a). In contrast, rats that used a response strategy spent a similar amount of time in all parts of the maze during the probe trial as they did during their last 3 training trials indicating that they behaved similarly even though the start location had changed ($p > 0.10$, see Figure 3b). This analysis suggests that place strategy users have a representation of the environment to which they must reorient when they solve the task from the new location. In contrast, response strategy users simply employ the same motor response acquired during training (e.g., "take a left") and therefore spend a similar amount of time in each segment of the maze on the probe as the last few training trials. Change in latency to reach the middle of the maze on a probe trial is a measure of strategy learning that is independent of arm choice. Therefore, this measure is a useful for assuring that rats have learned the task and are employing a strategy on the probe trial rather than choosing a goal arm at random or by mistake. This measure may be used in the future to confirm strategy use.

Statistical analysis

All analyses were calculated using an α value of 0.05. Because strategy choice was a binary dependent measure and group n's were small, we used two-tailed exact binomial tests to determine whether strategy use of each group differed from the null

hypothesis that there was no strategy bias (that is, 50% of the group used a place strategy and 50% used a response strategy). In addition, two-tailed Liebermeister's quasi-exact test for small sample sizes, which is an appropriate Bayesian alternative to Fisher's exact test when the frequency of one variable is not fixed (in this case, strategy used; see Seneta & Phipps, 2001), was used to determine differences in strategy use across groups in order to evaluate the effects of developmental age, estradiol treatment in adolescence, and estrous status in adulthood on strategy bias. Repeated measures ANOVAs were performed with latencies on the 10 training trials as the dependent measures and strategy (place or response) and estradiol status (estradiol- vs. oil-treated or estrous phase) as the independent measures to examine any differences in the rate of learning between groups. Because path length and latency measures were highly correlated, only latency measures are reported. T-tests were used to determine whether rats spent a significantly different amount of time in each part of the maze on the probe than on the last three training trials.

Results

Juvenile strategy use and learning

There were no significant differences in strategy use between estradiol- and oil-treated rats at any juvenile age ($p > 0.15$). However, 26-day-old estradiol-treated rats showed a significant bias toward using a place strategy when compared to chance ($p = 0.021$; Figure 4), while no other oil- or estradiol-treated groups showed a strategy bias ($p > 0.15$), as shown in Figure 4b. In addition, PD26 rats administered estradiol were

significantly more likely to use a place strategy than PD21 rats ($p = 0.034$), but there was no change in strategy use between PD16 and PD21 rats given estradiol or between any developmental age in oil-treated rats ($p > 0.20$). Together, these data suggest that estradiol was able to bias females to use a place strategy by PD26, at least a week before puberty.

A repeated measures ANOVA with strategy and estradiol status as the independent variables and trial latency (trials 1-10) as the dependent variable were calculated for each age. The analysis for PD16 rats revealed a main effect of trial ($F(9,144) = 4.87$, $p < 0.0001$) and an estradiol status x strategy interaction ($F(1,16) = 5.65$, $p = 0.030$), but no other effects ($p > 0.05$; Figure 5a). Individual trials analyses revealed that PD16 rats given estradiol that were classified as response strategy users had significantly longer trial latencies than oil-treated rats using a response strategy on trials three ($t(7) = 3.41$, $p = 0.011$), four ($t(7) = 2.85$, $p = 0.025$), and five ($t(7) = 4.52$, $p = 0.003$), but no other trials ($p > 0.05$). There were no differences in trial latency between oil and estradiol-treated place strategy users. ANOVAs for PD21 and PD26 revealed main effects of trial on PD21 ($F(9,63) = 6.99$, $p < 0.0001$) and PD26 ($F(9,63) = 8.33$, $p < 0.0001$) but no other effects ($p > 0.10$), indicating that all groups learned the task at similar rates (see Figures 5b and c).

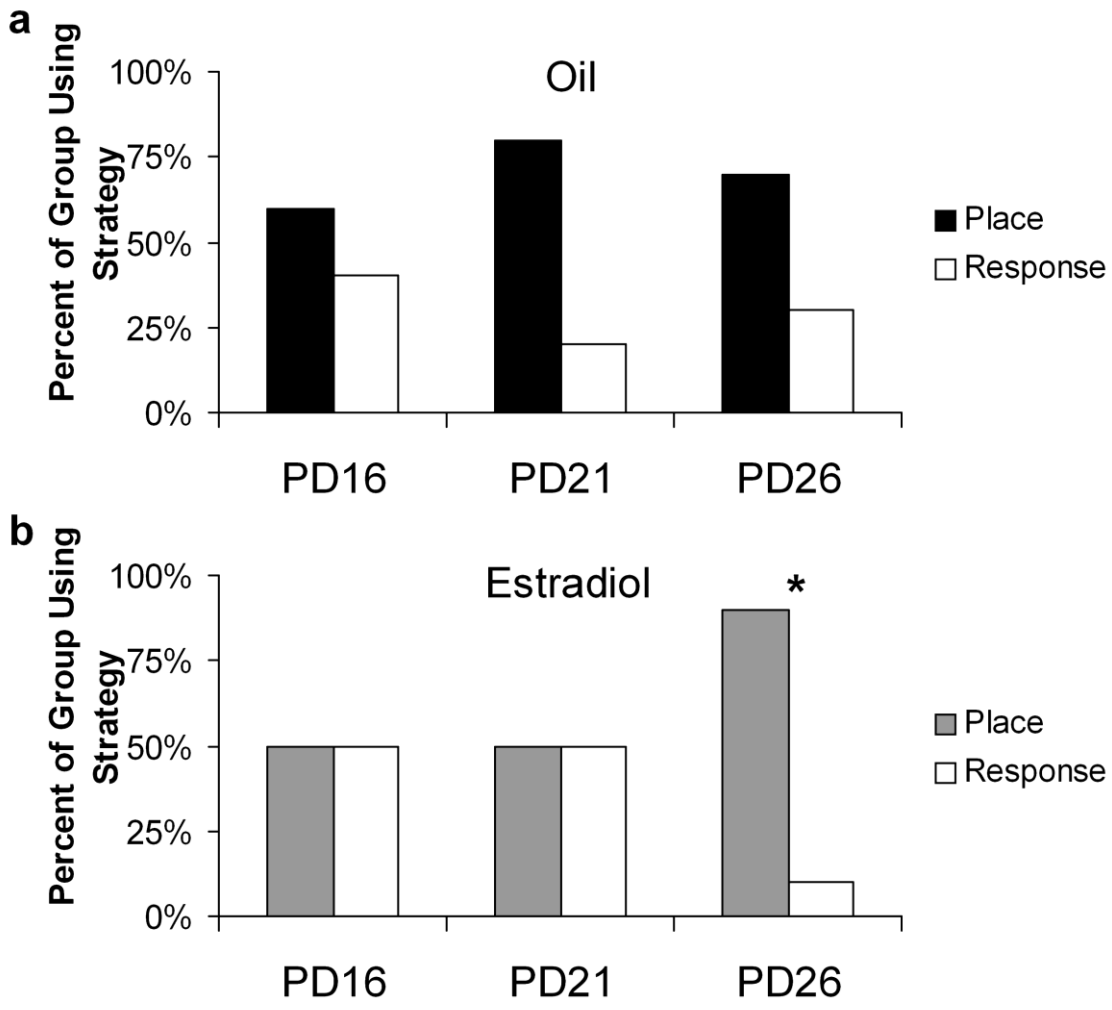

Figure 4: The proportion of rats in each age group that used place and response strategies in control (a) and estradiol-treated (b) conditions showing that estradiol biased PD26 rats to use a place strategy.

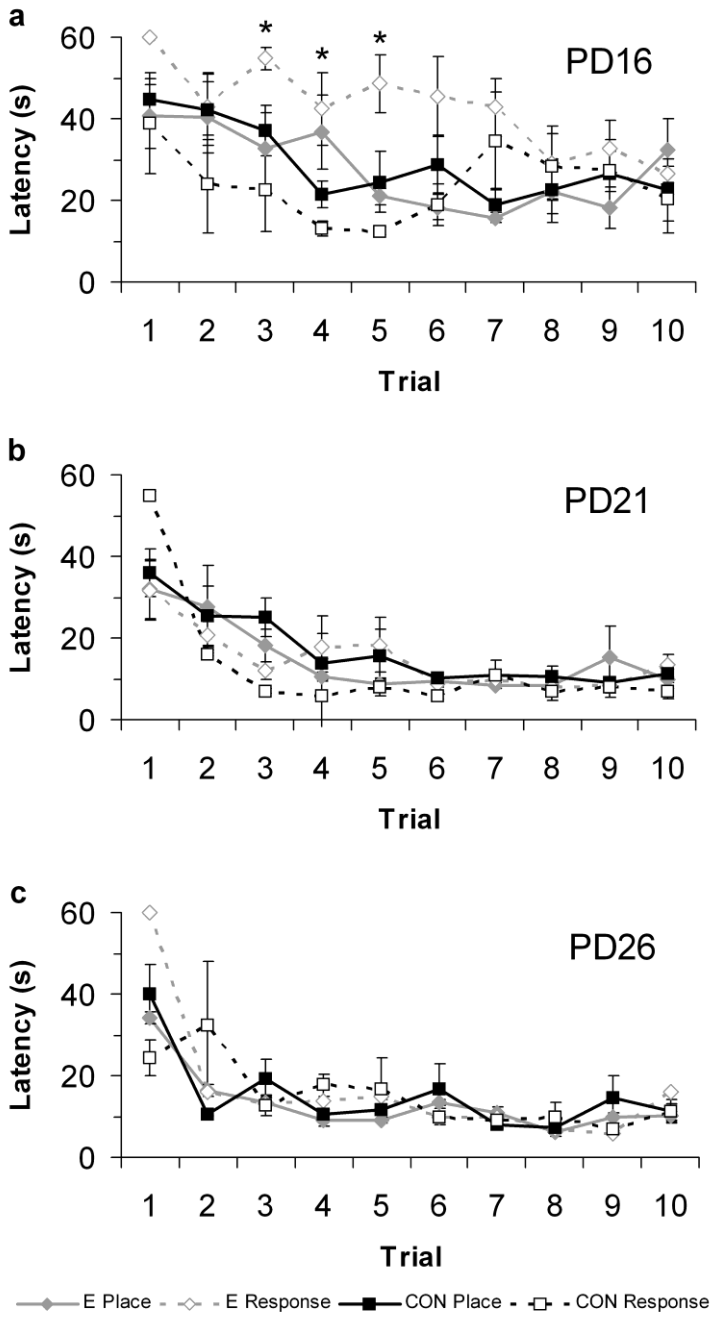

Figure 5: Latencies on the first 10 trials of PD16 (a), PD21 (b), and PD26 (c) rats. * indicates $p < 0.05$.

Adult strategy use and learning

Once control rats grew to adulthood, they underwent the same training and testing paradigm three times in different estrous phases but with different cue configurations. On adult test 1 (AT1), 66% of rats in estrus, 63% in diestrus, 71% in proestrus, and 50% of rats transitioning from proestrus to estrus used a place strategy. None of these values was different from chance ($p > 0.45$; Figure 6a).

Because we predicted that rats in proestrus would be more likely to use a place strategy than any other group, we directly compared the proestrus group to each other group and to all other groups combined, but none of these comparisons revealed any group differences ($p > 0.25$). To determine the effects of previous experience with the task, we evaluated the probability of using the same strategy on the first adult test that was used on the juvenile test. Regardless of estrous phase at AT1, rats were significantly likely to use the strategy on AT1 that they used as juveniles ($p = 0.024$; Figure 7). Together, these results suggest that previous experience, but not current estradiol status, strongly influenced choice behavior on AT1.

We also examined the effects of previous experience and estrous phase on the second (AT2) and third (AT3) adult tests using similar analyses. Neither strategy used as a juvenile nor strategy used on the previous test had a significant effect on strategy choice on either AT2 or AT3 ($p > 0.25$). When strategy selection was analyzed based on estrous phase, 60% of rats in estrus, 50% in diestrus, 90% in proestrus, and 50% transitioning from proestrus to estrus used a place strategy on AT2, and 40% in estrus, 0% in diestrus,

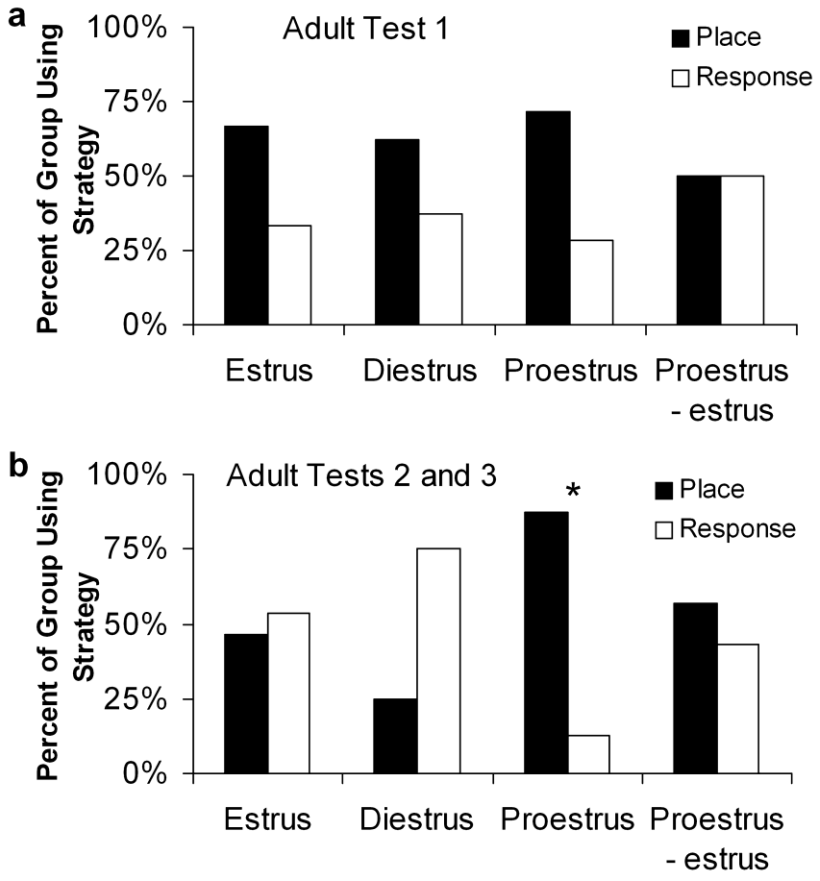

Figure 6: The proportion of rats on (a) adult test 1 and (b) adult tests 2 and 3 in each estrus cycle phase that used place and response strategies.
*** indicates $p < 0.01$.**

83% in proestrus, and 67% transitioning into estrus used a place strategy on AT3. Rats were biased to use a place strategy only during proestrus on AT2 compared to chance ($p = 0.021$), and proestrus rats were more likely to use a place strategy than rats in other estrous cycle phases ($ps < 0.10$) as well as to all other phases combined ($p = 0.048$).

While there was no significant bias toward using a place strategy during any estrous phase on AT3 ($p > 0.20$), proestrus rats were more likely to use a place strategy than those in estrus ($p = 0.060$) and diestrus ($p = 0.033$). In addition, because no rats were tested in the same estrous phase twice, choice behavior from AT2 and AT3 were combined to confirm that rats in proestrus were significantly more likely to use a place strategy than chance ($p = 0.004$) but no strategy bias was present during any other estrous phase ($p > 0.60$; Figure 6b). These results suggest that high estradiol levels, but not previous experience, modulated strategy use on AT2 and AT3.

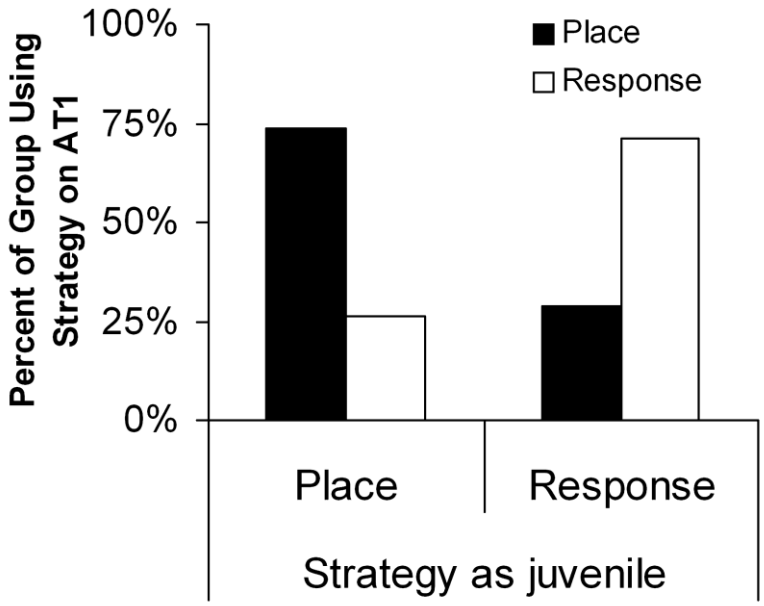

Figure 7: Comparison of strategy use across juvenile test and adult test 1 (AT1).

In addition to intact rats tested in adulthood, 60% of rats OVX at PD22 and 60% of rats OVX at PD44 used a place strategy to solve the task when they were tested once as young adults, revealing that there was no bias in either group ($p > 0.75$; Figure 8). These findings provide supporting evidence that rats with low or no circulating estradiol have no strategy bias in this task. Together, these results suggest that previous experience was the most influential factor on strategy choice at AT1, but high levels of estradiol biased rats to use a place strategy on AT2 and AT3.

Repeated measures ANOVAs were calculated for AT1, AT2, and AT3, with independent variables of strategy and estrous phase and dependent variables of latency on

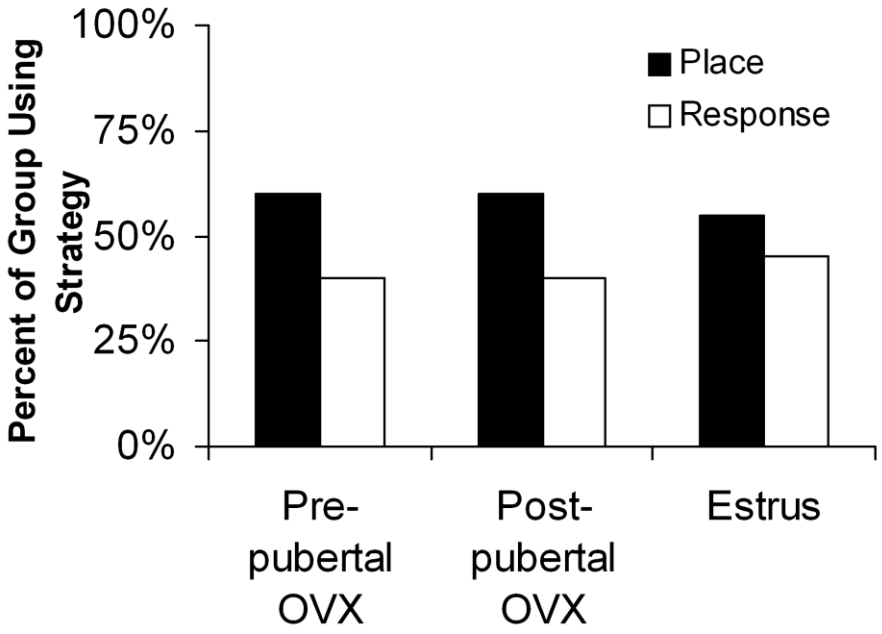

Figure 8: All low/no estradiol groups in adulthood displayed no strategy bias.

trials 1-10. On AT1, there was a main effect of trial, $F(9,171) = 33.1$, $p = 0.000$, and a trial x estrous phase x strategy interaction, $F(27,171) = 1.93$, $p = 0.007$, but no other effects, $p > 0.10$. Rats in proestrus that used a response strategy had significantly longer latencies on the first two training trials than those that used a place strategy (trial 1: $t(5) = 3.58$, $p = 0.016$; trial 2: $t(5) = 3.71$, $p = 0.014$), while response strategy users in estrus had significantly shorter latencies on the first training trial ($t(4) = 6.52$, $p = 0.002$). On AT2 and AT3, there were main effects of trial (AT2: $F(9, 99) = 5.44$, $p < 0.0001$; AT3: $F(9, 144) = 16.68$, $p < 0.0001$) but no other effects or interactions ($p > 0.10$).

Discussion

The results of the present study are consistent with previous findings that estradiol modulates spatial navigation strategy use in the adult female rat (Korol, 2004) and extend these findings by showing that prepubertal females can use either place or response strategies by PD21 but their choice of strategy is not altered by estradiol administration until PD26. Our data provide no support for the hypothesis that prepubertal females, because of their low circulating estradiol levels, are more likely to use a response strategy in a spatial navigation task. We have also shown for the first time that the experience of using one spatial navigation strategy biases what strategy is used on the next spatial navigation task, irrespective of current estradiol status. However, after females have experience with multiple estrous cycles and spatial navigation, estradiol gains control of navigation strategy use and its influence can override the effects of prior experience with

the task. Thus, we have found that both previous navigation strategy use and experience with cyclical estradiol influence the strategy used to navigate. Finally, we have shown that the normal hormonal transition of puberty is not required for the activational effects of estradiol on spatial navigation strategy use.

The development of estradiol's modulation of navigation strategy use

We found that estradiol administration may be able to bias female rats to use a place strategy by PD26, as it does in young adult females (Korol et al., 2004; Quinlan et al., 2008). Thus, while both male and female rats have the ability to use extra-maze cues to perform place navigation by 20 days of age (Rudy et al., 1987; Akers and Hamilton, 2007), hippocampal network sensitivity to estradiol in females is not functional for several more days. Interestingly, these results parallel the precocious ability of estradiol to elicit other estrogen-sensitive behaviors such as lordosis and ear wiggling (Williams, 1987), as well as physiological changes such as the induction of progesterone receptors (Williams and Blaustein, 1988). And, it is not surprising that hippocampal and/or striatal sensitivity to estradiol occurs before puberty, as there is evidence that estrogen receptors (ERs) are expressed in both regions in the juvenile rat. Estrogen receptor α (ERα) is expressed at low levels in the HPC at PD0 and stabilizes at an adult-like baseline by PD15 after a transient increase that peaks between PD7 and PD10 (O'Keefe et al., 1995; Orikasa et al., 2000; Solum and Handa, 2001; Perez et al., 2003). Estrogen receptor β (ERβ) does not appear to be present in the HPC until at least PD14 (Perez et al., 2003),

but it is present in the adult (Le Saux et al., 2006). There is little evidence that ERs are present in the juvenile rat striatum (Toran-Allerand et al., 1992), but both receptor types have been detected in the mouse striatum throughout development at greater or equal levels than that of the adult (Kuppers and Beyer, 1999). Given the many complex mechanisms through which estradiol may influence navigation strategy use and the lack of detailed examination of ER expression during the developmental window that we studied, it remains to be explained why estradiol did not have a behavioral effect until PD26. It is possible that ER sensitivity and/or distribution of expression changes throughout the developmental window between PD21 and PD26 contribute to the development of estradiol modulation of spatial navigation strategy use.

Previous research has shown that low levels of estradiol (as during estrus) bias adult females to use a response strategy to solve an appetitively motivated navigation task (Korol et al., 2004; Quinlan et al., 2008). These results suggest that a response strategy is the "default" for females with no or low estradiol and predict that prepubertal females, who have very low circulating estradiol, are naturally response-biased. Surprisingly, the absence of estradiol did not bias rats at any age to use a response strategy in our water plus maze task. Juveniles, rats ovariectomized shortly before or after puberty and tested in adulthood, and adult females in estrus and diestrus were not, as a group, biased toward using a response strategy. We speculate that our task parameters, room cues, or use of an aversively-motivated water escape task favored place navigation over response navigation. For example, our testing room may be richer with salient geometric and

landmark cues than those used by other labs examining this behavior or the use of aversive motivation may have increased rats' attention to the cues in the environment, increasing the probability of using a place strategy in all rats. In addition, it is possible, although unlikely, that the handling needed for oil injections of prepubertal rats influenced strategy use in adulthood.

While estradiol administration did not influence strategy use in PD16 or PD21 females, rats were able to learn the task quickly and successfully at PD21 and their behavior and learning rates were similar to that of adult rats. In contrast, PD16 rats showed only slight decreases in trial latency over the 10 training trials, and those categorized as using a place strategy did not spend more time in the start arm on the probe than during training as PD21 and PD26 rats did, suggesting that they did not learn to employ a strategy but instead randomly chose a goal arm on the probe trial. However, estradiol disrupted the learning rates of only those PD16 rats categorized as using a response strategy, such that trial latencies were higher than those of all other PD16 groups on several trials. These data suggest that there was some qualitative difference between rats classified as place and response users at PD16. In addition, PD16 rats were likely to use the same strategy on the first test in adulthood as they used as juveniles, just as PD21 and PD26 rats did, suggesting that some rats may have learned a strategy at PD16. Thus, it is possible that PD16 rats were learning the task but became tired, which caused their trial latencies to stay high. Together, our results are consistent with previous research that navigation strategies develop between PD16 and PD21 (Akers & Hamilton,

2007; Rudy et al., 1987) and show for the first time that estradiol is able to modulate navigation strategy use by PD26, before the pubertal onset of naturally-cycling estradiol.

Previous experience and high estradiol levels modulate strategy use in adulthood

Previous studies examining estradiol effects on place and response strategy use have employed between-subjects designs in which subjects have had no previous experience with the task. Because females cycle for a great deal of their adult lives and likely have many experiences in which they must navigate through space, we examined the modulatory roles of previous navigation experience and estrous phase on strategy use. Because estradiol was able to bias females to use a place strategy on a first navigation experience as early as PD26 but in adulthood, the strategy used on a single previous navigation experience before puberty was a better predictor of the strategy used on the first test in adulthood than current estrous phase, it seems that a salient navigation experience in adolescence can overshadow the modulatory effects of estradiol. However, with increased experience with the task, a female is more likely to use a place strategy when in a high estradiol state, or estradiol is more able to prevent the gradual shift to use of a response strategy that has been observed in males (Packard and McGaugh, 1996; Chang and Gold, 2003b). In our study rats received their initial navigation experience prior to puberty, though, such an experience may be extremely salient regardless of whether it occurs in adolescence or puberty. Our results suggest that the reason many

previous studies have not found within-subjects effects of estrous cycle phase on place navigation (Stackman et al., 1997) is that the previous experience with the task was more influential on behavior than current estrous status.

Potential mechanisms of estrogen-modulated spatial navigation

There is evidence that estradiol has its effects on spatial navigation strategy use in the adult female rat via ERs within the HPC and DS. Direct administration of estradiol to the DS impairs response learning, and direct administration to the HPC enhances place learning (Zurkovsky et al., 2007), and local administration of ER antagonists to the HPC prevents the enhancing effects of systemic estradiol on place learning (Zurkovsky et al., 2006). This likely occurs via modulation of one or more of the many mechanisms of plasticity described in the introduction, and the results of our study suggest that one or more of these estradiol-sensitive mechanisms in the HPC and/or DS become functional around 26 days of age. Our data also point to a mechanism that that is modified by experience. These findings support the view that hormone-responsive circuits for cognitive function may be organized and respond to experience in a similar fashion to hormone sensitive neural circuits for reproductive behaviors like lordosis (Beach and Orndoff, 1974) and maternal behavior (Maestripieri and Zehr, 1998), in which increased experience and length of exposure to ovarian hormones increases hormonal control of behavior.

CHAPTER 2: ESTRADIOL AND AGE MODULATE ACTIVATION OF HIPPOCAMPAL AND DORSAL STRIATAL ENSEMBLES DURING SPATIAL NAVIGATION

The results of Ch. 1 described the degree to which developmental and behavioral factors, including age and prior navigation experience, modulate the ability of estradiol to gain control over navigation strategy use. This chapter examines whether one functional mechanism by which estradiol influences navigation behavior is by modulating hippocampal and striatal activation. As described in the general Introduction, results from studies that have used lesions and activating/inactivating agents in male subjects (Olton and Samuelson, 1976; Olton and Papas, 1979; Mitchell and Hall, 1988; Devan et al., 1999; Chang and Gold, 2003a) support the hypothesis that the relative amount of hippocampal and striatal activation control spatial navigation strategy use (White and McDonald, 2002). And, several studies that have used the IEG *c-fos*, which is expressed when a cell is activated, and its protein, Fos, which peaks 90-120 min after the gene is activated (Hoffman et al., 1993; Hoffman and Lyo, 2002), to examine this hypothesis have found differential activation in the HPC and DS between male place and response strategy users (Colombo et al., 2003; Teather et al., 2005; Gill et al., 2007). However, these results have been inconsistent, with one study finding differential activation between place and response strategy users in the DLS but not in the HPC (Gill et al., 2007) and others studies finding differential activation in the DG and CA1 of the HPC but not in the DS (Colombo et al., 2003; Teather et al., 2005). Together these findings suggest that there is differential activation with the HPC and DS during place and

response navigation, but that specific results may depend on a number of factors, including task and sacrifice time point.

Several studies have examined the effects of estradiol on learning of spatial navigation tasks that are almost identical in procedure but differ only in the strategy that is required to solve the task (Korol and Kolo, 2002; Davis et al., 2005; Zurkovsky et al., 2007). For example, young adult ovariectomized female rats administered 10 μg 17 β-estradiol benzoate 48 and 24 hr prior to training took fewer trials to reach behavioral learning criterion than oil-treated controls when a place strategy was required to solve the task but more trials when a response strategy was required (Korol and Kolo, 2002). Estradiol administration has similar effects on place and response navigation when replacement is given chronically (e.g., a 0.5 mg 60-day release pellet) and when a more complex eight-arm radial maze is used to examine spatial learning (Davis et al., 2005). Because estradiol modulates the activation and plasticity of the HPC and DS under many circumstances (Pfaus et al., 1993; Rudick and Woolley, 2000; Dominguez-Salazar et al., 2006), these results suggest that estradiol may enhance place learning and impair response learning by a) differentially altering the baseline activation of the HPC and DS in the non-behaving rat so that it is biased to use the HPC at the onset of behavioral training or b) priming the HPC to become preferentially activated during navigation learning. Therefore this chapter assesses the effects of estradiol on hippocampal and striatal activation during navigation learning in young adult females.

In addition to examining a functional mechanism by which estradiol might modulate spatial navigation behavior in the young adult rat, this chapter also examines

whether the effects of estradiol on the activation of the HPC and DS during spatial navigation learning change as a function of adult aging. The results from Ch. 1 suggest that age influences the ability of estradiol to modulate spatial navigation strategy use during development, but very little is yet known about how aging during adulthood influences this effect (Markowska, 1999; Hogervorst et al., 2000). Therefore this study further examined age-related changes in the ability of estradiol to modulate spatial navigation behavior in adulthood. Several studies have shown that estradiol replacement improves spatial memory of ovariectomized, middle-aged female rats just as it does in when administered to young females (Markham et al., 2002; Acosta et al., 2008), but others have found that estradiol administration has no effect, suggesting that females may be less sensitive to the effects of estrogen by middle-age (Feng et al., 2004; Ziegler and Gallagher, 2005), perhaps because of age-related variation and/or decrease in circulating estradiol levels (LeFevre and McClintock, 1988). It is also likely that the DS loses sensitivity to estradiol (Roy et al., 1982), but little is known about how this affects navigation behavior. Therefore, in this chapter, we also examine hippocampal and striatal activation in the HPC and DS during navigation learning in middle-aged females.

Along with potential age-related decline in sensitivity to estradiol, ovarian production of estradiol also begins to decline during middle age (LeFevre and McClintock, 1988). Long-term hormone deprivation has been shown to be detrimental to general cognition and hippocampal function in particular (Frick et al., 2000; Markowska and Savonenko, 2002), however, aged rats ovariectomized 1.5-6 months before behavioral testing have better spatial memory than when ovariectomized less than one

month prior to being tested (Bimonte-Nelson et al., 2003). Almost no research has examined the effects of long-term hormone deprivation on striatal-dependent behavior, so the effects of long-term hormone deprivation on the function of the HPC and DS for navigation in middle-aged females are in need of further investigation. Therefore this chapter includes a group of long-term OVX middle-aged rats.

The current experiment examines whether one functional mechanism by which estradiol, age, and long-term hormone deprivation modulate navigation learning rate during spatial navigation is by altering hippocampal and striatal activation. To do this, we quantified Fos expression in several subregions of the HPC and DS in young adult and middle-aged rats that were OVX and replaced with estradiol or oil and then trained on place and response tasks.

Materials and Methods

Subjects

Subjects were 4- and 12-month-old female Sprague-Dawley CD strain rats purchased from Charles Rivers Laboratories (Kingston, NY) at 3 months of age. They were paired-housed in individually-ventilated cages and given *ad libitum* access to water and a standard diet (Rodent Diet 5001, PMI Nutrition International, Inc., Brentwood, MO). The temperature-controlled colony room was maintained on a 12:12 hr light:dark cycle with lights on at 7 a.m. daily, and all behavioral training took place during the lights-on phase of the day. Rats were handled daily for 10 days prior to ovariectomy and then an additional 7 days before behavioral testing.

Experimental design

Rats in three hormonal condition were trained on place or response versions of the water maze or were used as explore controls (see Figure 9): a) Rats ovariectomized (OVX) at 3 mo and behaviorally trained at 4 mo; b) Rats OVX at 11 mo and behaviorally trained at 12 mo; and c) Rats OVX at 3 mo and behaviorally trained at 12 mo. Half of the short-term OVX rats (groups a and b) received two estradiol injections prior to behavioral training, as illustrated in Figure 9. Thirty min following the probe trial, rats were perfused transcardially and their brains harvested for quantification of Fos-immunoreactivity (Fos-IR) in several hippocampal and dorsal striatal subregions, as delineated in Figure 10. This sacrifice time point was chosen because Fos expression peaks 90-120 min after induction (Morgan et al., 1987; Sagar et al., 1988; Hoffman et al., 1993; Hoffman and Lyo, 2002), and a previous study has shown that sacrificing rats 30 min after training on similar place and response tasks captures differential Fos expression in response to learning (Gill et al., 2007).

Hormonal manipulations

Ovariectomies and vaginal smears were conducted as described in Ch. 1. Forty-eight and 24 h before behavioral testing, rats received two subcutaneous injections of 10 μg 17 β-estradiol (Sigma-Aldrich, St. Louis, MO) dissolved in 0.1 mL sesame oil (Sigma-Aldrich, St. Louis, MO) or the oil vehicle alone.

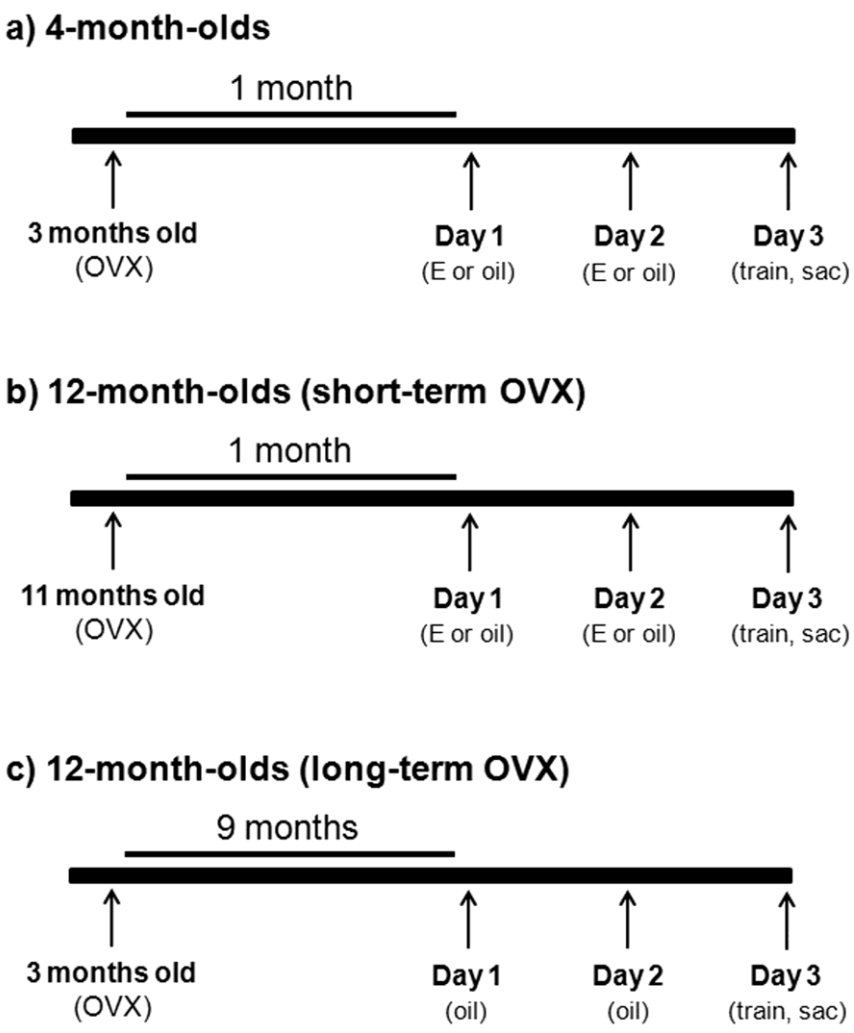

Figure 9: Experimental timeline of hormonal manipulations and behavioral training for rats trained at 4 months of age (a) and 12 months of age (b and c).

Apparatus and testing room

All behavioral training took place in a black plastic pool with a diameter of 1.8m filled with approximately 30 cm of water mixed with black water-based paint maintained at 24-25 ºC. A black escape platform with diameter 10.2 cm was either hidden just below the water's surface (place task) or raised above the surface of the water and wrapped with white tape in order to be visible (response task). The maze was located in a 6.5 m x 3.8 m rectangular room (1.3 m from the North wall and 0.7 m from the East wall) that was rich with cues including a curtain, table with computer, cart with cages, counter, shelves, and walls with posters and objects with high-contrast patterns.

Behavioral training

Behavioral training and probe testing took place within a single session. Rats in each hormonal condition were randomly assigned to receive training on either a place or response version of the water maze or to serve as explore controls. Cage mates were transported to the test room in individual holding cages on a cart that remained in a stable location in the test room throughout training. Each rat was placed in the pool and allowed to swim for 30 s. A curtain was drawn around the pool so that room cues were not visible.

During training, rats were placed in the water at one of four locations (N, S, E, and W) at the beginning of each trial in a pseudo-random order. Each trial ended when the rat climbed onto the escape platform. If the rat failed to find the platform at the end of 60 s, the experimenter guided it through the water to the platform. The rat remained on the escape platform for 10 s and was then returned to its holding cage, at which time its

cage mate received a training trial. Cage mates alternated trials in this fashion throughout the session until each had reached the learning criterion (see below) or 40 trials were completed. All rats completed training within 60-100 min of the beginning of training. Upon the next trial, the experimenter conducted one probe trial during which the platform was removed and the rat was allowed to swim for 60 s. During all training and probe trials, latencies to reach the end of any arms entered and latency to mount the platform were recorded by the experimenter and the path of the animal was recorded in real time using HVS Image (Buckingham, WH, UK). Some rats were randomly assigned to serve as controls for motor activity and latent learning associated with exploration while swimming in the pool in a novel environment. Each explore control was yoked to a behaviorally-trained rat so that it swam for the same amount of time and the same number of trials. Explore controls were yoked to trained rats that took the average number of trials to reach criterion.

Place task

Place training required rats to find an escape platform that was hidden underneath the surface of the water in a fixed location throughout training. The platform location was randomly assigned to the center of one of the four quadrants and was counterbalanced within and across experimental groups. Because the start location varied from trial to trial, rats could only navigate using landmarks and room geometry cues. Criterion for learning was achieved when the rat performed four consecutive trials with latencies under 10 s. After criterion was met, the rat was given one probe trial as described above.

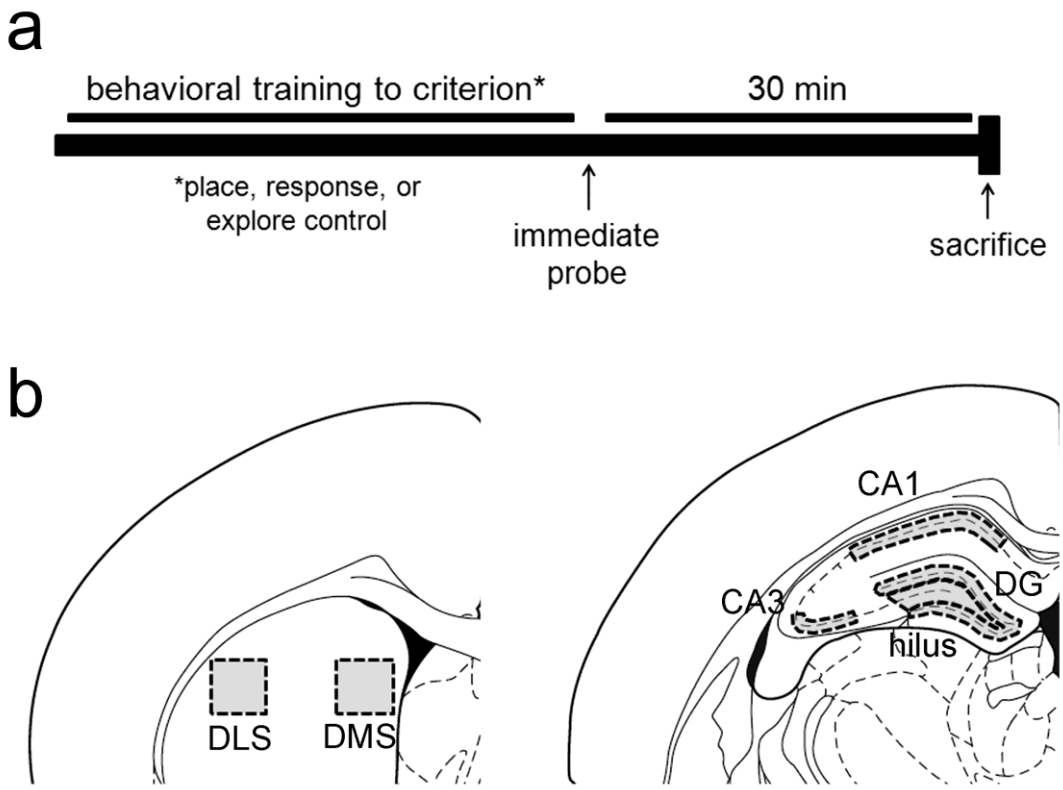

Figure 10: Timeline of behavioral training, probe testing, and sacrifice (a) and illustration of samples taken from left hemisphere of DLS and DMS (b, left) and DG, hilus, CA1, and CA3 of the hippocampus (b, right). (Panel b is modified from Paxino and Watson, 1998.)

Response task

Response training required rats to find a visible escape platform when environmental cues were hidden by a curtain drawn around the pool. The platform was always located in the quadrant either to the left or right of the random start position of the rat, so that it was always in the same relative position to the rat's start location. Thus, rats

could navigate to the platform by using a visual strategy and/or acquiring a response strategy (e.g., go to the left). Criterion for learning was reached when the rat completed four consecutive trials in which the latencies were within 10 s of each other and the rat made a similar path or turn sequence to reach the platform. A probe trial with no platform was conducted on the following trial.

Perfusion and immunohistochemistry

Because training took 60-100 min and Fos expression peaks 90-120 min after induction of *c-fos* (Morgan et al., 1987; Sagar et al., 1988; Hoffman et al., 1993; Hoffman and Lyo, 2002), rats were sacrificed 30 min after the probe trial (Figure 10a). Previously, this sacrifice time point was shown to uncover differences between place and response task learners in Fos-IR in males that were not present when rats were sacrificed 90 min after behavioral testing (Gill et al., 2007). Rats were anesthetized with an intraperitoneal injection of 80 mg/kg ketamine and 10 mg/kg xylazine and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. Brains were removed and stored in 4% paraformaldehyde for 4 days and then sodium azide at 4ºC until sectioned.

Brains were blocked to include the entire HPC and DS and sectioned on a microtome. Every fifth 50µm section was collected and used for immunohistochemistry. Tissue was transferred to 50 mM phosphate buffered saline (PBS) and washed, incubated in 1% hydrogen peroxide in PBS to remove endogenous peroxidase, and washed in PBS. Tissue was blocked with PBS containing 0.1% bovine serum albumin (BSA) and 0.2% TX-100 and then incubated in the primary antibody solution containing 1:5000 c-Fos

polyclonal rabbit IgG (Sigma, St. Louis, MO) at 4ºC overnight. The following day, tissue was washed PBS and then incubated in a secondary solution comprised of 0.1% BSA and 1:500 concentration of biotinylated anti-rabbit made in goat. Tissue was washed in PBS, incubated in an avidin biotin complex (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA) solution with 0.1% BSA, and washed again in PBS. Tissue was incubated in a 3,30-diaminobenzidine (DAB) solution (SigmaFast DAB tablets, Sigma, St. Louis, MO) at RT until brown (approximately 10 min), indicating that Fos- IR had occurred. Then sections were washed and stored in PBS. Tissue slices were mounted onto gelatin-coated glass slides in 50 mM potassium PB. The following day, tissue was dehydrated and coverslipped.

Quantification of Fos-immunoreactivity

The optical fractionator method was used to estimate the number of Fos-IR cells in subregions of the HPC and DS using StereoInvestigator software (MicroBrightField, Colchester, VT). Using unbiased stereology, cells were only counted as Fos-IR if they were in focus in the inner 20 µm block of tissue and the solid reaction product covered at least half of the nucleus. In the DLS and DMS, a 150 µm x 150 µm sample was counted within a 187.5 µm x 187.5 µm grid throughout a uniform-sized square sample outlined by the experimenter, illustrated in Figure 10b (left). In CA1, CA3, DG, and hilus of the HPC, a 120µm x 120 µm sample was counted within a 150 µm x 150 µm grid throughout the extent of each subregion as outlined by the experimenter, illustrated in Figure 10b (right). Three left-hemisphere sections were analyzed for each subregion of each rat at an

interval of five sections, between +1.2 mm anterior to bregma and -0.2 mm posterior to bregma for DS and between -2.8 and -4.0 mm posterior to bregma for the dorsal HPC according to Paxino & Watson's rat brain atlas (Paxino and Watson, 1998). Estimated counts based on section thickness were divided by estimated volume to attain a density measure of Fos-IR for each brain subregion of each rat.

Statistical analysis

All analyses were calculated using an alpha value of 0.05. Similar but separate analyses were calculated for 4- and 12-month-old rats treated with oil and estradiol, as well as for short- and long-term OVX 12-month-old rats.

Effects of estradiol on learning place and response tasks

To examine the effects of task and estradiol replacement on learning, the number of trials to reach behavioral criterion was examined using ANOVAs with estradiol status (oil vs. estradiol) and task (place, response, explore control) as independent variables. Because behavioral criteria were based on latency to find the platform, mean swim speed across the first 10 trials (before any rats reach criterion) was compared between oil- and estradiol-treated rats within each task using t-tests to determine whether estradiol influenced swim speed in each task. T-tests were also used to compare oil- and estradiol-treated rats in the number of platform crossings and percent time spent in each maze quadrant during the place task probe to determine the accuracy and precision of the platform location learned by rats trained on the place task. In the response task, rats that

made a similar turn sequence on the probe trial as the last four training trials were categorized as having acquired a response strategy during training, while those that searched randomly for the platform during the probe were categorized as having used a visual strategy.

Effects of estradiol and task on Fos-immunoreactivity in the hippocampus and dorsal striatum

To examine effects of estradiol and task on hippocampal and striatal activation, we used ANOVAs with the number of Fos-IR cells/mm^3 as the dependent variable and task (place, response, control) and estradiol treatment (oil, estradiol) as the independent variables for each hippocampal and striatal subregion quantified (DG, hilus, CA1, and CA3 in the HPC; DMS and DLS). T-tests were calculated to determine whether strategy used on the response probe affected Fos-IR, but only hippocampal analyses in 4-month-olds were significant, so they are the only ones reported in the results (all others: ps > 0.15).

In order to compare the relative activation of striatal and hippocampal subregions during place and response learning, ratios between Fos-IR in different subregions of HPC and DS were compared using ANOVAs with the ratio between the Fos-IR in the two subregions as the dependent variable and estradiol status and task as the independent variables. We also evaluated whether the strategy used on the response probe trial affected the relative activation of hippocampal and striatal subregions in a similar manner. Ratios compared included DG:DMS, DG:DLS, CA1:DMS, CA1:DLS, and DMS:DLS.

We calculated correlations between subregions to determine if there was a linear relationship between Fos-IR in different subregions for all rats combined, all place task learners, and all response task learners. Correlations were calculated between the following subregions: DMS vs. DLS, DG vs. CA1, DG vs. DMS, DG vs. DLS, CA1 vs. DMS, and CA1 vs. DLS. We also examined the relationship between Fos-IR in each subregion and the number of trials the reach behavioral criterion for all rats, all place task learners, and all response task learners to determine whether any effects in activation might be related to the amount of training or motor behavior rather than only task or estradiol replacement, but these correlations were significant and so are not reported below ($ps > 0.05$). Because explore controls used in the place and response tasks did not differ in any measure analyzed, they were combined for data analysis. All analyses described above were also used to determine the effects of duration of OVX in 12-month-old rats.

Results

Effects of estradiol replacement in 4-month-olds are presented first, followed by effects of estradiol replacement in 12-month-olds, and then the effects of duration of ovariectomy in 12-month-olds are described. For each age x hormonal condition comparison, behavioral results (number of trials to reach criterion, trial latency on the first 10 trials, mean speed, and probe trial analysis) are presented first. Then, Fos-IR in hippocampal and striatal subregions is presented, followed by ratios and correlations between Fos-IR in different subregions.

Effects of estradiol treatment on learning and Fos-immunoreactivity in 4-month-old rats

Learning

Surprisingly, estradiol replacement did not improve rate of place learning or performance on the probe trial. However, as predicted, estradiol impaired rate of response learning, as shown in Figure 11a. Once response-trained rats reached behavioral criterion, a majority of both oil and estradiol-treated rats used a response strategy on the probe trial, suggesting that only learning rate was impaired by estradiol. These findings were supported by an ANOVA for the number of training trials to reach behavioral criterion that revealed a main effect of task ($F[1,14] = 6.29$, $p = 0.025$) and an estradiol status x task interaction ($F[1,14] = 4.71$, $p = 0.048$) but no main effect of estradiol status ($p > 0.40$; Figure 11a). Estradiol-treated rats required more trials to reach criterion that oil treated rats when they were trained on the response task ($t[19] = 4.02$, $p = 0.001$) but estradiol replacement did not influence rate of learning of the place task ($p > 0.15$), and task did not affect learning rate in oil-treated rats ($p > 0.55$). T-tests comparing mean speed across the first 10 trials within each task revealed no difference between oil and estradiol-treated place task learners or response task learners (p's > 0.80), suggesting that effects of estradiol on response learning rate were not related to differences in swim speed.

Analysis of the percent time spent in each quadrant of the maze and the number of platform location crossings during the 60 s probe trial (where no platform was present) in

the place task revealed that both oil- and estradiol-treated rats learned the platform location to with a high level of accuracy and precision ($p > 0.80$). Path analysis from the response probe trial revealed that a majority of oil-treated rats (90%) and estradiol-treated rats (67%) used a response strategy to locate the target in the absence of a visible platform during the probe trial. That is, they traveled the same path on the probe trial as they did on the last 4 criterion trials, even though there was no visible platform. The remainder of the rats swam randomly throughout the maze on the probe trial, indicating that they had probably learned a visual strategy. There were no differences in the number of trials to reach criterion between rats that used a response strategy on the probe trial and those that used a visual strategy ($p > 0.10$), suggesting that strategy use did not affect learning rate within the response task.

Fos-immunoreactivity

Estradiol replacement did not affect Fos-IR in any hippocampal or striatal subregion analyzed in rats that simply swam in the pool (explore controls), and task did not affect Fos-IR in any subregion. However, there were a few small task-specific effects of estradiol replacement in the DS, as shown in Figure 11b-d, and Fos-IR in the DLS and DMS were correlated for all rats.

Hippocampus

Neither estradiol replacement or task increased Fos-IR in any hippocampal subregions analyzed, as shown in Figure 11b and Table 1 (p's > 0.20), nor were there any

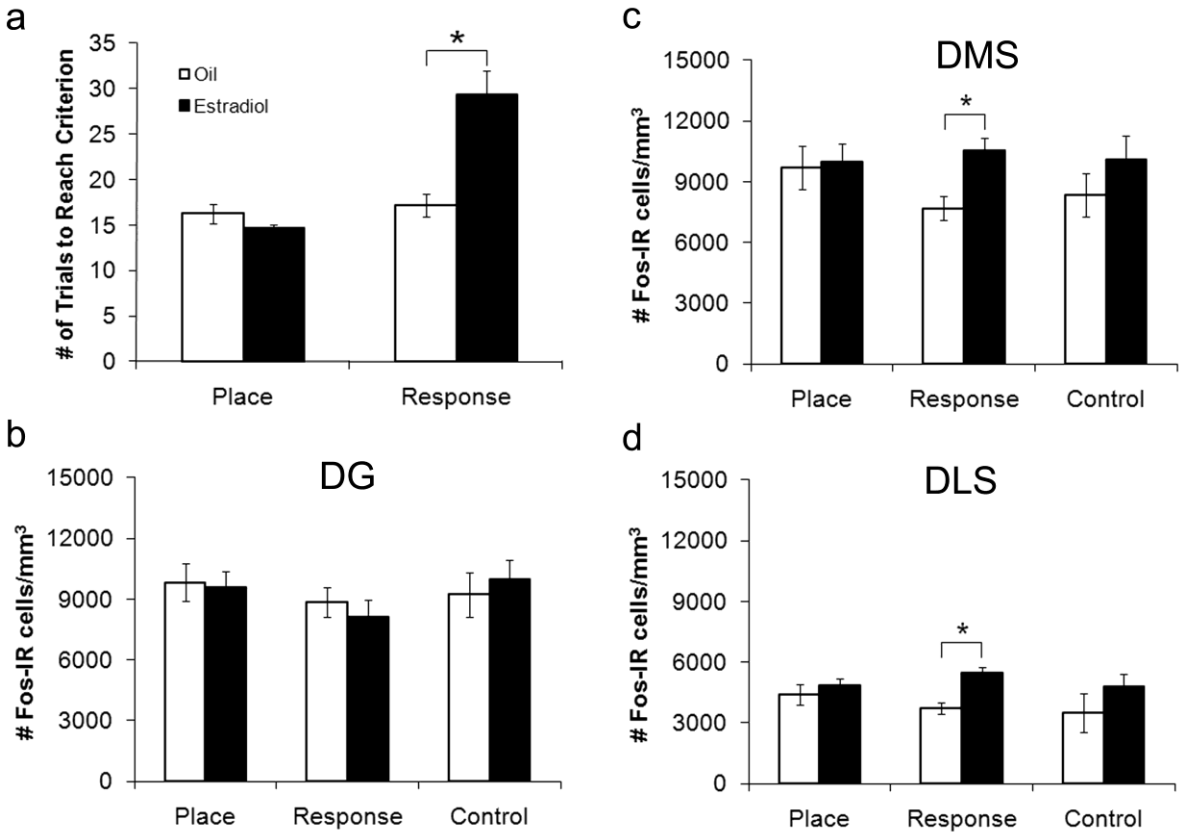

Figure 11: The mean number of trials to reach behavioral criterion in the place and response tasks (a) and Fos-IR in the DG (b), DMS (c), and DLS (d) of 4-month-olds. * indicates $p < 0.05$.

interactions between the two. However, response task learners that used a response strategy on the probe trial had more Fos-IR in the DG and hilus than rats that used a visual strategy (DG: $t[17] = 2.84$, $p = 0.011$; hilus: $t[17] = 2.45$, $p = 0.025$). In contrast, no differences in Fos-IR between these two groups were detected in CA1 or CA3 (p's >

0.10). These data suggest that learning a motor response strategy to navigate may engage some regions of the HPC more than learning to use a visual strategy to locate a target.

Table 1: The number of Fos-IR cells/mm^3 ± SEM in CA1, CA3, and hilus of 4-month-old OVX rats replaced with oil or estradiol.

	Place		Response		Control	
	Oil	Estradiol	Oil	Estradiol	Oil	Estradiol
CA1	7087 ± 466	6729 ± 491	6733 ± 498	6885 ± 307	6071 ± 754	5774 ± 407
CA3	2610 ± 355	2614 ± 405	2409 ± 311	2456 ± 296	2469 ± 290	1693 ± 570
hilus	7034 ± 1068	7407 ± 628	7030 ± 832	6583 ± 674	6782 ± 1679	5323 ± 1629

Dorsal striatum

Overall, estradiol replacement increased Fos-IR in the DMS ($F[1,40] = 4.68$, $p = 0.037$) and DLS ($F[1,40] = 10.23$, $p = 0.003$), but this effect was carried by response task learners (DMS: $t[20] = 3.31$, $p = 0.003$; DLS: $t[20] = 4.55$, $p = 0.0001$), as shown in Figure 11c and d. Estradiol replacement did not affect Fos-IR in place task learners or explore controls (p's > 0.30). Task did not affect Fos-IR and there were no interactions between estradiol status and task (p's > 0.20), suggesting that estradiol effects on Fos-IR were slight.

Ratios and correlations between Fos-IR in hippocampal and dorsal striatal subregions

To determine the effects of estradiol replacement and task on the relative engagement of hippocampal and striatal subregions during navigation learning, ratios of Fos-IR between several subregions were analyzed using ANOVAs with estradiol status and task as independent variables (data not shown). We found a main effect of estradiol status in several of these ratios (DG:DLS ($F[1,40] = 8.32$, $p = 0.006$), CA1:DLS ($F[1,40] = 9.51$, $p = 0.003$), CA1:DMS ($F[1,40] = 4.17$, $p = 0.048$), and DMS:DLS ($F[1,40] = 4.61$, $p = 0.038$), but no other effects nor any interactions (p's > 0.10). These significant differences in ratio were likely driven by the fact that estradiol replacement increased striatal but not hippocampal activation. Therefore we also correlated Fos-IR in these subregions for all place task learners, all response task learners, and all 4-month-old rats combined, to determine if there was a linear relationship between activation in these areas. Fos-IR in the DMS and DLS were positively correlated for all response task learners ($r = 0.791$, $p < 0.0001$) and all place task learners ($r = 0.699$, $p = 0.003$), as well as all 4-month-old rats combined ($r = 0.760$, $p < 0.0001$), suggesting direct communication between and possibly similar functions of these two subregions. No other correlations were significant (p's > 0.15).

Effects of estradiol treatment on learning and Fos-immunoreactivity in 12-month-old rats

Learning

Similar to 4-month-olds, estradiol replacement did not affect the number of training trials needed to reach behavioral criterion in the place task, and all rats performed similarly on the probe trial. Unlike 4-month-olds, estradiol did not impair response learning rate but did prevent a majority of rats from acquiring a response strategy during training. That is, most estradiol-treated rats acquired a visual strategy but never learned a response strategy to locate the platform. This may be the reason that it took response task learners significantly more trials to reach criterion than place task learners, as shown in Figure 12a. These results suggest that 12-month-olds found the response task considerably more difficult than 4-month-olds, and estradiol further impaired response learning in 12-month-olds by preventing response strategy acquisition.

These findings are supported by the following analyses. An ANOVA for the number of training trials to reach behavioral criterion revealed a main effect of task ($F [1, 18] = 19.06$, $p = 0.0004$) but no other effects (p's > 0.60), as shown in Figure 12a. Direct comparisons confirmed that there were no differences between oil and estradiol-treated rats that learned either the place ($p > 0.40$) or response task ($p > 0.75$) but response task learners took significantly more trials than place task learners to reach criterion ($t[9] = 2.83$, $p = 0.020$). And, there were no differences between oil and estradiol-treated rats in either the place or response tasks in mean speed across the first 10 trials (p's > 0.10).

When we analyzed probe trials for the place task, we found that the percent time spent in each quadrant of the maze and the number of platform location crossings revealed that both oil- and estradiol-treated rats learned the platform location with a high level of accuracy and precision ($p > 0.30$). When the probe trials for the response trained rats were analyzed, we found that while a majority of oil-treated rats (67%) used a response strategy to locate the target in the absence of a visible platform during the probe trial, only 17% of estradiol-treated rats used a response strategy. However, there was no difference in the number of trials to reach criterion between those that used a response strategy on the probe and those that used a visual strategy ($p > 0.10$). This suggests that at 12 months of age, estradiol impairs response learning to the point where rats are unable to acquire a response strategy.

Fos-immunoreactivity

Similar to 4-month-olds, estradiol replacement did not affect Fos-IR in any subregion analyzed in 12-month-old explore controls. However, performing the response task increased Fos-IR in the DMS, and estradiol modulated Fos-IR in a task-specific manner in the DG, DMS, and DLS (shown in Figure 12b-d), but neither estradiol nor task affected Fos-IR in CA1, CA3, or hilus (shown in Table 2). In addition, Fos-IR in the DG, DMS, and DLS were positively correlated with one another (shown in Table 3), suggesting that there is similar activation across hippocampal and striatal systems during navigation learning.

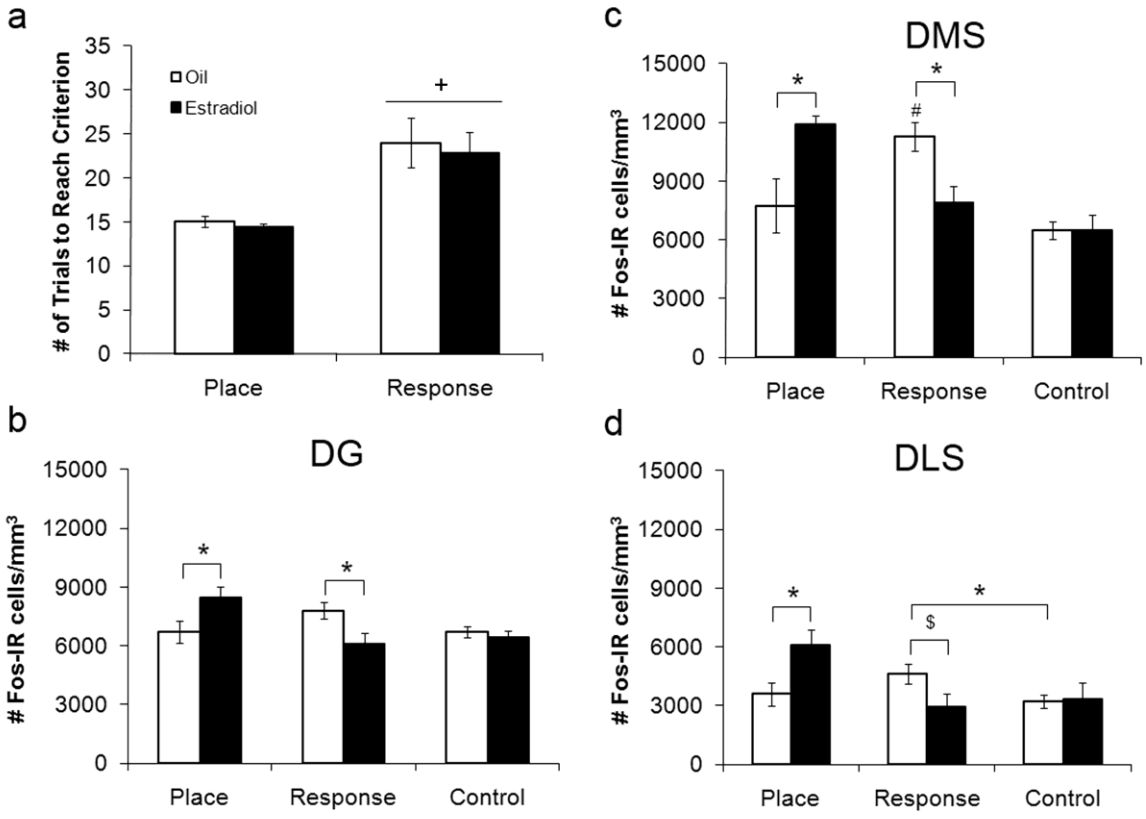

Figure 12: The mean number of trials to reach behavioral criterion in the place and response tasks (a) and Fos-IR in the DG (b), DMS (c), and DLS (d) of short-term OVX 12-month-olds.
+ indicates main effect of task with $p < 0.001$, * indicates $p < 0.05$, # indicates significantly different from all other oil-treated groups with $p < 0.05$, and $ indicates $p < 0.10$.

Hippocampus

Neither estradiol nor task alone affected Fos-IR in any hippocampal subregion examined (p's > 0.25). However, estradiol and task interacted to modulate Fos-IR in the DG ($F[2,20] = 6.36$, $p = 0.007$) but no other hippocampal subregions (p's > 0.15), as

shown in Figure 12b and Table 2. Direct comparisons revealed that estradiol treatment increased Fos-IR in the place task ($t[8] = 2.34$, $p = 0.048$) but prevented the increase in Fos-IR that occurred in oil-treated rats in the response task ($t[10] = 2.50$, $p = 0.032$; Figure 12b). However, oil-treated groups did not differ from each other in Fos-IR (p's > 0.10). Overall, these results suggest that estradiol modulated DG Fos-IR in a task-specific manner.

Table 2: The number of Fos-IR cells/mm^3 ± SEM in CA1, CA3, and hilus of 12-month-old short-term OVX rats replaced with oil or estradiol.

	Place		Response		Control	
	Oil	Estradiol	Oil	Estradiol	Oil	Estradiol
CA1	4505 ± 670	5022 ± 595	4287 ± 152	4195 ± 629	4564 ± 219	4093 ± 408
CA3	1723 ± 187	1906 ± 302	1515 ± 239	1576 ± 156	2581 ± 644	1548 ± 345
hilus	5155 ± 866	5294 ± 522	4931 ± 438	3610 ± 690	3853 ± 585	3884 ± 935

Dorsal striatum

Similar to the HPC, estradiol replacement did not affect Fos-IR in either subregion of the DS (p's > 0.85). However, task affected Fos-IR in the DMS ($F[2,20] = 4.15$, $p = 0.031$), with response task learners having greater Fos-IR than explore control rats ($t[6] = 3.55$, $p = 0.012$) and place task learners ($t[9] = 2.38$, $p = 0.041$), as illustrated in Figures 12c and 13. Task did not affect Fos-IR in the DLS ($p > 0.20$). Similar to effects

on Fos-IR in the DG, estradiol status and task interacted to modulate Fos-IR in the DS (DMS: $F[2,20] = 9.26$, $p = 0.001$; DLS: $F[2,20] = 5.23$, $p = 0.015$), as shown in Figure 12c and d. Estradiol replacement increased Fos-IR in rats that learned the place task (DMS: $t[8] = 2.85$, $p = 0.021$; DLS: $t[8] = 2.43$, $p = 0.041$) but prevented the increase in Fos-IR that occurred in oil-treated rats in the response task (DMS: $t[10] = 2.95$, $p = 0.015$; DLS: $t[10] = 2.04$, $p = 0.069$), displayed in Figure 12c and d. Thus, the patterns of Fos-IR in the DMS and DLS were similar to the pattern observed in the DG but not other hippocampal subregions.

Ratios and correlations between Fos-immunoreactivity in hippocampal and dorsal striatal subregions

Neither estradiol replacement nor task affected the ratios of activation between any hippocampal and striatal subregions (p's > 0.15). However, Fos-IR in the DG, DMS, and DLS were positively correlated with one another for all rats, as shown in Table 3, but CA1 Fos-IR was not significantly correlated with Fos-IR in any of these subregions (p's > 0.20). When place and response task learners were analyzed separately, similar orrelations were significant for place task learners (DMS vs. DLS, DG vs. DMS, DG vs. DLS; all others—p's > 0.10), as shown in Table 3. There were fewer significant correlations for response task learners (DMS vs. DLS, DG vs. DMS; all others—p's > 0.10), but similar patterns of and correlations between Fos-IR across tasks suggest that activation of these three subregions are related during navigation, regardless of the strategy used.

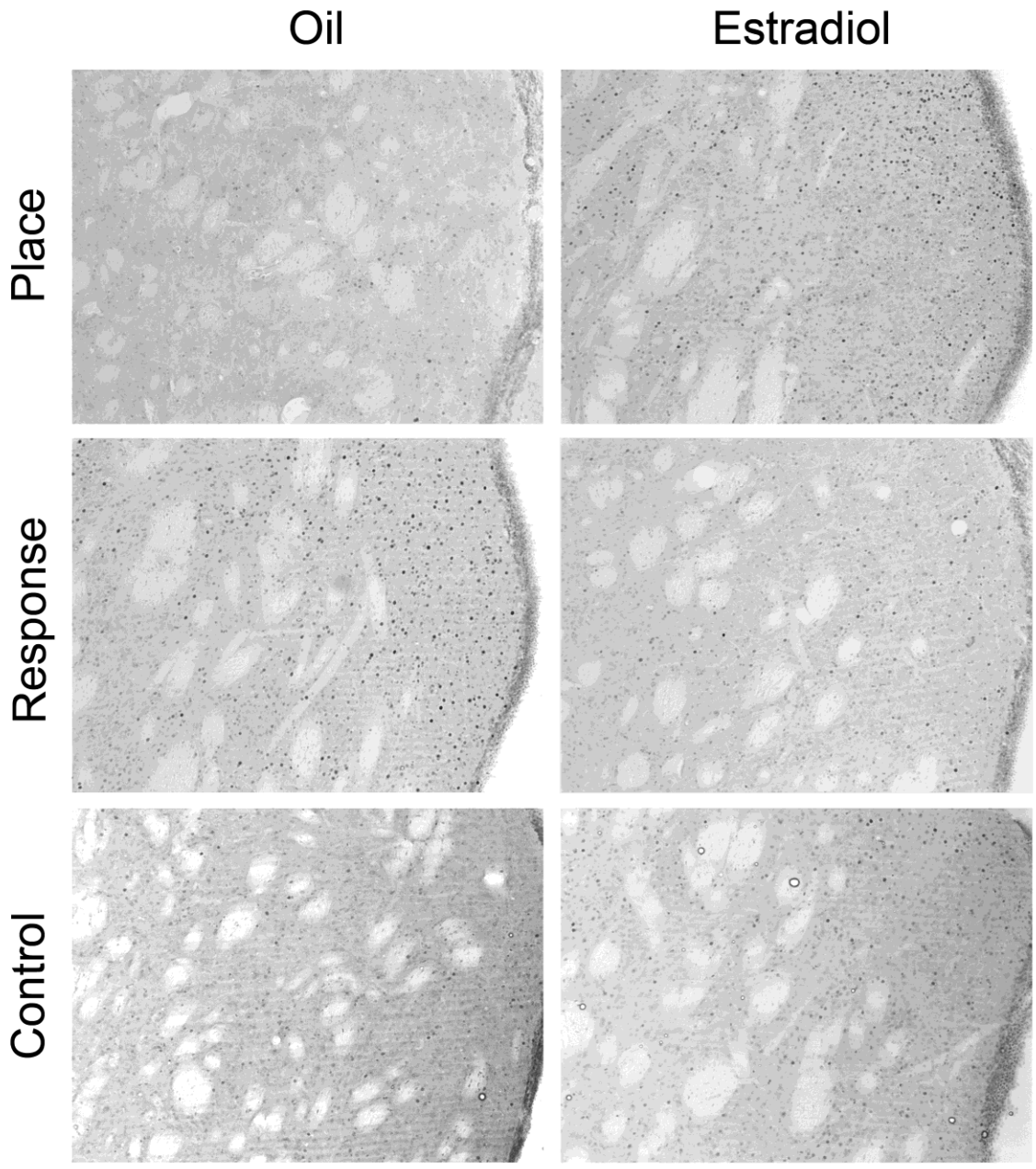

Figure 13: Examples of Fos-IR in the dorsomedial striatum of short-term OVX 12-month-old rats.

Table 3: Pearson's R values for correlations between Fos-IR in hippocampal and striatal subregions for 12-month-old short term OVX rats.

	All	Place	Response
DG vs. CA1	0.280	0.167	0.298
DMS vs. DLS	**0.740****	**0.844***	**0.660***
DG vs. DMS	**0.690*****	**0.789***	**0.617***
DG vs. DLS	**0.583****	**0.649***	0.470
CA1 vs. DMS	0.105	0.120	0.030
CA1 vs. DLS	0.255	0.299	0.135

*** indicates p < 0.05. ** indicates p < 0.01; *** indicates p < 0.001, **** indicates p < 0. 0001.**

Effects of long-term ovariectomy on learning and Fos-immunoreactivity in 12-month-old rats

We hypothesized that estradiol replacement to rats that were OVX for a short period of time (1 month) would enhance place learning and related activation in the HPC and DS. Because we were unable to enhance place learning with estradiol replacement following short term ovariectomy at either 4 or 12 months of age, we also included a group of 12-month-old rats that had been without ovarian hormones for 9 months (long-term OVX) to compare to 12-month-old short-term OVX rats. We asked if long-term deprivation of ovarian hormones would impair learning compared to short-term hormone deprivation, and whether this deficit would be correlated with activation levels in hippocampal and striatal subregions.

Learning

Compared to short-term OVX rats at 12 months of age, long-term OVX rats were impaired in learning rate on the place task, as shown in Figure 14a, but all rats learned the platform location to the same degree. Response learning and strategy use were unaffected by long-term OVX. An ANOVA for the number of training trials to reach behavioral criterion revealed a main effect of task ($F[1, 19] = 8.33$, $p = 0.009$) and a main effect of duration of OVX ($F[1, 19] = 5.93$, $p = 0.025$) but no interaction ($p > 0.45$). Direct comparisons showed that long-term OVX rats were significantly impaired in learning rate compared to short-term OVX rats on the place task ($t[8] = 2.33$, $p = 0.048$), but not on the response task (short-term: 24.0 ± 2.8, long-term: 28.3 ± 2.5 trials, respectively; $p > 0.25$). There were no differences in mean swim speed between short-term and long-term OVX rats in either the place or response tasks (p's > 0.10), suggesting that the effect of long-term OVX on place learning rate was not related to differences between groups in swim speed.

For the place task, analysis of the percent time spent in each quadrant of the maze and the number of platform location crossings during probe trial revealed that both short-term and long-term OVX rats learned the platform location with a high level of accuracy and precision ($p > 0.55$). Path analysis from the response probe trial revealed that a majority of long-term OVX rats (67%) and short-term OVX rats (67%) used a response strategy to locate the target in the absence of a visible platform during the probe trial. These results suggest that long-term OVX impaired place learning rate but not the degree

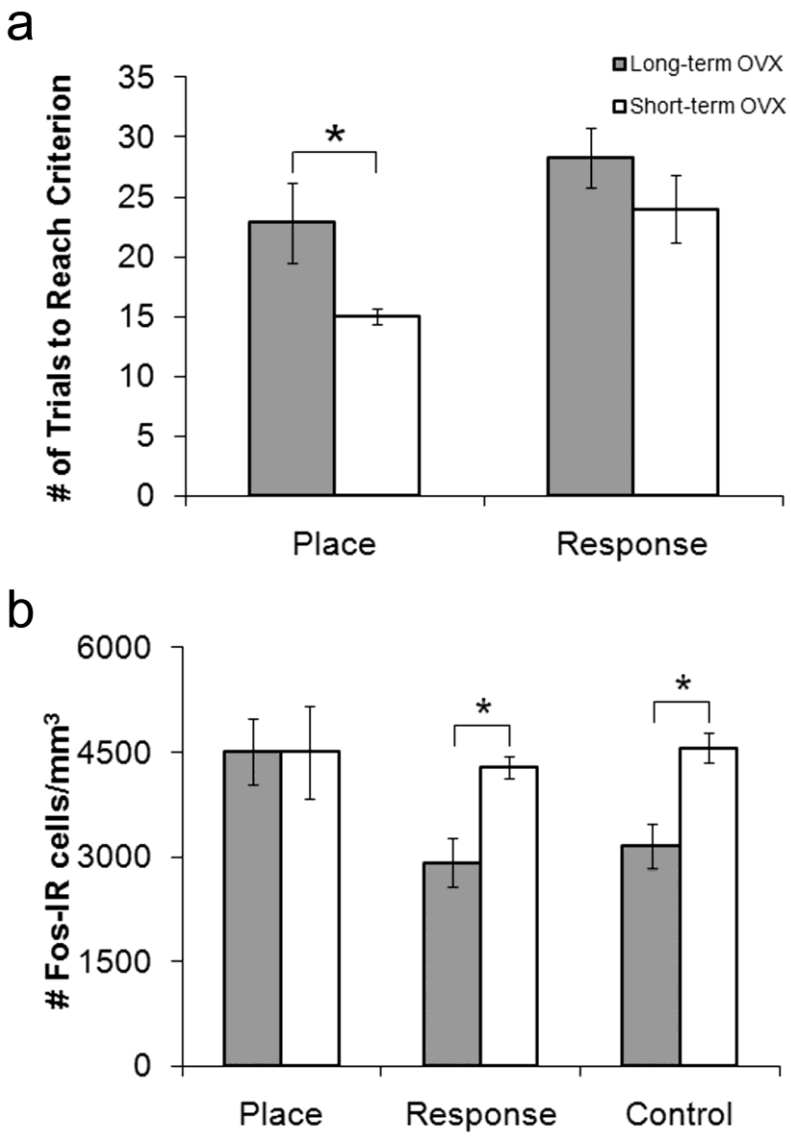

Figure 14: The mean number of trials to reach behavioral criterion in the place and response tasks (a) and Fos-IR in CA1(b) of short-term and long-term OVX 12-month-olds administered oil.
*** indicates $p < 0.05$.**

to which rats learned the platform location in the place task, and it did not affect any aspect of response learning.Fos-immunoreactivity

Long-term OVX decreased Fos-IR in CA1 of explore controls, as shown in Figure 14b, but did not affect Fos-IR in any other hippocampal or striatal subregions. Task had small effects on Fos-IR in several subregions. Similar to other groups, Fos-IR in the DG, DMS and DLS were correlated with one another. In addition, Fos-IR in the DG and CA1 were also positively correlated.

Hippocampus

Long-term OVX explore controls had significantly less Fos-IR than short-term OVX rats in CA1 ($p = 0.05$) but not other hippocampal subregions (p's > 0.45). Task did not affect Fos-IR in any subregion, but estradiol and task interacted to influence Fos-IR in the DG ($F[2,25] = 3.43$, $p = 0.048$), which was carried by response task learners (data not shown). Direct comparisons revealed that long-term OVX response task learners had less DG Fos-IR than short-term OVX response task learners ($t[10] = 3.07$, $p = 0.012$) but short-term OVX and long-term OVX rats had similar Fos-IR in the place task and in the control condition (p's > 0.70). In CA1, there was a main effect of duration of OVX ($F[1,25] = 6.69$, $p = 0.016$) and a trend of task ($p = 0.075$), but no interaction ($p > 0.15$). While response and control long-term OVX rats had less Fos-IR than short-term OVX rats ($t[10] = 3.70$, $p = 0.004$; $t[6] = 2.39$, $p = 0.05$, respectively), place task learners did not ($p > 0.95$), as shown in Figure 14b. All other ANOVAs revealed no effects or interactions (p's > 0.10). These results suggest that long-term OVX rats that learned the

place task increased CA1 Fos expression up to short-term OVX levels to learn the place task.

Dorsal striatum

There were no substantial effects of duration of OVX or task on DS Fos-IR. An ANOVA for Fos-IR in the DMS revealed a trend of task ($p = 0.088$) but no other effects (p's > 0.25; data not shown). However, direct comparisons revealed no effects (p's > 0.45). In the DLS, there was a main effect of task ($F[2,25] = 4.20$, $p = 0.027$) and OVX duration ($F[1,25] = 5.54$, $p = 0.027$) but no interaction ($p > 0.80$). However, no direct comparisons were significant (p's > 0.05), suggesting that long-term OVX did not alter activation in the DS.

Ratios and correlations between Fos-immunoreactivity in hippocampal and dorsal striatal subregions

Neither long-term OVX nor task affected the relative amount of activation in the hippocampal and striatal subregions examined. An ANOVA on the DG:DLS ratios revealed a main effect of duration of OVX ($F[1,25] = 4.91$ $p = 0.036$) and a trend of task ($p = 0.054$), but no interaction ($p > 0.10$). There was a main effect of duration of OVX in the DMS:DLS ratio ($F[1,25] = 7.56$, $p = 0.011$), but no other effects (p's > 0.10). The CA1:DLS ratio comparison revealed a main effect of task ($F[2,25] = 3.50$, $p = 0.046$), but no other effects (p's > 0.25). However, direct comparisons were not significant for any of these ANOVAs (p's > 0.15). Other ANOVAs revealed no significant effects ($p > 0.05$). Several correlations for all rats combined were positive and significant or nearly

significant (DMS vs. DLS, DG vs. DMS, DG vs. DLS, DG vs. CA1), as shown in Table 4. However, CA1 was not correlated with either the DMS or DLS (p's > 0.25). When place and response task learners were analyzed separately, similar correlations were significant or nearly significant for response task learners (DMS vs. DLS, DG vs. DMS, DG vs. DLS, DG vs. CA1; all others—p's > 0.10). There were few significant correlations for place task learners (DG vs. CA1, DG vs. DMS; all others—p's > 0.25). These results suggest that as in other groups, activation in the DG, DMS, and DLS were related, but additionally, CA1 activation corresponded with these subregions.

Table 4: Pearson's R values for correlations between Fos-IR in hippocampal and striatal subregions for 12-month-old short-term OVX and long-term OVX rats administered oil.

	All	Place	Response
DG vs. CA1	**0.662*** **	**0.689***	**0.712****
DMS vs. DLS	**0.541****	0.310	**0.632***
DG vs. DMS	**0.574*** **	**0.692***	**0.605***
DG vs. DLS	0.365	0.306	0.514
CA1 vs. DMS	0.302	0.370	0.466
CA1 vs. DLS	0.339	0.301	0.467

*** indicates p < 0.05. ** indicates p < 0.01; *** indicates p < 0.001.**

Discussion

The current results suggest a complex interaction between hippocampal and striatal subregions activated during place and response learning that is influenced by aging and is highly modulated by estradiol. However, our data do not support the contention that the HPC and DS compete for control over navigation behavior via selective activation (White and McDonald, 2002), nor that activation in the HPC and DS are directly related to ease of place or response learning (Packard and McGaugh, 1996). We found that estradiol replacement increased Fos-IR in the DG, DMS, and DLS of 12-month-old ovariectomized rats that learned the place task but prevented the increase of Fos-IR in these subregions that was observed in oil-treated ovariectomized rats that learned a response task. Estradiol's effects on Fos-IR required rats to be engaged in navigation task, because estradiol did not alter activation in any hippocampal or striatal subregions in rats that simple swam and explored the pool. These large effects of estradiol replacement on hippocampal and striatal activation were easily detectable in 12-month-old rats that may have found the tasks more difficult, but were not detectable in 4-month-old rats. In contrast to previous published findings (e.g., Korol and Kolo, 2002; Markham et al., 2002; Daniel et al., 2006a), estradiol replacement did not improve place learning at either age. However, consistent with previous work (e.g., Korol and Kolo, 2002), estradiol impaired response learning. And, we showed for the first time that age impairs response learning in females and that estradiol exacerbates this impairment in middle age. These results suggest that under some conditions, estradiol modulates both learning and neural activation during spatial navigation, but we found no evidence that

the HPC and DS compete for behavioral control in females via selective subregional activation.

Estradiol affects learning and Fos-immunoreactivity differently in 4 and 12-month-olds

Estradiol has been shown to increase baseline levels of neural activation in the HPC and DSs (Rudick and Woolley, 2000; Dominguez-Salazar et al., 2006). However, in this study, estradiol and oil-treated rats showed the same level of neural activation in hippocampal and striatal subregions when they simply swam in the maze without behavioral training. Interestingly, estradiol replacement and task were able to interact to modulate activation in the DG, DLS and DMS beyond explore control levels in 12-month-olds but not 4-month-olds. Together, these data suggest that in 4-month-olds, the level of neural activation in relevant brain regions that occurred during swimming/exploring alone may have already been sufficient to support learning. In 12-month olds, activation levels induced by swimming/exploring may not have been sufficient to support learning, so estradiol may have modulated neural activation during training to assure quick learning. This proposed role of estradiol is similar to its known function in mediating lordosis in the female rodent. While it is possible to elicit lordosis with strong tactile stimulation (Rodriguez-Sierra et al., 1975), priming with estradiol (and progesterone) sensitizes the neural circuit such that gentle flank stimulation is sufficient to elicit lordosis (Pfaff et al., 1977).

Surprisingly, we did not find that estradiol replacement enhanced the rate of place learning in either 4 or 12-month-olds. Our task may not have been demanding enough to elicit obvious differences in place learning between 4- and 12-month-olds because water maze training took place in a single day (as in Berry et al., 1997; Warren and Juraska, 1997; Chesler and Juraska, 2000). But, 12-month-olds might have found the task sufficiently difficult to require an estradiol-induced increase in Fos-IR in order to reach the same level of task performance, while the task was easy enough for 4-month-olds not to require this increase. Therefore, an alternative possibility for why we found different effects of estradiol on hippocampal and striatal activation in 4 and 12-month-olds is that although we sacrificed rats 30 min after the completion of training and probe testing in order to capture peak Fos expression induced by the learning phase of the task, we may have actually observed the neural correlate for later memory. Several studies have shown that estradiol enhances the consolidation of spatial memory in female rodents (Packard and Teather, 1997b, 1997a; Gresack and Frick, 2006). If we had probed rats for their memory of the platform location 24 hr after training, 4-month-olds may have all easily remembered the trained platform location while only estradiol-treated 12-month-old rats may have remembered. Previous research has shown that estradiol administration just after learning aids in the consolidation of spatial memory in an age-dependent manner (Packard and Teather, 1997b, 1997a; Gresack and Frick, 2006; Gresack et al., 2007). Thus it is possible that while learning did not appear to be correlated with observed patterns of Fos-IR, we may have found behavioral effects of estradiol on memory that correlated with the activation patterns we observed.

In the response task, 12-month-olds clearly found the task more difficult than 4-month-olds, and estradiol further impaired response learning in 12-month-olds to the point that few learned a response strategy during training. While oil-treated rats may have been able to compensate for this difficulty to a certain degree by increasing Fos-IR, estradiol may have prevented this compensation, and therefore, estradiol-replaced 12-month-olds failed to acquire a response strategy during acquisition. This is consistent with previous studies showing that during spatial navigation, estradiol acts like or facilitates the actions of dopamine receptor antagonists in the DS (Daniel et al., 2006b; Quinlan et al., 2008). Because direct administration of estradiol to the DS impairs response learning (Zurkovsky et al., 2007), these results suggest that one possible mechanism by which estradiol impairs response learning is by locally blocking dopamine receptors in the DS, which are required for several forms of plasticity (Lovinger, 2010; Yin et al., 2009) needed for response learning.

Learning rate is not related to activation above a threshold

The patterns of striatal Fos-IR in 12-month-olds are somewhat consistent with the view that response learning requires DS activation. For example, oil-treated response task learners had greater DS Fos-IR than controls but place task learners did not, and estradiol prevented this increase in Fos-IR and impaired learning, suggesting a relationship between striatal activation and learning. However, much of the current data suggests that striatal activation is not related to ease of learning of a response task. Estradiol impaired response learning at 4 months of age as it did at 12 months of age, but it did not alter Fos-

IR in the DS. And, there were no significant correlations between Fos-IR in any subregions analyzed and learning rate on either task for any 4-month-old groups, suggesting that striatal activation is not related to ease of learning a response task.

We also found that training, task, and estradiol did not have any effects on Fos-IR in any hippocampal subregion of 4-month-olds or in CA1, CA3, or hilus of 12-month-olds. These null effects do not allow us to examine the relationship between level of activation in any hippocampal subregion and place learning rate. However, results from 12-month-olds suggest that there is no relationship between place learning and activation in the DG. Estradiol administration increased DG Fos-IR in place task learners but did not alter learning. And, long-term ovariectomy impaired place learning but did not affect DG Fos-IR. While these results suggest that learning rate is not related to hippocampal activation, there was some evidence that successful place learning required CA1 activation to be at a threshold. While all groups of oil-treated short-term OVX 12-month-olds had similar levels of Fos-IR in CA1, long-term OVX explore controls and response task learners had lower level of Fos-IR in CA1 than short-term OVX rats, but long-term OVX place task learners had similar levels to short-term OVX place task learners. Thus, long-term OVX rats had to increase CA1 activation in order to learn place task, suggesting that some threshold of CA1 activation may be required for successful place learning. This also suggests that the reason long-term OVX rats required more training trials than short-term OVX rats to reach behavioral criterion in the place task is because they did not have sufficient CA1 activation at the onset of training to support learning as short-term OVX rats did. Because DG and CA1 Fos-IR were correlated with one another

in these rats, a threshold of DG activation may also be required for successful place learning, but this hypothesis could not be specifically tested because all groups had similar levels of DG activation.

Functions of and coordination between hippocampal and dorsal striatal subregions during navigation learning

Together, our results suggest that the effects of estradiol on hippocampal and striatal activation patterns are related to task but not learning rate. They suggest very different functions of hippocampal and striatal subregions during learning in females than what has previously been reported in males. While we found that DLS activation is higher in response task learners than other groups (like Gill et al., 2007), we found a more robust effect in the DMS. In contrast to all male studies, the pattern of activation in the DS was also observed in the DG. These results do not support the theory that the HPC and DS compete for control during navigation learning via selective activation as predicted (Colombo et al., 2003; Teather et al., 2005). These differences highlight the importance of task and sacrifice time point on activation of the HPC and DS, and they suggest that there may be marked sex differences in the roles of hippocampal and striatal subregions during navigation learning, with females highly tuned to the effects of estradiol on these functions.

The DMS forms an association between an action and its outcome (Yin et al., 2005a; Yin et al., 2005b), which is especially important during learning. As the action becomes more habitual and the outcome is no longer a part of the representation, the

representation anatomically shifts to the DLS (Yin et al., 2004; Yin and Knowlton, 2006). Because the timing of our learning, probe and animal sacrifice attempted to capture peak Fos-IR during the learning phase of the task, and both types of training required subjects to form a representation that included the outcome, it is not surprising that we found the greatest activation in the DMS and a similar pattern in the DLS in 12-month-old rats. A similar pattern of activation was observed in the DG in 12-month-olds, suggesting that Fos-IR measured here reflects the similar sensory inputs received by the DG and DS from the EC (Finch et al., 1995; Finch, 1996); (Dolorfo and Amaral, 1998) rather than similar functions of all three subregions. Surprisingly, we did not observe a correlation between activation in the DG and any other hippocampal subregion, even though the DG and CA3 have been shown to similar encode information during spatial navigation, and the DG is thought to assist with CA3 function in a number of spatial contexts (see Kesner, 2007). Had we sacrificed rats at a later timepoint, such as 90 min after criterion was met, we might have observed a dissociation between the HPC and DS and similar effects across the HPC because we would have captured Fos expression in response to asymptotic performance when task demands were clear and a specific strategy was being employed by each rat. While Fos-IR allowed us to determine the amount of engagement from hippocampal and striatal subregions during navigation learning, it does not tell us how these subregions contribute to learning.

Measuring activation versus plasticity

Results from the current study and other previous studies indicate that both the HPC and DS are engaged during navigation tasks and that the degree of engagement and responses of specific neurons vary depending on the parameters of the task (Markus et al., 1994; Jeffery and O'Keefe, 1999; Mizumori et al., 2000a; Mizumori et al., 2000b; Ragozzino et al., 2001; Ragozzino et al., 2002; Mizumori et al., 2004; Yeshenko et al., 2004; Eschenko and Mizumori, 2007). As discussed in the General Introduction, theta rhythm in the HPC and DS is necessary for neural plasticity needed for learning and memory (Mehta et al., 1997; Mehta et al., 2000; Buzsaki, 2002), suggesting that reliable plasticity is crucial for the successful strategy use during spatial navigation. And, high circulating estradiol modulates spatial navigation learning (Pleil and Williams, 2010; Korol and Kolo, 2002) as well as hippocampal and striatal plasticity (Woolley and McEwen, 1992; Cordoba Montoya and Carrer, 1997; Cyr et al., 2000), possibly via effects on theta rhythm generated within the thalamus and projected to the HPC and DS (Leranth and Shanabrough, 2001). These results suggest that estradiol may influence navigation strategy use by modulating the plasticity and reliability of hippocampal and striatal ensembles during navigation rather than simply increasing activation.

CHAPTER 3: ESTRADIOL MODULATES PLASICITY AND RELIABILITY OF HIPPOCAMPAL AND DORSAL STRIATAL ENSEMBLES DURING SPATIAL NAVIGATION

Studies over the past decade have shown that the IEG *Arc* is required for NMDA receptor-dependent synaptic plasticity and memory consolidation (Steward et al., 1998; Guzowski et al., 2000; Steward and Worley, 2001b; Plath et al., 2006; Miyashita et al., 2008) that is needed for neural representations required for learning and memory of spatial navigation tasks (Guzowski et al., 2000; Daberkow et al., 2007). For example, *Arc* is induced in the HPC during place navigation tasks including the Morris water maze (Guzowski et al., 2001a; Fletcher et al., 2006) and spatial exploration (Guzowski et al., 1999; Vazdarjanova et al., 2002; Ramirez-Amaya et al., 2005; Vazdarjanova et al., 2006)., and in the DS during response navigation tasks (Daberkow et al., 2007). As discussed in the General Introduction, while *c-fos* is expressed in neurons when they become activated, *Arc* is expressed when neurons undergo synaptic plasticity. Ch. 2 showed that spatial navigation strategy use was not related to the activation levels of hippocampal and striatal ensembles. However, it is possible that the amount of plasticity occurring in these ensembles controls navigation strategy use, and measuring *Arc* during spatial navigation may be a useful tool for examining this hypothesis.

There is also evidence that reliability in the expression of plasticity is necessary for successful strategy use (Ranck, 1973; O'Keefe, 1976; McNaughton et al., 1983; Skaggs et al., 1995; Redish and Touretzky, 1997; Jog et al., 1999; Mizumori et al., 2004; Mizumori et al., 2009). *Arc* transcription within individual CA1 pyramidal neurons is

highly reliable across two exposures to the same context separated by 20-30 min (Guzowski et al., 1999; Vazdarjanova and Guzowski, 2004), similar to electrophysiological recordings of place cells that remain stable across exposures to the same location within an environment (Thompson and Best, 1990; Wilson and McNaughton, 1993; Leutgeb et al., 2004; Leutgeb et al., 2005). And, high-frequency theta rhythm, which synchronizes primary neural firing in the HPC and DS during spatial navigation (O'Keefe and Recce, 1993; Gengler et al., 2005; DeCoteau et al., 2007b; Maurer and McNaughton, 2007), is required for the neural plasticity needed for learning and memory (Mehta et al., 1997; Mehta et al., 2000; Buzsaki, 2002, , 2005). Theta rhythm becomes more coherent within the neural system guiding navigation behavior across trials of a spatial navigation task (DeCoteau et al., 2007b), suggesting that the reliability of primary neuronal firing is essential for the successful use of navigation strategies. These data suggest that the reliability of plasticity occurring at synapses within hippocampal and striatal ensembles may be more important for controlling navigation strategy use than the level of ensemble activation *per se*, a hypothesis that is supported by the findings in Ch. 2.

If this hypothesis is correct, it predicts that estradiol modulates navigation strategy use by differentially altering hippocampal and striatal plasticity and reliability (see General Introduction and Ch. 2 Discussion). In this case, either a) all rats with high estradiol levels display one pattern of hippocampal and striatal plasticity, while all rats with low estradiol levels display a different pattern, or b) the pattern of plasticity that predicts the use of each strategy is different when a rat has low estradiol levels than when

it has high estradiol levels. Alternatively, estradiol might not influence hippocampal and striatal plasticity during spatial navigation, so all place strategy users display one pattern of plasticity, while all response strategy users display a different pattern. These possibilities are discussed in more detail in the General Introduction. To test the hypothesis that strategy use is determined by hippocampal and striatal plasticity and reliability, and that estradiol modulates strategy use by altering these patterns of plasticity, we used catFISH for *Arc* to measure the plasticity and reliability of hippocampal and striatal ensembles in cycling female rats during performance on a water-based navigation task where either a place or response strategy could be successfully used. We found that estradiol state differentially modulated *Arc* expression in subregions of the HPC and DS and that the pattern of *Arc* expression that predicted the use of place and response strategies depended on rats' estradiol state.

Materials and Methods

Subjects

Subjects were 40 three-month-old female Sprague-Dawley CD strain rats acquired from Charles Rivers Laboratories (Raleigh, NC). They were given ad libitum access to water and a standard diet (Rodent Diet 5001, PMI Nutrition International, Inc., Brentwood, MO). The temperature-controlled colony room was maintained on a 12:12 hr light:dark cycle with lights on at 7 a.m. daily. Rats were handled and monitored for estrous cycle phase daily for 10 days prior to behavioral testing.

Monitoring estrous cycle

Estrous cycle phase was determined as described in Ch. 2. Rats classified as being in estrus or metestrus on the day of sacrifice were categorized as being in a "low estradiol state" (low E) and rats in proestrus were categorized as being in a "high estradiol state" (high E).

Apparatus and testing room

Training and probe testing were conducted in the same apparatus and testing room as described in Ch. 1 (Figure 2). However, no additional cues were hung from the ceiling.

Behavioral training and probe testing

Behavioral training and probe testing was similar to that in Ch. 1, but it took place in two sessions over two consecutive days. On the first day, each rat received two blocks of five training trials each, where the second block of training was initiated 25 min after the start of the first block. On the second day, each rat received five training trials followed 25 min later by a single probe trial (Figure 15). This timing of training, probe, and sacrifice was chosen because it is optimal for the use of *Arc* catFISH to measure the reliability of individual neuronal activity across multiple behavioral epochs (Guzowski et al., 1999, , 2001b). Between trials, all walls of the maze were wiped with ethanol to remove intramaze odor cues. Between trial blocks, the rat was returned to its holding cage.

On the probe trial, when rats were started from the opposite side of the maze as during training trials, rats that swam to the location where the hidden platform had previously been were categorized as place strategy users, while rats that swam the same path that they did on the training trials were considered response strategy users. During all training and probe trials, latencies to reach the end of any arms entered and latency to mount the platform were recorded by the experimenter and the path of the animal was recorded in real time using HVS Image software (Buckingham, WH, UK). The latencies of all rats trained to find the escape platform reached asymptote within the first ten trials ($F(9,126) = 23.9$, $p < 0.0001$), regardless of their estradiol status or the strategy used on the probe trial (p's > 0.05), with average latencies of 33.3 ± 4.3 s on trial 1 and 7.6 ± 1.0 s on trial 10.

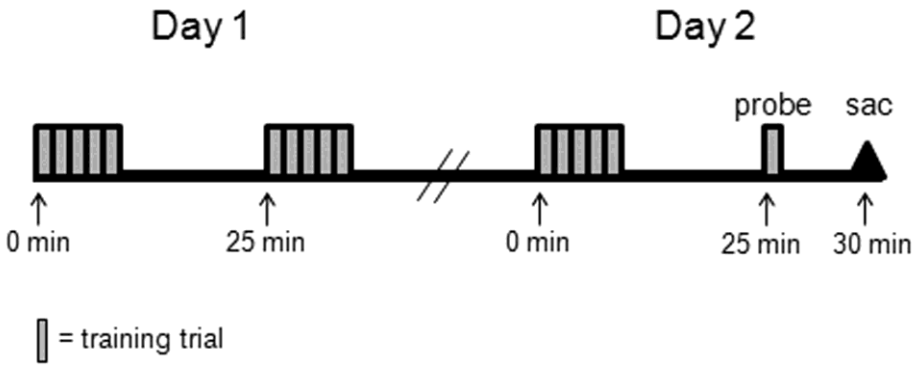

Figure 15: Experimental timeline for training, probe testing, and sacrifice. On Day 1, rats received two blocks of 5 training trials started 25 min apart; on Day 2, rats received a block of 5 training trials and a single probe trial started 25 min apart, and then were sacrificed 5 min after the start of the probe trial.

In Ch. 1, we observed that rats that use a place strategy to solve this ambiguous task have greater latencies on the probe trial than on the last several training trials, while rats that use a response strategy have similar latencies on the probe trial and last several training trials. In the present experiment, rats that selected the "place" arm had probe trial latencies that were approximately 40% longer than latencies on the last three training trials, and most rats that selected the "response" arm on the probe trial had similar latencies on the probe trial and the last several training trials, as previously shown. Rats that chose the response arm but whose latencies were at least 40% longer on the probe trial than the training trials, indicating that they had either attended to the external cues as rats that chose the place arm did rather than simply using a motor response to navigate or had not learned, were excluded (low E: n = 3; high E: n = 3). Because our lab and other labs have previously described the behavior of female rats as they use place and response strategies during ambiguous navigation tasks, the focus of this study was on the effects of estradiol state on the patterns of plasticity in the HPC and DS during navigation. Therefore, we behaviorally trained and probe tested rats in platoons until there were at least four rats in each estradiol state x task condition.

In addition to behaviorally-trained rats, we included two types of controls that were transported to the testing room with the behaviorally-trained rats. Transport controls remained in their transparent holding cages in the testing room throughout the session to control for *Arc* expression during transport and exposure to the testing room. Explore controls were allowed to swim in the maze with the arms open without a platform present for the same amount of time as a behaviorally-trained rat on all trials, including the

probe, to control for effects of motor activity, stress, motivation to escape the water, and latent learning associated with exploring the novel environment. Explore controls did not differ from behaviorally-trained rats in swimming speed or distance (p's > 0.15), so the only difference between explore controls and behaviorally-trained rats was learning of a goal-directed navigation task. All control rats were sacrificed using the same time parameters and in the same manner as behaviorally-trained animals. All procedures were approved by the Institutional Animal Care and Use Committee of Duke University.

Arc catFISH

Twenty-five min after the start of the training trials and five min after the start of the probe trial, each rat was placed in a chamber containing isofluorane until deeply anesthetized and immediately sacrificed by decapitation. Brains were rapidly extracted, hemisected, flash-frozen in isopentane that was equilibrated in ethanol and dry ice, and stored at -80ºC. This sacrifice time point allowed us to visualize *Arc* mRNA present in the nucleus and cytoplasm, which indicated induction occurred in response to the probe trial and training trials, respectively, according the kinetic of *Arc* previously described (Guzowski et al., 1999, , 2001b; Vazdarjanova et al., 2002). Half-brains were blocked at -20ºC to include both the DS and HPC of 6-8 subjects using Tissue-Tek OCT (Miles, Elkhart, IN) so that all groups were represented in each block. Blocks were sectioned on the coronal plane at 20 µm on a cryostat and placed on Superfrost plus slides. An *Arc* cDNA plasmid of ~3kbp was used to produce the riboprobe used. The cDNA was converted into linearized template using Not1. This template was used to make a cRNA

probe using T7 RNA polymerase from a commercial transcription kit (MaxiScript, Ambion, Austin, TX) and digoxigenin RNA labeling mix (Roche Molecular Biochemicals). The riboprobe was purified on a G-50 spin column (Roche Molecular Biochemicals).

In situ hybridization for *Arc* mRNA was conducted using a modified version of the procedure previously described (Guzowski et al., 1999). Slides containing the dorsal HPC and DS were fixed in 4% paraformaldehyde (pH 7.5) at 4ºC, rinsed in 2x saline-sodium citrate (SSC), and then washed in acetic anhydride. Slides were dipped briefly in diethylpyrocarbonate (DEPC) water and then placed in an acetone/methanol solution at -20ºC and rinsed in 2x SSC. Slides were then covered with 110 µL of prehybridization buffer, coverslipped, and placed in a humid chamber with formamide/2x SSC for 30 min. Coverslips were removed with 2x SSC and hybridized with an *Arc* mRNA probe (110 µL/slide) tagged with digoxigenin in hybridization buffer (Sigma, St. Louis, MO), coverslipped, and incubated overnight in a sealed chamber with formamide/2x SSC. The next day, tissue was rinsed in 2x SSC and then washed in 2x SSC with RNase A at 37ºC, rinsed in 2x SSC followed by 0.5x SSC at RT for 10 min, 56ºC for 30 min, and RT for 5 min. Slides were quenched in 1% H_2O_2 in 1x SSC-T for 15 min, rinsed in 1x SSC-T, and washed in TBS. Slides were then blocked with TSA blocking buffer with 5% normal sheep serum, coverslipped, and placed in a humid chamber with TBS for 30 min at RT before being incubated in anti-digoxigenin in a 0.5% TSA blocking buffer at a 1:200 ratio overnight at 4ºC. The following day, tissue was rinsed in TBS-T, and the stain was visualized by incubating the tissue in a 1:50 Cy3:diluent solution for 30 min. The slides

were washed in TBS-T, counterstained with a 1:500 concentration of DAPI to visualize cell nuclei, washed in TBS, and coverslipped with Vectashield (Vector Laboratories, Burlingame, CA) and sealed with nail polish.

Confocal microscopy

Two slides containing each region of interest for each rat were imaged and analyzed for *Arc* mRNA in the following manner. Image stacks composed of 1-µm-thick optical slices were collected for the entire 20 µm-thick tissue in several samples from CA1, CA3, and DG of the HPC, as well as for DLS and DMS, using 405 nm and 561 nm lasers on a Zeiss 510 inverted confocal microscope with a 20x objective. One sample per slide was taken for each of the DLS and DMS at approximately 0.7 mm from bregma for a total of two samples per rat (Figure 16a, left). For CA1 and CA3 regions, two sample images were taken per region per slide at approximately -3.4 mm from bregma, for a total of four samples per rat (Figure 16a, right). For each sample, one image was taken that optimized the visualization of *Arc* within the nucleus as one or two intensely-stained foci, and another image was taken that optimized *Arc* detection in the cytoplasm by altering only the amplifier offset setting. The entire DG was imaged using serial images, and the middle plane of each image stack taken was used to reconstruct a two-dimensional, flat image of the entire structure using Adobe Photoshop (Adobe Systems, San Jose, CA). For all images, intensity and contrast parameters were set using control sections on each slide and maintained for all brains on that slide.

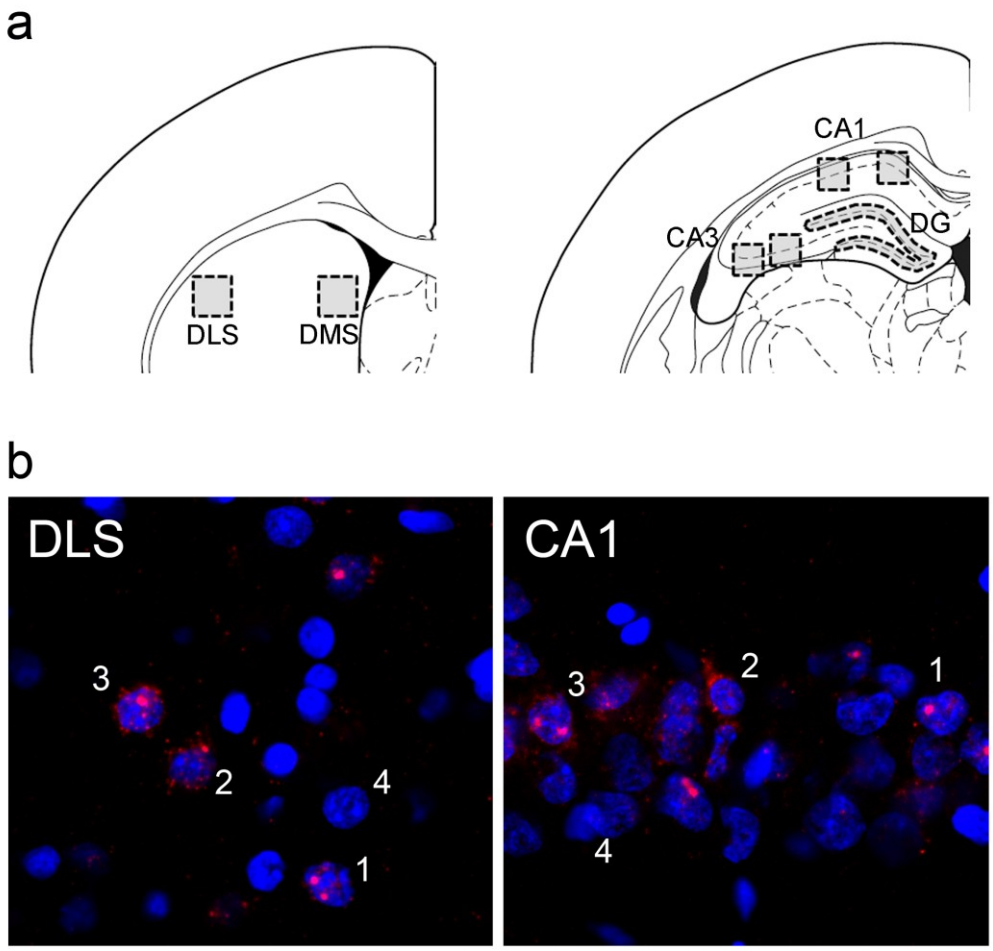

Figure 16: a) Regions analyzed for *Arc* mRNA expression in the dorsal striatum at 0.7 mm bregma (left) and hippocampus at -3.4 mm bregma (right). b) Examples of neurons categorized as having *Arc* mRNA in the nucleus only (1), cytoplasm only (2), nucleus and cytoplasm (3), and neither (4) in the DLS (left) and CA1 (right). Images were taken with an inverted confocal microscope using a 40x objective.

Image analysis

Neurons with *Arc* mRNA in the nucleus, cytoplasm, both, or neither was manually counted using MetaMorph imaging software. Glia and neurons were easily distinguished because glial nuclei are small and express the counterstain much more

intensely and evenly. All cells classified as neurons whose nuclei were present in the middle 20% of the z-stack were counted. For all brain regions except the DG, neurons were considered positive for intranuclear foci if they had one or two discrete *Arc* foci that were coexpressed with the nuclear stain in three consecutive planes. Neurons were considered *Arc*-positive in the cytoplasm if at least 60% of the nucleus was surrounded by a halo of staining and both stains were visible in at least three consecutive planes. All neurons were classified as either: a) positive for nuclear *Arc* foci only, b) positive for cytoplasmic *Arc* only, c) positive for both nuclear foci and cytoplasmic, or d) negative for *Arc* in both cytoplasm and nucleus, as illustrated in Figure 16b. Counts from all samples for a region were summed to obtain one value in each category per rat for each region. For each rat, the mean number of neurons quantified was 387 for CA1, 260 for CA3, 274 for DLS, and 351 for DMS. There was no correlation between the proportion of neurons that expressed *Arc* mRNA and the number of neurons quantified (p's > 0.20). Because *Arc* transcription in the DG is sustained for up to two hours after it is induced (Ramirez-Amaya et al., 2005; Bramham, 2007; Messaoudi et al., 2007), intranuclear foci and cytoplasmic *Arc* could not be distinguished. Using the reconstructed image, *Arc*-positive neurons were identified, the area of the DG was traced, and the proportion of *Arc*-positive neurons was then calculated using these values.

Statistical Analysis

We trained cycling female rats on an ambiguous navigation task and sacrificed them 25 min after training and 5 min after the probe trial so that we could examine the

effects of estradiol state and navigation strategy use on plasticity and reliability of hippocampal and striatal subregions during goal-directed navigation. We also included transport and explore control groups to control for factors including exposure to the testing room, motor activity, stress, and latent learning associated with exploring the maze. Three specific dependent measures were analyzed for CA1, CA3, DMS, and DLS using counts generated during data collection: 1) proportion of all sampled with *Arc* mRNA signal (number of neurons with *Arc* signal divided by the total number of neurons sampled = TOTAL) as a measure of synaptic plasticity; 2) proportion of neurons with signal in both compartments (number of neurons with intranuclear *and* cytoplasmic *Arc* mRNA signal divided by the total number of neurons sampled = BOTH) as a measure of the size of a reliable network; and 3) the signal-to-noise ratio (number of neurons with intranuclear *and* cytoplasmic *Arc* mRNA signal divided by the number of neurons with *Arc* mRNA signal in only one compartment (intranuclear *or* cytoplasmic signal, but not both) = SNR) as a measure of reliability. For the DG, only TOTAL was calculated because intranuclear and cytoplasmic *Arc* mRNA signal could not be distinguished, as described in the Image Analysis section. In order to evaluate the effects of exploration and estradiol state on these measures, ANOVAs included control condition (explore and transport) and estradiol state (high E and low E) as independent variables. To examine the effects of explicit learning and strategy use on these measures, ANOVAs included behavioral condition (place, response, explore control) and estradiol state as independent variables. Planned comparisons were made between low E groups in different behavioral conditions as well as between low and high E rats within each behavioral condition.

Results

In order to examine if exploring the maze and/or estradiol state affected plasticity and reliability in the female HPC and DS, we compared *Arc* expression of rats that were just transported to the test room with rats that were allowed to swim in the pool but were not explicitly trained to learn a navigation task. We found that exploration but not estradiol alone increased plasticity and reliability in almost all subregions of HPC and DS analyzed. We then addressed whether estradiol state interacted with spatial navigation learning and/or navigation strategy use to alter patterns of *Arc* expression. We found that estradiol increased CA1 plasticity in all rats that were explicitly trained to find the escape platform compared to rats that explored freely (explore controls). And, when rats performed the same behaviors but did so using distinct strategies, estradiol state and strategy used interacted to alter the reliability of *Arc* expression in CA1 and the plasticity and reliability of *Arc* expression in the DLS. These findings are explained in more detail below.

Effects of exploration and estradiol state on *Arc* mRNA expression

Exploration but not estradiol increased *Arc* expression to approximately two times the levels displayed by transport controls in CA1, DG, DMS and DLS but not CA3 (see Figure 17). In CA1, there was a main effect of control condition (transport vs. explore) for the proportion of neurons that expressed *Arc* mRNA (TOTAL; $F(1, 11) = 10.50$, $p = 0.008$; Figure 17a and Figure 19) but no main effect of estradiol state or interaction between control condition and estradiol state (p's = 0.057). There was also a main effect

of control condition for the proportion of neurons that expressed *Arc* in both the nucleus and cytoplasm (BOTH; $F(1, 11) = 5.34$, $p = 0.041$) but no main effect of estradiol state or interaction between control condition and estradiol state ($p > 0.40$). There were no effects of or interaction between estradiol state or control condition on the signal-to-noise ratio (SNR) in CA1 (p's > 0.20). In CA3, there were no effects of or interaction between estradiol state or control condition on any of the three dependent measures (p's > 0.10; Figure 17b). In the DG, there was a main effect of control condition for TOTAL ($F(1, 11) = 11.58$, $p=.0059$) but no effect of estradiol state and no interaction between estradiol state and control condition ($p > 0.65$; Figure 17c). These results suggest that plasticity in CA1 and DG ensembles, but not CA3, are increased by exploration.

Similar to CA1, exploration increased *Arc* expression in both subregions of the DS. Explore controls had greater TOTAL (DMS: $F(1, 11) = 15.91$, $p = 0.002$; DLS: $F(1, 11) = 4.98$, $p = 0.047$), BOTH (DMS: $F(1, 11) = 11.05$, $p = 0.007$; DLS: $F(1, 11) = 10.144$, $p = 0.009$), and SNR (DMS: $F(1, 11) = 5.73$, $p = 0.0357$; DLS: $F(1, 11) = 6.10$, $p = 0.031$) than transport controls, but there were no main effects of estradiol state and no interactions between control conditions and estradiol state ($p > 0.20$; see Figure 17d and e for TOTAL and Figure 20 for illustration). Overall, these results suggest that exploration increased plasticity and reliability in all regions analyzed except for CA3, but the presence of estradiol did not affect plasticity or reliability in any region analyzed.

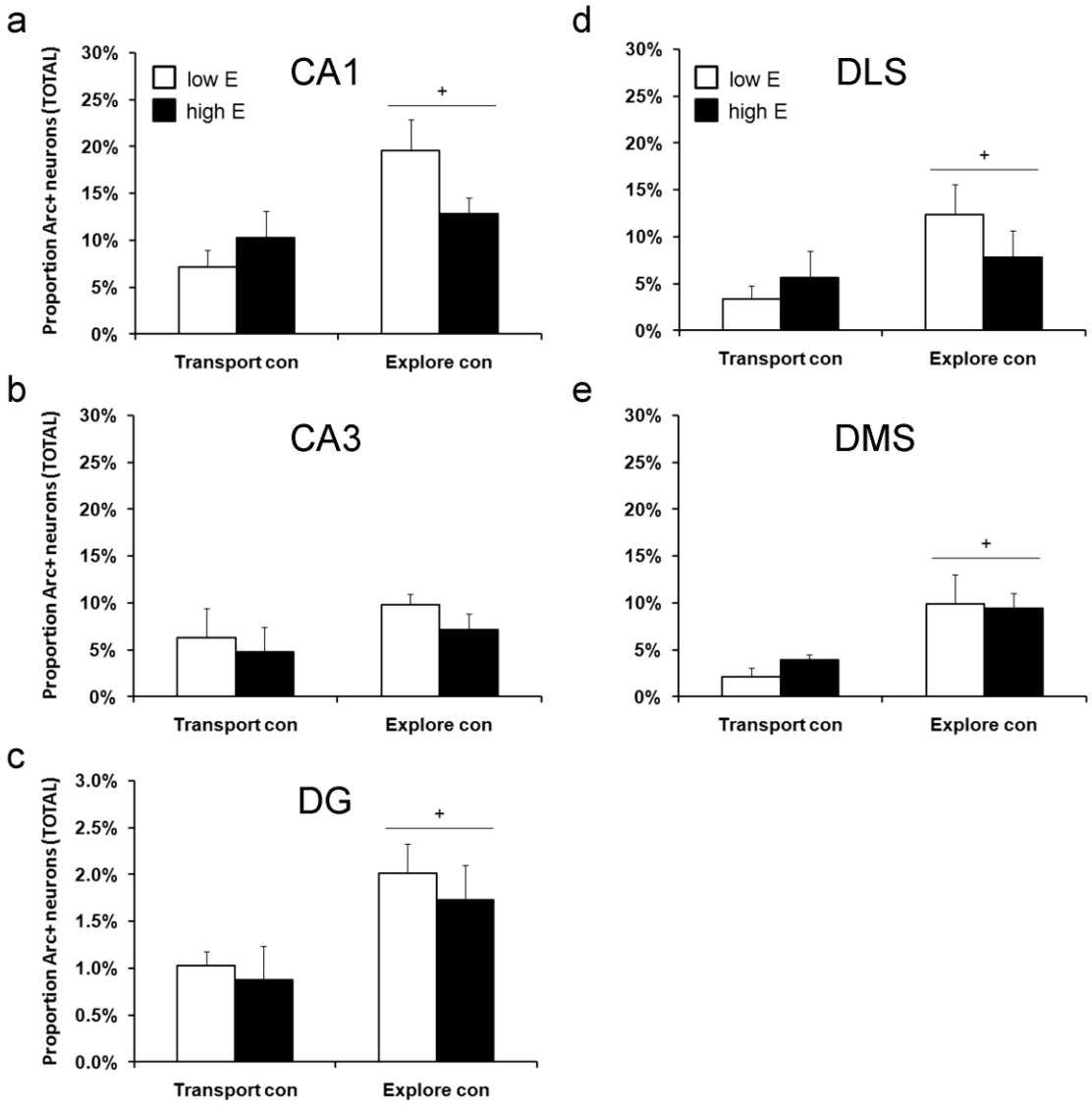

Figure 17: Proportion of neurons with *Arc* mRNA signal (TOTAL) in hippocampal (a-c) and dorsal striatal (d-e) subregions.
Note that scale for y-axis for DG (c) is different. + indicates significant main effect of control condition with $p < 0.05$.

Effects of explicit training, strategy used, and estradiol state on *Arc* mRNA expression

Because exploration of the maze increased plasticity in the HPC and DS, we next asked whether explicit training and/or specific strategy use affected hippocampal and striatal plasticity and reliability beyond the levels produced by exploration. We found that neither estradiol state nor learning increased *Arc* expression in DG, CA3, or DMS above the levels induced by exploration. However, in CA1 and DLS, both estradiol state and strategy use interacted to modulate *Arc* expression and reliability (see Figure 18). In CA1, estradiol increased *Arc* expression in all rats that learned to locate an escape platform (behaviorally-trained) beyond that induced by exploration alone. Interestingly, estradiol only increased the reliability of *Arc* expression in rats that learned to use a place strategy and not those that learned a response strategy (Figures 18a-c and 19). In contrast, only response strategy users in a low estradiol state had increased plasticity and reliability in the DLS compared to all other groups (Figures 18d-f and 20). These findings are detailed below.

ANOVAs and planned comparisons performed for the DG, CA3, and DMS showed that neither behavioral condition nor estradiol state affected any dependent measure examined (p's > 0.05), suggesting that all groups that swam in the maze displayed similar plasticity and reliability in these subregions (data not shown).

In CA1, for TOTAL number of neurons expressing *Arc*, there was a main effect of estradiol state ($F(1, 19) = 4.68$, $p = 0.043$), no main effect of behavioral condition ($p > 0.10$), and a behavioral condition x estradiol state interaction ($F(2, 19) = 6.62$, $p = 0.007$).

In low E rats, regardless of behavioral condition, 15-20% of neurons expressed *Arc* (TOTAL; p's > 0.30). In contrast, high E place and response strategy users had more than 25% of neurons express *Arc*, which was significantly higher than the low E place and response strategy users (place: $t(6) = 2.34$, $p = 0.058$; response: $t(8) = 3.25$, $p = 0.012$), suggesting that estradiol increased CA1 plasticity only in rats that learned to navigate to a hidden platform (see Figure 18a and Figure 19).

For BOTH in CA1, there were main effects of estradiol state ($F(1, 19) = 14.34$, $p = 0.001$) and behavioral condition ($F(2, 19) = 7.54$, $p = 0.004$), as well as a behavioral condition x estradiol state interaction ($F(2, 19) = 7.35$, $p = 0.004$). Low E place and response strategy users had similar BOTH to explore controls (approximately 2%; p's > 0.95), while high E place and response strategy users displayed between a two and five-fold increase in this measure compared to low E rats (place: $t(6) = 5.78$, $p = 0.001$; response: $p = 0.091$; Figure 18b). A post-hoc comparison revealed that high E place strategy users had greater BOTH than response strategy users ($t(8) = 2.86$, $p = 0.021$), suggesting that while estradiol increased the size of a reliable network in all behaviorally-trained rats, this was greater in place strategy users than response strategy users.

For SNR in CA1, there were main effects of estradiol state ($F(1, 19) = 8.09$, $p = 0.010$) and behavioral condition ($F(2, 19) = 7.44$, $p = 0.004$), as well as a behavioral condition x estradiol state interaction ($F(2, 19) = 5.44$, $p = 0.014$). Low E place and response strategy users had similar SNR to explore controls (p's > 0.70), but high E place strategy users had three times greater SNR than low E place strategy users ($t(6) = 3.57$, p

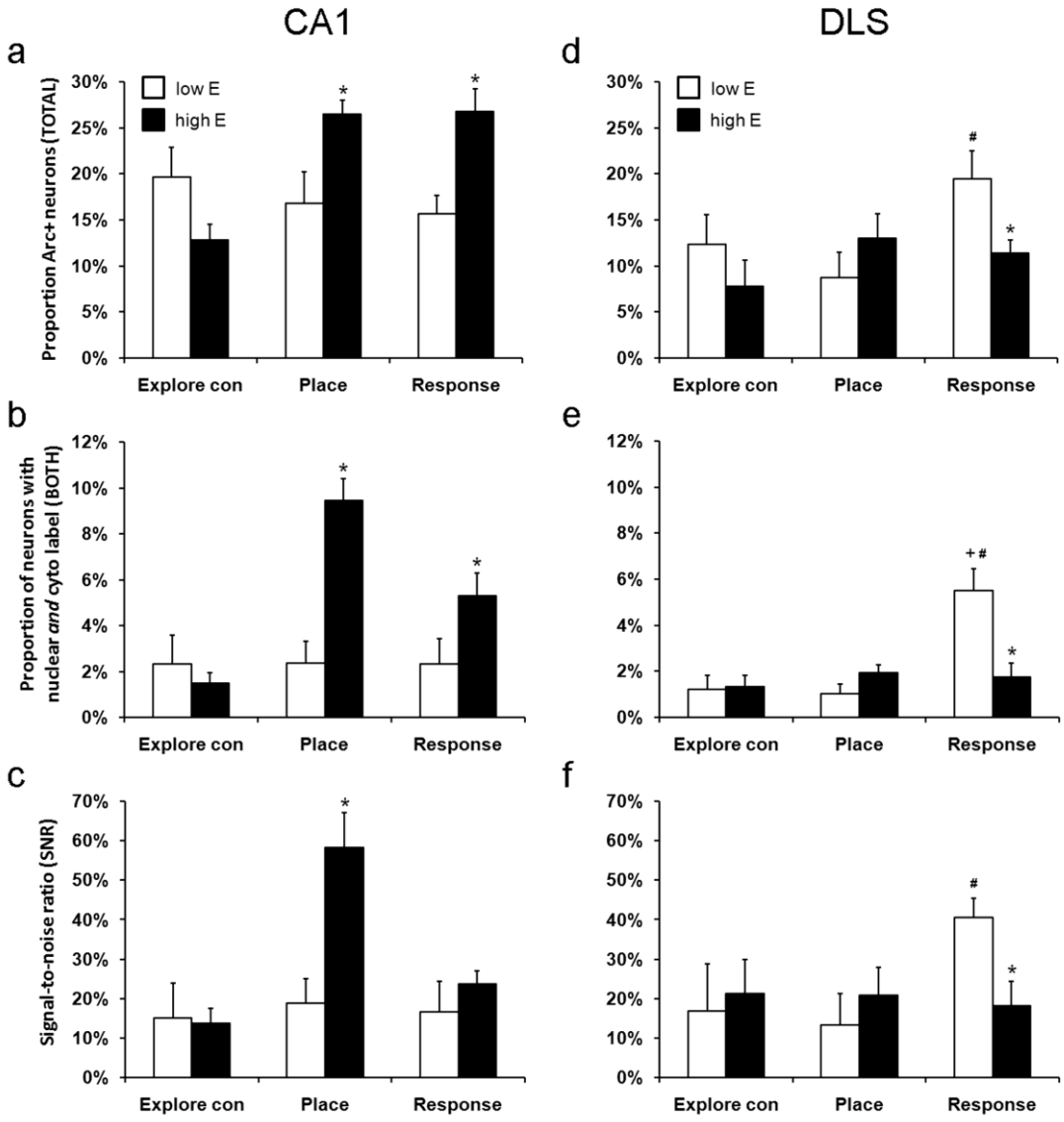

Figure 18: *Arc* expression in CA1 (a-c) and DLS (d-f) during spatial navigation. Estradiol increased TOTAL *Arc* expression (a) and the proportion of neurons with *Arc* in BOTH the nucleus and cytoplasm (b) in behaviorally-trained rats, and the SNR of place strategy users (c). Response strategy users in a low estradiol state had high TOTAL *Arc* expression (d), BOTH (e), and SNR (f) in the DLS. + indicates different from low E transport controls with $p < 0.05$. # indicates different from low E place strategy users with $p < 0.05$. * indicates different from low E rats in same behavioral condition with $p < 0.05$.

= 0.012), while SNR did not differ between high E and low E response strategy users ($p > 0.35$; Figure 18c). These results show that high E place strategy users had greater CA1 reliability than all other groups, who displayed similar, low levels of reliability.

In the DLS, an ANOVA for the TOTAL number of neurons expressing *Arc* revealed only trends toward a main effect of behavioral condition ($p = 0.098$) and an interaction between behavioral condition and estradiol state ($p = 0.066$) and no main effect of estradiol state ($p > 0.20$). However, planned comparisons showed that low E response strategy users had 1.5-2 times more *Arc* expression than high E response strategy users ($t(8) = 2.69$, $p = 0.028$ and low E place strategy users ($t(6) = 2.61$, $p = 0.040$); Figure 18d). An ANOVA on the number of neurons expressing *Arc* in BOTH cytoplasm and nucleus revealed a main effect of behavioral condition ($F(2, 19) = 9.16$, $p = 0.002$), no effect of estradiol state ($p = 0.097$), and a significant interaction between behavioral condition and estradiol state ($F(2, 19) = 8.40$, $p = 0.002$). Low E response strategy users had more than three times as many neurons with *Arc* in BOTH the nucleus and cytoplasm than low E place strategy users ($t(6) = 4.25$, $p = 0.005$), high E response rats ($t(8) = 3.49$, $p = 0.008$), and low E explore controls ($t(5) = 3.44$, $p = 0.018$; Figure 18e). And, while the ANOVA for SNR revealed no significant effects or interactions ($p > 0.10$), planned comparisons showed that low E response strategy users displayed an SNR that was twice as large as low E place strategy users ($t(6) = 2.95$, $p = 0.026$) and high E response strategy users ($t(8) = 2.60$, $p = 0.031$; Figure 18f). Together, these results reveal that the strategy-specific effects of *Arc* expression are modulated by estradiol state in CA1 and DLS, as illustrated in Figures 19 and 20, respectively.

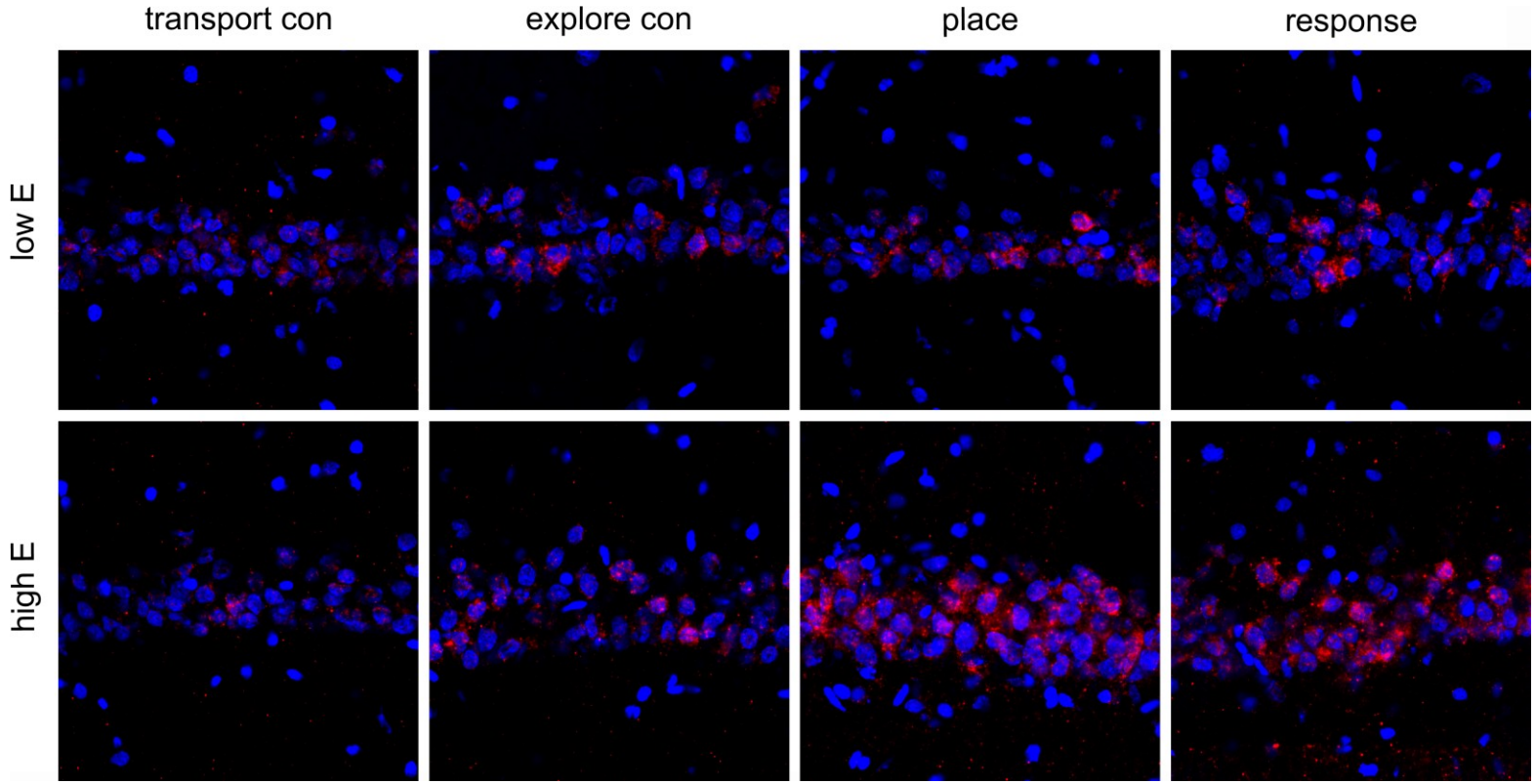

Figure 19: *Arc* mRNA expression in CA1 during training and probe testing on the ambiguous navigation task.

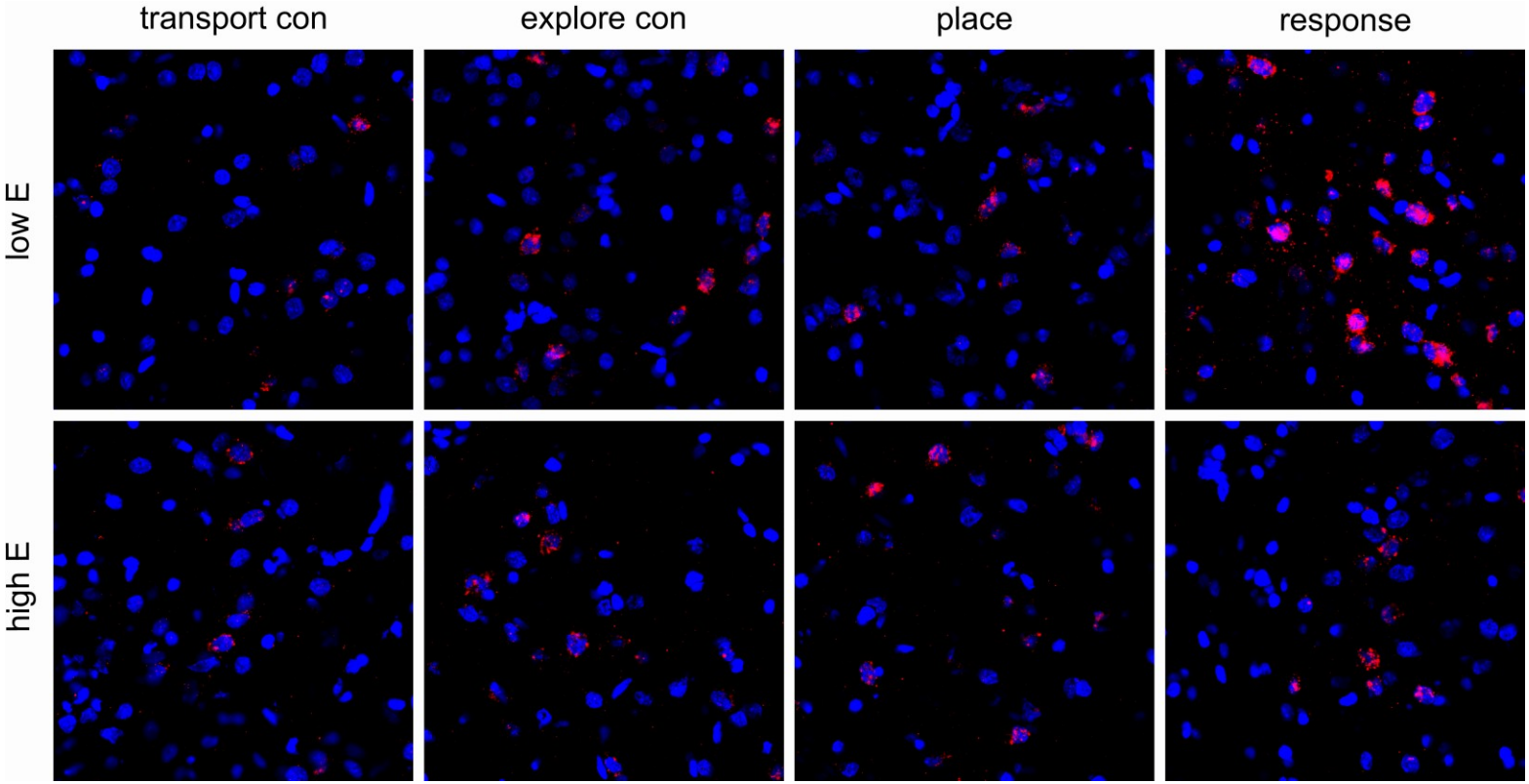

Figure 20: *Arc* mRNA expression in the DLS during training and probe testing on the ambiguous navigation task.

Correlations between *Arc* mRNA expression in hippocampal and dorsal striatal subregions

To determine whether there was a relationship between the plasticity and/or reliability occurring in hippocampal and striatal subregions, and whether learning a navigation task altered this relationship, we calculated correlations between plasticity and reliability measures in different subregions, presented in Table 5. Surprisingly, even though *Arc* expression in CA3 was not increased by exploration, estradiol, or navigation, CA3 *Arc* expression was positively correlated with expression in CA1 in control rats, and this relationship was maintained in behaviorally-trained rats. Similar positive correlations were observed between the DLS and DMS, even though navigation learning and estradiol did not significantly alter DMS *Arc* expression. Interestingly, plasticity and reliability measures in CA1 and DLS were correlated in control rats but not behaviorally-trained rats. These results suggest that similar plasticity events occur within and across neural systems when rats are not explicitly trained on a navigation task, but these relationships are only maintained within (and not across) neural systems in rats that learn a navigation task.

Discussion

The current results reveal marked differences in plasticity and reliability of hippocampal and striatal networks while rats perform the same physical behavior but use different strategies to navigate the maze and are in different estradiol states. While

Table 5: Pearson's R values for correlations between plasticity and reliabilty measures in hippocampal and striatal subregions of all control rats and all behaviorally-trained rats.

a) TOTAL

	Control	Behaviorally-trained
CA1 vs. CA3	0.410	**0.485***
CA1 vs. DG	0.342	0.085
CA1 vs. DLS	**0.526***	0.114
DLS vs. DMS	**0.720****	0.370

b) BOTH

	Control	Behaviorally-trained
CA1 vs. CA3	**0.787*****	**0.522***
CA1 vs. DLS	0.413	0.236
DLS vs. DMS	**0.893******	0.295

c) SNR

	Control	Behaviorally-trained
CA1 vs. CA3	**0.592***	0.399
CA1 vs. DLS	**0.565***	0.002
DLS vs. DMS	**0.843******	**0.530***

*** indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, and **** indicates $p < 0.0001$.**

exploration of the maze with no explicit navigation training increased plasticity, as indexed by *Arc* expression, in all hippocampal and striatal subregions analyzed except CA3, estradiol state and strategy used to solve the task only affected plasticity in rats explicitly trained to find a hidden escape platform, and these effects were specific to CA1 and DLS. Rats trained in proestrus, when estradiol levels are high, had increased plasticity in hippocampal CA1 ensembles following learning compared to rats with low estradiol levels, but this increased CA1 plasticity was only reliable in rats that used a place strategy to locate the platform. In addition, high estradiol state appeared to prevent the increase in DLS plasticity and reliability that we observed in low E response strategy users. Therefore the patterns of hippocampal and striatal *Arc* expression that predicted the use of place and response strategies differed depending on rats' estradiol state.

Exploration but not estradiol state increases plasticity and reliability in hippocampal and dorsal striatal ensembles

Similar to nine-month-old Fisher 344 male rats in land-based guided spatial exploration studies, three-month-old Sprague-Dawley females in this study that swam in the maze (explore controls) displayed significantly more *Arc* expression in the DG and CA1 subregions of the HPC, as well as greater reliability in this expression in CA1, than transport controls. However, exploration-induced *Arc* expression and reliability in CA1 was surprisingly lower than levels males have displayed. While we observed that 15-20% of CA1 neurons sampled expressed *Arc* during exploration of the maze, with 3% of

neurons displaying *Arc* in both the nucleus and cytoplasm, males in passive or guided exploration tasks have displayed proportions of 30-40% of neurons expressing *Arc* and most of these having *Arc* in both the nucleus and cytoplasm (Guzowski et al., 1999; Vazdarjanova et al., 2006). More surprisingly, we did not find that exploration of the maze increased *Arc* expression in CA3 compared to transport controls. We observed that all groups in this study had 5-10% of CA3 neurons with *Arc*, compared to 15-20% in males in passive explorations tasks (Guzowski et al., 1999; Vazdarjanova et al., 2006). In contrast, TOTAL *Arc* expression in the DS (~10%) and the DG (~2%) during exploration were similar to that previously published in males (Vazdarjanova et al., 2006). These results suggest that our results are due to one or more of the following possible factors: a) there is a sex or strain difference in exploration-induced hippocampal *Arc* expression, b) the strenuous motor behavior or stress of swimming in our task suppressed *Arc* induction, and c) *Arc* is not as easily induced during the lights-on (sleep) phase of rats' sleep-wake cycle as it is during their active phase. Swim stress has been shown to impair other forms of hippocampal plasticity, including long-term potentiation (Kavushansky et al., 2006), but its direct effects on *Arc* expression have not been examined. However, restraint stress does not affect *Arc* expression in CA1 (Mikkelsen and Larsen, 2006), and previous exploration studies in males have provided some evidence that *Arc* induced in the HPC could not be attributed to stress or motor activity (Guzowski et al., 1999; Guzowski et al., 2001a). *Arc* expression has been shown to decline in the HPC during the sleep phase of the day (Cirelli and Tononi, 2000), so it is likely that circadian effects contribute to the

low levels of *Arc* expression observed here, possibly in conjunction with sex differences in *Arc* expression. Possible effects of motor activity, stress, and circadian rhythm on *Arc* expression during exploration are addressed in Ch. 4.

While exploration of the maze increased *Arc* expression in this study, estradiol alone did not modulate hippocampal or striatal plasticity. No previous studies have examined estradiol's effects on baseline *Arc* expression, but this lack of effect of estradiol on *Arc* expression in transport controls was somewhat surprising because estradiol has been shown to increase a number of mechanisms of plasticity in the HPC and suppress some forms of plasticity in the DS in the non-behaving rat and in vitro, as discussed in the General Introduction. This suggests that estradiol does not modulate navigation strategy use by simply altering hippocampal and striatal plasticity at baseline. It was even more surprising that estradiol did not modulate the hippocampal and striatal plasticity induced by exploration (as it modulated plasticity in these regions during explicit learning). These results suggest that either a) the stress and/or motor behavior during the forced exploration masked possible effects of estradiol on *Arc* expression or b) explicit learning is required for estradiol's modulation of hippocampal and striatal plasticity. Either way, the current results do not support the hypothesis that estradiol biases rats to use a place strategy by priming their HPC to display plasticity and/or preventing the DS from displaying plasticity during navigation experience. These issues are further examined in Ch. 4.

Effects of estradiol state on *Arc* mRNA expression in the hippocampus and dorsal striatum are strategy and subregion-specific

The results of the current study suggest estradiol increases CA1 plasticity only during spatial navigation learning, and estradiol-induced place strategy bias only occurs when there is also an increase in the reliability of this heightened plasticity. Estradiol is almost always able to increase this reliability. However, an increase in plasticity without accompanying reliability in this plasticity may impair hippocampal function and consequently produce a response strategy bias, as observed in response strategy users in a high estradiol state. Estradiol may also contribute to a place bias by preventing increases in plasticity and reliability in the DLS. However, when plasticity and reliability are low in both the DLS and CA1, rats appear to default to using a place strategy, suggesting that geometry and landmark cues in the external environment are still more salient than internal cues in this case. Together, these results suggest that the amount and nature of plasticity occurring in CA1 and the DLS are related to the use of place and response strategies in a manner that is dependent upon estradiol levels. The interactions between estradiol, hippocampal and striatal plasticity and reliability, and navigation strategy use are further explored in the General Discussion.

While the effects of estradiol and strategy use were generally opposite in CA1 and the DLS, there were several differences in the observed patterns that suggest that estradiol has different mechanisms in these subregions to influence strategy use. All rats with high estradiol levels that were trained to find the hidden platform displayed

increased plasticity in CA1 compared to their behaviorally-trained counterparts with low estradiol; in contrast, only low E response strategy users (and not low E place strategy users) had greater DLS plasticity than their high estradiol counterparts (and all other groups). And, the interaction between estradiol state and strategy was largest in the reliability measure (SNR) in CA1, while this interaction was greatest in the size of the reliable network (BOTH) in the DLS. This suggests that estradiol may modulate navigation strategy use by predominantly influencing the amount of plasticity in the DLS and the reliability of plasticity in CA1 during spatial navigation.

The specificity of estradiol's effects on plasticity to the DLS and CA1, among all of the hippocampal and striatal subregions examined, support previous findings that estradiol acts locally via ERs within these hippocampal and striatal regions to modulate plasticity and consequent behaviors (for review, see Morissette et al., 2008; for review, see Spencer et al., 2008). ERs are located within cell bodies and at extranuclear sites in the HPC in a number of different types of cells including pyramidal and granule principle neurons and inhibitory interneurons (Loy et al., 1988; Orikasa et al., 2000; Hart et al., 2001; Milner et al., 2001; Mehra et al., 2005; Milner et al., 2005; Romeo et al., 2005). This distribution allows estradiol to have many effects on plasticity, such as transiently modulating neurotransmitter systems such as the dopaminergic system, which is required for late-phase LTP in CA1 (Frey et al., 1990), and altering gene expression that lead to structural changes at synapses. In contrast to the HPC, ERs are not easily detectable in the DS using standard histological and autoradiography methods (Shughrue et al., 1997;

Kuppers and Beyer, 1999). However, administration of ERβ agonists produce rapid increases in dopamine receptor and dopamine transporter (DAT) expression (Morissette and Di Paolo, 1993; Le Saux et al., 2006), as well as striatal-dependent behaviors (e.g., (Becker, 1990; Castner et al., 1993; Xiao et al., 2003), suggest non-genomic mechanisms of estradiol in the DS that may work via membrane-bound ERs. Together, these findings suggest that the effects of estradiol on hippocampal plasticity may occur via a number of mechanisms, but estradiol effects on CA1 reliability might be attributed to genomic mechanisms that alter the activity of neurons across some period of time that cannot be completely accounted for by transient non-genomic mechanisms. In the DLS, however, because ERs are not easily detected, the primary effects observed in this study on the size of the activated ensemble during navigation may be largely produced by rapid and transient non-genomic influences of estradiol on neuronal excitability and neurotransmission.

Subregions of the hippocampus and dorsal striatum perform unique roles during spatial navigation

Strategy-specific effects of estradiol state were only observed in CA1 and the DLS, suggesting that the use of place and response strategies rely on the interaction between plasticity occurring in these two structures. In the DMS and DG, exploration in the maze elicited an increase in plasticity in the DMS and DG that was not further increased by behavioral training. DG plasticity induced by exploration in this maze is not surprising given increases in DG plasticity that have been reported during exploration of

novel land-based environments (Guzowski et al., 1999; Ramirez-Amaya et al., 2005). However, the lack of increased *Arc* expression in the DG during navigation strategy use suggests that the DG is not specifically required for the employment of a place strategy. DMS plasticity induced by exploration and not furthered by behavioral training supports previous reports that the DMS is engaged during spatial navigation in a distinct manner from that of the DLS and that the DLS but not DMS is required for successful response learning (Devan et al., 1999; Yin and Knowlton, 2004). However, plasticity and reliability in the DMS and DLS were correlated in control and behaviorally-trained rats, suggesting that plasticity occurring within the DMS is related to the patterns of plasticity observed in the DLS that predicted strategy use and is therefore important for the successful employment of spatial navigation strategies. A similar case can be made for the importance of CA3 plasticity in the function of CA1 plasticity and reliability that was essential for strategy use. This relationship between CA1 and CA3 is intriguing because within the HPC, the highest concentration of both ERα and ERβ is in CA3 (Laflamme et al., 1998; Mehra et al., 2005), suggesting that estradiol may have some effect in CA3 that alters the processing of spatial information in CA1. These results also suggest that being brought into the testing room was sufficient to induce CA3 plasticity that supports successful navigation.

Conclusions

Electrophysiological studies have shown reliable firing of property-specific neurons, such as place cells, in the DS and HPC during spatial navigation (Mizumori et al., 2004), and plasticity has been examined in the HPC and DS separately during spatial navigation (Guzowski et al., 2001a; Daberkow et al., 2007). This is the first study to examine both plasticity and reliability in hippocampal and striatal ensembles, illustrate that the plasticity occurring in these memory systems interacts to determine strategy use, and show that estradiol may influence strategy use by modulating these properties during spatial navigation. While previous studies have compared the activation levels of hippocampal and striatal subregions using IEGs such as *c-fos* (Colombo et al., 2003; Teather et al., 2005; Gill et al., 2007), this study shows that plasticity and reliability in hippocampal and striatal ensembles are much more important for the successful use of place and response strategies and suggests that estradiol modulates strategy use by altering hippocampal and striatal plasticity and reliability in a subregion-specific manner. These complex findings suggest that estradiol state determines how you utilize the HPC and DS to use a navigation strategy. Estradiol state primes the HPC and DS to display some specific aspects of plasticity and reliability during navigation; for example, rats in a high estradiol state always display high CA1 plasticity and low DLS plasticity and reliability compared to rats in a low estradiol state. Therefore, the pattern of CA1-DLS plasticity that predicts the use of each strategy is different in a high estradiol state than a low estradiol state. These findings are discussed further in the General Discussion.

CHAPTER 4: ESTRADIOL DOES NOT MODULATE PLASTICITY OR RELIABILITY OF HIPPOCAMPAL AND DORSAL STRIATAL ENSEMBLES DURING SPATIAL EXPLORATION

Previous research has demonstrated that estradiol modulates hippocampal and striatal plasticity in the non-behaving rat (Morissette et al., 2008; Spencer et al., 2008), suggesting that estradiol may modulate spatial navigation strategy use by priming the female brain to preferentially use the HPC or DS. However, Ch. 3 surprisingly showed that estradiol did not increase plasticity in any hippocampal and striatal subregions examined in rats that were transported to the testing room or in those that swam in the maze. Rather, estradiol only modulated *Arc* expression in rats that were explicitly trained to navigate to a hidden platform. Together these findings suggest that learning during navigation is required for estradiol's modulation of hippocampal and striatal plasticity and reliability. As discussed in the Ch. 3 Discussion, there are several factors that may have prevented us from observing a possible estradiol-modulated increase in *Arc* expression. It is possible that transport controls' experience, including being brought to the testing room and being able to examine the testing room from the transparent holding cage, altered *Arc* expression sufficiently to mask the effects of estradiol on baseline levels of plasticity and reliability. In addition, an estradiol-induced increase in *Arc* expression during exploration may have been masked by effects of swimming in the maze in the absence of an escape platform, including stress and motor activity. Therefore, it is possible that estradiol biases rats to use a place strategy during spatial navigation by

either modulating hippocampal and/or striatal baseline plasticity or by altering the plasticity induced by spatial exploration, but we could not observe these effects in our paradigm. Therefore, the current experiment uses a paradigm that controls for the possible effects of stress and motor activity to determine whether goal-directed navigation is required for estradiol's modulation of hippocampal and striatal plasticity or whether estradiol biases the female brain to use a place strategy by priming the HPC to be preferentially used during exposure to an environment even when strategy use is not required. In order to ensure that any hormonal effects examined could be specifically attributed to estradiol and not to other cyclic ovarian hormones, we ovariectomized females and administered rats either oil or estradiol.

In Ch. 3, we observed lower levels of total *Arc* expression and reliability in CA1 and CA3 than we expected. Previous research that has examined reliability during passive exploration of a novel spatial environment in males during the lights-off, active phase of the day has found that there is extremely high CA1 and CA3 reliability across two epochs of passive exploration of a single land-based environment (Guzowski et al., 1999; Vazdarjanova et al., 2006). However, in Ch. 3, when females were forced to explore the water-based maze during the lights-on phase of the day, CA1 plasticity and reliability were low, and exploration did not increase CA3 *Arc* expression at all. Therefore, in this experiment, females passively explored a land-based task during the lights-off phase of the day to examine whether CA1 and CA3 *Arc* expression and reliability would be high across two exposures to the same environment.

This experiment also examined the effects of estradiol on the reliability of hippocampal ensembles during spatial exploration. If estradiol biases the female brain to use a place strategy by altering hippocampal plasticity during exposure to an environment even when strategy use is not required, then it might do this by altering a female's attention to environmental cues. Females have been shown to use both geometry and landmark cues during place navigation (Williams et al., 1990). And in males, altering the cues or geometry of a familiar environment produces changes in hippocampal place cell activity (Lee et al., 2004) and decreases the reliability of *Arc* expression in CA1 (Guzowski et al., 1999), in addition to disrupting place navigation (Williams et al., 1990). Therefore, the current study examined whether females with estradiol attend to environmental cues more than or differently than females without estradiol in order to determine whether one way that estradiol might bias females to use a place strategy is by altering a rat's attention to environmental cues and the corresponding reliability of hippocampal ensembles.

To examine whether estradiol primes the brain to preferentially use the HPC by altering hippocampal and/or striatal plasticity and reliability during exposure to a novel environment even before goal-directed learning, we quantified *Arc* mRNA expression in OVX females with and without estradiol replacement after two exposures to a single novel environment separated by 25 min. To examine the effects of cue change and evaluate whether potential differential attention to cues affected plasticity and reliability

in the HPC and DS, we included a condition in which the second exposure was to the same room but with altered landmark cues.

Materials and Methods

Subjects

Subjects were 3.5-month-old female CD strain Sprague-Dawley rats purchased from Charles Rivers Laboratories (Kingston, NY) at two months of age. They were paired-housed in individually-ventilated cages and given ad libitum access to water and a standard diet (Rodent Diet 5001, PMI Nutrition International, Inc., Brentwood, MO). The temperature-controlled colony room was maintained on a 12:12 hr light:dark cycle with lights on at 6 a.m. daily, and all behavioral testing took place during the lights-off phase of the day. Rats were handled daily for 10 days prior to ovariectomy, eight days before water maze training, and twelve days before the spatial exploration task to minimize any effects of stress from being handled during behavioral testing. All procedures were approved by the Institutional Animal Care and Use Committee of Duke University.

Experimental design

Three weeks after arrival to our colony, vaginal samples were taken daily to confirm that rats were cycling normally. Rats were then ovariectomized and allowed to recover for two weeks before being trained on a standard water maze task. Ten days later, rats received either two subcutaneous injections of estradiol or oil 48 and 24 hrs before

being placed in a spatial exploration paradigm. Immediately after the exploration task, rats were sacrificed and their brains harvested for *Arc* mRNA analysis.

Hormonal manipulations

Vaginal samples, ovariectomies, and estradiol replacement were conducted as described in Chs. 1 and 2.

Water maze training

In order to confirm that all rats were capable of using both the HPC and DS to navigate successfully, they were trained on place and visible platform versions of the water maze. Water maze training took place in the same apparatus and testing room described in Ch. 2. A black escape platform was either hidden just below the water's surface or raised above the surface of the water and wrapped with white tape in order to be visible, depending on the demands of the task (specifics described below). The visible platform had a diameter of 10 cm, and the hidden platform had a diameter of 19 cm.

The day before water maze training began, rats were habituated to the maze in pairs. Each rat was placed on the platform in the middle of the pool for 30 s and returned to its holding cage while another rat underwent the same procedure. Then the first rat was placed in the pool facing the pool's edge and allowed to swim for 10 s before being guided to the platform by the experimenter. The rat remained on the platform for 15 s before being returned to its home cage, and the second rat underwent the same procedure.

The following three days, rats were trained on a standard water maze task. On the first two days of training, each rat performed two blocks of 4 training trials each. Four rats were brought into the behavioral testing room in individual holding cages. Rats were placed in the water at one of four locations (N, S, E, and W) at the beginning of each trial in a pseudo-random order. Each trial ended when the rat climbed onto the escape platform. If the rat failed to find the platform at the end of 60 s, the experimenter guided it through the water to the platform. The rat was left on the escape platform for 10 s and then placed back in its transport cage while the other rat perfomed one trial. Two rats alternated trials within each trial block, and after the first pair completed one block, they remained in their holding cages while another pair performed one block of trials. Then this process was repeated. After all four rats completed both trial blocks, they were placed back into their colony room. On day three, instead of a second block of training trials, rats performed one probe trial during which the platform was removed and the rat was allowed to swim for 60 s followed by one trial in which the curtain was drawn around the pool to remove environmental cues. The probe trial served to measure the accuracy and precision with which rats learned the platform location, and the curtain trial was conducted to ensure that rats used the cues to locate the platform during training. On the fifth day, rats received eight trials in which the platform was visible, to assure that all rats could see and swim well enough to navigate in the water. During all trials, latencies to reach the platform were recorded by the experimenter and the path of the animal was recorded in real time using HVS Image (Buckingham, WH, UK).

Guided spatial exploration task

Guided exploration took place in a 60 cm x 60 cm plexiglass exploration box with 6 cm high walls. Tape on the floor of the box demarcated 9 squares of 20 cm x 20 cm. The box was placed in the center of a 6.8 m x 5 m room with several salient geometric and landmark cues, including a curtain along one wall, a metal cart with rats in cages on it, a radio playing white noise, and a black cart with several large objects on it wrapped in cellophane (see Figure 21). Rats were brought into the testing room in an opaque bucket with fresh corn cob bedding and placed directly into the exploration box to begin the first exploration epoch in context A. The rat was placed into a different square by the experimenter every 15 s for 5 min. The order of squares was assigned randomly before the session and was held constant for all rats. Immediately after the exploration of context A, the rat was returned to its home cage in the bucket. Twenty-five min after the start of the first exploration, the rat was brought back into the testing room for a second exploration epoch; half of the rats explored context A again, and the other half explored context B, which was in the same room but the positions of the large landmark cues were altered within the room (see Figure 21). The order of squares that the rat was placed in remained the same for all rats.

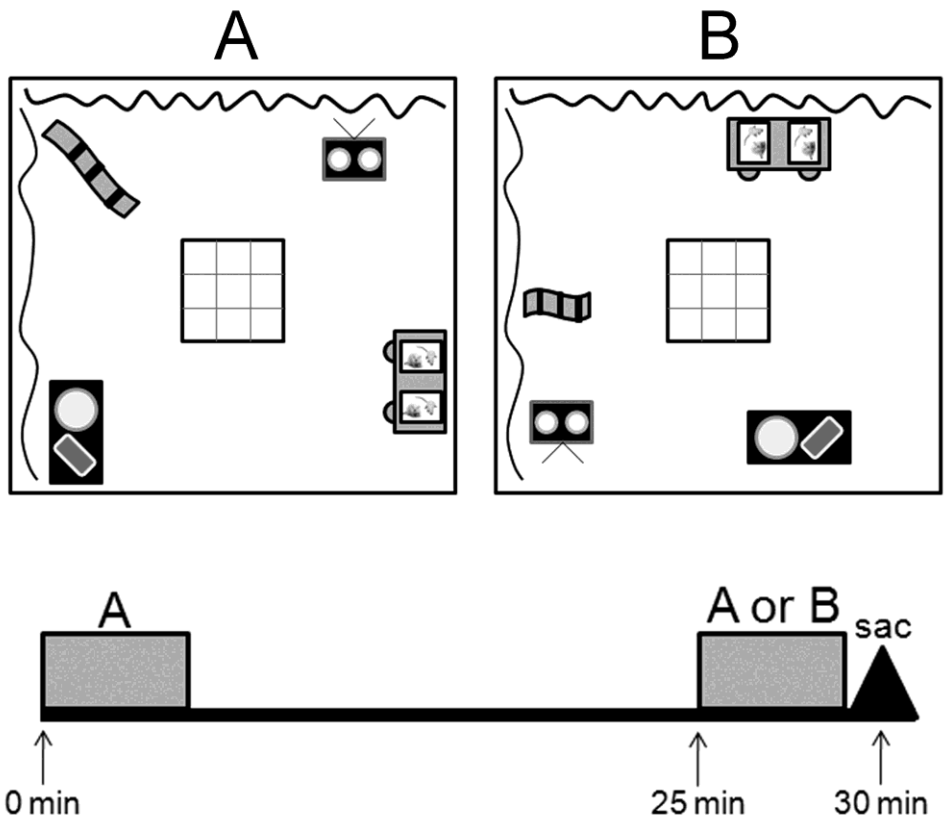

Figure 21: Contexts and experimental design for guided spatial exploration task. All rats were exposed to context A for 5 min. Twenty min later, rats were exposed to either context A again or to context B, which was in the same room but the locations of the four large landmark cues were altered. Immediately after the second exploration epoch, rats were sacrificed for Arc mRNA analysis.

In situ hybridization, confocal microscopy and image analysis

Immediately following the second behavioral epoch, the rat was anesthetized with isofluorane and sacrificed by rapid decapitation. All procedures for brain extraction, *in situ* hybridization, confocal microscopy, and image analysis were conducted as described

in Ch. 3 and illustrated in Figure 16. For each rat, the mean number of neurons quantified was 337 for CA1, 136 for CA3, 176 for DLS, and 249 for DMS. There was no correlation between the proportion of neurons that expressed *Arc* mRNA and the number of neurons quantified (p's > 0.05).

Statistical analysis

Behavioral measures including learning rate during training water maze training in blocks of four trials and visible platform trials in blocks of two trials, as well as time spent in each quadrant during the probe trial, were analyzed using one-way repeated-measures ANOVAs. Latency on the curtain trial was also compared to the average latency on the last block of training trials with a paired-samples t-test. Dependent measures for brain data were the same as described in Ch. 3. ANOVAs were conducted for each dependent measure with independent variables of estradiol treatment (oil vs. estradiol) and environment (A/A, A/B, or caged control).

Results

All rats displayed successful learning using hippocampal and dorsal striatal strategies

Because we measured *Arc* expression during a passive spatial exploration task that did not require rats to explicitly use their HPC or DS, we wanted to behaviorally confirm that all rats could successfully use these brain regions during the exploration

task. Therefore we trained rats to use place and response strategies two weeks before the spatial exploration task. Rats showed successful learning during water maze training ($F(4, 104) = 40.8$, $p < 0.0001$) and spent significantly more time in the target quadrant than other quadrants of the maze during the probe trial ($45.4 \pm 3.2\%$; $F(3,78) = 23.1$, $p < 0.0001$; data not shown). They also had significantly longer latencies on the curtain trial than on the last block of training trials ($t(26) = 7.74$, $p < 0.0001$). These results indicate that rats successfully learned the location of the hidden platform using a hippocampal-dependent strategy. Rats also showed successful learning of the visible platform task ($F(3,78) = 39.0$, $p < 0.0001$), indicating that they could also use a striatal-dependent strategy.

Exploration but not estradiol or cue change affects *Arc* mRNA expression in hippocampal and dorsal striatal subregions

In order to determine the effects of estradiol, exploration, and environmental cue change on plasticity and reliability in hippocampal and striatal subregions, we examined *Arc* expression in these regions in ovariectomized females replaced with oil and estradiol across two exposures to the same context or two different contexts that only differed in landmark cue arrangement. Exploration increased TOTAL *Arc* expression in all hippocampal and striatal subregions analyzed and increased the proportion of neurons that had *Arc* expression in BOTH the nucleus and cytoplasm and the SNR in all

subregions examined except the DLS, as shown in Figures 22 and 23. However, neither estradiol nor cue change altered any of these measures.

ANOVAs for TOTAL, BOTH, and SNR in CA1 all revealed main effects of task (TOTAL: $F(2,18) = 9.36$, $p = 0.002$; BOTH: $F(2,18) = 6.35$, $p = 0.008$; SNR: $F(2,18) = 3.75$, $p = 0.044$) but no effects of estradiol or any interactions (p's > 0.50). Rats in both explore conditions had three times as much TOTAL *Arc* expression, four times as many neurons that expressed *Arc* in BOTH the nucleus and cytoplasm, and twice as large SNRs as caged controls (p's < 0.05; see Figure 22a-c). However, there were no differences between rats in the A/A and A/B conditions for any of these measures (p's > 0.25). These results suggest that exploration increased CA1 plasticity but that neither cue change nor estradiol affected plasticity.

The same pattern was revealed for CA3. ANOVAs for TOTAL, BOTH, and SNR in CA1 all revealed main effects of task (TOTAL: $F(2,19) = 7.13$, $p = 0.005$; BOTH: $F(2,19) = 5.95$, $p = 0.010$; SNR: $F(2,18) = 4.76$, $p = 0.021$) but no effects of estradiol or any interactions (p's > 0.30). Rats in both explore conditions had twice the TOTAL *Arc* expression, four times as many neurons that expressed *Arc* in BOTH the nucleus and cytoplasm, and twice as large SNRs as caged controls (p's < 0.05; see Figure 22d-f). However, there were no differences between rats in the A/A and A/B conditions for any of these measures (p's > 0.35). These results suggest that exploration increased CA3 plasticity and reliability but that neither estradiol nor cue change altered these levels of *Arc* expression.

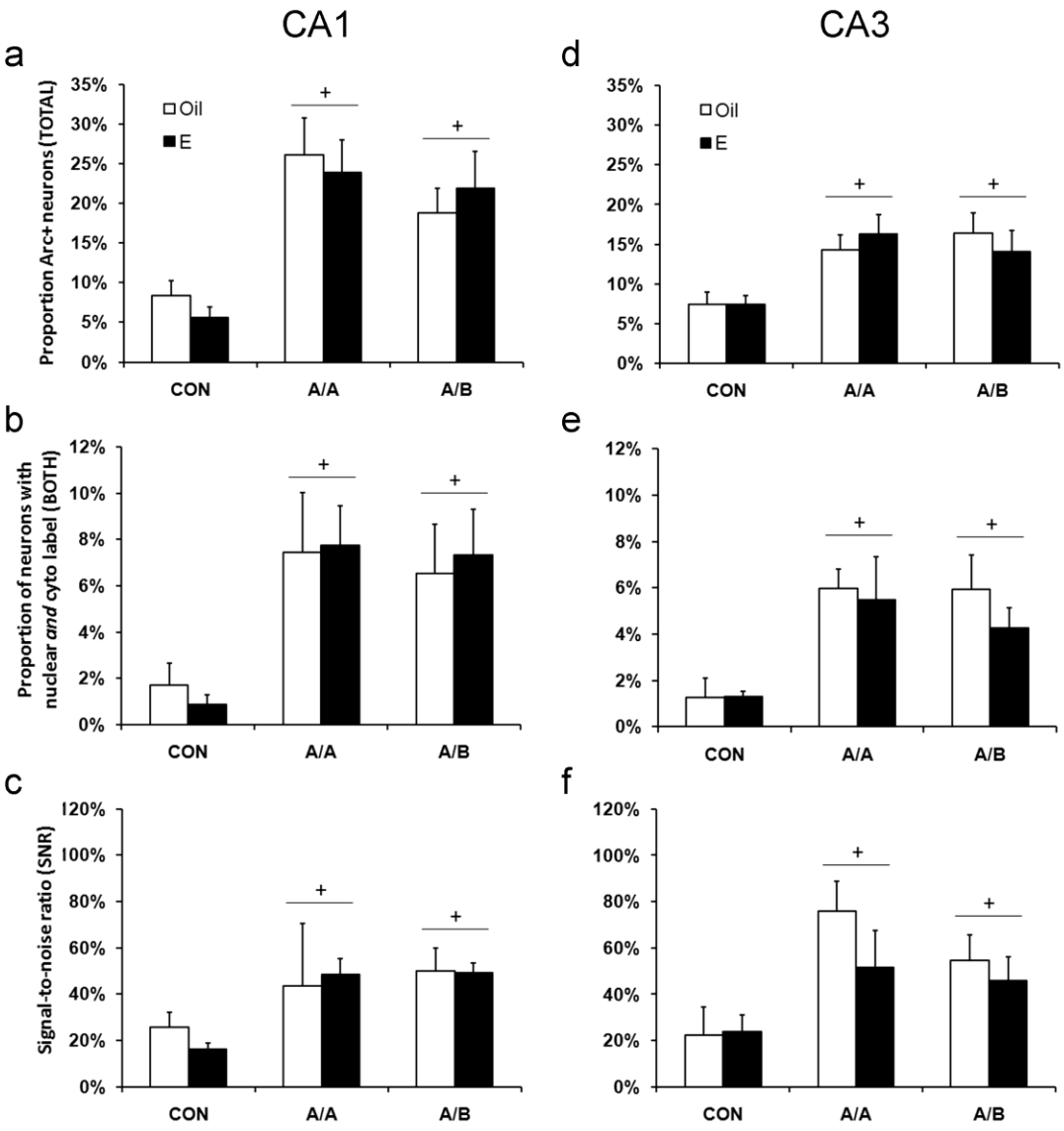

Figure 22: *Arc* expression in CA1 (left column) and CA3 (right column) during guided spatial exploration.

TOTAL *Arc* mRNA signal (top row), porortion of neurons with Arc signal in BOTH the nucleus and cytoplasm (middle row), and the signal-to-noise ratio (SNR; bottom row).+ indicates significantly different from controls with $p < 0.05$.

Again, a similar pattern of plasticity and reliability emerged in the DMS. ANOVAs for TOTAL, BOTH, and SNR in CA1 all revealed main effects of task (TOTAL: $F(2,17) = 11.68$, $p = 0.0006$; BOTH: $F(2,17) = 6.51$, $p = 0.008$; SNR: $F(2,17) = 5.80$, $p = 0.012$) but no effects of estradiol or any interactions (p's > 0.30). Rats in both explore conditions had four times the TOTAL *Arc* expression, five times as many neurons that expressed *Arc* in BOTH the nucleus and cytoplasm, and four times greater SNRs as caged controls (p's < 0.05; see Figure 23a-c). However, there were no differences between rats in the A/A and A/B conditions for any of these measures except TOTAL *Arc* expression, with A/A rats having greater TOTAL than A/B rats ($t(15) = 2.17$, $p = 0.047$; all other p's > 0.05). Overall, these results suggest that explorers had greater plasticity and reliability than caged controls but neither cue change nor estradiol affected plasticity.

In the DLS, exploration only increased TOTAL *Arc* expression ($F(2,17) = 7.44$, $p = 0.005$), but not BOTH or SNR (p's > 0.15), and there was no effect of estradiol or an interaction between task and estradiol for any ANOVAs (p's > 0.30). Rats in both explore conditions had three times as much TOTAL *Arc* expression as caged controls (p's < 0.01; see Figure 23c), but there were no differences between rats in the A/A and A/B onditions in this measure ($p > 0.65$). These results suggest that exploration increased plasticity but not reliability in the DLS.

Similar to Ch. 3, plasticity and reliability in hippocampal and striatal subregions were correlated with one another for all comparisons except SNR for CA1 vs. DLS (see

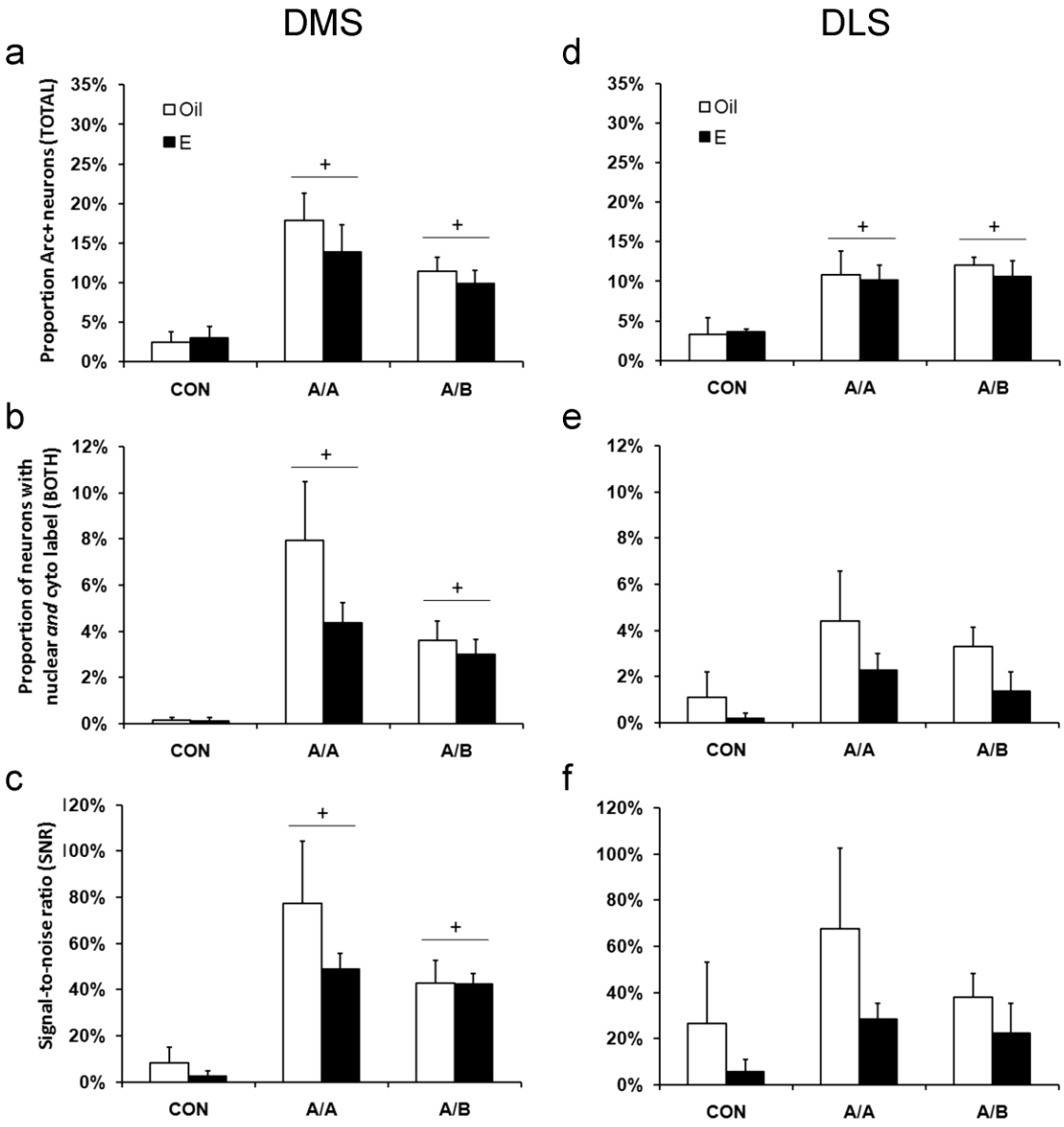

Figure 23: *Arc* expression in the DMS (left column) and DLS (right column) during guided spatial exploration.
TOTAL *Arc* mRNA signal (top row), porortion of neurons with *Arc* signal in BOTH the nucleus and cytoplasm (middle row), and the signal-to-noise ratio (SNR; bottom row).+ indicates significantly different from controls with $p < 0.05$.

Table 6). This exception is likely a consequence of task increasing reliability in CA1 but not DLS. However, overall, these results suggest that the motor activity and exploration performed by explorers increased plasticity across neural systems.

Table 6: Pearson's R values for correlations between plasticity and reliability measures in hippocampal and striatal subregions of all rats.

	TOTAL	BOTH	SNR
CA1 vs. CA3	0.657***	0.550**	0.410*
CA1 vs. DLS	0.652***	0.478*	0.307
DLS vs. DMS	0.598***	0.513**	0.515**

*** indicates p < 0.05, ** indicates p < 0.01, *** indicates p < 0.001**

Discussion

The results from the current experiment support and expand those from Ch. 3. Passive exploration of a novel environment increased plasticity across subregions of the HPC and DS compared to the plasticity displayed by cage controls, but estradiol did not further increase this plasticity, as indexed by TOTAL *Arc* expression. In Ch. 3, it was possible that a potential increase in baseline plasticity by estradiol was masked by being in the testing room. However, transport controls in Ch. 3 and home cage controls in this experiment displayed similar *Arc* expression that was not modulated by estradiol,

suggesting that being in the testing room and remaining in the transport cage likely did not mask the effects of estradiol on baseline *Arc* expression. It was also possible that we did not observe modulation of plasticity during exploration by estradiol because the motor behavior or stress of not being able to produce a behavior that predicted escape masked this effect. However, the levels of *Arc* expression induced by passive, guided exploration in this experiment were similar across hippocampal and striatal subregions to the levels induced by exploration of the water maze in Ch. 3. These findings suggest that estradiol likely influences *Arc* expression only during goal-directed behavior and not during undirected exploration. The implications of goal-directed behavior being necessary for the effects of estradiol on hippocampal and striatal plasticity are discussed in the General Discussion.

Alterations in landmark cues did not elicit changes in hippocampal reliability across context exposures

Previous studies have shown that the reliability of *Arc* expression in hippocampal subregions in male rats is greater across two exposures to the same environment than across exposures to different environments (Guzowski et al., 1999; Lee et al., 2004; Vazdarjanova et al., 2006). And, gradual alterations in spatial cues in an environment are sufficient to elicit changes in hippocampal place cell activity (Lee et al., 2004). Here, we did not observe changes in hippocampal or striatal ensembles when landmark cues were altered within the same geometric frame. Thus, either rats did not notice the change in

cues or this cue change was not sufficient to alter *Arc* expression even though it might have altered place cell activity. Given that patterns of hippocampal *Arc* expression appear to be so similar to those of place cell firing (Muller et al., 1987; Thompson and Best, 1990; Wilson and McNaughton, 1993; Leutgeb et al., 2004), it is more likely that the cue change was not noticed. Interestingly, our laboratory has previously shown that at asymptotic performance after several weeks of training on a radial-arm maze, females without estradiol use both landmarks and geometry to solve the task (Williams et al., 1990). Therefore, our results suggest that either more exposure to the environment or explicit learning is required for females to display behavioral or neural evidence that landmark cues are utilized for female spatial navigation. Had explicit navigation learning been required, we may have observed differences in hippocampal ensembles between contexts.

Possible effects of stress, motor behavior, and circadian rhythm on the plasticity and reliability of hippocampal and dorsal striatal ensembles

The levels of *Arc* expression in hippocampal and striatal subregions observed in Ch. 3 and this study were surprisingly similar to each other. However, there were a few notable differences. First, while neither explore controls nor behaviorally-trained rats showed increased CA3 plasticity or reliability above transport control levels in Ch. 3, explore rats in this study showed greater CA3 plasticity and reliability than caged controls (but this was still only approximately 5% more *Arc* expression than observed in

Ch. 3). Because transport controls from Ch. 3 and caged controls from Ch. 4 displayed similar levels of CA3 plasticity, these results suggest that the stress of swimming, motor activity, habituation to the context, or circadian effects likely caused a slight suppression of CA3 *Arc* expression in Ch. 3. In addition, the levels of *Arc* expression observed here were comparable to that previously reported in males, suggesting that when no explicit learning is required, there may not be sex differences in *Arc* expression in CA3. However, it would be important to test this within the same study using the identical parameters.

Another difference between the results of Ch. 3 and the current study is that forced exploration in Ch. 3 increased DLS plasticity and reliability, while guided exploration in Ch. 4 increased DLS plasticity but not reliability. These results are not surprising because in Ch. 4, rats did not have to perform the same motor behaviors during both exposures to the task, but in Ch. 3, rats were forced to swim along the path of the maze (only straight swimming and 90° turns) even if this swimming was random. Altogether, the results of the current study suggest that the female HPC and DS display reliable plasticity during exploration that is not modulated by estradiol but may be slightly influenced by the effects of stress, motor behavior, and circadian rhythm. These results confirm the finding of Ch. 3 that goal-directed behavior may be required for estradiol to modulate *Arc* expression in the HPC and DS during spatial navigation. The implications of the current results for the roles of hippocampal and striatal subregions during spatial exploration and navigation are considered in the General Discussion.

GENERAL DISCUSSION

Previous research has found that estradiol tends to bias rats to use a hippocampal-dependent place strategy over a striatal-dependent response strategy (Korol et al., 2004; Quinlan et al., 2008). While the behavioral effects of estradiol on spatial navigation have been well-described in the young adult female rat, the functional mechanisms by which estradiol has these behavioral effects is not well understood. And, because research has focused on the young adult female rat, there is currently almost no understanding about what factors, such as age and previous experience, modulate the presence and robustness of this effect. The studies in this dissertation aimed to examine the functional mechanisms by which strategy use is determine by examining the activation, plasticity, and reliability of ensembles within subregions of the HPC and DS during spatial navigation and to understand how estradiol modulates the function of these ensembles to influence navigation behavior. In addition, these studies examined how age and previous navigation experience alter the ability of estradiol to modulate navigation behavior in order to further understand estradiol's role in spatial navigation behavior across the lifespan.

The findings from chapters 2, 3, and 4 are consistent with the hypothesis that estradiol modulates spatial navigation strategy use by modifying the plasticity and reliability of hippocampal and dorsal striatal neuronal ensembles during spatial navigation. In Ch. 2, we observed similar effects of estradiol and task on activation of the DG, DMS, and DLS, indicating that the HPC and DS may be similarly activated by

common sensory and modulatory input from the entorhinal cortex (EC). Ch. 3 showed that these inputs are processed differently within hippocampal and striatal subregions depending on estradiol status, and that multiple distinct patterns of CA1-DLS plasticity/reliability are associated with the use of each navigation strategy. For example, a specific pattern of CA1-DLS was observed when rats used a place strategy and were in a high estradiol state, but a different pattern was observed in place strategy users that were in a low estradiol state. Finally, Ch. 4 confirmed findings from Ch. 3 that goal-directed movement through space (strategy use) is necessary to produce these distinct patterns of plasticity that are modulated by estradiol. While estradiol may prime CA1 to be more plastic and the DLS to be less plastic at the onset of goal-directed navigation behavior, use of a learned strategy is required to elicit these changes in plasticity. These results have important implications for the effects of estradiol on spatial navigation across development and adult aging, behaviorally described in Ch. 1 and Ch. 2.

Summary of Findings

Chapter 1

In order to investigate how estradiol's modulation of navigation strategies in females develops we examined the developmental onset of the use of spatial navigation strategies and the age at which estradiol can first modify strategy preference. We also investigated the stability of navigation strategy use across pre and post-pubertal time points and multiple navigation experiences. Consistent with other studies (Rudy et al.,

1987; Akers and Hamilton, 2007), we found that rats used either a place or a response strategy successfully by PD21 but that estradiol did not bias rats to use a place strategy until PD26. On the first adult navigation experience, rats were significantly more likely to use the same navigation strategy they used prepubertally, regardless of current estrous cycle phase. On the second and third adult tests, after rats had more experience with the task, previous navigation experience did not predict strategy use. Rats in proestrus, with high estradiol levels, were significantly more likely to use a place strategy, while rats in estrus and diestrus, with lower estradiol levels, were not biased to use either strategy. These results suggest that while estradiol can modulate spatial navigation strategy use before puberty, hormonal modulation interacts with previous navigation experience. This study sheds light on when and under what circumstances estradiol gains control over spatial navigation behavior in the female rat. Place and response navigation strategies can be used from a very early age and may be modulated by estradiol well before puberty, but strategy preference is influenced by a combination of experiential and hormonal factors.

Chapter 2

In order to examine whether the use of place and response strategies requires hippocampal and striatal activation, respectively, and whether estradiol modulates spatial navigation learning by altering activation in the HPC and DS, expression of the immediate early gene *c-fos* was used to examine neural activation in these brain regions in ovariectomized female rats with or without estradiol replacement during spatial

navigation. We quantified Fos protein expression 30 min after rats learned place or response tasks in order to measure activation that occurred during learning. Based on previous work in males (Colombo et al., 2003; Teather et al., 2005; Gill et al., 2007), we hypothesized that rats trained to learn a place task would have more Fos expression in the HPC and less Fos expression in the DS than rats trained to learn a response task. We also hypothesized that estradiol would increase hippocampal Fos and/or decrease striatal Fos and enhance place learning rate and impair response learning rate. However, we found that in 4-month-old rats, neither task nor estradiol increased Fos-IR above motor control levels in any subregion analyzed, even though estradiol impaired response learning. In 12-month-olds, estradiol increased Fos-IR in the DG, DMS, and DLS in place task learners, while the absence of estradiol increased Fos-IR in these regions in response task learners. Surprisingly, learning rate was not affected by estradiol in either task. Together, these results suggest that relative hippocampal and striatal activation are not related to learning rate or to the strategy used during spatial navigation learning in females, but that estradiol may enhance activation of hippocampal and dorsal striatal ensembles in response to inputs from common structures including the entorhinal cortex.

Chapter 3

In order to examine an alternate neural mechanism for place and response navigation in female rats, we used catFISH for *Arc* mRNA, which allowed us to visualize the plasticity of individual neurons within neuronal ensembles across multiple behavioral

experiences, to examine the effects of estradiol on the plasticity and reliability of hippocampal and dorsal striatal neuronal ensembles during spatial exploration navigation. Exploration of the maze increased *Arc* mRNA expression over cage control levels in all hippocampal and striatal subregions analyzed except CA3, but estradiol did not increase *Arc* expression alone or during spatial exploration. These results suggest that while exploration induces plasticity in several hippocampal and striatal subregions, estradiol alone does not enhance plasticity.

Neither behavioral training nor estradiol increased *Arc* expression in the DG or DMS above the level induced by exploration alone. All rats with high circulating estradiol that were trained to find a hidden platform rats had increased CA1 *Arc* expression compared to trained rats with low estradiol as well as control rats that simply explored the maze. However, CA1 reliability was only high is rats that had high estradiol levels and used a place strategy. In contrast, when rats with low estradiol used a response strategy, *Arc* expression in the DLS was high and reliable across training and probe testing compared to rats with low estradiol and rats that used a place strategy and high E response strategy users. Thus, the pattern of CA1-DLS plasticity and reliability that predicted the use of each strategy was different when females had low estradiol than when they had high estradiol. Together, these results suggest that estradiol modulates *Arc* expression only during goal-directed behavior and that estradiol may influence spatial navigation strategy use by altering subregion-specific plasticity and reliability in the HPC and DS.

Chapter 4

In order to examine the effects of estradiol on baseline plasticity and reliability as well as on females' attention to environmental cues during exploration, we used catFISH for *Arc* mRNA to examine plasticity and reliability of hippocampal and dorsal striatal neuronal ensembles during passive spatial exploration of the same environment twice or to two environments that differed only in the location of salient landmarks within the same geometric frame. Prior studies with males have found that alteration of cues within a familiar environment causes a decrease in hippocampal reliability (Guzowski et al., 1999; Vazdarjanova and Guzowski, 2004). We found that spatial exploration increased plasticity in all hippocampal and striatal subregions analyzed, but neither estradiol nor landmark cue changes modulated this plasticity. In addition, exploration increased reliability in all subregions examined except the DLS. Together with Ch. 3, these results suggest that estradiol's modulation of hippocampal and striatal plasticity requires goal-directed behavior. These findings also suggest that females' attention to landmark cues during navigation likely requires explicit spatial navigation training or more exposure to the environment.

The findings of the studies in this dissertation suggest that subregions of the HPC and DS have unique roles during spatial navigation, and that multiple patterns of hippocampal-striatal plasticity and reliability are associated with the use of a single strategy. These findings do not support the dogmatic view that greater hippocampal activity/plasticity than striatal activity/plasticity results in efficient use of a place strategy

and the opposite pattern results in efficient use of a response strategy. Rather, our findings suggest that the modulation of these specific subregions by each other and other brain areas, as well as modulatory substances like estradiol, alter the way that females use their brains to use place and response strategies. Therefore, the following discussion focuses on 1) the roles of hippocampal and striatal subregions during navigation, 2) the relationship between navigation strategy use and observed patterns of hippocampal and striatal plasticity and reliability, 3) possible functional mechanisms of navigation strategy use in the low estradiol brain, 4) potential local and distributed effects of estradiol that may contribute to a place strategy bias, and 5) the specificity of estradiol's modulation of hippocampal and striatal function during navigation to goal-directed behavior.

Roles of Hippocampal and Striatal Subregions During Spatial Navigation

We found that navigation task and estradiol modulated the activation of the DG, DMS, and DLS in a similar fashion during spatial navigation learning. The similarities in activation patterns across these specific hippocampal and dorsal striatal subregions suggest that activation measured here may reflect similar input to these subregions rather than their unique roles during spatial navigation. The primary inputs to both the HPC and DS are the entorhinal cortex (EC) and subcortical areas via the fimbria-fornix pathway (as seen in Figure 25), and both of these projections enter the HPC and DS via the DG and DMS, respectively (Andersen et al., 1966b, 1966a; Hjorth-Simonsen, 1972; Hjorth-

Simonsen and Jeune, 1972; Steward and Scoville, 1976; Sorensen and Witter, 1983; Groenewegen et al., 1990). The EC sends information from sensory cortices, as well as top-down modulatory input from the PFC, and subcortical structures including the thalamus, hypothalamus, and ventral striatum (VS) send sensory and modulatory information about factors such as motivation (Lewis et al., 1967; Fonnum, 1970; Lindvall and Bjorklund, 1974; Amaral and Kurz, 1985; Wainer et al., 1985). Because these inputs project primarily to the DG in the HPC and to the DMS, it is likely that the increases in activation in these target regions caused by both exploration and estradiol reflect increased responses to input from this projection pathway. Because the DMS and DLS directly project to one another, it is not surprising that the DLS shows a similar activation pattern to the DMS during navigation.

Interestingly, active exploration, navigation strategy use, and estradiol did not modulate CA3 activation or plasticity beyond that induced by exposure to the testing room. This finding suggests that either CA3 is unnecessary for successful navigation in these water-based tasks or that plasticity induced by exposure to the room was sufficient to support successful navigation behavior. We also found that exploration-induced plasticity in the DG and DMS but neither estradiol nor strategy use modulated this plasticity. Again, these results suggest that the DG and DMS are critical parts of the hippocampal and striatal ensembles needed for spatial navigation (e.g., Devan et al., 1996; Lee and Kesner, 2004; e.g., Goodrich-Hunsaker et al., 2008) and this processing is likely crucial for the subsequent processing in CA1 and DLS that modulate navigation

strategy use, but that plasticity in the DG and DMS is not necessary for the employment of a specific strategy during spatial navigation. In contrast to the other hippocampal and striatal subregions examined, CA1 and DLS plasticity and reliability were altered by estradiol and strategy use across training and probe trials, and specific patterns of CA1-DLS plasticity and reliability predicted place and response strategy use. Together, these results suggest that strategy use is ultimately determined by the plasticity in CA1 and the DLS but that the DG and DMS are also engaged during spatial navigation behavior.

Multiple Patterns of Hippocampal and Dorsal Striatal Plasticity and Reliability Predict the Use of Place and Response Strategies

In this dissertation, we observed that when estradiol levels were low, females were just as likely to use a place strategy as they were to use a response strategy (Ch. 1) on an ambiguous navigation task, and estradiol did not affect learning rate of explicit place and response tasks (Ch. 2). These results were surprising given previous reports that adult females with low estradiol are biased to use a response strategy (Korol et al., 2004). However, they are consistent with one another and suggest that our testing room may be richer with landmark and geometry cues than testing rooms in other laboratories. Thus, in our experiments, under low estradiol conditions (when presumably no other modulatory factors differed between rats), half of the rats defaulted to using a place strategy and half defaulted to using a response strategy. And, in Ch. 1 and other previously published studies, estradiol biased a significant majority of females to use a

place strategy, but some rats still used a response strategy (e.g., Korol et al., 2004; Quinlan et al., 2008). The possible neural mechanisms driving default strategy use and estradiol-induced place strategy bias are considered below.

We observed four distinct patterns of CA1-DLS plasticity and reliability that were associated with the use of place and response navigation strategies, depicted in Figure 24. When estradiol was low, CA1 plasticity and reliability were never increased above exploration levels, so strategy use was always associated with the amount of DLS plasticity and reliability. Rats that used a response strategy also displayed high DLS plasticity and reliability, while rats that used a place strategy also displayed low DLS plasticity and reliability. In contrast, when estradiol levels were high, CA1 plasticity was always increased above exploration levels, and DLS plasticity and reliability were always low. In this case, the amount of CA1 reliability was associated with the use of each strategy. Rats that used a place strategy displayed high reliability in CA1, while rats that used a response strategy displayed low CA1 reliability. These results show that multiple patterns of neural plasticity and reliability were able to predict the use of a single strategy, but these patterns depended on estradiol levels. For example, place strategy users with high estradiol levels displayed a different pattern of CA1-DLS plasticity and reliability than place strategy users with low estradiol. And, there was only one pattern of plasticity/reliability per estradiol state that was associated with the use of each strategy. Thus, it was not case that all place strategy users displayed one pattern of CA1-DLS plasticity and reliability, while all response strategy users displayed another pattern. It

was also not the case that all rats with high estradiol displayed one pattern of plasticity and reliability, while all rats with low estradiol displayed another pattern. Rather, each pattern of CA1-DLS plasticity and reliability was associated with the use of a specific strategy in a specific estradiol state. These results were surprising given that the current hypotheses in the literature suggest that all place strategy users display high activity/plasticity in the HPC and low activity/plasticity in the DS, and vice versa for response strategy users (White and McDonald, 2002; Colombo et al., 2003). Rather, they suggest a complex interaction between the HPC, DS, and inputs from other brain regions during spatial navigation in females that is modified by estradiol.

While the use of place and response strategies was correlated with specific patterns of CA1-DLS plasticity and reliability, these results do not allow us to determine whether the patterns of plasticity and reliability in CA1 and the DLS drive the use of place and response strategies or whether the observed patterns are a consequence of using these navigation strategies. However, previous research has demonstrated that inhibition of hippocampal *Arc* expression via administration of antisense oligonucleotides to the HPC blocks late-phase LTP and the memory consolidation of a goal location learned during a place task (Guzowski et al., 2000; Plath et al., 2006), suggesting that *Arc* expression is required for place navigation rather than a consequence of navigation. And, demonstrations that *Arc* catFISH in CA1 measures the neural activity of place cells required for successful place navigation (Steward et al., 1998; Steward and Worley, 2001a; Steward and Worley, 2001b) suggest that *Arc* expression in CA1 may code for

spatial navigation behavior. In Ch. 3, estradiol state had local, stable effects on plasticity in CA1 and the DLS that occurred regardless of the strategy used during navigation, but these effects appeared to establish that CA1 reliability would determine strategy use (see Figure 24). Together, these results suggest that the patterns of CA1-DLS plasticity and reliability may drive the use of place and response strategies, and that one way that estradiol may bias rats to use a place strategy is by modulating *Arc* expression in CA1 and the DLS during spatial navigation. A model of how estradiol state predicts strategy use is depicted in Figure 24.

One way to test the hypothesis that specific patterns of *Arc* expression in CA1 and the DLS drive the use of place and response strategies would be to experimentally manipulate *Arc* expression in CA1 and the DLS and then assess navigation strategy use. For example, because protein kinase A (PKA) activation is necessary for the expression of *Arc* mRNA (Waltereit et al., 2001), we could block *Arc* transcription locally in CA1 and the DLS by directly administering the PKA inhibitor H-89 to both subregions before spatial navigation behavior. If the pattern of CA1-DLS plasticity and reliability drives spatial navigation strategy use, then rats in this condition, with low CA1 and DLS plasticity and reliability, should use a place strategy (see Figure 24). If we administered H-89 to CA1 but administered brain-derived neurotrophic factor (BDNF), which stimulates *Arc* expression (Zheng et al., 2009), to the DLS, rats should use a response strategy. Together, these results would support the previous studies that have shown that *Arc* expression is necessary for place navigation and suggest that navigation strategy use

is determined by the pattern of plasticity and reliability in CA1 and the DLS. These patterns of reliability and plasticity observed in females in high and low estradiol states can be seen in Figure 24.

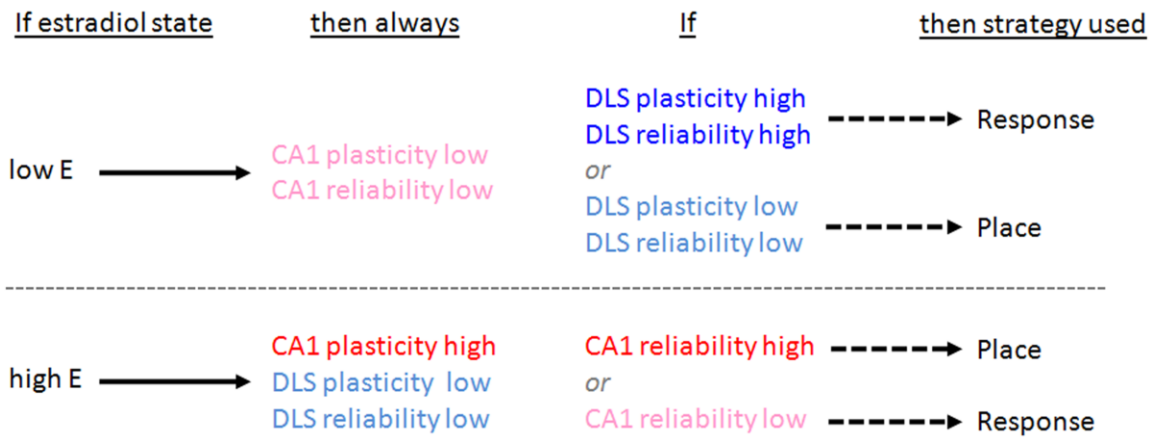

Figure 24: A model of the patterns of CA1-DLS plasticity and reliability that predict the use of place and response strategies.

Possible Top-down and Bottom-up Influences on Hippocampal and Dorsal Striatal Plasticity and Reliability

Our results suggest that when estradiol is low, the strategy that an individual rat defaults to using may be determined by the amount of plasticity and reliability in the DLS, as illustrated in Figure 24. There are two possibilities for why rats in the same hormonal condition might display different patterns of DLS plasticity/reliability that lead to the use of different default strategies: a) natural variation of DLS plasticity/reliability might allow some rats to more usefully engage the DLS during navigation, or b) another brain region could have a top-down influence on DLS plasticity/reliability to influence the rat's default strategy. One likely candidate for top-down modulation of CA1 and DLS ensembles is the prefrontal cortex (PFC). It has been suggested that the PFC serves as the comparator between the outputs of the HPC and DS during spatial navigation, which then determines the strategy that should be used (White and McDonald, 2002). However, PFC lesions impair spatial navigation learning and working memory (McDonald et al., 2008; Kennerley and Wallis, 2009). And, the PFC has been shown to monitor ongoing strategy use and code for strategy switches across learning and during changes of task demands (Ragozzino et al., 1999; Rich and Shapiro, 2007; Rich and Shapiro, 2009), suggesting that it has top-down influences on the function of CA1 and DLS via relatively direct connections through the EC, as depicted in Figure 25. Along with evidence of the PFC's role in decision-making and the organization of behavior more broadly (e.g., Kennerley et al., 2009), its activity during spatial navigation suggests that the PFC may exert top-

down influences to directly modulate hippocampal and striatal plasticity and reliability rather than simply comparing the two outputs and "deciding" between them.

The PFC may constantly try to modulate hippocampal and striatal plasticity so that a particular default strategy is used to solve the task. I hypothesize that when estradiol is low, the PFC is always able to control strategy use by modulating DLS plasticity, and when estradiol is high, estradiol almost always becomes a stronger modulator of strategy use, either via its local effects on CA1 and DLS plasticity and reliability or by modulating the activity of brain regions that project to CA1 and the DLS, ideas that will be explored later in the Discussion. One way that the PFC might influence the reliability of hippocampal and striatal ensembles across trials of a navigation task is to modulate the timing of firing of primary neurons in the HPC and DS that code for spatial and movement information (Koene et al., 2003), which is essential to maintaining temporally and spatially organized neural representations that produce successful navigation (e.g., Tort et al., 2008). High-frequency theta rhythm is extracellular sinusoidal (oscillatory) EEG activity with frequency between 7 and 12 Hz with low amplitude and takes place in both the HPC and DS to modulate the probability of neural firing (Berke et al., 2004; DeCoteau et al., 2007a, 2007b). Theta rhythm is one mechanism that regulates the timing of neural firing within the HPC and DS as they process and integrate incoming sensory, reward, and top-down modulatory information (O'Keefe and Recce, 1993; Berke et al., 2004). It occurs only when the rat is moving through space and during the rapid eye movement phase of sleep, when memory

consolidation is thought to occur (Whishaw and Vanderwolf, 1973; Black, 1975). In the HPC, theta rhythm becomes synchronized with place cell firing (Maurer and McNaughton, 2007) to orchestrate the timing of spatial coding (O'Keefe and Recce, 1993; Gengler et al., 2005). Similarly, theta modulates the timing of neuronal firing in the DS during performance of learned motor sequences (DeCoteau et al., 2007a, 2007b). Consequently, local theta rhythm is important for neural plasticity needed for learning and memory (Winson, 1978; Mehta et al., 1997; Buzsaki, 2002; Mehta et al., 2002; Buzsaki, 2005). For example, when a single burst of high frequency stimulation is applied to hippocampal tissue at the peak of theta oscillation, LTP is induced; stimulation at the trough induces LTD (Pavlides et al., 1988; Huerta and Lisman, 1995; Holscher et al., 1997; Pittenger et al., 2006). Local theta activity in the HPC is NMDA receptor-dependent (Buzsaki, 2002), suggesting that it is involved with many of the same mechanisms of synaptic consolidation for which *Arc* is required, so modulation of theta rhythm in the HPC and DS might be one way that the reliability of *Arc* can be regulated.

High-frequency theta rhythm is generated by subcortical structures including the rostral pontine nucleus and supramammillary area of the hypothalamus and is transmitted to the HPC and DS via the medial septum and EC (Winson, 1978, , 1980; Mizumori et al., 1989), as depicted in Figure 25. Theta rhythm is strongest in CA1 of all hippocampal subregions (Ranck, 1973), and EC input is the strongest modulator of and necessary for theta rhythm in CA1 (Kramis et al., 1975; Buzsaki, 2002). The EC receives theta rhythm from the septum as well as top-down modulatory influences from the PFC, suggesting

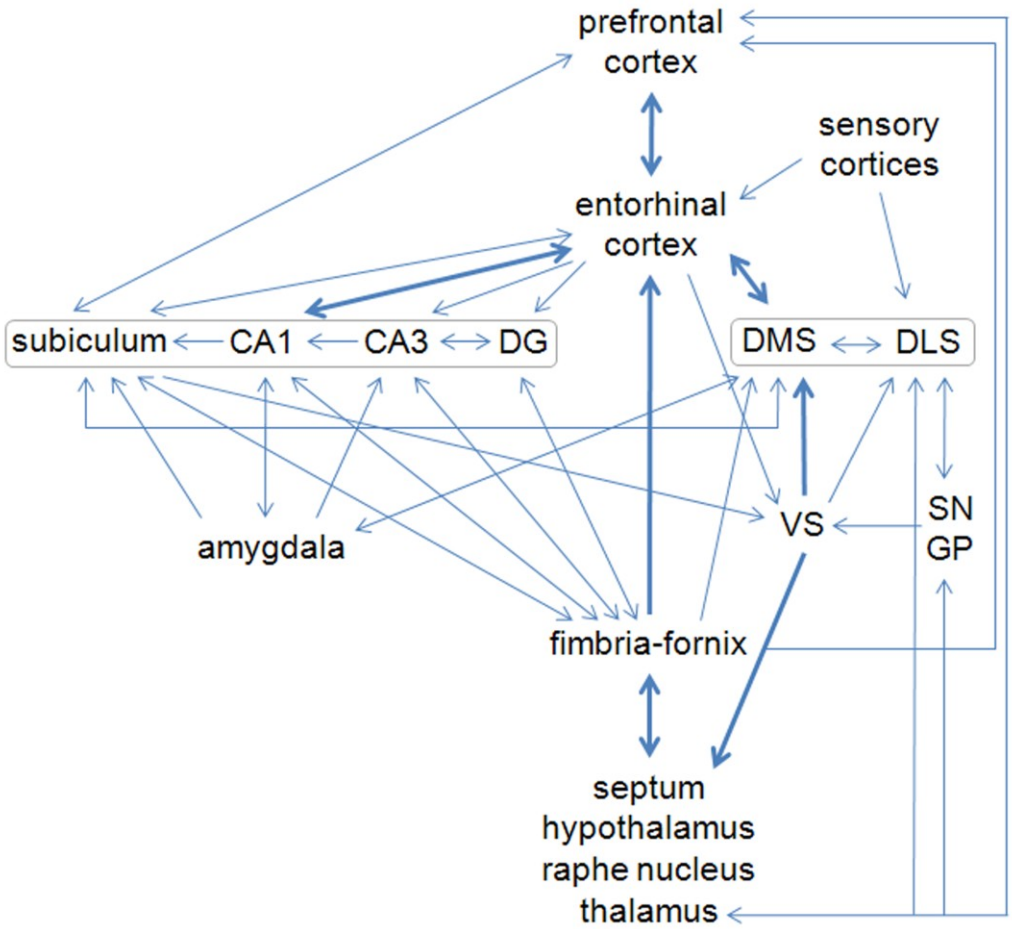

Figure 25: Important circuitry that may contribute to the modulation of spatial navigation strategy use.

that the PFC may modulate theta rhythm before it enters the HPC and DS. Alternatively, the PFC may modulate hippocampal and striatal principle neuron activity in response to theta rhythm directly or indirectly via the EC. By possibly altering the degree to which primary neuronal firing synchronizes with theta rhythm, the PFC may influence the reliability of CA1 and DLS ensembles during spatial navigation to influence the strategy used.

Possible Mechanisms of Estradiol that Alter Plasticity and Reliability in the Hippocampus and Dorsal Striatum

Results from Ch. 3 suggest that estradiol increases plasticity and reliability in CA1 and decreases plasticity and reliability in the DLS. However, it is unclear which of these effects occur locally within these subregions and which might be downstream effects of estradiol's actions in other brain regions that project to the HPC and DS. Given that estrogen receptors (ERs) are distributed broadly throughout the brain and estradiol has been shown to alter many distributed neural functions involved in learning and memory (e.g., Cyr et al., 2000), this section proposes several mechanisms by which estradiol might alter CA1 and DLS plasticity and reliability during spatial navigation, including local effects on plasticity in CA1 and the DLS, effects on the supramammillary area of the hypothalamus to alter theta rhythm projected to CA1, and local response of CA1 primary neuronal firing in response to incoming theta rhythm.

Previously reported modulatory effects of estradiol in CA1 and the DLS support the hypothesis that estradiol locally modulates hippocampal and striatal plasticity during spatial navigation and suggest that estradiol may do so by binding to local ERs to alter NMDA receptor expression and binding (as described in Cyr et al., 2000; Morissette et al., 2008). Estradiol increases NMDA receptor subunit expression and binding in the HPC, particularly in CA1 (Weiland, 1992; Gazzaley et al., 1996; Romeo et al., 2005). And, several NMDA receptor-dependent mechanisms of plasticity are increased by estradiol in the HPC, including spine density (Gould et al., 1990; Woolley et al., 1990; Woolley and McEwen, 1992, , 1993; Murphy and Segal, 1996; Woolley et al., 1997), which has been shown to be correlated with hippocampal-dependent learning (Daniel and Dohanich, 2001; Leuner et al., 2003). Therefore, it is not surprising that *Arc*, which is necessary for NMDA receptor-dependent plasticity (Lyford et al., 1995; Lanahan and Worley, 1998; Plath et al., 2006), was upregulated by estradiol in CA1 during a task that requires NMDA receptor-dependent plasticity, as depicted in Figure 24. Conversely, estradiol suppresses NMDA receptor expression and binding within the DLS (Cyr et al., 2000). Similarly, we observed that when estradiol was high, *Arc* expression in the DLS was not increased by goal-directed spatial navigation above levels induced by exploration (see Figure 24). Thus, the current results are consistent with the hypothesis that NMDA receptor modulation is involved in the effects of estradiol on hippocampal and dorsal striatal plasticity that influence navigation strategy use.

The effects of age on estradiol's modulation of spatial navigation behavior observed in chapters 1 and 2 support the hypothesis that estradiol alters NMDA receptor activity via ERs. In Ch. 1, we showed that estradiol could bias females to use a place strategy by PD26, well before estradiol begins to naturally cycle at the onset of puberty at PD34. This finding suggests that the brain mechanisms for estradiol effects on the HPC and DS are functional well before they are needed in adulthood and is supported by evidence that ER expression and NMDA receptor subunits expressed in adulthood are present in the juvenile brain at adult levels by three weeks of age (Watanabe et al., 1992; O'Keefe et al., 1995; Orikasa et al., 2000; Solum and Handa, 2001; Perez et al., 2003). During natural aging in adulthood, although ER expression declines (Mehra et al., 2005; Yamaguchi-Shima and Yuri, 2007), NMDA receptor expression and binding increase in the HPC (Adams et al., 2001). In Ch. 2, we found that the middle-aged HPC and DS were more responsive to the effects of estradiol than young adult females, suggesting that their increased NMDA receptor expression contributed to this increased neural responsivity. Together, these findings support the hypothesis that estradiol has local effects on hippocampal and striatal plasticity that modulate spatial navigation behavior and suggest that alterations in NMDA receptor expression may contribute to age-related changes in estradiol's effects on spatial navigation behavior.

In addition to local effects of estradiol on hippocampal plasticity during spatial navigation, estradiol might also alter CA1 reliability to contribute to a place strategy bias. Hippocampal theta rhythm (Creutzfeldt et al., 1976; Del Rio-Portilla et al., 1997) and

place cell firing rate (Tropp et al., 2005) have been shown change across the estrous cycle. Therefore, effects of estradiol on CA1 reliability could be due to local mechanisms or actions in one or more regions that project to the HPC. Estradiol may alter theta activity in the subcortical structures where theta rhythm is generated or alter the synchronization of CA1 pyramidal neurons or the local primary neuronal firing with local theta rhythm. The supramammillary area, which is one subcortical structure that generates theta rhythm (Kocsis and Vertes, 1994), has neurons that express ERα (Leranth and Shanabrough, 2001). Administration of estradiol to the supramammillary area increases synapse density in CA1 (Leranth et al., 2000; Leranth and Shanabrough, 2001), suggesting that estradiol may affect the plasticity and reliability of CA1 ensembles via its effects on incoming theta rhythm. Estradiol may also alter the synchronization of pyramidal neuron firing that leads to the synaptic plasticity needed for learning and memory. For example, the probability of LTP induction in CA1 pyramidal neurons by stimulation at high theta frequency is significantly greater when females are primed with estradiol than oil (Cordoba Montoya and Carrer, 1997). Together, these results suggest that estradiol may be able to increase the reliability of CA1 ensembles and bias rats to use a place strategy via bottom up and/or local effects of estradiol in CA1 that interact with top-down modulation by the PFC (see Figure 25). The effects of estradiol may supersede those of top-down influences from the PFC, for example, estradiol's control over theta rhythm in the supramammillary area may be greater than the PFC's top-down modulation of theta activity at the EC and therefore lead to increased CA1 reliability.

One could determine whether potential effects of E on CA1 reliability that contribute to a place strategy bias are local within the HPC or perhaps caused by effects in the supramammillary area of the hypothalamus by locally blocking ERs in CA1 of a rat with high circulating estradiol. If the rat displays low plasticity and reliability in CA1, this would suggest that estradiol effects are local in CA1. However, if the rats still shows high CA1 plasticity and reliability and uses a place strategy, then this suggests that alterations of hippocampal plasticity are only downstream effects of estradiol's actions in another region. Antagonism of ERs in the supramammillary area may abolish the effects of circulating estradiol on CA1 reliability to reveal that locus of these effects is in the region that generates theta rhythm. Together, the evidence provided in this section suggests that estradiol may influence navigation behavior via a) local effects on plasticity in CA1 and the DLS, as well as either b) effects on the supramammillary area of the hypothalamus to alter theta rhythm projected to CA1 or c) local response of CA1 primary neuronal firing in response to incoming theta rhythm. While I consider these three mechanisms the most likely candidates for estradiol modulation of CA1 and DLS plasticity and reliability related to alterations in spatial navigation strategy use, it is possible that spatial navigation behavior feeds back to alter estradiol levels in the brain during navigation behavior. While this has not previously been shown in spatial navigation, it has been demonstrated in male quail and mice during sex behavior. Sexual experience with a female for 5 min excites the preoptic area and stimulates aromatase activity, which in turn leads to greater estradiol levels and more display of sex behavior

(as reviewed in Balthazart et al., 2009). Therefore, it is possible that during spatial navigation, the behavior itself or specific strategy use alters estradiol levels in the brain to alter behavior in the near future.

Goal-directed Behavior is Required for Estradiol's Modulation of Activity and Plasticity During Spatial Navigation

During goal-directed spatial navigation, reward information must be integrated into the representation of the task in order for a rat to learn what actions and locations lead to the most beneficial outcomes (Recce and Harris, 1996; Gerstner and Abbott, 1997; Redish and Touretzky, 1998; Gaussier et al., 2002; Hasselmo et al., 2002; Chavarriaga et al., 2005). In Ch. 3 and Ch. 4, while active and passive spatial exploration increased *Arc* expression, we found no evidence that estradiol modulated hippocampal or striatal *Arc* expression in rats that actively explored the water maze in Ch. 3 or passively explored an open field in Ch. 4 but did not have an explicit goal and were removed from the apparatus at random. Rather, estradiol only modulated *Arc* expression in rats that were explicitly trained to locate a hidden escape platform in the water maze and learned an association between either a spatial location or their physical behavior and the goal of escaping the water. These results suggest that goal-directed behavior may be essential for estradiol's modulation of *Arc* expression in the HPC and DS during navigation. At first glance, it seemed somewhat surprising that estradiol did not modulate hippocampal and striatal plasticity under baseline conditions because estradiol has previously been shown

to modulate activity and a number of mechanisms of plasticity in these regions in the naïve rodent, including dendritic spine density (Creutzfeldt et al., 1976; Cordoba Montoya and Carrer, 1997; Cyr et al., 2000; Rudick and Woolley, 2000). However, this finding is supported by evidence that Arc transcription is only induced above control levels by highly-stimulating behavioral or electrical events (Vazdarjanova et al., 2006).

Because goal-directedness was required for estradiol's effects on IEGs in the HPC and DS, it is likely that input from brain areas that code for reward modulate the ability of estradiol to influence hippocampal and striatal plasticity/reliability and consequent navigation strategy use. Several subcortical structures convey information about reward and motivational state to the HPC and DS via the septum on the fimbria fornix pathway, including the hypothalamus, thalamus, amygdala, ventral tegmental area, substantia nigra, and VS (Mitrano et al., 2010; Martin and Ono, 2000; Reynolds et al., 2001; Ferreira et al., 2008; Jones et al., 2009), as shown in Figure 25. This reward information is also projected to the PFC via the EC, where it is integrated. The PFC conveys this integrated information to the HPC and DS, which may contribute to its top-down influences during spatial navigation (Robertson, 1989; Robertson and Laferriere, 1989; Groenewegen et al., 1990; Kita and Kitai, 1990; McDonald, 1991; Pratt and Mizumori, 2001; Kennerley and Wallis, 2009). This information is also sent to the VS, which also integrates this information with subcortical input and interacts directly and indirectly with the HPC and DS to guide goal-directed navigation (Mogenson et al., 1980; Groenewegen et al., 1982; Cador et al., 1989; Lavoie and Mizumori, 1994; Chavarriaga et al., 2005;

Mizumori et al., 2009). It has not been demonstrated that the VS is specifically involved in the use of either a place or a response strategies. However, the VS is necessary for the encoding of reward properties during acquisition of spatial navigation tasks (Annett et al., 1989; Sutherland and Rodriguez, 1989; Seamans and Phillips, 1994; Gal et al., 1997; Sargolini et al., 2003). And, human neuroimaging data suggest that reward-associated memories are better remembered when the VS is highly activated during encoding (Wittmann et al., 2005; Adcock et al., 2006). Interestingly, midbrain dopaminergic input to the HPC is primarily received at CA1 (Gasbarri et al., 1993), and late-phase LTP in CA1 is highly dependent on this input (Frey et al., 1990). In addition, the DLS receives much more dopaminergic input that the DMS (Haber et al., 2000; Martinez et al., 2003). And, estradiol increases dopamine transporter levels in the VS and dopamine levels in the ventral tegmental area (Chavez et al., 2010; Russo et al., 2003), which is necessary for phasic dopamine release that increases the salience of reward during spatial navigation (Zweifel et al., 2008; Zweifel et al., 2009). Because CA1 and the DLS appear to be most critical for spatial navigation strategy use and are also most modulated by dopaminergic input within the HPC and DS, these data support the hypothesis that the representation of reward contributed by the VS and PFC during spatial navigation may be an important component of estradiol's modulatory influences on spatial navigation strategy use and are themselves modulated by several other subcortical and cortical inputs.

Conclusions and Future Directions

The experiments in this dissertation have revealed marked effects of estradiol and age on hippocampal and striatal activity, plasticity, and reliability, and their relationship to spatial navigation strategy use. While the use of place and response navigation strategies are not related to the relative amount of hippocampal and striatal activation, specific strategy use is associated with particular patterns of CA1-DLS plasticity and reliability that depend on estradiol status. As discussed above, establishing a causal link between patterns of plasticity and reliability in CA1 and the DLS and navigation strategy use is a logical next step that can be accomplished by experimentally manipulating *Arc* expression in these specific hippocampal and striatal subregions and then assessing navigation strategy use. Furthermore, this dissertation shows that estradiol influences several aspects of plasticity and reliability in CA1 and the DLS that may contribute to a place strategy bias. Future studies that systematically block estrogen receptors in these hippocampal and striatal subregions may reveal converging evidence that estradiol's local effects on hippocampal and striatal plasticity during spatial navigation contribute to a place strategy bias. Future work might also examine the proximate mechanisms of this effect, for example the potential role for NMDA receptors in this function, and how these mechanisms change with age.

These studies contribute knowledge about the circuitry involved in the use of place and response strategies during spatial navigation and the functional mechanisms by which estradiol may bias female rats to use a place strategy. However, the effects

observed in these experiments suggest several possible internal and external factors coded by other brain areas that might interact with estradiol in the HPC and DS to influence navigation strategy use. For example, input from the VS and PFC may be integral to successful spatial navigation and estradiol's modulation of navigation strategy use. And, the fact that we found that the pattern of plasticity and reliability across the HPC and DS was related to specific strategy use suggests that one function of a modulatory region, such as the PFC, may be to modulate the interaction between the HPC and DS during spatial navigation.

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BIOGRAPHY

Kristen Elizabeth Pleil was born in Durham, NC on April 1, 1983. She attended Emory University, where she received a Bachelor's of Arts degree in Psychology in May 2005. She attended graduate school at Duke University, where she received a Doctor of Philosophy in May 2010.

Publications and Awards

Pleil KE, Williams CL (2010). The development and stability of estrogen-modulated spatial navigation strategies in female rats. Horm Behav. 57:360-367.

Williams CL, Pleil KE (2008) Toy story: why do monkey and human males prefer trucks? Comment on "Sex differences in rhesus monkey toy preferences parallel those of children" by Hassett, Siebert and Wallen. Horm Behav 54:355-358.

Annie Laurie Aiken fellowship in Neuroscience (2007 and 2009)

Professional Affiliations

Society for Neuroscience (SfN)

Society for Behavioral Neuroendocrinology (SBN)

Duke University Women in Science and Engineering (WISE)

Psi Chi, The National Honor Society in Psychology