December 2017

The Role of Hippocampal and Medial Prefrontal Interactions in the Estrogenic Regulation of Memory

Jennifer Tuscher
University of Wisconsin-Milwaukee

THE ROLE OF HIPPOCAMPAL AND MEDIAL PREFRONTAL INTERACTIONS IN THE ESTROGENIC REGULATION OF MEMORY

by

Jennifer Tuscher

A Dissertation Submitted in

Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

in Psychology

at

The University of Wisconsin-Milwaukee

December 2017

ABSTRACT

THE ROLE OF HIPPOCAMPAL AND MEDIAL PREFRONTAL INTERACTIONS IN THE ESTROGENIC REGULATION OF MEMORY

by

Jennifer Tuscher

The University of Wisconsin-Milwaukee, 2017
Under the Supervision of Dr. Karyn M. Frick

Dendritic spine plasticity is thought to be essential for the formation and storage of memories. The sex-steroid hormone 17β-estradiol (E_2) increases dendritic spine density in 2 brain regions necessary for memory formation, the dorsal hippocampus (DH) and medial prefrontal cortex (mPFC), but the mechanisms through which it does so remain largely unknown. Further, the extent to which these brain regions interact to mediate E_2's effects on memory is also unclear. Recently, we found that infusion of E_2 directly into the DH also increases dendritic spine density in the DH and mPFC, and that these effects depend upon rapid activation of the extracellular signal-regulated kinase (ERK) and mammalian target of rapamycin (mTOR) cell-signaling pathways in the DH (Tuscher et al., 2016). These intriguing findings highlighted a previously unexplored interaction between the DH and mPFC that may have important implications for understanding how E_2 regulates memory. As such, these data led us to question what the role of the mPFC is during object memory formation, and whether interactions between the DH and mPFC are necessary for the E_2-induced memory enhancements we have previously observed in our object memory tasks (Fernandez et al., 2008, Boulware et al., 2013, Fortress et al., 2013).

Therefore the overall goal of the dissertation was to examine the role of the DH and mPFC in object memory consolidation both in the presence and absence of exogenous E_2 infusions, and to examine how E_2 regulates spine density changes in these regions, which may ultimately strengthen the synaptic connections involved in the formation of such memories. To this end, we first utilized inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to inactivate the DH, the mPFC, or both brain regions simultaneously immediately after novel object training to assess the role of each of these regions individually and in combination during object memory consolidation. Next, we asked whether E_2 can act directly in the mPFC to enhance object memory consolidation and increase spine density in the mPFC and DH. Finally, we combined DREADD-mediated inhibition of the mPFC with direct infusion of E_2 into the DH to examine whether DH-mPFC interactions are necessary for the beneficial mnemonic effects of DH infused E_2. Our results collectively suggest that individual and simultaneous activation of both the DH and mPFC is required for the successful consolidation of object recognition and spatial memories. We also found that infusion of E_2 directly into the mPFC increases mPFC apical spine density and facilitates object memory consolidation. Finally, we demonstrate that activation of the mPFC is necessary for the memory-enhancing effects of DH-infused E_2. Together, these studies provide critical insight into how the DH and mPFC work in concert to facilitate E_2-mediated memory enhancement in female mice. Further, this work will enable future studies investigating circuit and cellular-level questions regarding how E_2 mediates cognition across the lifespan.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

CNO: clozapine-N-oxide

DH: dorsal hippocampus

DREADD: designer receptor exclusively activated by designer drug

ERα: estrogen receptor alpha

ERβ: estrogen receptor beta

ERK/MAPK: extracellular-signal-regulated kinase or mitogen-signal regulated kinase

GPER: G protein-coupled estrogen receptor

hM4Di: human muscarinic receptor 4 DREADD

KORD: Kappa opiod receptor DREADD

mTOR: mammalian target of rapamycin

mGluR: metabotropic glutamate receptor

mPFC: medial prefrontal cortex

NMDAR: N-Methyl-D-aspartate receptor

SALB: salvinorin-B

ACKNOWLEDGEMENTS

I would first like to express sincere gratitude to my mentor and advisor, Dr. Karyn Frick, for granting me the opportunity to conduct my graduate studies in her laboratory. Without her guidance none of this work would have been possible, and I am truly grateful for her time, patience, advice, and mentoring throughout the past 6 years. I am also thankful for my committee members, Drs. Fred Helmstetter, Debbie Hannula, Cecelia Hillard, and Sue Lima for their time, advice, patience, and invaluable feedback during my dissertation and throughout my time at UWM. Each of my committee members has shown me, by example, what it means to be an exceptional scientist and mentor. I would like to extend an additional thank you to Dr. Fred Helmstetter for the generous use of his confocal microscope to collect fluorescent images, and to Dr. Jim Moyer for the use of his light microscope to conduct spine counting. I would also like to thank Drs. Maya Frankfurt and Wendy Koss, for their advice for optimizing the golgi staining protocol and spine density analyses used to collect data for this dissertation.

Next, I would like to thank Dr. Ashley Fortress and Dr. Reverend Patrick Cullen for their continuous mentorship, guidance, and friendship during my time at UWM. A specific thank you to Patrick for his training and assistance in using the confocal microscope, which was integral to the completion of my dissertation. I am also grateful to all those whom I have had the pleasure of working with in the UWM department of Psychology, specifically Madalyn Hafebriedel, Elizabeth Doncheck, Hanna Yousuf, Lisa Taxier, Nicole Ferrara and Jaekyoon Kim. My time in Milwaukee would not have been the same without their wise council, stimulating discussions, technical assistance, and moral support. It has been a privilege to work with each of these

individuals. I would also like to thank our undergraduate students Jacquie Haertel and Sarah Philippi, for their assistance with behavior, and Jayson Schalk, for his assistance with spine density counting for this dissertation. I am also truly appreciative for technical assistance provided by Pat Reilly; especially when important equipment malfunctioned. I would also like to thank my parents, for always encouraging me to achieve the goals I set for myself, and for instilling me with a strong work ethic. Finally, thank you to my husband Tyler, for his continuous love and support, endless patience, understanding, and good humor.

CHAPTER ONE: Introduction and Background Information

The role of the hippocampus and prefrontal cortex in memory

Perhaps one of the most well-known historical examples for the role of temporal lobe structures, such as the hippocampus, in memory comes from observations of memory loss in Henry Molaison, more commonly known as patient H.M. In 1953, H.M. underwent a surgical procedure to reduce the frequency and severity of the debilitating seizures from which he often suffered (Squire, 2009). His neurosurgeon, William Scoville, bilaterally removed a sizable portion of tissue from his medial temporal lobes, including his hippocampus. Although the surgery did reduce his seizure activity, H.M. experienced profound anterograde memory impairment, as well as some retrograde memory loss (Schmolck et al., 2002). Several observations of H.M.'s abilities post-surgery provided critical insight into the existence of different types of memory, which are distributed across several brain regions. For example, despite his severe anterograde memory impairment, his skill retention for certain motor tasks (i.e., mirror-drawing) improved across days, even though he had no memory of previously attempting the task. His intact ability to remember facts and recall remote memories from early in life and childhood, suggested that the medial temporal structures removed were likely critical for consolidation of memory, but were not the final repository for long-term memories (Squire and Wixted, 2011). Overall, the observations gave rise to a theory positing two larger classes of memory: declarative and non-declarative. Declarative memory includes conscious knowledge of events (episodic memory) and facts (semantic memory). What we now call declarative memory was largely impaired in H.M., and as such, the involvement of medial

temporal structures removed during his surgery has been the focus of most research on declarative memory. However, his non-declarative memory—which includes skill and habit learning, simple conditioning, priming, and perceptual learning—was largely spared. The contrast between H.M.'s loss of certain types of memory, but not others, formed the basis of our current understanding of memory systems, and supported the notion that different types of memory might be stored in different neural networks. Thus, early observations of patient H.M. created a foundation for future investigations into the structures that subserve different memory systems, and ushered a new era of systems-level experimental research in clinical and preclinical models.

Since the initial insights provided by H.M. a half century ago, a great deal of information has been gleaned from studying the neural circuitry that supports our memory systems, including the prefrontal cortex, amygdala, and hippocampal region. The "hippocampal region" is defined here to include CA fields, subiculum, and dentate gyrus (see Fig. 1; (Squire, 2008; Squire and Wixted, 2011). Three primary pathways are utilized to transfer information into and throughout the hippocampus. Information first enters from the entorhinal cortex and is directed to granule cells in the dentate gyrus via the perforant pathway. Granule cells in the dentate gyrus then relay information through the mossy fiber pathway to the pyramidal cells of CA3. Next, information travels from the CA3 subfield to the CA1 subfield via the Schaffer collateral pathway (Squire, 2008). Finally, information flows out of the hippocampus through the adjacent subiculum. The hippocampal formation and adjacent parahippocampal regions are largely conserved in terms of anatomical and circuit connectivity across most mammalian species (Manns and Eichenbaum, 2006). Together, these structures collaborate and operate in

parallel to support numerous cognitive functions, including the formation and storage of memories.

Figure 1

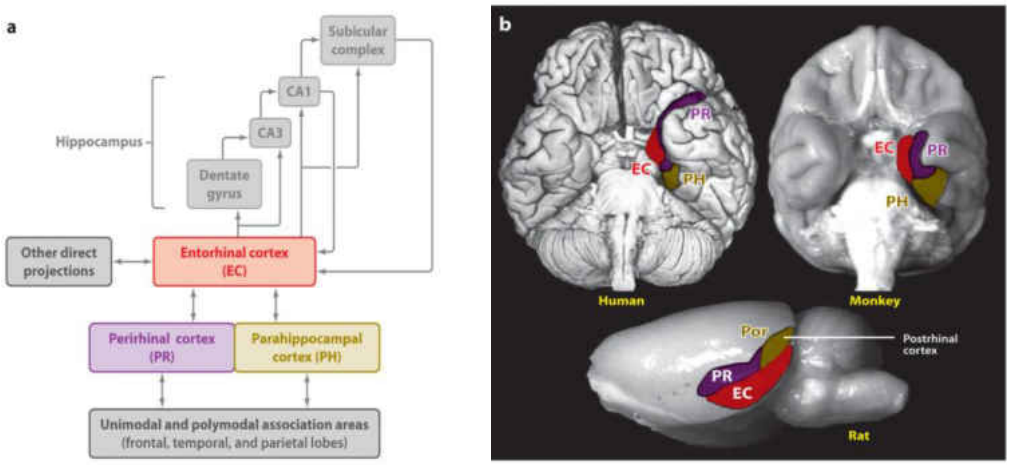


Figure 1. Overview of the medial temporal lobe memory system for declarative memory, which includes the hippocampus and the perirhinal, entorhinal, and parahippocampal cortices. (a) Schematic of the medial temporal lobe memory system. (b) Ventral view of a human brain (upper left), monkey brain (upper right), and a sagittal view of a rat brain (lower center). In humans, the hippocampus lies beneath the cortex of the medial temporal lobe. Its anterior region lies below the posterior entorhinal (shown in red) and perirhinal (shown in purple) cortices, and the main body of the hippocampus lies beneath the parahippocampal cortex. The parahippocampal cortex is also known as the postrhinal cortex in the rat brain. Abbreviations: EC, entorhinal cortex; PH, parahippocampal cortex (shown in brown); Por, postrhinal cortex; PR, perirhinal cortex. Figure from Squire and Wixted, 2011.

In addition to the subiculum, the hippocampus also projects directly and indirectly to the medial prefrontal cortex (mPFC; Jay and Witter, 1991; Burwell and Amaral, 1998; Hoover and Vertes, 2007; Vertes et al., 2007), and these DH-mPFC connections will be discussed in greater detail in the following section (Potential interactions between the hippocampus and mPFC). Evidence suggests that the mPFC, which is composed of functionally distinct regions along its dorsal-ventral gradient, may also contribute to long-term memory formation. The functional distinctions within the mPFC likely arise due to unique afferent and efferent

projections to the dorsal vs. ventral subregions of this structure. For example, ventral mPFC has reciprocal projections with regions involved in adaptive responses to stress or reward, such as the amygdala, striatum, dorsal raphe, ventral tegmental area, and locus coeruleus (Schultz, 2001; Maier and Watkins, 2005; Itoi and Sugimoto, 2010; Kranz et al., 2010). The ventral mPFC also coordinates with the hypothalamus, which regulates homeostatic drives (i.e., hunger, thirst), the endocrine system, and autonomic responses (Gabbott et al., 2005). Therefore, the ventral mPFC is thought to be essential for regulation of autonomic and emotional control. The dorsal mPFC however, has greater connectivity to regions that guide motor function (Gabbott et al., 2005; Hoover and Vertes, 2007) and projects directly to the spinal cord. As such, the dorsal mPFC is thought to be more involved in control of actions. Together, the unique pattern of connectivity for the dorsal and ventral portions of the mPFC suggest this region is poised to receive information sent to the ventral mPFC about rewarding or aversive stimuli within a particular context, which then guides adaptive behavioral responses coordinated by the dorsal mPFC. The mPFC may then interact with the hippocampus to store and retrieve this information as necessary to guide behavioral responses that are advantageous to the organism during future events based on previous experience. Chapter two of this dissertation will focus on the role of the mPFC, as well as its interactions with the dorsal hippocampus (DH), in the formation of object memories.

Potential interactions between the hippocampus and mPFC

How might the hippocampus and mPFC interact to facilitate memory formation? A growing body of evidence supports the existence of direct projections from the DH to the mPFC

(Hoover and Vertes, 2007; Xu and Sudhof, 2023; DeNardo et al., 2015; Ye et al., 2017). Alternatively, there are also data indicating indirect reciprocal connections exist between the DH and mPFC routed through the nucleus reunions of the thalamus or lateral entorhinal cortex (Burwell and Amaral, 1998; Hoover and Vertes, 2007; Vertes et al., 2007). Other anatomical studies examining connectivity between these regions have reported unilateral projections between the ventral hippocampus (VH) and subiculum of the hippocampus to the mPFC (Ferino et al., 1987; Jay et al., 1989; Jay and Witter, 1991; Cenquizca and Swanson, 2007). Evidence for the functional relevance of these connections is supported by electrophysiological studies demonstrating that tetanic stimulation in the ventral CA1/subiculum of anesthetized rats results in stable long-term potentiation (LTP) in prefrontal neurons (Laroche et al., 1990; Jay et al., 1992). Further, LTP between hippocampal and prefrontal synapses leads to a persistent (several day) increase in synaptic strength in awake behaving rats (Jay et al., 1996), suggesting direct excitatory input from the hippocampus to the mPFC.

Behavioral studies aimed at addressing the functional relevance of prefrontal-hippocampal interactions involve disrupting unilateral projections between the hippocampus and mPFC by lesioning the mPFC in one hemisphere and the hippocampus in the contralateral hemisphere. These functional disconnections reportedly impair memory in rodents in the water maze (Wang and Cai, 2008), T-maze (Wang and Cai, 2006), and spatial win shift radial arm maze (Floresco et al., 1997; Goto and Grace, 2008). Other lesion studies in which the hippocampus was disconnected from the mPFC have demonstrated impaired performance in episodic-like memory tasks, such as the object-in-place recognition memory task and temporal order memory task (Barker et al., 2011). However, the same functional disconnection lesion

that impaired memory in the object-in-place and temporal order memory tasks failed to impair object location and object recognition memory (Barker et al., 2011). Taken together, these findings suggest that the DH and mPFC may interact directly to mediate the formation of memory in some episodic memory tasks, but not others. For example, perhaps more complex processing of object information in conjunction with spatial information, or object information in conjunction with temporal information, requires a more direct collaborative effort between DH and mPFC. Alternatively, these findings may suggest that a single lesion targeting the unilateral projections between the hippocampus and mPFC is not sufficient to disrupt memory in all episodic-like tasks, as the brain may be able to compensate by utilizing indirect projections routed through the nucleus reunions, perirhinal or entorhinal cortices (Burwell and Amaral, 1998; Hoover and Vertes, 2007; Vertes et al., 2007). Further, temporary inactivation of these structures (i.e., pharmacological, chemogenetic or optogenetic inhibition) may yield different behavioral results than permanent disruption (i.e., lesions), which would likely also influence initial learning and memory acquisition. Chapter 2 of this dissertation will discuss whether temporary inactivation of the DH alone, mPFC alone, or simultaneous inactivation of both these structures is sufficient to impair object memory consolidation in female mice. Chapter 3 will then expand on these issues to examine the necessity of prefrontal-hippocampal interactions in the estrogenic regulation of memory.

A great deal of research has been dedicated to understanding how external (i.e., behavioral experiences, lesions, pharmacological or genetic manipulations) or internal factors (i.e., changes in growth factors or hormonal milieu) influence the cognitive processes regulated by the hippocampus and the mPFC. In recent years, the sex steroid hormone 17β-estradiol (E_2)

has been shown to play an important role in mediating learning and memory. Although our laboratory and others have discovered much about how E_2 regulates neural plasticity and memory, many important questions remain. Chief among these involve determining the cellular and molecular mechanisms through which E_2 regulates memory formation and storage in the hippocampus, and how the hippocampus may interact with other brain regions such as the prefrontal cortex to support different types of memory. The experiments conducted for this dissertation were designed to address these important issues. To provide context to frame the importance of these questions, the following sections will discuss evidence supporting the role of E_2 in learning and memory at the behavioral and molecular level.

Overview of E_2 across the lifespan

Sex steroid hormones such as E_2 are potent regulators of brain function across the lifespan. They begin exerting their effects on the brain prenatally, where hormone concentrations differ between males and females from the first trimester of gestation (Nelson, 2000). This *in utero* exposure to estrogens guides the organization of sexually dimorphic brain regions involved in regulating reproductive behavior, gonadotropin secretion, and cognitive function in both males and females (Schwarz and McCarthy, 2008). Neural networks 'organized' during this early developmental window can then be 'activated' later in life by another surge of hormones, for example during puberty or adulthood. In females, natural fluctuations in E_2 levels occur across the month-long menstrual cycle in humans, and the 4-5 day estrous cycle in rodents (Long and Evans, 1922). Performance in mnemonic tasks and changes in neural plasticity have been reported to fluctuate along with these normal changes in endogenous hormone levels (Phillips and Sherwin, 1992; Frick and Berger-Sweeney, 2001;

Tuscher et al., 2014), and are also regulated by exogenous E_2 treatment in ovariectomized female rodents (Daniel et al., 2006; Galea et al., 2008; Frick, 2009; Gibbs, 2010; Choleris et al., 2012; Frick, 2012; Luine and Frankfurt, 2012; Maki, 2012), the latter of which will be discussed in greater detail below. Finally, a precipitous loss of sex steroid hormones occurs in females during middle age, and a considerable amount of research has investigated how this hormone loss may contribute to age-related cognitive decline (Foster et al., 2003; Frick, 2009; Daniel and Bohacek, 2010; Rodgers et al., 2010; Smith et al., 2010) and susceptibility to neurodegenerative diseases, such as Alzheimer's disease (Zandi et al., 2002; Li et al., 2012; Long et al., 2012; Li et al., 2014).

E_2 and hippocampal memory

The earliest work to demonstrate that the adult hippocampus is sensitive to ovarian hormones examined how dendritic spine density in the CA1 region of the hippocampus is affected by hormonal fluctuations during the estrous cycle or exogenous administration of E_2 and progesterone (P_4). This work, published in the early 1990s by Bruce McEwen and colleagues, showed that dendritic spine density was 30% higher in the hippocampus during proestrus when E_2 levels peak, relative to estrus, when E_2 levels wane (Woolley et al., 1990). Further, bilateral removal of the ovaries significantly decreased spine synapse density relative to intact females, an effect that could be reversed within hours by acute E_2 treatment alone or E_2 + P_4 (Gould et al., 1990). In addition to dramatic changes in spine number, spine synapses were also increased during proestrus (Woolley and McEwen, 1992) and by exogenous E_2 treatment (Woolley and McEwen, 1993). In the years since, these findings have been replicated and expanded upon by numerous labs (Segal and Murphy, 2001; Li et al., 2004; MacLusky et al.,

2005; Phan et al., 2011; Luine and Frankfurt, 2012; Phan et al., 2012; Phan et al., 2015; Tuscher et al., 2016a). In fact, our own lab recently reported that an infusion of E_2 directly into the DH increases spine density in CA1 30 minutes and 2 hours post-infusion (Fig. 2A-B; Tuscher et al., 2016). Surprisingly, we also found that DH-E_2 infusion significantly increased basal spine density in the mPFC 2 hours after infusion (Fig. 2D-E), but not in other E_2-sensitive regions such as the dentate gyrus or ventromedial nucleus of the hypothalamus (VMN; Fig. 2C and 2F).

Figure 2: DH infusion of E_2 increases spine density in CA1 and the mPFC

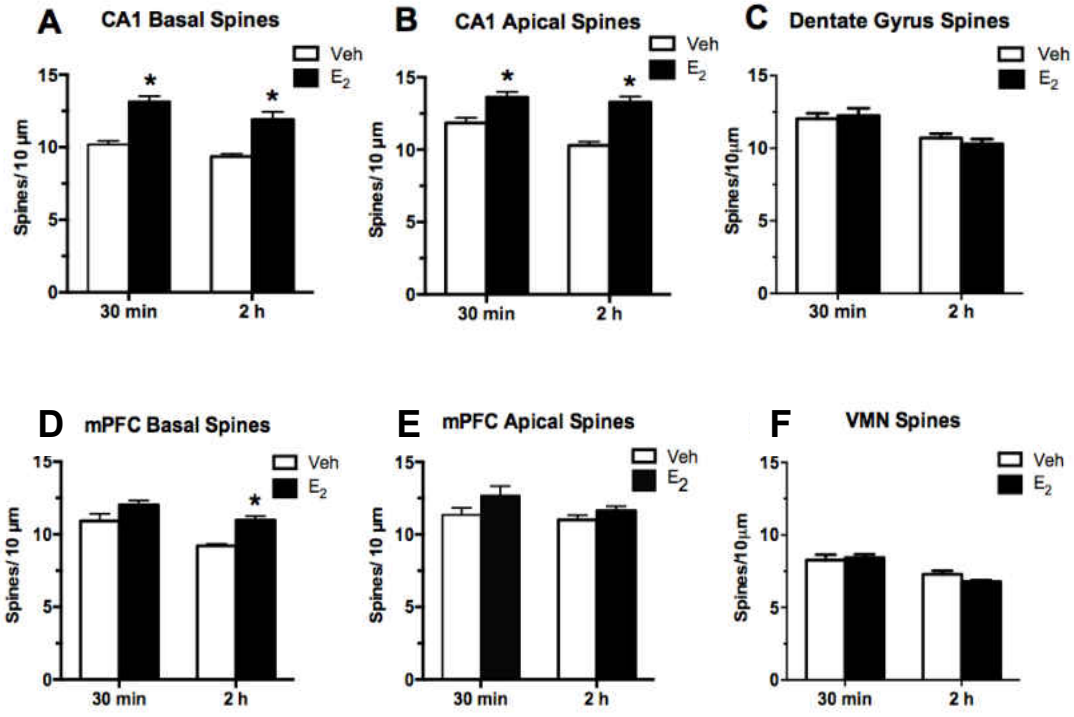


Fig. 2: DH E_2 infusion increases spine density in CA1 and mPFC. Relative to vehicle, basal (A) and apical (B) CA1 spine density were increased 30 min and 2 h after DH infusion of 5 µg/hemisphere E_2. (C) E_2 did not significantly alter dentate gyrus spine density at either time point. In the mPFC, basal spine density was significantly increased relative to vehicle 2 h after DH E_2 infusion (D). E_2 did not significantly alter spine density on mPFC apical dendrites (E) or in the VMN (F) at either time point. Bars represent the mean ± SEM, *$p < 0.05$. Adapted from Tuscher et al., 2016.

In a subsequent experiment, we found that ICV-E_2 infusion also increased spine density in CA1 and the mPFC 2 hours later, and that these increases were blocked by a DH infusion of U0126 or rapamycin, which are inhibitors of the extracellular signal-regulated kinase (ERK) and mammalian target of rapamycin (mTOR) cell signaling pathways (Fig. 3; Tuscher et al., 2016).

Fig. 3: E_2-induced increases in CA1 and mPFC spine density are blocked by ERK or mTOR inhibition in the DH

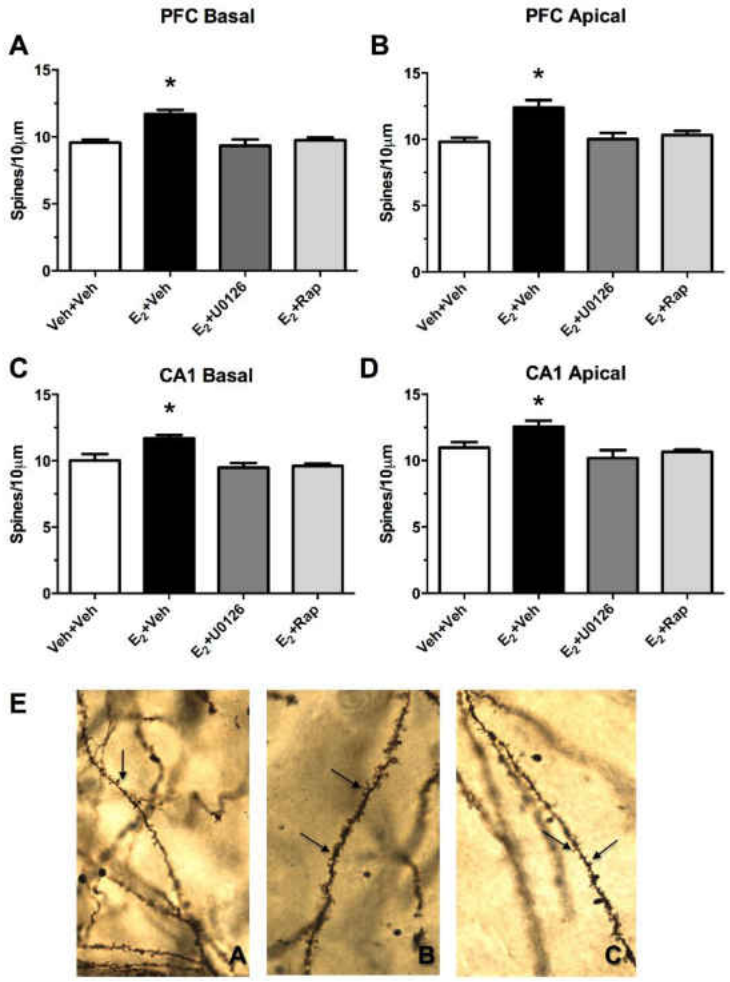


Fig. 3. E_2-induced increases in CA1 and mPFC spine density are blocked by ERK or mTOR inhibition in the DH. Two hours after ICV E_2 infusion, basal and apical spine density was significantly increased in CA1 **(A, B)** and mPFC **(C, D)** relative to vehicle. These effects were blocked by DH infusion of U0126 or rapamycin. Bars represent the mean $\pm$ SEM, $*p < 0.05$ relative to all other groups. **(E)** Photomicrograph of Golgi-impregnated secondary basal dendrites of CA1 pyramidal cells (panel A = vehicle+vehicle, panel B = E_2+vehicle, panel C = E_2+U0126). Arrows denote spines. Under oil 63x. Adapted from Tuscher et al., 2016.

These unexpected findings suggested a potential interaction between the DH and mPFC that may be critical for E_2-mediated memory enhancements previously observed by our lab. These intriguing data, as well as the landmark findings described above, provide evidence that ovarian hormones can rapidly modify synaptic morphology, and laid the foundation for many of the questions addressed in this dissertation.

In the nearly three decades since the initial demonstration that ovarian hormones regulate CA1 dendritic spine density, numerous studies have reported that E_2 can enhance learning and memory in tasks that require the hippocampus (Packard and Teather, 1997; Fader et al., 1998; Daniel et al., 1999; Luine et al., 2003; Daniel, 2006; Daniel et al., 2006; Fernandez et al., 2008; Lewis et al., 2008; Walf et al., 2008; Fan et al., 2010; Zhao et al., 2010); see Daniel et al., 2006 and Tuscher et al., 2014 for review), and this effect has been observed in adults of a variety of species, including songbirds, rodents, non-human primates, and humans (for review see: (Frick, 2009; Bimonte-Nelson et al., 2010; Hammond and Gibbs, 2011; Frick, 2012; Maki, 2012; Schlinger and Remage-Healey, 2012). For example, young ovariectomized rodents treated with exogenous E_2 exhibit enhanced spatial memory in the object placement (OP), Morris water maze, radial arm maze, and T-maze tasks (Daniel et al., 1997; Fader et al., 1998; Luine et al., 1998; Fader et al., 1999; Bimonte et al., 2002; Bowman et al., 2002; Sandstrom and Williams, 2004). E_2 can also facilitate memory in a number of non-spatial tasks, as demonstrated in the object recognition (OR; Fernandez et al., 2008; Boulware et al., 2013; Fortress et al., 2013), social recognition (Phan et al., 2012), inhibitory avoidance (Singh et al., 1994; Rhodes and Frye, 2004), fear conditioning (Chang et al., 2009; Barha et al., 2010; Milad et al., 2010; Zeidan et al., 2011; Lebron-Milad and Milad, 2012), and trace eyeblink conditioning

(Leuner et al., 2004) tasks. Collectively, these studies provide converging evidence that E_2 treatment can benefit hippocampal memory in numerous behavioral tasks. However, it should also be noted that several other factors impact the effects of E_2 on memory, including dose, duration of treatment, age, length of ovarian hormone deprivation prior to treatment, type of memory being tested, timing of administration relative to testing, task difficulty, and reproductive history (Daniel, 2006; Acosta et al., 2009; Frick, 2009; Acosta et al., 2010; Luine, 2014). Nonetheless, the balance of studies supports the conclusion that E_2 facilitates hippocampal memory. The molecular mechanisms through which E_2 exerts these beneficial effects will be discussed in greater detail below in a later section ("Molecular mechanisms through which E_2 impacts memory").

E_2 and prefrontal memory

The effects of E_2 within the mPFC are not as well characterized as those in the hippocampus, however tasks that recruit the mPFC, such as the Morris water maze (Leon et al., 2010), radial arm maze (Maviel et al., 2004), delayed alternation (Wang and Cai, 2006; Izaki et al., 2008), inhibitory avoidance (Holloway and McIntyre, 2011; Zhang et al., 2011), fear conditioning (Runyan et al., 2004; Zhao et al., 2005; Corcoran and Quirk, 2007), and extinction of drug-associated memories (Peters et al., 2008; LaLumiere et al., 2010) are enhanced by systemic E_2 administration (Luine and Rodriguez, 1994; Singh et al., 1994; Daniel et al., 1997; Packard, 1998; Rhodes and Frye, 2004; Zeidan et al., 2011; Twining et al., 2013). Although the systemic route of administration cannot lead to definitive conclusions about E_2 acting directly within the mPFC, electron microscopy has revealed that estrogen receptors (e.g., ERα, ERβ, G

Protein-Coupled Estrogen Receptor; GPER) are expressed throughout the mPFC (Almey et al., 2014). This expression provides a potential mechanism of action through which E_2 could bind and initiate cell-signaling cascades within the mPFC (i.e., ERK and mTOR) that are known to be essential for memory (Atkins et al., 1998; Blum et al., 1999; Fernandez et al., 2008; Hoeffer and Klann, 2010; Fortress et al., 2013). Systemic E_2 also enhances memory in object-based memory tasks (see Luine, 2015 and Tuscher et al., 2014 for review), which some studies suggest requires the involvement of the mPFC (Akirav and Maroun, 2006; Warburton and Brown, 2010, 2015). Studies delivering systemic E_2 immediately after object training have reported concomitant increases in mPFC spine density within 30 min after injection (Inagaki et al., 2012; Luine, 2015), which suggests that changes in prefrontal spine density may contribute to E_2-mediated memory enhancement in these tasks. Additional support for this notion comes from our recently reported observation that DH-infusion of E_2, which enhances object memory consolidation, also leads to an increase in spine density in the mPFC (Tuscher et al., 2016). However, whether these increases in mPFC spine density contribute to the memory-enhancing effects of DH-E_2 infusion remains unknown. Few studies have infused E_2 directly into the mPFC to assess E_2-mediated changes in memory. However, at least one study has shown that E_2 infusion into the mPFC rapidly biases female rats to use a place rather than a response strategy in a modified plus maze task, demonstrating that E_2 can act directly within the mPFC to alter performance in mnemonic tasks (Almey et al., 2014). Similarly, one other study demonstrated E_2infusion into the mPFC improved spatial working memory in the win shift task (Sinopoli et al., 2006). Nevertheless, no one to date has examined whether E_2 acts directly within the mPFC to enhance object memory formation, or whether activation of the mPFC is necessary for E_2

infused into the DH to enhance object memories. Chapter 3 of this dissertation will discuss experiments conducted to address both of these questions.

Molecular mechanisms through which E_2 impacts memory

Classical estrogen receptors and genomic mechanism of action

There are two isoforms of the classical intracellular estrogen receptors (ERs), ERα and ERβ, which are found within the cytoplasm and nucleus of the cell. Localization of ERα, which was the first isoform characterized, dates back to the 1960s, when radioactively labeled E_2 was used to detect it's presence within cell nuclei of a variety of rat tissues (Jensen, 1962b). The original mapping of ERα focused on its distribution in the uterus, mammary glands, pituitary glands, and brain, but later investigations examined expression in specific brain regions (McEwen, 2001), which will be described in greater detail below. It was not until several decades later that ERβ, an ER with a distinct but similar binding affinity to ERα, was discovered in rat uterus (Kuiper et al., 1997). ERα and ERβ have similar ligand-binding domains and affinity for E_2, but regulate different gene targets in a tissue and cell-specific manner (Tee et al., 2004). Outside of the central nervous system, both ERα and ERβ are highly expressed in the ovaries, testes, and uterus, although each receptor has its own unique pattern of distribution. For example, ERα is expressed at moderate to high levels in the pituitary, kidney, epididymis, and adrenals, whereas ERβ is more highly expressed in prostate, lung, and bladder (McEwen, 2001).

Within the central nervous system, many of the brain regions that support memory formation and storage also express these classical intracellular ERs. Both ERα and ERβ have their own distinct patterns of expression in the cerebral cortex, basal forebrain, amygdala,

prefrontal cortex and hippocampus in a variety of species, including mouse, rat, non-human primates, and humans (Shughrue et al., 1997; Osterlund et al., 2000; Shughrue and Merchenthaler, 2000; Shughrue et al., 2000; Milner et al., 2001; Milner et al., 2005). In the basal forebrain, which sends cholinergic projections to the hippocampus and neocortex, both ERα and ERβ are expressed, although ERα is more abundant (Shughrue et al., 2000). ERα and ERβ are also both expressed in the dorsal and ventral hippocampus, predominantly in pyramidal neurons of the CA1 and CA3 subfields, although ERβ is more prevalent than ERα in these subfields (Shughrue and Merchenthaler, 2000). Studies examining the ultrastructural localization of ERs within neurons indicate the presence of ERα in the nuclei and cytoplasm of GABAergic interneurons, and in the cytoplasm of pyramidal and granule cells (Milner et al., 2001). Both receptors are found in dendritic spines and axon terminals of pyramidal neurons, however ERβ is more prevalent at these extranuclear sites (Milner et al., 2001; Milner et al., 2005). In addition, both ERs are also expressed in extranuclear sites (i.e., axons, terminals, dendritic shafts, and spines) in the rat mPFC (Almey et al., 2014), which receives direct and indirect projections from the DH (Hoover and Vertes, 2007; Burwell and Amaral, 1998; Agster and Burwell, 2009; Ye et al., 2017).

Classical intracellular hormone receptors, such as ERα and ERβ, are located within the cytosol and consist of 3 essential domains: the C- terminal (the site for hormone binding), the central domain (which binds DNA), and the N-terminal (which interacts with DNA binding proteins to affect transcription activation) (Nelson, 2000). The classical "genomic" action of E_2 is initiated once the hormone dissociates from a carrier protein at the site of its target tissue, diffuses through the target cell's outer membrane, and binds ERα or ERβ within the cytoplasm

(Nelson, 2000). Once the estrogen-receptor complex is formed, it translocates to the nucleus, where it binds to estrogen response elements on the DNA. Here, the complex acts as a transcription factor, and can initiate the transcription of E_2-sensitive genes that help maintain the neural circuitry that ultimately influences behavior and cognition (Jensen, 1962a; Heldring et al., 2007). Changes in gene expression elicited by such nuclear receptor-hormone interactions, occur slowly (on the scale of hours - days), and are thought to yield long lasting changes.

Membrane ERs and rapid cell-signaling mechanisms

E_2 can also influence cell function in a non-classical manner, by binding to membrane-associated estrogen receptors (mERs; e.g., GPER, Gq-mER;(Srivastava and Evans, 2013), or by interacting with neurotransmitter receptors near the cell membrane (e.g., mGluRs, NMDARs; (Boulware et al., 2005; Lewis et al., 2008; Boulware et al., 2013; Smith et al., 2009), to rapidly activate intracellular signaling pathways on the order of seconds to minutes (Gillies and McArthur, 2010). Although these mechanisms are often referred to as "non-genomic", this designation should be thought of as way to distinguish a separate mode of action of membrane-associated ERs from classical nuclear receptor activation, and should not be taken literally, as activation of mERs can ultimately influence gene transcription. For example, E_2-induced activation of membrane receptors rapidly initiates cell-signaling cascades like ERK and PI3K (Fernandez et al., 2008; Fan et al., 2010), whose downstream effects result in activation of transcription factors like CREB (Wade and Dorsa, 2003; Boulware et al., 2005). Rapid activation of cell-signaling cascades such as ERK also induce post-translational epigenetic modifications

such as histone acetylation and DNA methylation (Zhao et al., 2010), and initiate mTOR-mediated protein synthesis and spinogenesis (Fortress et al., 2013; Tuscher et al., 2016a). Therefore, binding of these non-classical receptors can still ultimately result in modified expression of genes important for neural plasticity and cognition.

Studies investigating the rapid cell-signaling mechanisms initiated by E_2 have focused primarily on their actions within the DH. Relatively few studies have investigated the mnemonic effects of E_2 in the mPFC, and even fewer have examined whether this region might collaborate with the DH to enhance the consolidation of object memories. This relatively novel area of neuroendocrinology research was the focus of this dissertation, and data collected to address each of these issues will be described in greater detail in Chapters 2 and 3.

DREADDS: Using a chemogenetic approach to examine prefrontal-hippocampal interactions in the E_2-mediated enhancement of object memory formation

The chemogenetic approach known as DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) was used in several experiments described in this dissertation to inactivate the DH, the mPFC, or both structures to examine their roles in object memory formation. This technique utilizes an adeno-associated virus (Saavedra et al.) host to deliver a mutated human G-coupled muscarinic (e.g., hM4, hM3) receptor into neurons, which can lead to net suppression of neuronal firing (hM4Di), or induce burst neuronal firing (hM3Dq; Fig. 4) (Armbruster et al., 2007). Point mutations in the muscarinic receptor prevent binding of its normal endogenous ligand, acetylcholine. Although originally thought to be activated exclusively by the designer synthetic ligand clozapine-N-oxide (CNO), recent evidence has

emerged indicating that DREADD receptors are instead activated by clozapine, which is rapidly metabolized from CNO *in vivo* (Gomez et al., 2017). Once bound by clozapine converted CNO, the hM4Di receptors inhibit intracellular adenylate cyclase signaling, and activate G-coupled inward rectifying K^+ (GIRK) channels to hyperpolarize the cell, resulting in net suppression of excitatory neurotransmission (Farrell and Roth, 2013). Conversely, hM3Dq receptors increase Ca^{2+} influx, leading to depolarization and, thus, an increase in neuronal firing (Farrell and Roth, 2013). In addition to mutated muscarinic DREADD receptors, a new G_i-coupled Kappa-opioid receptor DREADD (KORD) has recently been developed to allow for multiplexing of behavior. Similar to muscarinic-based DREADDs, the modified inhibitory KORD receptor is no longer activated by its natural endogenous ligand (i.e., opioids), and is instead exclusively activated by the synthetic ligand salvinorin B (SALB; Fig. 4). SALB is posited to be otherwise pharmacologically inert, and does

Figure 4.

Designer **R**eceptors **E**xclusively **A**ctivated by **D**esigner **D**rugs

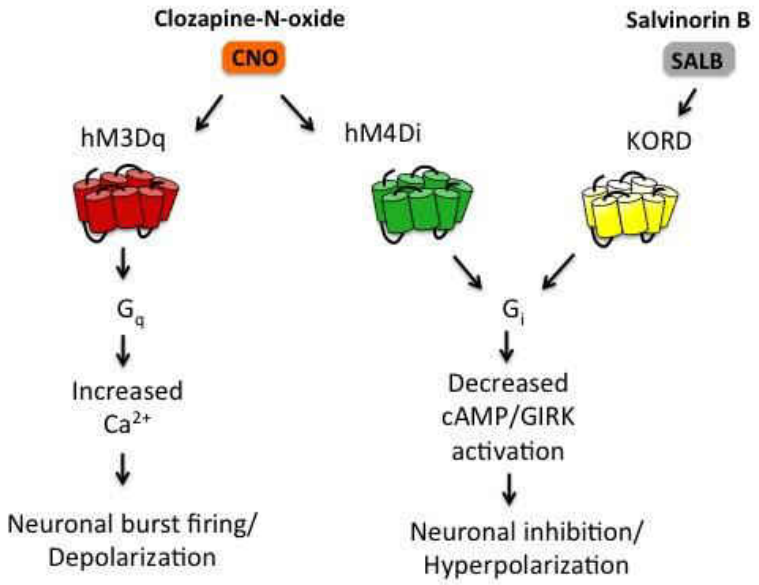


Fig. 4: General overview of DREADD- and KORD-mediated effects on neuronal activity. Clozapine-N-oxide can act on either the mutated G_q-coupled M3 receptor (hM3Dq) to induce burst firing in neurons, or the G_i-coupled M4 receptor (hM4Di) to suppress neuronal firing. Ligand Salvinorin B binds the Kappa-opiod receptor DREADD (KORD), which also inhibits neuronal activity.

not act on other endogenously expressed receptors (Vardy et al., 2015). Thus, SALB-activated DREADDs can be paired with CNO-activated DREADDs to bidirectionally modulate behavior in the same animal, or discretely manipulate different structures within a circuit in the same animal at different points in time. Experiments described in the following chapters utilized both CNO- and SALB-activated DREADDs to investigate the role of the DH, the mPFC, and their interactions during E_2-mediated enhancement of object memory formation. The specific details of the experimental designs used to address these questions will be outlined in the methods section of each chapter.

Summary and significance

E_2 is an essential modulator of cognitive function across the lifespan. It exerts numerous beneficial effects on brain regions that regulate learning and memory, including the prefrontal cortex and hippocampus. Despite decades of neuroendocrinology research on E_2's beneficial mnemonic effects, many important questions remain. The overall goal of this dissertation was to gain a better understanding of the role of the DH and mPFC in the formation of object memory consolidation both in the presence and absence of exogenous E_2 infusions, and to examine how E_2 regulates spine density changes in these regions, which may ultimately strengthen the synaptic connections involved in the formation and storage of such memories. The DH and mPFC were the focus of this research because these brain regions are critical for many types of learning and memory, their function is modulated by E_2, and these regions deteriorate significantly in aging and AD. First, we utilized a chemogenetic approach to examine the necessity of the DH, the mPFC, and potential DH-mPFC interactions during object

memory formation in female mice. We posited that disruption of neural activity in either brain region alone, or both of these regions in unison, would impair object memory consolidation. The experimental approach, methods, and results for this series of experiments will be discussed in Chapter 2. In Chapter 3, we asked whether E_2 can act directly in the mPFC to enhance object memory formation and increase spine density, and whether DH-mPFC interactions are necessary for the memory-enhancing effects of E_2 infused into the DH. We hypothesized that E_2 would act directly in the mPFC to enhance memory consolidation and increase spine density in the mPFC, and that mPFC activation would be necessary for the memory-enhancing effects of E_2 infused into the DH. Our results suggest that both the DH and mPFC, as well as simultaneous activation of these brain regions, is necessary for the successful consolidation of object recognition and spatial memories. We also found that infusion of E_2 directly into the mPFC increases mPFC apical spine density and facilitates object memory consolidation. Finally, we demonstrate that activation of the mPFC is necessary for the memory-enhancing effects of DH-infused E_2. Together, the experiments described herein provide much needed insight into the role of the mPFC and it's interactions with the DH during E_2-mediated memory enhancement in females. Further, this work will enable subsequent studies investigating the molecular mechanisms through which E_2 mediates cognition in these brain regions across the female lifespan.

CHAPTER TWO: The role of the dorsal hippocampus, medial prefrontal cortex, and their interactions in object recognition memory formation

INTRODUCTION

Memory formation for a particular event or episode involves the integration of information regarding *what* was encountered, *when* it happened, and *where* the encounter occurred. The acquisition of such information for episodic memories, as well as the successful consolidation of, and subsequent ability to retrieve, this information requires coordinated effort between the hippocampus and prefrontal cortex. In humans, episodic memory is impaired during the course of normal aging (Shing et al., 2010; Tulving et al., 1983), in certain neurodegenerative disorders such as AD and PD (Dubois et al., 2007; Williams-Gray et al., 2006), and in psychiatric disorders such as depression and PTSD (Dere et al., 2010; Kleim and Ehlers, 2008; McNally 2006; Williams et al., 2007; Moore and Zoellner, 2007). Given the substantial public health implications of these disorders and limited therapeutic options currently available, it is of great interest and relevance to define the neurobiological basis of episodic memory formation. Mechanistic approaches for studying episodic memory are not feasible in humans, therefore rodents provide a useful model for studying the functional connectivity and subcellular properties of the neuronal populations that support memory formation.

Numerous species, including rodents, can encode and store episodic-like memories. Increasingly, object recognition (OR) and object placement (OP) tasks have been used to model

what (i.e., an object) and where (i.e., context or location within the behavioral arena) components of memory in rodents (Eichenbaum, 2017; Barker et al., 2017, Ennaceur, 2010; Dere et al., 2005; Ennaceur and Delacour, 1988). As such, OR and OP are useful behavioral approaches for identifying the functional circuitry that supports episodic memory formation, as well as the cellular and molecular processes within each structure that subserve the circuit. Further, these behavioral tasks allow for the measurement of multiple types of memory without the confounds inherent to behavioral paradigms that involve appetitively or aversely motivating stimuli (McGaugh, 1989).

Interactions between the hippocampus and medial prefrontal cortex (mPFC) have been implicated in episodic-like memory (Warburton and Brown, 2015) and delayed spatial working memory (Churchwell and Kesner, 2011) tasks, but the specific role of mPFC alone, and its interactions with the dorsal portion of the hippocampus during OR and OP memory formation remains controversial. For example, some data suggest that mPFC activation is required for spatial object tasks, such as OP, but not for OR or temporal order object tasks (DeVito and Eichenbaum, 2010). Yet others have reported that mPFC inactivation after object training does impair OR memory consolidation (Akirav and Maroun, 2006). Behavioral studies aimed at addressing the functional relevance of prefrontal-hippocampal interactions often involve a "functional disconnection" approach, which uses lesions in the mPFC and hippocampus to disrupt either ispi- or contralateral projections between the two structures (Barker et al., 2017; Barker and Warburton, 2011, Floresco et al., 1997; Wang and Cai, 2008). One study using this functional disconnection approach reported impaired performance in certain episodic-like memory tasks, such as the object-in-place recognition memory task and the temporal order

memory task, but not in object location and object recognition tasks (Barker et al., 2011). These findings suggest that a single lesion targeting the unilateral projections between the hippocampus and mPFC may not be sufficient to disrupt memory in all episodic-like tasks, as the brain may be able to compensate by utilizing indirect projections routed through the nucleus reunions or entorhinal cortex to maintain hippocampal-prefrontal communication (Burwell and Amaral, 1998; Hoover and Vertes, 2007; Vertes et al., 2007). Further, temporary inactivation of these structures (i.e., pharmacological or chemogenetic inhibition) may yield different behavioral results than permanent disruption (i.e., lesions).

In the present study, we utilized the chemogenetic approach known as DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) to examine whether temporary inactivation of the dorsal hippocampus (DH) alone, mPFC alone, or simultaneous inactivation of both these structures disrupts episodic memory formation in female mice. This technique utilizes an adeno-associated virus (Saavedra et al.) host to deliver a mutated human G_i-coupled muscarinic receptor (hM4-DREADD; hM4Di) or kappa opioid receptor (KOR-DREADD; KORD) into neurons, which leads to net suppression of neuronal firing once these receptors are bound by their respective synthetic ligands (e.g., clozapine-n-oxide, CNO; salvinorin-B, SALB; Armbruster et al., 2007; Vardy et al., 2015). We report that hM4Di-mediated inhibition of the DH before or immediately after training impairs spatial, but not object recognition, memory. In a subsequent experiment, we utilized a multiplexed approach with hM4Di injected into the mPFC and KORD injected into the DH. We found that hM4Di-mediated inhibition of the mPFC, or KORD-mediated inhibition of the DH, were each sufficient to impair spatial and object recognition memory. Finally, we found that simultaneous subthreshold suppression of neural

activity in both the mPFC and DH was sufficient to disrupt object memory formation, suggesting that both regions coordinate to regulate OR and OP memory consolidation. These findings provide new insight into the neural circuitry that supports episodic memory formation, a type of memory whose function is compromised during aging and in numerous neuropsychiatric diseases.

MATERIALS AND METHODS

Subjects. Our previous work in ovariectomized female mice demonstrated that a bilateral dorsal hippocampal infusion of a memory-enhancing dose of 17β-estradiol increases dendritic spine density in both the DH and mPFC (Tuscher et al., 2016), suggesting potentially important interactions between the DH and mPFC in mediating memory consolidation. To maintain consistency with our previous work, all experiments used young (9-12 week-old) female C57BL/6 mice (Taconic, Cambridge City, IN) who were ovariectomized as described previously (Tuscher et al., 2016; Kim et al., 2016). Mice were housed in groups of up to 5 until surgery, after which they were singly housed. Mice were maintained on a 12 h light/dark cycle with ad libitum access to food and water. All experimental protocols and procedures were approved by the University of Wisconsin-Milwaukee Institutional Animal Care and Use Committee and are in accordance with National Institutes of Health guidelines or Guide for the Care and use of Laboratory Animals.

Surgery

General. Surgeries were conducted at least 3 weeks prior to behavioral testing. Mice were anesthetized with isoflurane (5% for induction, 2% for maintenance) in 100% oxygen and placed

in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Female mice were ovariectomized and injected with virus during the same surgical session.

DH DREADD surgeries. Following ovariectomy, an incision was made in the scalp and small perforations were made in the skull with a 26 ½ GA needle to create an opening for bilateral injection of virus into the DH using a 10-µl Hamilton syringe and metal needle (Hamilton, Reno, NV). For our first experiment, hM4Di virus (AAV-CamKIIα-HA-hM4Di-IRES-mCitrine, $2.1x10^{12}$ particles/ml, serotype 8, UNC Vector Core, Chapel Hill, NC), eGFP control virus (AAV-CamKIIα-eGFP, $2.1x10^{12}$ particles/ml, serotype 8, UNC Vector Core, Chapel Hill, NC), or saline was injected into the DH (-1.7 mm AP, ±1.5 mm ML, -2.3 mm DV; 1.2 µl/hemisphere). Injection volume and flow rate were controlled by a syringe pump (KD Scientific, Holliston, MA). The Hamilton syringe was first lowered to -2.3 mm ventral to the surface of the skull and held in place for two minutes to create a pocket for the first viral infusion. Three 0.4 µl injections were delivered per hemisphere, one at -2.2 mm, one at –2.1 mm, and one at -1.9 mm DV. The Hamilton syringe was left in place 2 minutes after each injection to allow for diffusion of the virus, and was then slowly retracted before the process was repeated in the contralateral hemisphere. Mice received carprofen MediGel one day prior to surgery, as well as a s.c. injection of 5 mg/kg Rimadyl at the completion of surgery. Mice were allowed a minimum of 3 weeks for the virus to express and for surgical recovery prior to behavioral testing.

Double DREADD surgeries. For double DREADD surgeries, 2 types of inhibitory DREADDs were used (e.g., hM4Di, KORD), each activated by a unique ligand, to examine the role of the mPFC,

the DH, and their interactions during memory formation. For virus injections into the mPFC, the same hM4Di DREADD described above, eGFP control virus, or saline was injected into the mPFC (1.8 mm AP, ±0.3 mm ML, -2.7 mm DV). mPFC virus injections occured at the same rate as described for the DH (0.4 µl/2 min), however only 0.8 µl total was delivered per hemisphere (two 0.4 µl injections, one at -2.7 mm DV, one at -2.4 mm DV). These infusions targeted both the prelimbic and infralimbic regions of the mPFC. In the mPFC, injections were separated by 8 minutes to allow for diffusion of the virus. During the same surgical session, mice were also bilaterally injected with an inhibitory KORD virus (AAV-CamKIIα-HA-KORD-IRES-mCitrine, $2.1x10^{12}$ particles/ml, serotype 8, UNC Vector Core), eGFP control virus (as described above), or saline into the DH (-1.7 mm AP, ±1.5 mm ML, -2.3 mm DV; 1.2 µl/hemisphere). This viral construct also targets the CaMKIIα promoter, and similar to the hM4Di DREADD, can be used to effectively suppress excitatory neurotransmission (Vardy et al., 2015). Unlike the hM4-DREADD, the KORD-DREADD is activated by the synthetic ligand Salvinorin B (SALB), and can therefore be used for multiplexed modulation of behavior with CNO-activated DREADDs (Vardy et al., 2015). Thus, the use of both DREADDs permits determination of whether activation of mPFC alone, DH alone, or both mPFC and DH simultaneously is critical for memory formation in the same set of mice. Mice received carprofen MediGel 1 day prior to surgery, as well as a s.c. injection of 5 mg/kg Rimadyl at the completion of surgery, and were allowed a minimum of 3 weeks for the virus to express and for surgical recovery prior to behavioral testing.

Drugs and Infusions. Stock solutions of CNO and SALB (Cayman Chemical, Ann Arbor, MI) were dissolved in 100% dimethyl sulfoxide (DMSO; Fisher Scientific, Pittsburgh, PA) at a

concentration of 100 mg/ml, and stored in 10 µl aliquots at -20 ^{o}C. On the day injections were administered, CNO stock was thawed and diluted to a concentration of 1 or 2 mg/ml in a solution of sterile 0.9% saline containing 2% DMSO. SALB stock was thawed and diluted in 100% DMSO to a concentration of 5 or 10 mg/ml.

Behavioral Testing. Object recognition (OR) and object placement (OP) were used to measure object recognition and spatial memory as we have described previously (Boulware et al., 2013; Fortress et al., 2013; Kim, 2016). Previous work from our laboratory (Gresack and Frick, 2006; Fernandez et al., 2008) and others (Luine et al., 2003; Li et al., 2004; Walf et al., 2008) has established that each of these tasks involves the DH (see Tuscher et al., 2015 for review). Three weeks after surgery, mice were handled for 1 minute/day for 3 days prior to habituation. After the first day of handling, a Lego was placed in each home cage to habituate the mice to objects during the remaining handling days and habituation period (Fig. 5). After 3 days of handling, mice were habituated to the behavioral apparatus for 2 consecutive days by allowing them to explore the empty white arena (60 cm x 60 cm x 47 cm) for 5 minutes/day. For the OR task (Fig. 5A), mice are required to accumulate 30 seconds exploring 2 identical objects placed 5 cm from the upper left and right corners of the arena during the training phase. Mice that did not accumulate a total of 30 seconds exploring the objects during training were not infused or injected, and were not included in testing. Either 30 minutes prior to or immediately after training, mice were injected i.p. with CNO, SALB, or both in two separate syringes. Pre-training injections were used first to examine the effects of DREADD-mediated inhibition on memory acquisition and consolidation. Post-training injections were next used to pinpoint the effects of

DREADD-mediated inactivation specifically to the memory consolidation period, while minimizing potential confounding effects on performance factors (e.g., motivation, anxiety) during training or retention testing (McGaugh, 1989; Frick and Gresack, 2003). OR memory was then tested 24 hours later by measuring the amount of time spent with the novel and familiar object. Intact OR memory consolidation is demonstrated if the mice spend more time than chance (15 seconds) with the novel object during testing. At the 24-hour time point, vehicle-infused ovariectomized females show intact object recognition (Fortress et al., 2013, Boulware et al., 2013), thereby permitting observation of the potential memory-impairing effects of DREADD-mediated inactivation.

Fig. 5: Overview of the object memory testing protocols

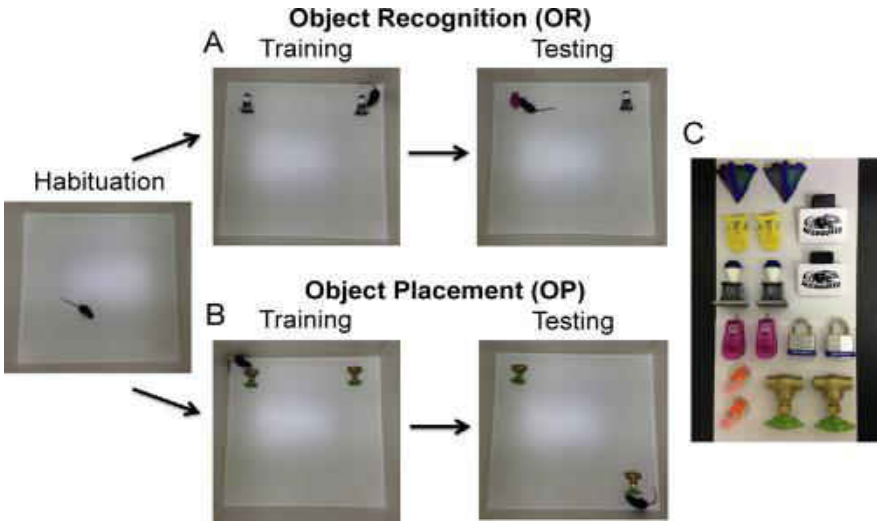


Fig. 5. Overview of object memory testing protocols. Mice are first habituated to an empty arena prior to beginning behavioral training (habituation). **(A)** In object recognition (OR), mice are then allowed to explore two identical novel objects placed in the arena (training). Finally, retention is tested by presenting mice with one novel and one familiar object (testing). Mice who remember the familiar object from training spend more time exploring the novel object relative to the familiar object or to chance (15 sec). **(B)** Object placement (OP) uses the same apparatus and general procedure, but during testing, one training object moves to a new location in the arena, rather than being replaced with a new object. Mice who remember the object in the familiar location spend more time exploring the moved object relative to the unmoved object or to chance. **(C)** Object pairs used in our laboratory's OR and OP protocols.

Training and testing for OP was identical to OR, except that testing was conducted 4 hours after training, and involved moving one of the identical training objects to a new location in the arena (lower right or lower left corner) during testing (Fig. 5B). Intact spatial memory was demonstrated if mice spent more time than chance with the moved object. At the 4-hour delay, vehicle-infused ovariectomized females show intact OP memory (Boulware et al., 2013; Kim et al., 2016), which allowed any DREADD-mediated spatial memory impairments to be observed. All mice were trained and tested in both behavioral tasks. To counterbalance the order in which behavior was completed, half of the mice completed OR first, followed by OP, and the other half completed OP first, followed by OR. OR and OP training were separated by one week, and mice were trained with a unique set of objects for each task.

Histological verification of DREADD expression. Histology was performed to verify expression of hM4Di and KORD in the mPFC and DH, respectively. Three weeks after surgery, mice (n=3/group) were anesthetized with isoflurane and perfused with 4% paraformaldehyde (PFA) in 1X PBS. Whole mouse brains were then removed and post-fixed in 1X PBS/4% PFA overnight, followed by dehydration in a 1X PBS/30% sucrose solution until brains sank. Tissue was then sectioned on a cryostat (40 µm) and free-floated in 1X PBS until mounted onto microscope slides (VWR, Arlington Heights, IL) using aqueous mounting medium containing the nuclear stain DAPI. Fluorescent images were captured using an Olympus Fluoview FV1200 confocal microscope and accompanying software.

Data analysis. All statistical analyses were conducted using GraphPad Prism 6 (La Jolla, CA). To determine whether each group demonstrated intact memory for each behavioral task, OR and OP data were first analyzed using one sample *t*-tests to determine if the time spent with the novel or moved object differed significantly from chance (15 seconds; Kim et al., 2016, Fortress et al., 2013, Boulware et al., 2013). This analysis was used because time spent with the objects is not independent; time spent with 1 object reduces time spent with the other object (Frick and Gresack, 2003). Student's *t* tests were then used to determine significant differences in performance between control and DREADD mice. Statistical significance for all analyses was determined as $p \leq 0.05$.

RESULTS

hM4Di-mediated inhibition of the DH impairs OP but not OR memory

Three weeks after surgery, brain tissue was collected from a subset of mice to verify eGFP and hM4Di expression in the DH at the initiation of behavioral testing (Fig. 6A-D). To test whether hM4Di-mediated inactivation of the DH impairs OP and OR memory formation, mice injected with saline, eGFP, or hM4Di into the DH received 2 mg/kg of CNO i.p. 30 minutes before OP or OR training (Fig. 7A&B). OP memory was tested 4 hours after training. Because Sham and eGFP controls did not differ, they were combined into a single control group and compared to the hM4Di group. Sham and eGFP mice administered 2 mg/kg CNO 30 minutes prior to training spent significantly more time than chance exploring the displaced object during OP testing (Sham+eGFP: $t_{(12)} = 5.80$, $p < 0.0001$; Fig. 7C), demonstrating intact spatial memory and suggesting that 2 mg/kg CNO is a behaviorally-ineffective subthreshold dose that does not

impair OP memory on its own. However, hM4Di mice did not spend significantly more time than chance with the displaced object (hM4Di: $t_{(8)}$ = 0.09, p = 0.93; Fig. 7C), suggesting that spatial memory was impaired by hM4Di-mediated inhibition of the DH. Mice expressing hM4Di in the DH also spent significantly less time with the moved object than control mice ($t_{(20)}$ = 3.24, p = 004; Fig. 7C), providing further evidence that spatial memory was impaired by DREADD-mediated suppression of the DH.

OR memory was evaluated 24 hours after training. In contrast to the OP task, Sham, eGFP, and hM4Di mice all spent significantly more time than chance with the novel object during testing (Sham+eGFP: $t_{(10)}$ = 4.39, p = 0.001; hM4Di: $t_{(6)}$ = 3.41, p < 0.01; Fig. 7D), suggesting that all groups had intact object recognition memory. Together, these data suggest that hM4Di-mediated inhibition of the DH, as driven by 2 m/kg CNO, impairs spatial memory but not object recognition memory.

Fig. 6: hM4Di and eGFP expression in the DH 3 weeks after injection

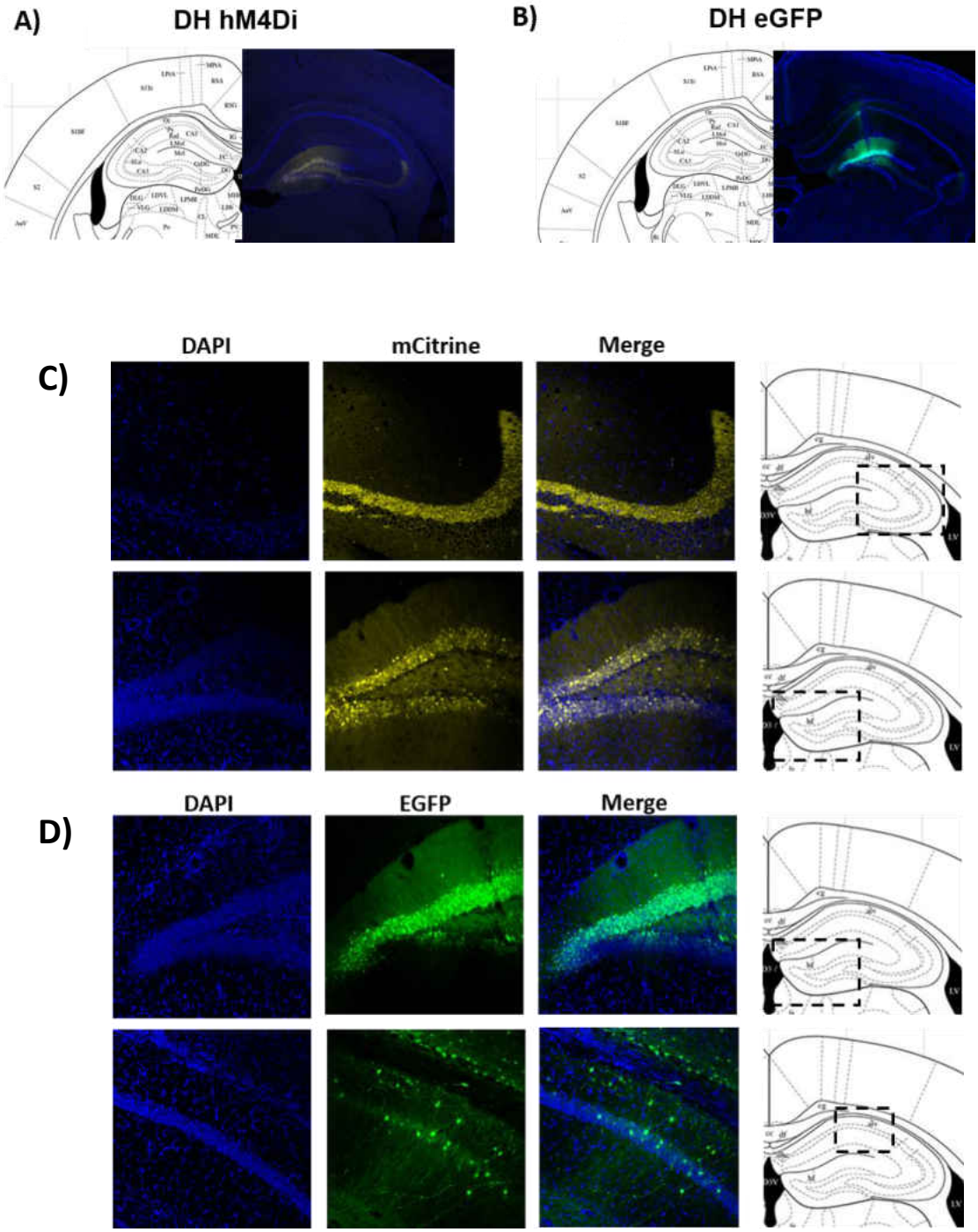


Fig. 6: (A&C) Coronal sections (40 µm) of CaMKIIα-hM4Di-mCitrine DREADD or **(B&D)** CaMKIIα-EGFP control virus in female mouse brain 3 weeks post-injection demonstrate high levels of expression in the dentate gyrus, as well as weaker expression in CA1 and CA3. Blue puncta: DAPI; yellow: mCitrine-tagged DREADD virus; green: eGFP-tagged control virus.

Fig. 7: Pre-training hM4Di-mediated inhibition of the DH impairs OP, but not OR, memory

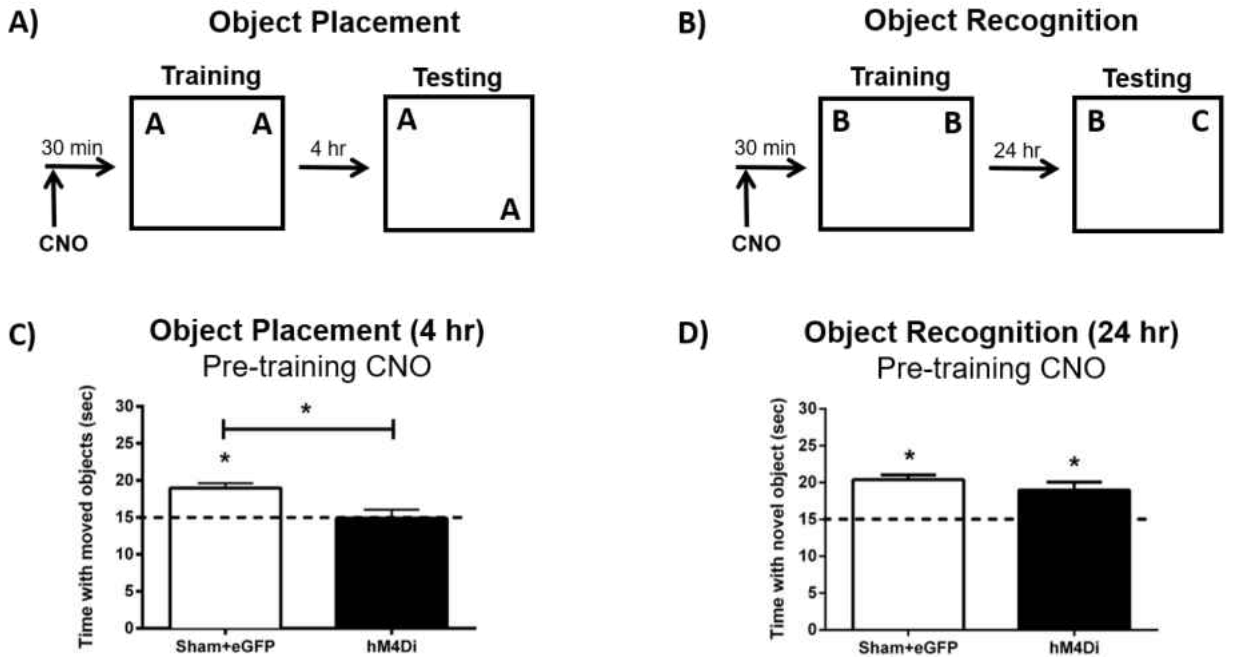


Fig. 7: Experimental design for pre-training CNO injections using the object placement **(A)** and object recognition task **(B). (C)** In the object placement task, DH sham and eGFP control mice administered 2 mg/kg CNO 30 minutes before training spent significantly more time than chance (15 s) with the moved object 4 hours after training, whereas DH-hM4Di-expressing mice administered 2 mg/kg CNO did not. **(D)** In the object recognition task, DH Sham, eGFP, and hM4Di mice administered 2 mg/kg CNO 30 minutes prior to training all spent significantly more time than chance (15 s) with the novel object during testing, suggesting intact object recognition memory 24 hours after training. This finding suggests that hM4Di-mediated inactivation of the DH impairs spatial memory formation. Bars represent the mean ± SEM, $*p < 0.05$.

Because our CNO injections were administered prior to training, it was not clear if DREADD-mediated inhibition of the DH impaired acquisition or consolidation of OP memory formation. To target the consolidation period of memory formation, the same mice were trained one week later with a new set of objects in the OP task and were injected with 2 mg/kg CNO immediately after training (Fig. 8A) to pinpoint effects on memory consolidation. Sham and eGFP control mice spent significantly more time than chance with the moved object,

demonstrating intact OP memory (Sham+eGFP: $t_{(13)} = 4.84$, $p = 0.0003$; Fig. 8C), whereas hM4Di expressing mice administered 2 mg/kg CNO did not, suggesting that hM4Di-mediated inactivation of the DH impaired OP memory consolidation (hM4Di: $t_{(8)} = 0.08$, $p = 0.94$; Fig. 8C). Sham and eGFP control mice also spent significantly more time with the moved object during testing than the hM4Di group ($t_{(21)} = 3.07$, $p = 0.006$; Fig. 8C), further supporting the notion that DREADD-mediated inhibition of the DH disrupts spatial memory consolidation.

To examine whether post-training hM4Di-mediated inactivation of the DH also impairs OR memory consolidation, we trained the same mice in the OR task with novel objects, and administered 2 mg/kg immediately after training (Fig. 8B). Unlike OP, 2 mg/kg CNO did not impair OR memory consolidation in either group (Sham+eGFP: $t_{(10)} = 4.39$, $p = 0.001$; hM4Di: $t_{(6)} = 3.41$, $p = 0.01$; Fig. 8D). To test if higher doses of CNO could impair OR memory consolidation in mice expressing hM4Di DREADDs in the DH, we also administered 4 or 8 mg/kg immediately after object recognition training. Neither the 4 mg/kg (Sham+eGFP: $t_{(9)} = 2.71$, $p = 0.02$; hM4Di: $t_{(8)} = 2.60$, $p = 0.03$; Fig. 8E), or 8 mg/kg (Sham+eGFP: $t_{(11)} = 6.58$, $p < 0.0001$; hM4Di: $t_{(8)} = 3.78$, $p = 0.01$; Fig. 8F) dose of CNO impaired OR performance in the Sham, eGFP, or hM4Di groups. Collectively, these data suggest hM4Di-mediated suppression of neurotransmission in the DH is sufficient to impair spatial, but not object recognition, memory consolidation.

Fig. 8: Post-training hM4Di-mediated inhibition of the DH impairs OP, but not OR, memory

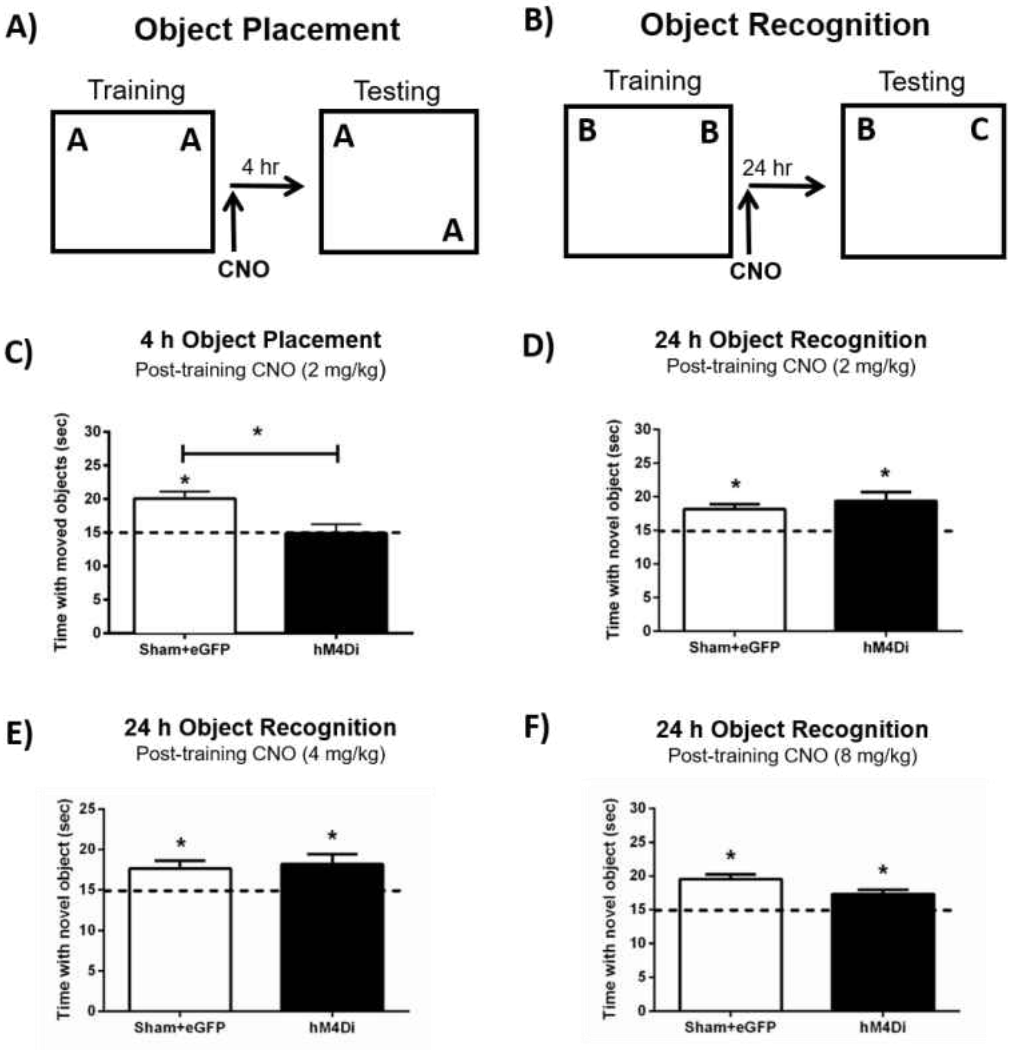
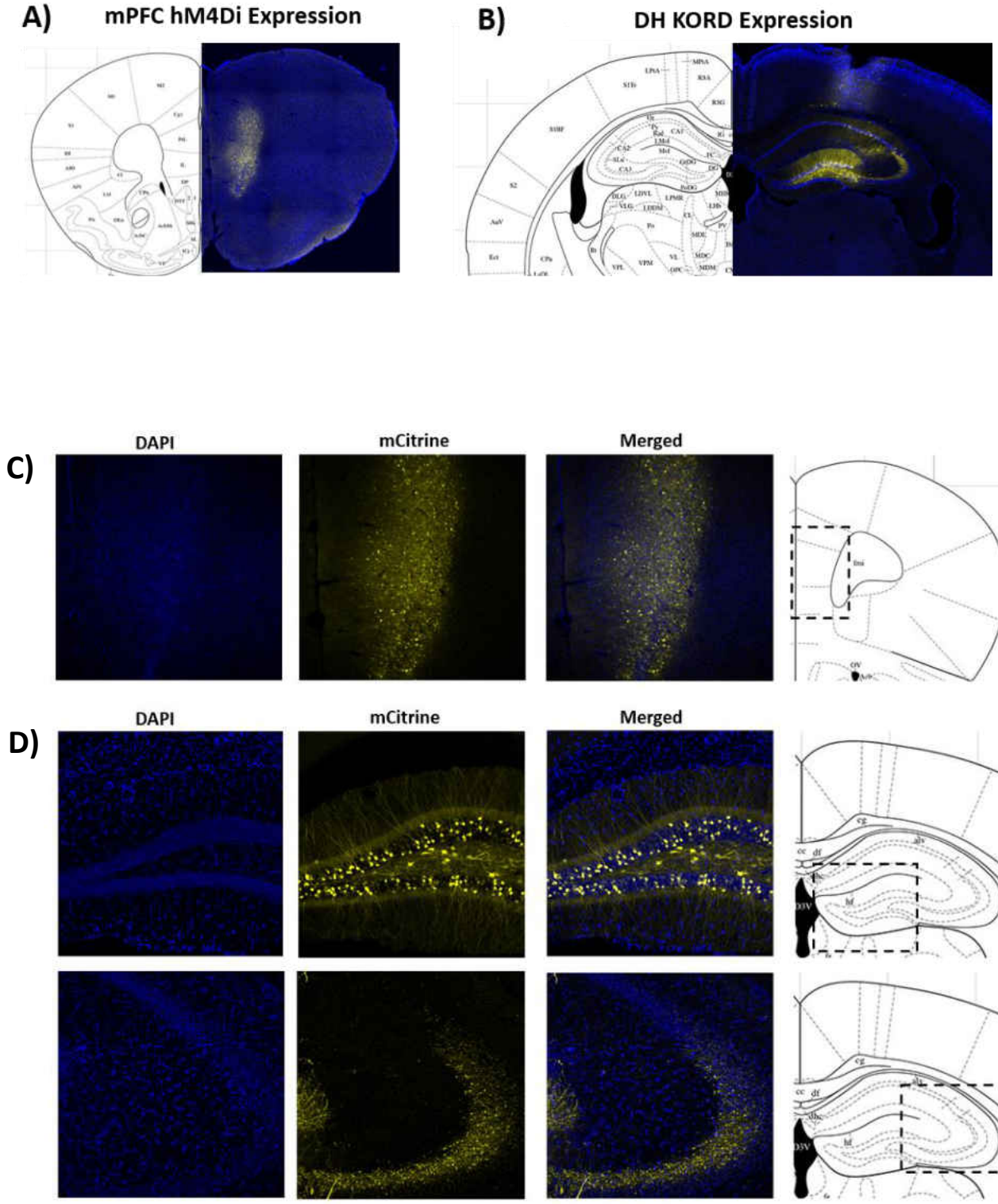


Fig. 8: (A&C) In the object placement task, DH sham and eGFP control mice administered 2 mg/kg CNO immediately post-training spent significantly more time than chance (15 s) with the moved object 4 hours after training, whereas DH-hM4Di-expressing mice administered 2 mg/kg CNO did not. **(B)** In the object recognition task, DH Sham, eGFP, and hM4Di mice administered 2 mg/kg **(D)**, 4 mg/kg **(E)**, or 8 mg/kg **(F)** CNO immediately post-training all spent significantly more time than chance (15 s) with the novel object during testing, suggesting intact object recognition memory 24 hours after training. This finding suggests that hM4Di-mediated inactivation of the DH impairs spatial memory consolidation. Bars represent the mean ± SEM, $*p < 0.05$.

hM4Di-mediated inhibition of the mPFC impairs OP and OR memory consolidation

To investigate the role of the mPFC alone, and its interactions with the DH, during object memory consolidation, a new set of mice was injected with the hM4Di inhibitory DREADD into the mPFC and another G_i-coupled inhibitory DREADD (kappa opioid receptor-DREADD; KORD) into the DH. Unlike the hM4-DREADD, the KOR-DREADD is activated by the synthetic ligand salvinorin-B (SALB), and can therefore be used for multiplexed modulation of behavior with CNO-activated DREADDs, such as hM4Di (Vardy et al., 2015). We used these two constructs to determine in the same mice whether activation of the mPFC alone, DH alone, or both mPFC and DH simultaneously is critical for memory formation. This approach yielded three experimental groups: 1) mPFC-hM4Di + DH-KORD, 2) mPFC-eGFP + DH-eGFP, and 3) mPFC-Sham + DH-Sham. Expression of eGFP (Fig. 9E&G) or hM4Di in the mPFC (Fig. 9A&C) and eGFP (Fig. 9F&H) or KORD (Fig. 9B&D) in the DH was verified by fluorescence microscopy 3 weeks after surgery. Expression of mPFC-hM4Di and DH-KORD DREADDs were also detected at 6 weeks (Fig. 10A&B) and 18 weeks (Fig. 10C&D) post-injection.

<u>Fig. 9:</u> mPFC-hM4Di, DH-KORD, and eGFP expression 3 weeks post-injection



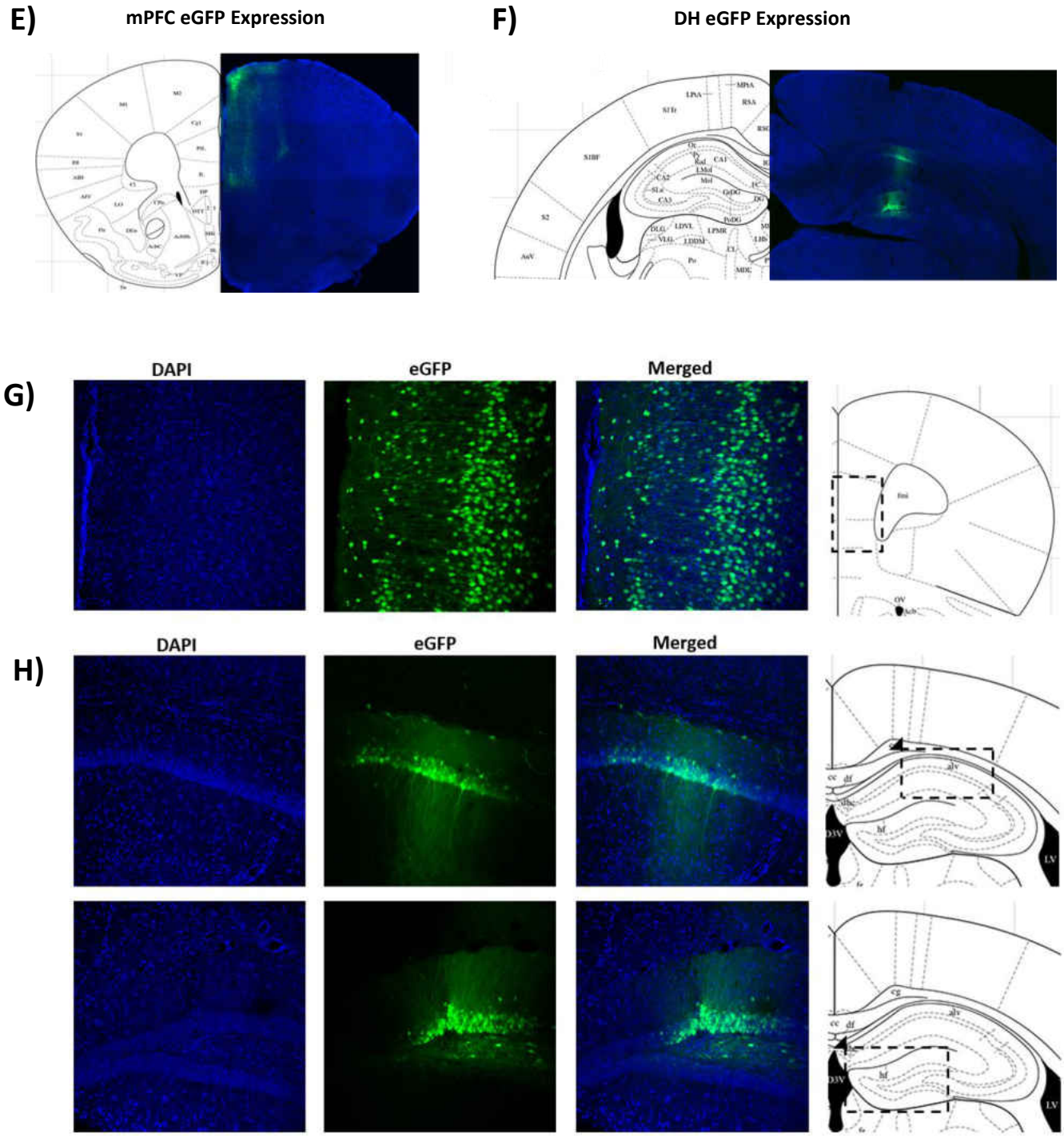
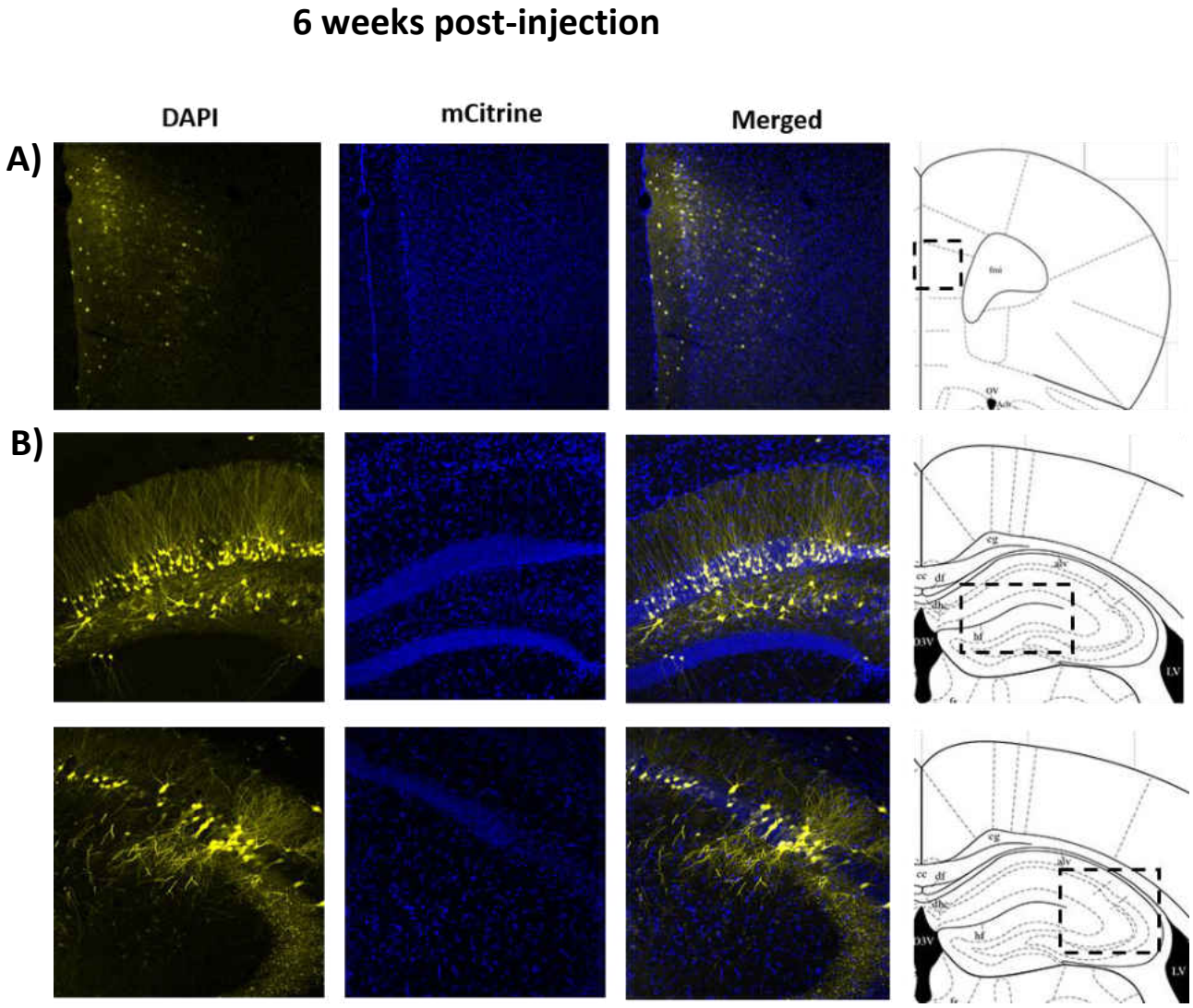


Fig. 9: Representative coronal sections (40 µm) of CaMKIIα-hM4Di-mCitrine DREADD in the mPFC **(A&C)**, CaMKIIα-KORD-mCitrine DREADD in the DH **(B&D),** and CaMKIIα-eGFP control virus in the mPFC **(E&G)** or DH **(F&H)** in female mouse brain 3 weeks post-injection. Blue puncta: DAPI; yellow: mCitrine-tagged DREADD virus; green: eGFP-tagged control virus.

Fig. 10: mPFC-hM4Di and DH-KORD expression 6 and 18 weeks post-injection



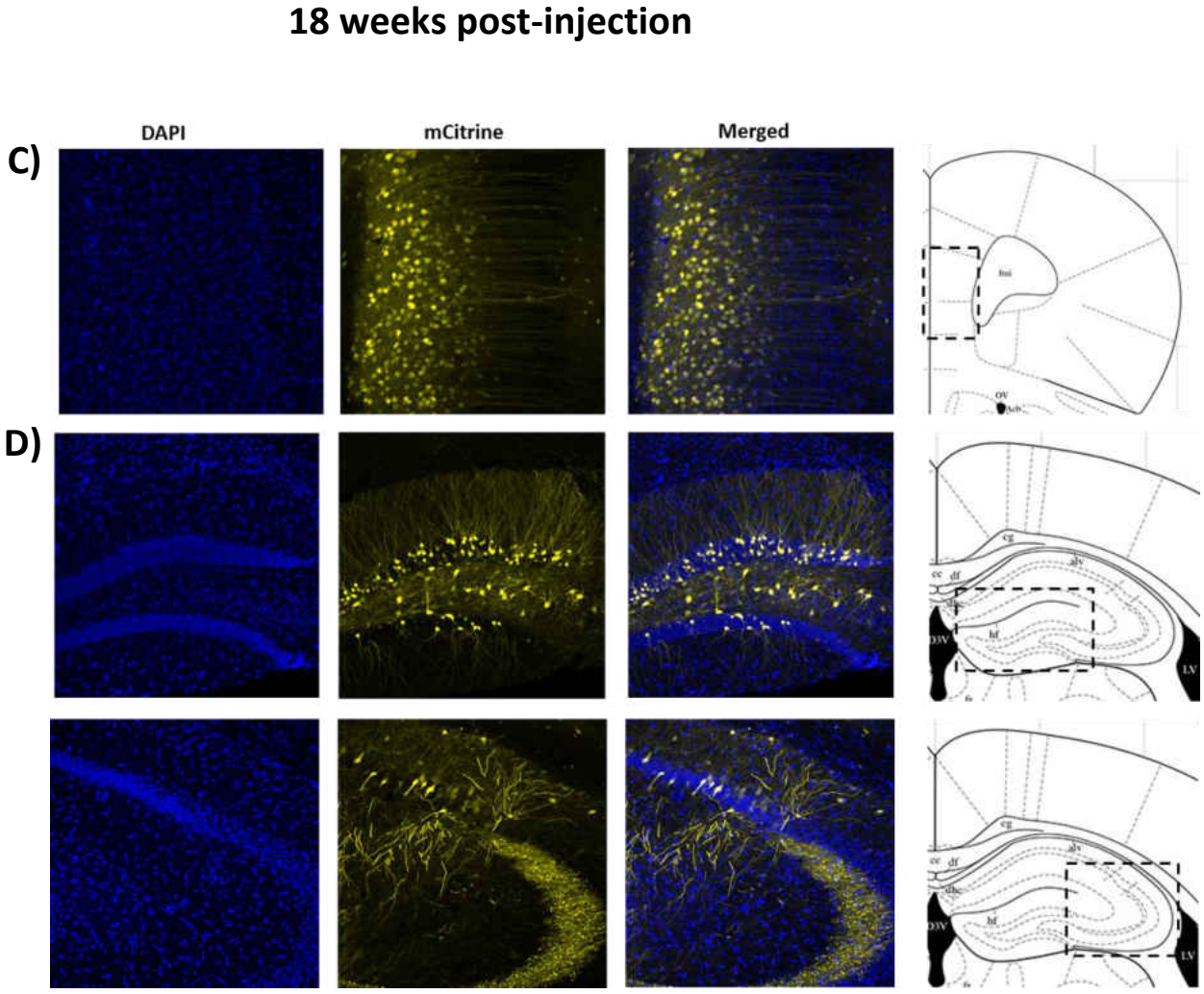


Fig. 10: Representative coronal sections (40 µm) of CaMKIIα-hM4Di-mCitrine DREADD in the mPFC **(A&C)** or CaMKIIα-KORD-mCitrine in the DH **(B&D)** in female mouse brain 6 weeks **(A&B)** and 18 weeks **(C&D)** post-injection. Blue puncta: DAPI; yellow: mCitrine-tagged DREADD virus; green: eGFP-tagged control virus.

To examine if mPFC activation alone is necessary for spatial memory consolidation, mice were trained in OP and then received an i.p. injection of CNO immediately after training (Fig. 11A). Mice expressing hM4Di in the mPFC spent no more time than chance with the displaced object during testing 4 hours later when injected with 2 mg/kg CNO (hM4Di: $t_{(5)} = 0.40$, $p = 0.71$;

Fig. 11C), but not 1 mg/kg, CNO (hM4Di: $t_{(8)} = 5.04$, $p = 0.001$; Fig. 4E). The Sham+eGFP group demonstrated intact spatial memory after i.p. injection of either 1 mg/kg CNO (Sham+eGFP: $t_{(14)} = 6.51$, $p < 0.0001$; Fig. 11E) or 2 mg/kg CNO (Sham+eGFP: $t_{(12)} = 6.34$, $p < 0.0001$; Fig. 11C), suggesting that both doses were subthreshold for controls. Further, Sham+eGFP control mice and hM4Di expressing mice injected with 1 mg/kg CNO did not differ in the amount of time spent with the displaced object ($t_{(22)} = 0.24$, $p = 0.81$; Fig. 11E). These findings suggest that hM4Di-mediated inhibition of the mPFC impairs OP memory after administration of 2 mg/kg, but not 1 mg/kg, of CNO. Mice expressing hM4Di in the mPFC also spent significantly less time with the displaced object during testing than control mice when injected with 2 mg/kg CNO ($t_{(17)} = 3.10$, $p = 0.006$; Fig. 11C), further demonstrating DREADD-induced suppression of the mPFC disrupts spatial memory consolidation.

We next examined object recognition memory consolidation, and found that 2 mg/kg of CNO administered immediately after OR training impaired object recognition memory consolidation in mice expressing hM4Di in the mPFC, as these mice did not spend more time than chance with the novel object during testing (hM4Di: $t_{(8)} = 0.47$, $p = 0.65$; Fig. 11D). In contrast, the Sham+eGFP group was not impaired by 2 mg/kg CNO when tested 4 hours later (Sham+eGFP: $t_{(13)} = 2.64$, $p = 0.02$; Fig. 11D). mPFC-hM4Di mice injected with 2 mg/kg CNO immediately post-training also tended to spend less time with the novel object during testing than controls ($t_{(21)} = 1.58$, $p = 0.12$; Fig. 11D), suggesting suppression of the mPFC impaired OR memory. Post-training injection of 1 mg/kg CNO did not impair OR memory consolidation in any treatment condition (hM4Di: $t_{(5)} = 3.32$, $p = 0.02$; Sham+eGFP: $t_{(12)} = 4.80$, $p = 0.0004$; Fig. 11F), and the amount of time spent with the novel object was not statistically different

between Sham+eGFP control mice and hM4Di expressing mice injected with 1 mg/kg CNO ($t_{(17)}$ = 0.83, p = 0.42; Fig. 11F). Collectively, these data suggest that suppression of mPFC neurotransmission by 2 mg/kg CNO disrupted both spatial and object recognition memory consolidation.

Fig. 11: hM4Di-mediated inhibition of the mPFC impairs OP and OR memory consolidation

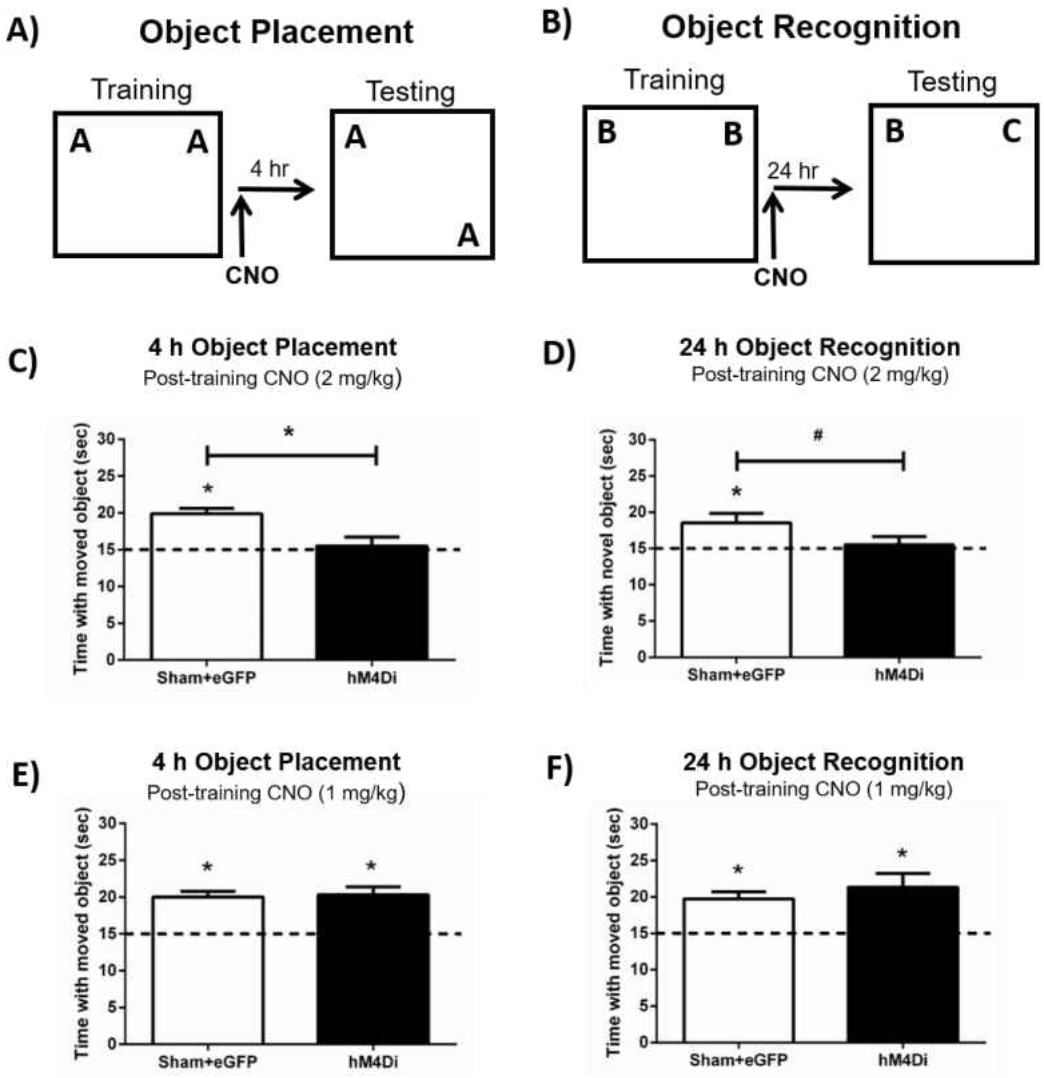


Fig. 11: Experimental design for the object placement **(A)** and object recognition task **(B)**. DREADD-mediated inhibition of the mPFC impaired both object placement **(C)** and object recognition **(D)** memory in mice expressing hM4Di in the mPFC that were administered 2 mg/kg CNO immediately after training. A 1 mg/kg dose of CNO did not impair memory in the object placement **(E)** or object recognition **(F)** task. This finding suggests that hM4Di-mediated inactivation of the mPFC impairs spatial and object recognition memory consolidation. Bars represent the mean ± SEM, $^{*}p < 0.05$, $^{\#}p \leq 0.1$.

KORD-mediated inhibition of the DH impairs OP and OR memory consolidation

Our first series of experiments examining hM4Di-mediated inactivation of the DH indicated that DH activity is necessary for OP, but not OR, memory consolidation. However, numerous pharmacological studies suggest that DH activity is necessary for consolidation in these tasks (Baker and Kim, 2002; Broadbent et al., 2004; Hammond et al., 2004; Fernandez et al., 2008; Zhao et al., 2012; Cohen et al., 2013; Fortress et al., 2013), and so we examined the effects of KORD-mediated DH inhibition on OR and OP memory to determine if the effects observed with the hM4Di DREADD would generalize to another viral construct. The same mice described above were trained in OP and OR with new sets of objects (Fig. 12A & B). Sham and eGFP mice injected immediately post-training with 10 mg/kg SALB (Sham+eGFP: $t_{(16)} = 4.10$, $p = 0.001$; Fig. 12C) or 5 mg/kg SALB (Sham+eGFP: $t_{(14)} = 4.77$, $p = 0.0003$; Fig. 12E) spent significantly more time than chance with the moved object during testing, demonstrating that SALB does not impair OP memory consolidation on its own at these doses. In mice expressing KORDs in the DH, 10 mg/kg SALB impaired spatial memory consolidation, as these mice did not spend more time than chance with the displaced object during testing (KORD: $t_{(8)} = 1.35$, $p = 0.21$; Fig. 12C). However, OP memory consolidation was not impaired in DH KORD-expressing mice by 5 mg/kg SALB (KORD: $t_{(8)} = 3.45$, $p = 0.01$; Fig. 12E), suggesting this dose was insufficient to trigger KORD-mediated inactivation. Further, Sham+eGFP control mice and KORD expressing mice injected with 5 mg/kg SALB did not differ in the amount of time spent with the displaced object ($t_{(22)} = 0.69$, $p = 0.50$; Fig. 12E).

As in the OP task, Sham and eGFP mice injected with 10 mg/kg SALB (Sham+eGFP: $t_{(12)}$ = 2.22, p = 0.04; Fig. 12D) or 5 mg/kg SALB (Sham+eGFP: $t_{(7)}$ = 5.23, p = 0.001; Fig. 12F), demonstrated intact OR memory. Also similar to OP, immediate post-training injection of 10 mg/kg SALB prevented DH-KORD mice from spending more time than chance with the novel object during testing 24 hours later (KORD: $t_{(8)}$ = 1.14, p = 0.29; Fig. 12D), suggesting impaired object recognition memory consolidation. Again, OR was not impaired by 5 mg/kg SALB in DH-KORD mice (KORD: $t_{(9)}$ = 4.15, p = 0.002; Fig. 12F), and the amount of time spent with the novel object was not statistically different between Sham+eGFP control mice and KORD expressing mice injected with 5 mg/kg SALB ($t_{(16)}$ = 1.41, p = 0.18; Fig. 12F). Collectively, these findings show KORD-mediated suppression of the DH impairs both OP and OR memory consolidation. The fact that OR memory consolidation was impaired in the DH by KORD-mediated inactivation, but not hM4Di-mediated inactivation, suggests potentially interesting differences in the effects of these viruses and/or their relative expression in these two studies.

Fig. 12: KORD-mediated inhibition of the DH impairs OP and OR memory

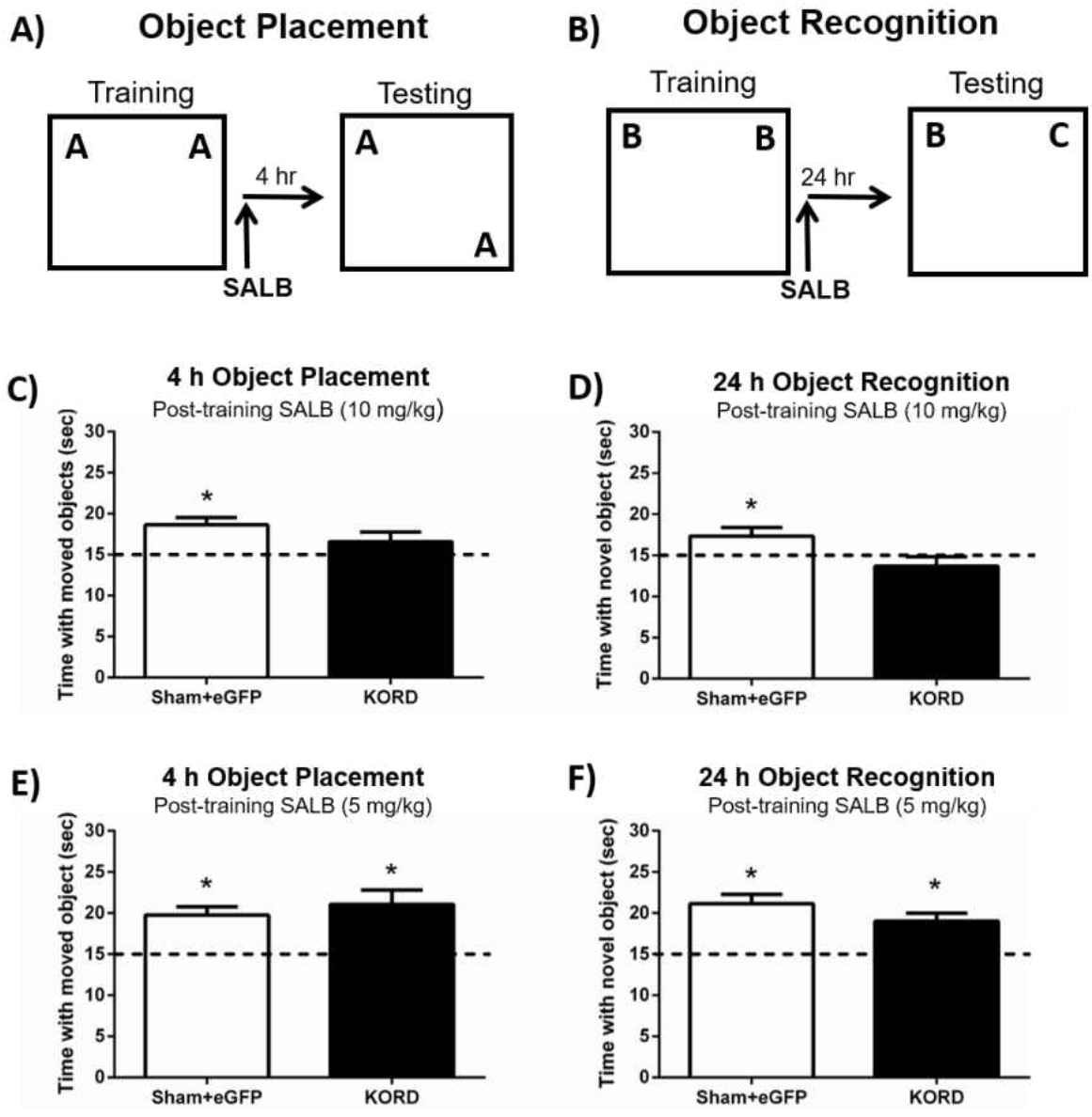


Fig. 12: Experimental design for the object placement **(A)** and object recognition task **(B)**. DREADD-mediated inhibition of the DH impaired both object placement **(C)** and object recognition **(D)** memory in mice expressing KORD in the DH that were administered 10 mg/kg SALB immediately after training. A 5 mg/kg dose of SALB did not impair memory in the object placement **(E)** or object recognition **(F)** task. This finding suggests that KORD-mediated inactivation of the DH impairs spatial and object recognition memory consolidation. Bars represent the mean ± SEM, $*p < 0.05$.

Simultaneous subthreshold inhibition of the mPFC and DH impairs OP and OR memory

Finally, to examine the potential interaction between the DH and mPFC during object recognition and spatial memory consolidation, we used subthreshold doses of CNO and SALB to simultaneously suppress neurotransmission in the DH and mPFC. Importantly, neither dose of CNO (1 mg/kg; Fig.11E & F) or SALB (5 mg/kg; Fig. 12E & F) used for this experiment was sufficient to impair memory in either OP or OR when administered on its own. Thus, any memory impairments observed should be a result of combined disruption of the DH and mPFC. To this end, immediately after training with a new set of objects, mice were injected i.p. with 1 mg/kg CNO and 5 mg/kg SALB delivered in separate syringes. We found that Sham and eGFP mice administered this combined subthreshold injection protocol spent more time than chance with the moved object in OP (Sham+eGFP: $t_{(5)} = 4.61$, $p = 0.006$; Fig. 13C) and the novel object in OR (Sham+eGFP: $t_{(11)} = 5.43$, $p = 0.02$; Fig. 13C), suggesting spatial and object recognition memory was not impaired in our control groups. However, mice expressing hM4Di in the mPFC and KORD in the DH spent no more than chance amount of time with the moved object during OP testing (mPFC-hM4Di + DH-KORD: $t_{(8)} = 1.33$, $p = 0.22$; Fig. 13C) and the novel object during OR testing (mPFC-hM4Di + DH-KORD: $t_{(8)} = 0.01$, $p = 0.99$; Fig. 13D) when injected with 1 mg/kg CNO and 5 mg/kg SALB immediately after training. Further, mPFC-hM4Di + DH-KORD mice administered 1 mg/kg CNO and 5 mg/kg SALB post-training also spent significantly less time with the moved object than the Sham and eGFP control groups during OP testing ($t_{(13)} = 3.58$, $p = 0.003$; Fig. 13C), and trended toward spending less time with the novel object than Sham and eGFP control mice in the OR task ($t_{(19)} = 1.75$, $p = 0.09$; Fig. 13D). These findings suggest

simultaneous disruption of neurotransmission in the mPFC and DH impairs spatial and object recognition memory consolidation.

Fig. 13: Simultaneous subthreshold inhibition of the mPFC and DH impairs OP and OR memory

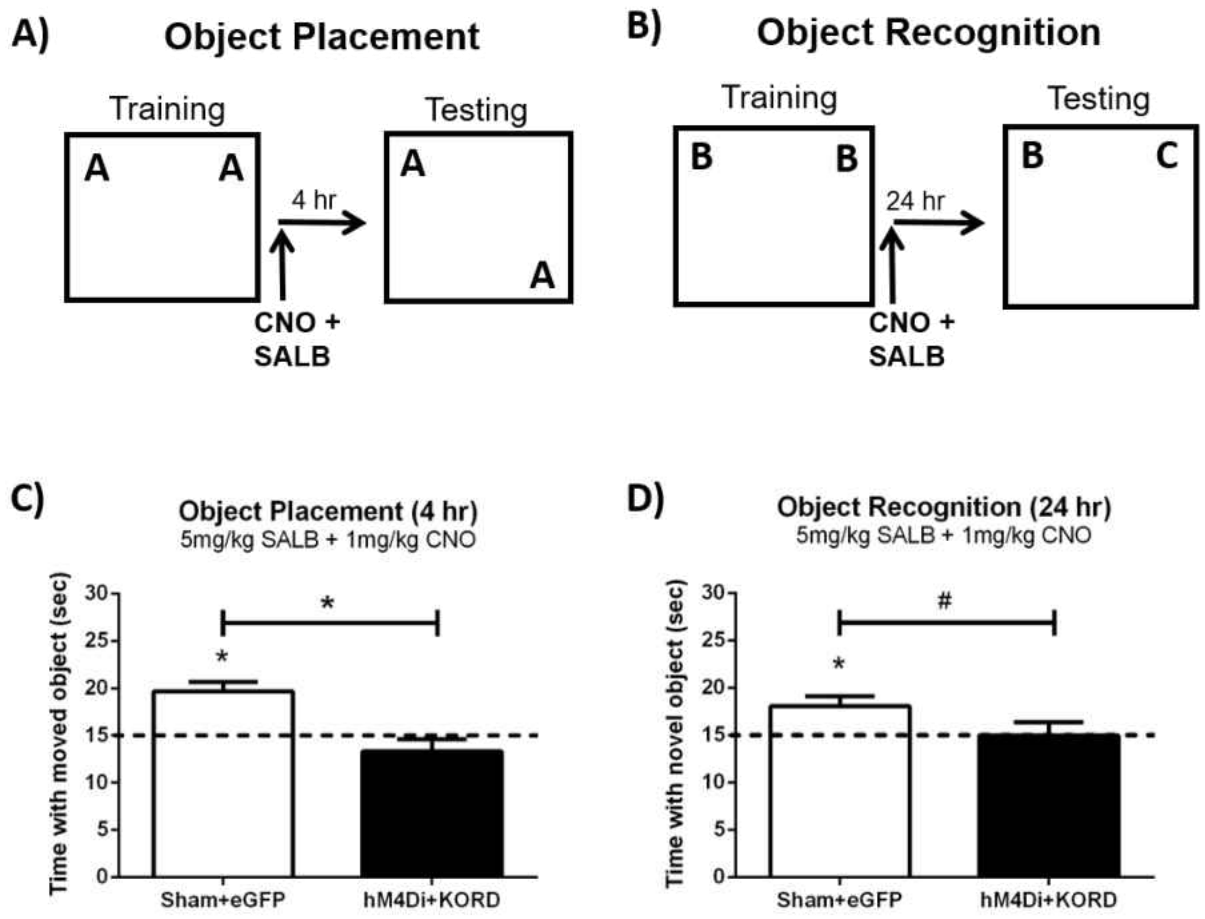


Fig. 13: Experimental design for the object placement **(A)** and object recognition **(B)** subthreshold inactivation experiments. Doses of CNO and SALB that do not impair memory on their own impair OP **(C)** and OR **(D)** memory when administered simultaneously in mice expressing KORD in the DH and hM4Di in the mPFC. Subthreshold doses of SALB and CNO do not impair memory in Sham and eGFP control mice in either task. This finding suggests that simultaneous neural activity in both the DH and the mPFC is necessary for the consolidation of spatial and object recognition memories. Bars represent the mean ± SEM, $^{*}p < 0.05$, $^{\#}p < 0.10$.

DISCUSSION

The goal of the present study was to determine the roles of the mPFC and DH, independently and in combination, in object recognition and spatial memory consolidation. Using two different inhibitory DREADD constructs, we found that inhibition of either the mPFC or DH impaired the consolidation of both types of memory, although DH inhibition impaired object recognition only when using the KORD construct. These data suggest that both the mPFC and DH play key roles in mediating object recognition and spatial memory consolidation. Importantly, these brain regions appear to work in concert to mediate memory in the OR and OP tasks, as simultaneous disruption of neurotransmission in both brain regions using subthreshold doses of DREADD ligand impaired memory consolidation in both tasks.

Our present findings that DH inactivation can disrupt OP memory using the hM4Di DREADD and both OP and OR using the KORD DREADD are consistent with previously published evidence demonstrating that inhibiting DH function in rodents impairs performance in object tasks. For example, OR memory consolidation is impaired when the hippocampus is lesioned or pharmacologically inhibited by $GABA_A$ agonists, NMDA antagonists, or inhibitors of ERK/MAPK cell signaling, histone acetylation, and protein synthesis (Baker and Kim, 2002; Broadbent et al., 2004; Hammond et al., 2004; Fernandez et al., 2008; Zhao et al., 2012; Cohen et al., 2013; Fortress et al., 2013). Given previous studies demonstrating that DH inactivation impairs OR memory consolidation, it is perhaps not surprising that suppression of excitatory neurotransmission in the DH impaired memory consolidation in both OR and OP. However, despite numerous studies showing that DH activation is necessary for OR memory formation, some have reported that DH inactivation does not impair OR (Mumby, 2001; Winters et al.,

2004; Forwood et al., 2005; Squire et al., 2007; Broadbent et al., 2010). Further, others have reported DH infusion of hM4Di DREADDs impaired performance in the OP task, but did not impair OR memory in male mice (Lopez et al., 2016). Similarly, we also found that hM4Di-mediated inactivation of the DH was only sufficient to impair OP, but not OR, memory consolidation. Although it is not entirely clear why one inhibitory DREADD impaired OR while another form of inhibitory DREADD driven by the same CaMKII promoter did not, there are a couple reasons why this might be the case. One possibility is that the proportion of neurons transfected by the DREADD may have differed by cohort. Although the percentage of DREADD-transfected cells was not quantified in each experiment to allow for direct comparison, there were notable differences in the pattern of DREADD expression between mice injected with hM4Di vs. KORD in the DH. Namely, we observed more KORD DREADD in the hilar region of the dentate gyrus and in CA2 compared to the hM4Di DREADD (see Fig. 6C, 9D, 10B&D). Therefore, it is possible that a larger population of neurons was inhibited during the KORD-mediated inactivation experiment, and as such, neurotransmission was disrupted in a larger proportion of the DH. Alternatively, spatial memory may be more easily disrupted by perturbations of the DH compared to recognition memory (Broadbent et al., 2004; Squire et al., 2007; Wilson et al., 2013). Thus, DREADD-mediated inactivation of the DH may be sufficient to impair spatial memory, whereas the entorhinal or perirhinal cortices may be able to compensate for partial DH disruption in recognition-based tasks.

Our data also suggest that hM4Di-mediated inactivation of the mPFC immediately after training impaired both OR and OP memory consolidation. Although at least one study has implicated the mPFC as a critical locus for OR and OP memory consolidation (Akirav and

Maroun, 2006), other mPFC inactivation studies have suggested that this region is involved in spatial object tasks but not OR (Warburton and Brown, 2015, DeVito and Eichenbaum, 2010). One potential factor that may contribute to this discrepancy is the length of the delay between training and testing. In studies concluding that mPFC activation was not necessary for OR, only a 50 minute or 2-hour delay was imposed between training and testing (DeVito and Eichenbaum, 2010, Barker, 2007). However, a study using a 24-hour delay between training and testing reported that mPFC inactivation impaired OR memory (Akirav and Maroun, 2006). Therefore, it may be that the mPFC is critical for consolidation of long-term memories (i.e., beyond 2 hours), but not short-term memories (i.e., less than 2 hours). This theory is consistent with our present findings, which indicate that recall at 4 or 24 hours is impaired when neurotransmission is disrupted in the mPFC immediately after training.

This report is the first to our knowledge using multiplexed inhibitory DREADDs to partially inactivate both the DH and mPFC during memory formation to address whether simultaneous activity in these regions is required for episodic-like memory consolidation. Given the numerous potential routes of communication between the DH and mPFC (Ye et al., 2017, Hoover and Vertes, 2007, Burwell and Amaral, 1998, Cenquizca and Swanson, 2007), this approach prevented potential compensatory effects through alternate indirect routes (i.e., nucleus reunions, entorhinal and perirhinal cortices) which could be utilized in functional disconnection studies that only disrupt either ipsi- or contralateral communication between these structures (Warburton and Brown, 2015). Importantly, we used doses of CNO (1 mg/kg; Fig. 11E & F) and SALB (5 mg/kg; Fig. 12E & F) that were not sufficient to impair object memory consolidation in either task when used alone to suppress neurotransmission in the mPFC or DH,

respectively. Although our findings cannot definitively attribute memory impairment to blockade of a direct, monosynaptic connection between DH and mPFC, these data do provide support that both regions must be functional for the successful consolidation of OR and OP memories. Future studies utilizing chemogenetic or optogenetic approaches to selectively target DH projection terminals in the mPFC (rather than silencing the entire mPFC) could be used to address whether direct DH efferent input into the mPFC is necessary for episodic-like memory formation.

The fact that simultaneous disruption of neurotransmission in the mPFC and DH impaired memory consolidation in the present experiments is consistent with other research reporting temporally-coordinated neuronal activity in these regions during periods of sleep and wakefulness in rodents is necessary for systems memory consolidation. For example, hippocampal input to the mPFC during sleep or slow-wave oscillations during rest periods after behavioral training are required for consolidation (Schwindel and McNaughton, 2011). During periods of wakefulness, neuronal firing in the DH and mPFC is phase-locked to hippocampal theta oscillations, and firing coherence is increased during spatial working memory tasks (Jones and Wilson, 2005, Hyman et al., 2005, 2010). Further, reduced theta rhythm coherence between CA1 and mPFC in mice is correlated with poor performance in a spatial working memory task (Sigurdsson et al., 2010). Given that hippocampal-prefrontal neural synchrony appears to be important for memory consolidation in the aforementioned studies, it follows that simultaneous chemogenetic suppression of the DH and mPFC in the present study may have disrupted functional connectivity between the DH and mPFC, which ultimately impaired OR and OP memory consolidation.

Our present findings also align with recent work demonstrating that direct input from the dentate gyrus into the mPFC during contextual fear conditioning is necessary for establishing immature engram cells within the mPFC (Kitamura et al., 2017). Disruption of these DH-mPFC interactions during fear conditioning also prevents spine density increases later observed on eYFP labeled engram cells in the mPFC at a remote memory test (Kitamura et al., 2017). This work and our current findings support the idea that communication between the DH and mPFC must be established during the consolidation period in order to support long-term memory formation. Other recent research investigating the necessity of DH-mPFC interactions during memory formation has shown that DREADD-mediated inhibition of DH projection terminals in the mPFC prior to reactivation sessions prevents reactivation-induced increases in fear memory expression and memory-associated proteins in the mPFC (e.g., Arc, pCREB, and pCofilin protein; (Ye et al., 2017). Taken together, these studies and our present findings lend additional support to the idea that the DH and mPFC individually contribute to, and also work together during, the successful consolidation of episodic-like memories.

SUMMARY AND CONCLUSION

In summary, the present study indicates that both the DH and the mPFC are required for the consolidation of object recognition and spatial memories, as suppressing neurotransmission in either brain region impairs performance in each of these tasks. In addition to the individual contribution of each brain region, our data also support the notion that these brain regions must act in concert to consolidate object recognition and spatial memories. Collectively, these findings provide additional insight into the neurobiological basis of episodic-like memory

formation, and may provide an important foundation for studying how hippocampal-prefrontal communication is compromised in certain neuropsychiatric disorders.

CHAPTER THREE: Dorsal hippocampal and medial prefrontal interactions in the estrogenic regulation of object memory formation

INTRODUCTION

Sex-steroid hormones have a broad impact on the neural circuitry that supports learning and memory, yet much remains unknown about the cellular, synaptic, and circuit-level mechanisms through which they exert their effects. The potent estrogen 17β-estradiol (E_2) can regulate neuronal excitability and spinogenesis in the dorsal hippocampus (DH) and medial prefrontal cortex (mPFC), brain regions important for cognitive function that are compromised during aging and in numerous neuropsychiatric disorders. Memory consolidation, a process which requires coordinated effort between the hippocampus and mPFC, is facilitated by systemic injection or direct infusion of E_2 into the DH of female rodents. However, the specific mechanisms through which E_2 enhances memory consolidation remain poorly understood, and little is known about how interactions between the DH and mPFC might contribute to the estrogenic regulation of memory.

The object recognition (OR) and object placement (OP) tasks involve the integration of *what* and *where* components of memory, and are commonly used to assess episodic-like memory in rodents (Dere et al., 2005; Ennaceur, 2010; Barker et al., 2017; Eichenbaum, 2017). Our laboratory has previously demonstrated that direct DH infusion of E_2 immediately after object training can extend the delay at which ovariectomized mice are able to recall training objects or locations, and that this enhanced memory consolidation depends on E_2-mediated activation of the extracellular signal-regulated kinase (ERK) and mammalian target of rapamycin

(mTOR) cell-signaling pathways in the DH (Fernandez et al., 2008; Fortress et al., 2013). Recently, we showed that E_2-induced increases in spinogenesis in both the DH and mPFC also rely on activation of ERK and mTOR signaling in the DH, highlighting putative systems-level interactions between these brain regions that may be important for memory formation in female rodents. However, the extent to which medial prefrontal-hippocampal interactions are necessary for the memory enhancing effects of DH-infused E_2 remains unknown. Further, it is also unclear whether E_2 can act directly in the mPFC to enhance memory consolidation.

Therefore, the present study sought to determine the extent to which E_2 in the mPFC regulates object recognition and spatial memory consolidation, and the necessity of DH-mPFC interactions for the memory-enhancing effects of DH E_2 infusion. We first delivered E_2 directly to the mPFC of ovariectomized mice immediately after object training to assess the effects of mPFC-E_2 infusion on episodic memory consolidation. Next, we utilized Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to inactivate the mPFC immediately prior to DH E_2 infusion to determine whether communication between the DH and mPFC is necessary for DH-infused E_2 to enhance memory consolidation and increase DH and mPFC dendritic spine density in ovariectomized mice. We found that E_2 enhanced object recognition and spatial memory consolidation when infused into the mPFC, and that chemogenetic inactivation of the mPFC prevented DH-infused E_2 from enhancing both types of memory. These data suggest an important role for E_2 in the mPFC in mediating memory formation, and indicate that the mPFC and DH interact to mediate the memory-enhancing effects of E_2 in ovariectomized mice.

MATERIALS AND METHODS

Subjects. All experiments used young (9-12 week-old) female C57BL/6 mice (Taconic, Cambridge City, IN). Mice were housed in groups of up to five until surgery, after which they were singly housed. Mice were maintained on a 12 h light/dark cycle with ad libitum access to food and water. All experimental protocols and procedures were approved by the University of Wisconsin-Milwaukee Institutional Animal Care and Use Committee and are in accordance with National Institutes of Health guidelines or Guide for the Care and use of Laboratory Animals.

Surgeries. All mice were bilaterally ovariectomized as previously described (Tuscher et al., 2016; Kim et al., 2016) and were then implanted with bilateral cannulae into the mPFC or DH as described below. DH-cannulated mice were also injected into the mPFC with DREADD virus as described below. Ovariectomy, cannulae implantation, and virus injections occurred during the same surgical session.

mPFC cannulation. Mice were anesthetized with isoflurane (5% for induction, 2% for maintenance) in 100% oxygen and placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Immediately after ovariectomy, mice were implanted with stainless steel bilateral guide cannulae (Plastics One, Roanoke, VA) aimed at the mPFC (1.8 mm AP, ±0.3 mm ML, -2.3 mm DV), and cannulae were fixed to the skull with dental cement (Darby Dental Supply, New York, NY) that served to close the wound. Dummy cannulae were used to prevent clogging of the cannula tracts. Mice received carprofen MediGel one day prior to surgery, as well as a s.c. injection of 5 mg/kg Rimadyl at the completion of surgery. Mice were given 1 week for recovery prior to behavioral testing.

mPFC DREADD delivery and DH cannulation. Immediately following ovariectomy, an incision was made in the scalp to expose the skull, and small perforations were made in the skull with a 26 ½ GA needle to create openings for mPFC viral injections and DH guide cannula implantation. Viral injections were made into the mPFC (1.9 mm AP, ±0.3 mm ML, -2.8 mm DV) using a 10-µl Hamilton syringe (Hamilton, Reno, NV), which was first lowered to -2.8 mm ventral to the surface of the skull and held in place for two minutes to create a pocket for the first virus injection. The syringe was then raised 0.1 mm, and hM4Di DREADD virus (AAV-CamKIIα-HA-hM4Di-IRES-mCitrine, $2.1x10^{12}$ particles/ml, serotype 8, UNC Vector Core, Chapel Hill, NC), eGFP control virus (AAV-CamKIIα-eGFP, $2.1x10^{12}$ particles/ml, serotype 8, UNC Vector Core, Chapel Hill, NC), or saline (Sham condition) was delivered at a rate of 0.2 µl/minute over a two-minute period, for a total of 0.4 µl per infusion. Injection volume and flow rate were controlled by a syringe pump (KD Scientific, Holliston, MA). After the first infusion, the syringe was raised 0.2 mm, and a second infusion of the same volume was delivered at the same rate for a total of 0.8 µl per hemisphere. The Hamilton syringe was left in place 8 minutes after each injection to allow for diffusion of the virus, and was then slowly retracted. After completion of virus injections, mice were implanted with stainless steel bilateral guide cannulae (Plastics One, Roanoke, VA) aimed at the DH (-1.7 mm AP, ±1.5 mm ML, -2.3 mm DV) as described previously (Tuscher et al., 2016, Kim et al., 2016). Cannulae were fixed to the skull with dental cement (Darby Dental Supply, New York, NY) and dummy cannulae were used to prevent clogging of the cannula tracts. Mice received pre- and post-surgical analgesia as described above. Mice were then given 3 weeks for recovery and to allow adequate time for the virus to express prior to behavioral testing.

Drugs and Infusions. Intracranial infusions into the mPFC or DH were conducted as described previously (Fernandez et al., 2008; Zhao et al., 2010; Zhao et al., 2012; Fortress et al., 2013). Briefly, cyclodextrin-encapsulated 17β-E_2 (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile 0.9% saline to a concentration of 10 μg/μl, and was infused bilaterally into the DH or mPFC immediately after object training. Vehicle infusions consisted of 2-hydroxypropyl-β-cyclodextrin (HBC; Sigma-Aldrich, St. Louis, MO) dissolved in saline to the same concentration of cyclodextrin present in the cyclodextrin-encapsulated E_2 solution. All infusions were conducted at a rate of 0.5 μl/min for 1 min per hemisphere as described previously (Fernandez et al., 2008; Zhao et al., 2010; Zhao et al., 2012; Fortress et al., 2013), resulting in a dose of 5 μg E_2 per hemisphere. Mice with mPFC or DH cannulae were infused with HBC vehicle or 5 μg E_2/hemisphere immediately after object training (see below).

For DREADD experiments, stock solutions of clozapine-n-oxide (CNO, Cayman Chemical, Ann Arbor, MI) were first prepared by dissolving CNO in 100% dimethyl sulfoxide (DMSO, Fisher Scientific, Pittsburgh, PA) at a concentration of 100 mg/ml, and stored in 10 μl aliquots at -20 ^{o}C. On the day injections were administered, CNO stock was thawed and diluted to a concentration of 1 mg/ml in a solution of sterile 0.9% saline containing 2% DMSO. Mice were injected i.p. with 1 mg/kg CNO immediately after object training, followed immediately by an infusion of HBC vehicle or 5 μg E_2/hemisphere into the DH.

Behavioral Testing. Object recognition (OR) and object placement (OP) were used to measure object recognition and spatial memory as we have described previously (Boulware et al., 2013;

Fortress et al., 2013; Kim, 2016). Work from our laboratory (Gresack and Frick, 2006; Fernandez et al., 2008) and others (Luine et al., 2003; Li et al., 2004; Walf et al., 2008) has established that each of these tasks is sensitive to E_2 and involves the DH (see Tuscher et al., 2015 for review). One week after mPFC cannula implantation surgery or three weeks after DREADD surgery, mice were handled for 1 minute/day for 3 days prior to habituation. Mice were then habituated to the apparatus for 2 consecutive days by allowing them to explore the empty white arena (60 cm x 60 cm x 47 cm) for 5 minutes/day. For object training, mice must first accumulate 30 seconds exploring 2 identical objects placed 5 cm from the upper left and right corners of the arena. Immediately after training, mice were injected or infused as described above and then returned to their home cage. These post-training injections allowed us to pinpoint the effects of infusions and DREADD-mediated inactivation specifically to the memory consolidation period while minimizing potential confounding effects of performance factors (e.g., motivation, anxiety) on the measurement of memory consolidation (McGaugh, 1989; Frick and Gresack, 2003). Mice that did not meet the 30 second criterion for object exploration were not infused or injected, and were not included in subsequent testing. OR memory was then tested 48 hours later by measuring the amount of time spent with the novel and familiar object. Intact OR memory consolidation was demonstrated if the mice spent more time than chance (15 seconds) with the novel object during testing. Training and testing for OP were identical to OR, except that testing was conducted 24 hours after training, and involved moving one of the identical training objects to a new location in the arena during testing. The 48- and 24-hour delays between training and testing in OR and OP, respectively, were used because mice infused with E_2 into the DH demonstrate enhanced OR and OP memory

consolidation at these time points relative to chance and vehicle-infused mice (Kim et al., 2016, Tuscher et al., 2016, Fortress et al., 2013, Boulware et al., 2013). All mice were trained and tested in both behavioral tasks. To counterbalance the order in which behavior was completed, half of the mice completed OR first, followed by OP, and the other half completed OP first, followed by OR.

Histological verification of DREADD expression. Three weeks after surgery, mice were anesthetized with isoflurane and perfused with 4% paraformaldehyde (PFA) in 1X phosphate-buffered saline (PBS). Whole mouse brains were then removed and post-fixed in 1X PBS/4% PFA overnight, followed by dehydration in a 1X PBS/30% sucrose solution until the brains had sunk. Tissue was sectioned on a cryostat (40 µm) and free-floated in 1X PBS until mounted onto microscope slides (VWR, Arlington Heights, IL) using aqueous mounting medium containing the nuclear stain DAPI (Santa Cruz, Dallas, TX). Fluorescent images were captured using an Olympus Fluoview FV1200 confocal microscope and accompanying software.

Golgi staining and spine counting. Two weeks after the completion of behavioral testing, mice (n=7-8/treatment group) were injected i.p. with 1 mg/kg CNO, followed by DH infusion of HBC vehicle or E_2. Mice were killed two hours later and whole brains were subjected to Golgi staining to assess E_2-mediated spine density changes in the DH and mPFC. Brains were collected 2 hours post-infusion because we have previously observed a significant increase in spine density in both brain regions two hours after DH E_2 infusion (Tuscher et al., 2016).

Therefore, this design allowed us to determine if hM4Di-mediated inhibition of the mPFC prevents E_2-mediated increases in spine density in the DH and mPFC.

Brain tissue for Golgi staining was collected as described above, two weeks after the completion of behavior (n=7-8/treatment group). Golgi impregnation was performed as described previously (Frankfurt et al., 2011) using the Rapid GolgiStain Kit (FD NeuroTechnologies, Columbia, MD). Briefly, secondary basal dendrites and tertiary apical dendrites were counted blindly from pyramidal neurons in dorsal hippocampal CA1 and layer II/III of the mPFC. Dendrites from 6 cells/region/brain were included in the analysis, and 5-7 brains were quantified/group. Neurons in all areas were chosen for analyses as follows: 1) cell bodies and dendrites were well impregnated, and 2) dendrites were continuous and clearly distinguishable from adjacent cells. Spines were counted on an Olympus BX51WI microscope under oil (100x) using Neurolucida, version 11.08 (MBF Bioscience, Williston, VT). Spine density was calculated by dividing spine number by dendrite length, and data expressed as number of spines/10 µm dendrite.

Data analysis. All statistical analyses were conducted using GraphPad Prism 6 (La Jolla, CA). To determine whether each group demonstrated intact memory for each behavioral task, OR and OP data were first analyzed using one sample *t*-tests to determine if the time spent with the novel or moved object differed significantly from chance (15 seconds; Kim et al., 2016, Tuscher et al., 2016b). This analysis was used because time spent with the objects is not independent; time spent with one object reduces time spent with the other object (Frick and Gresack, 2003). For mPFC-infusion experiments, student's *t*-tests were then used to determine significant

differences in performance between vehicle and E_2-infused groups, and effect of E_2 treatment on spine density within each brain region. Statistical significance for all analyses was determined as $p \leq 0.05$. For experiments with more than 2 treatment groups, between-group treatment differences for each task were measured using one-way ANOVAs with treatment as the independent variable, followed by Fisher's LSD *post hoc* tests when appropriate (Kim, 2016; Tuscher et al., 2016b). For spine density analyses, one-way ANOVAs or student's *t*-tests were used to determine the effect of E_2 treatment on spine density in each brain region, followed by *post hoc* tests when appropriate. Statistical significance for all analyses was determined as $p \leq 0.05$.

RESULTS

Infusion of E_2 into the mPFC immediately after training enhances memory consolidation and increases mPFC apical spine density

To determine if E_2 can act directly within the mPFC to enhance memory, young female mice were ovariectomized and implanted with bilateral guide cannulae aimed at the prelimbic region of the mPFC 1 week prior to beginning behavioral training. Mice were then trained in the OR or OP task, and bilaterally infused with HBC vehicle or 5 μg/hemisphere E_2 into the mPFC immediately after training (n=10-13/group). OR memory was tested 48 hours after training. Vehicle-infused mice spent no more time than chance with the novel object during testing ($t_{(11)} = 0.175$, $p = 0.865$; Fig. 14A), suggesting object recognition memory was not intact in our control group. In contrast, mice infused with E_2 spent significantly more time than chance with the novel object ($t_{(12)} = 3.402$, $p = 0.005$; Fig. 14A), demonstrating intact memory

for the familiar training object. In addition, E_2-infused mice spent significantly more time with the novel object than vehicle-infused mice ($t_{(23)} = 2.294$, $p = 0.031$; Fig. 14A), suggesting that E_2 in the mPFC enhances object recognition memory consolidation. Similarly, mice infused with E_2 immediately after OP training spent significantly more time than chance with the moved object 24 hours later ($t_{(10)} = 5.056$, $p = 0.001$; Fig. 14B), whereas vehicle-treated females did not ($t_{(9)} = 0.183$, $p = 0.859$; Fig. 14B). Moreover, E_2-infused mice spent significantly more time with the displaced object during testing than the vehicle-infused group ($t_{(19)} = 2.500$, $p = 0.022$; Fig. 14B), suggesting that E_2 in the mPFC can also enhance spatial memory consolidation. Together, these data demonstrate that direct infusion of E_2 in the mPFC enhances object recognition and spatial memory consolidation in ovariectomized female mice.

Two weeks after the completion of behavioral testing, mice were infused into the mPFC with their respective vehicle or E_2 treatments, and whole brains were collected two hours later for Golgi staining and spine density analyses. mPFC-E_2 infusion significantly increased mPFC apical ($t_{(12)} = 3.18$, $p = 0.008$; Fig. 15A), but not basal ($t_{(11)} = 1.13$, $p = 0.28$; Fig. 15B), spine density relative to vehicle-infused mice by 2 hours after infusion. No significant differences were observed in CA1 apical spine density between mice infused with vehicle or E_2 into the mPFC ($t_{(12)} = 0.43$, $p = 0.67$; Fig. 15C). These findings demonstrate that E_2 can increase spine density in the mPFC within two hours of infusion, but does not impact CA1 apical spine density at this time point.

Fig. 14: Infusion of E_2 into the mPFC immediately after training enhances memory consolidation

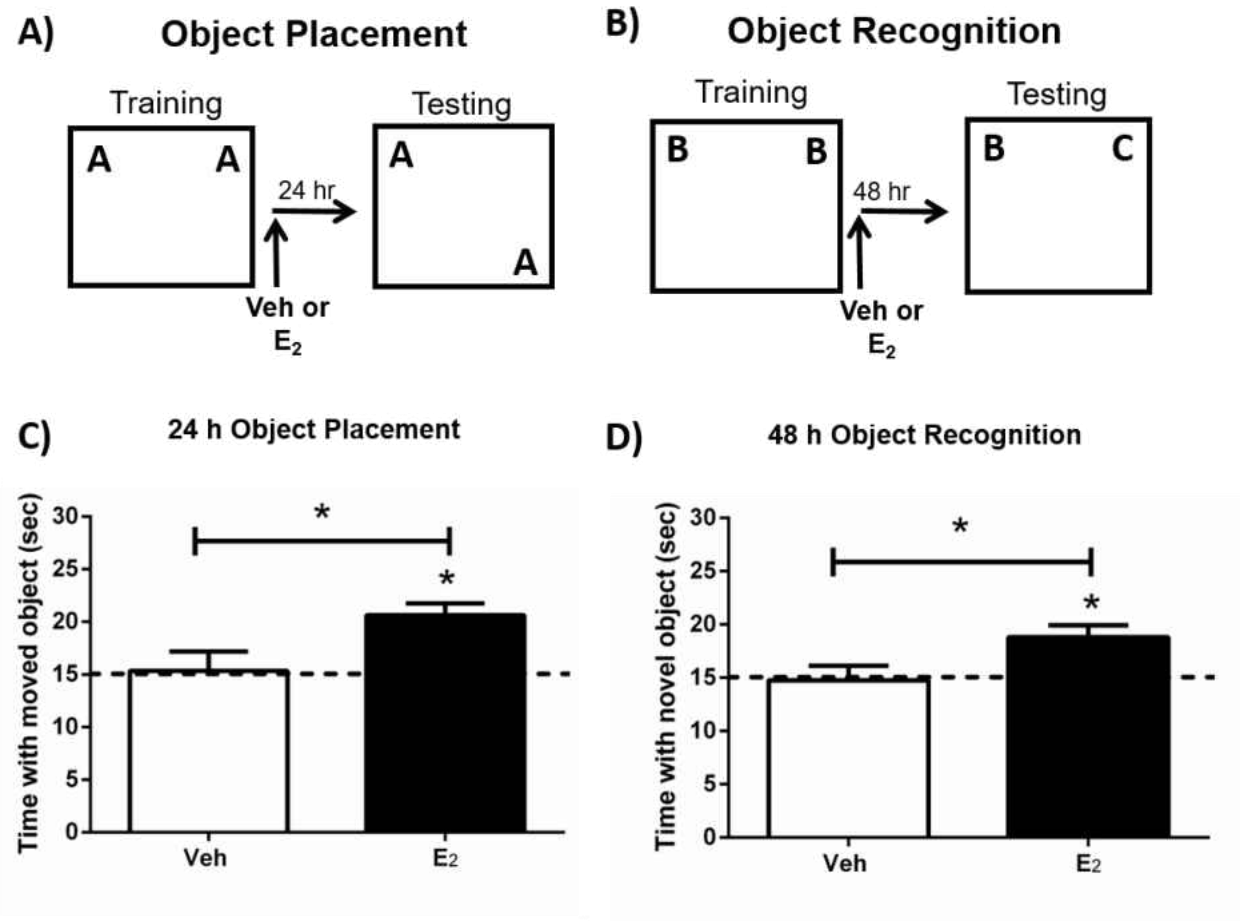


Fig. 14: Mice infused with E_2 directly into the mPFC spent significantly more time than chance (15 s) with the moved object **(A & C)** when tested 24 hours after training, or with the novel object **(B & D)** 48 hours after training. Mice infused with HBC vehicle into the mPFC did not spend more time than chance with the novel or moved objects. These data suggest that E_2 can improve the consolidation of object memories by acting directly in the mPFC. Bars represent the mean ± SEM, $*p < 0.05$ relative to chance and the vehicle group (n=10-13/group).

Fig. 15: mPFC E_2 infusion increases apical spine density in the mPFC 2 hours later

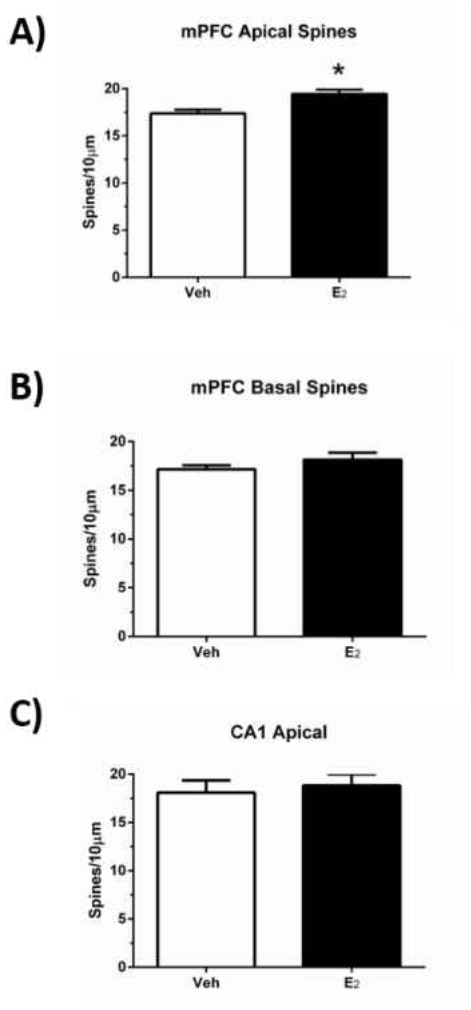


Fig. 15: Relative to vehicle, apical **(A)**, but not basal **(B)** spine density was significantly increased in the mPFC 2 hours after mPFC infusion of 5 µg E_2 per hemisphere. mPFC infusion did not alter apical spine density in CA1 **(C)**. Bars represent the mean ± SEM, *p < 0.05 relative to the vehicle group (n=6-7/group).

hM4Di and eGFP viral expression in the mPFC is present 3 weeks post-injection

DH infusion of E_2 increases dendritic spine density in the mPFC, raising the possibility that the DH and mPFC might interact to mediate the memory-enhancing effects of DH E_2 infusion. To determine if mPFC activation is necessary for the memory-enhancing effects of DH-E_2 infusion, we silenced the activity of mPFC excitatory neurons using DREADDs. Ovariectomzied mice were bilaterally injected with saline (Sham) or an AAV viral vector containing either eGFP or the hM4Di DREADD. This viral construct targets the CaMKIIα promoter, effectively blocking excitatory neurotransmission near the site of injection when bound by its ligand CNO (Armbruster et al., 2007; Zhu et al., 2014). The control viral construct AAV8-CaMKIIα-EGFP, which is driven by the same promoter as that used for the DREADD virus but lacks the hM4Di gene (Zhu et al., 2014), was used to control for nonspecific virus effects.

To verify the location and extent of expression of the virus near the injection sites in the mPFC, a subset of mice (n=3/group) was injected with the eGFP construct or hM4Di DREADD and perfused 3 weeks later (Fig. 9). EGFP (Fig. 9B & D) and mCitrine-tagged hM4Di (Fig. 9A & C) virus was detected throughout the dorsal/ventral extent of the mPFC (including the infralimbic and prelimbic regions).

Chemogenetic suppression of neurotransmission in the mPFC immediately after training prevents the memory enhancement induced by DH E_2 infusion

Once hM4Di DREADD expression was confirmed 3 weeks after injection, our next goal was to examine whether mPFC activation is necessary for the memory-enhancing effects of E_2 infused into the DH. To address this question, we first had to identify a dose of CNO that did

not impair memory on its own. This was an important first step, in light of recent data suggesting that the CNO metabolite clozapine can cross the BBB and affect behavior (Gomez et al., 2017). This step was also essential for ensuring that any DREADD-mediated disruption of the memory-enhancing effects of DH-infused E_2 resulted from an interaction between neural inactivation and E_2, rather than DREADD-mediated impairment of memory consolidation in general. To this end, a new set of ovariectomized mice received mPFC injections of saline, eGFP, or hM4Di DREADD 3 weeks prior to behavioral training (n=9-11/group). Mice were trained in OP and OR, and then received an i.p. injection of 1 mg/kg CNO immediately after training (Fig. 16A & B). Four-hour and 24-hour time points were used because previous work has established that vehicle-treated ovariectomized females show intact memory in OP and OR at these respective delays (Fortress et al., 2013, Boulware et al., 2013), which allowed us to observe any potential memory-impairing effects of DREADD-mediated inactivation. We found that post-training injection of 1 mg/kg CNO did not impair OP memory (hM4Di: $t_{(8)} = 5.04$, $p = 0.001$; Sham+eGFP: $t_{(14)} = 6.51$, $p < 0.0001$; Fig. 16C) or OR memory (hM4Di: $t_{(5)} = 3.32$, $p = 0.02$; Sham+eGFP: $t_{(12)} = 4.80$, $p = 0.0004$; Fig. 16D) in any treatment condition, as all groups spent significantly more time than chance with the moved or novel object during testing. Further, Sham+eGFP control mice and hM4Di expressing mice injected with 1 mg/kg CNO did not differ in the amount of time spent with the displaced object ($t_{(22)} = 0.24$, $p = 0.81$; Fig. 16D), or the novel object ($t_{(17)} = 0.83$, $p = 0.42$; Fig. 16C), during testing. Collectively, these data suggest that suppression of mPFC neurotransmission using 1 mg/kg CNO is not sufficient to impair either spatial or object recognition memory consolidation.

Fig. 16: CNO (1 mg/kg) does not impair memory consolidation in mice expressing hM4Di in the mPFC

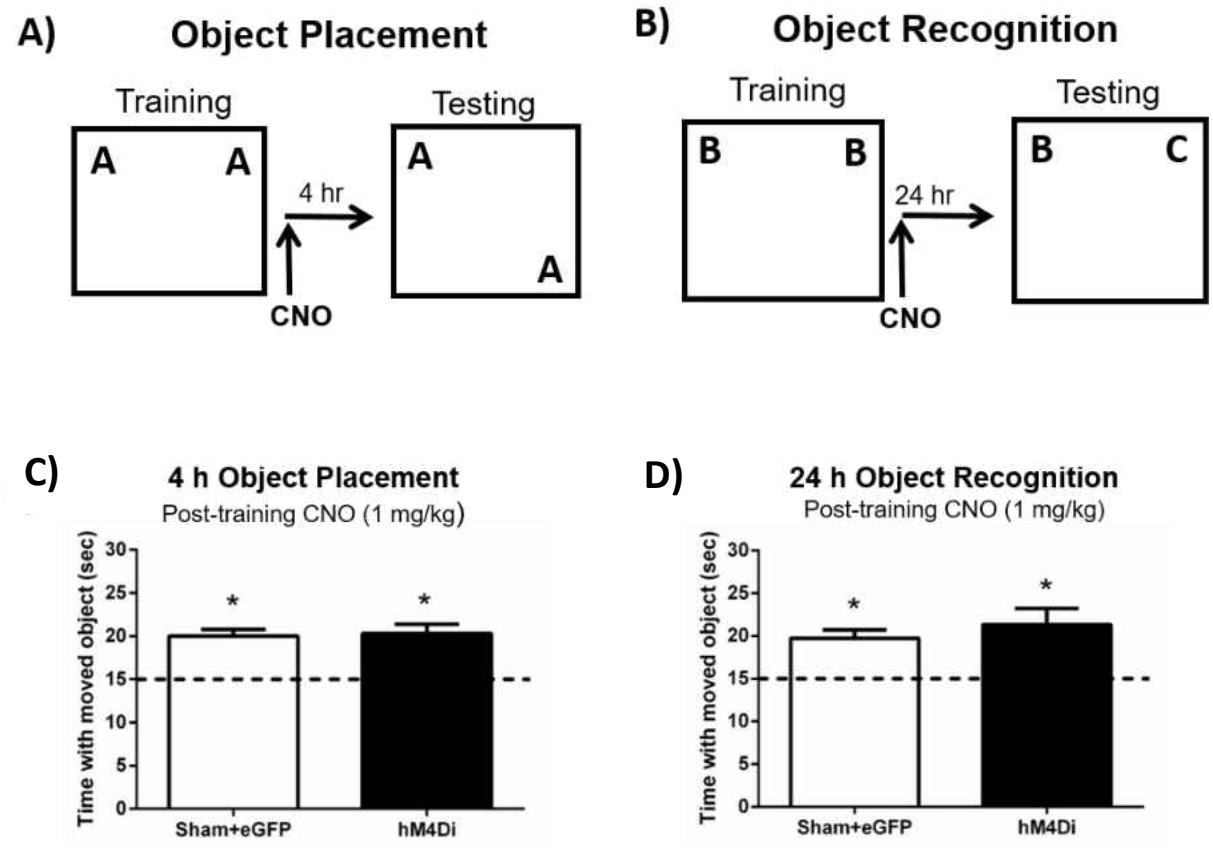


Fig. 16: Experimental design for the object placement **(A)** and object recognition task **(B)**. A 1 mg/kg dose of CNO did not impair memory consolidation in the object placement **(C)** or object recognition **(D)** tasks, as all mice spent significantly more time than chance (15 sec) with the moved or novel object. This finding suggests that 1 mg/kg CNO is not sufficient to cause enough hM4Di-mediated suppression of the mPFC to impair spatial or object recognition memory consolidation. Bars represent the mean ± SEM, $*p < 0.05$ relative to chance.

After a non-impairing dose of CNO was identified, we next asked if communication between the mPFC and DH was necessary for the memory enhancing effects observed after infusion of E_2 into the DH. To address this question, a new set of ovariectomized mice received mPFC injections of saline, eGFP, or hM4Di DREADD, and were implanted with bilateral DH guide

cannulae three weeks prior to behavioral training (n=9-11/group). Immediately after training, mice were injected with 1 mg/kg CNO to activate the DREADD, followed immediately by bilateral DH infusion of HBC vehicle or 5 µg/hemisphere E_2. We found that DREADD-mediated suppression of excitatory neurotransmission in the mPFC immediately after training blocked the E_2-mediated enhancement of OR memory. Specifically, Sham, eGFP, or hM4Di groups receiving CNO+HBC did not spend significantly more time than chance with the novel object when tested 48 hours after OR training (Sham: $t_{(10)} = 1.85$, $p = 0.09$; eGFP: $t_{(10)} = 1.42$, $p = 0.19$; hM4Di: $t_{(9)} = 0.14$, $p = 0.89$; Fig. 17D), suggesting a lack of intact OR memory in all groups. In contrast, Sham or eGFP mice receiving CNO+E_2 remembered the familiar training object 48 hours later (Sham: $t_{(8)} = 4.37$, $p = 0.002$; eGFP: $t_{(8)} = 2.78$, $p = 0.02$; Fig. 17D), indicating that sham surgery and the eGFP construct did not interfere with the ability of DH-infused E_2 to enhance OR memory consolidation. Importantly, the hM4Di group that received CNO+E_2 did not spend more time than chance with novel object (hM4Di: $t_{(9)} = 0.94$, $p = 0.37$; Fig. 17D) during testing, suggesting that excitatory neurotransmission in the mPFC is necessary for DH-infused E_2 to enhance OR memory consolidation. These findings were further supported by a one-way ANOVA, which demonstrated a significant main effect of treatment among the six groups ($F_{(5,54)} = 5.14$, $p = 0.001$). Fisher's LSD *post hoc* tests revealed that Sham and eGFP groups receiving DH E_2 infusions spent significantly more time with the novel object than E_2-treated hM4Di mice, or any group infused with HBC (ps < 0.05; Fig. 17D).

To determine if mPFC-DH interactions were also necessary for spatial memory consolidation, the same mice were tested in the object placement task. OP training was conducted just as OR, although the order of testing varied among mice as described in the

Methods. Immediately after training, mice were injected with 1 mg/kg CNO, followed by bilateral DH infusion of vehicle or 5 μg/hemisphere E_2. As with OR, we found that DREADD-mediated suppression of excitatory neurotransmission in the mPFC immediately after training blocked E_2-mediated enhancement of OP memory consolidation. Sham, eGFP, and hM4Di groups receiving CNO+HBC did not spend significantly more time than chance with the moved object when tested 24 hours after OP training (Sham: $t_{(9)} = 0.07$, $p = 0.94$; eGFP: $t_{(9)} = 0.56$, $p = 0.591$; hM4Di: $t_{(9)} = 0.56$, $p = 0.59$; Fig. 17C), suggesting a lack of spatial memory consolidation in all HBC-infused groups. In contrast, Sham and eGFP mice receiving CNO+E_2 displayed intact OP memory 24 hours after training (Sham: $t_{(9)} = 7.50$, $p < 0.0001$; eGFP: $t_{(9)} = 3.34$, $p = 0.01$; Fig. 17C), suggesting that E_2 enhanced spatial memory consolidation in both groups. However, as in OR, the DH E_2-mediated memory enhancement in OP was suppressed in the hM4Di group receiving CNO+E_2, as this group did not spend more time than chance with moved object (hM4Di: $t_{(9)} = 0.13$, $p = 0.90$) during testing. Similar to OR, this pattern of findings was further supported by a one-way ANOVA, demonstrating a significant main effect of treatment ($F_{(5,54)} = 2.79$, $p = 0.03$; Fig. 17C). Fisher's LSD *post hoc* tests revealed that Sham or eGFP mice receiving DH E_2 infusion spent significantly more time with the moved object than hM4Di mice infused with E_2, or sham mice infused with HBC (ps < 0.05; Fig. 17C). Taken together, these data suggest that mPFC activation is necessary for DH-infused E_2 to enhance memory in the OR and OP tasks in ovariectomized mice, and indicates the importance of systems-level interactions for hippocampal E_2 to regulate both spatial and object recognition memory consolidation in females.

Fig. 17: Chemogenetic suppression of neurotransmission in the mPFC immediately after training prevents the memory enhancement induced by DH E_2 infusion

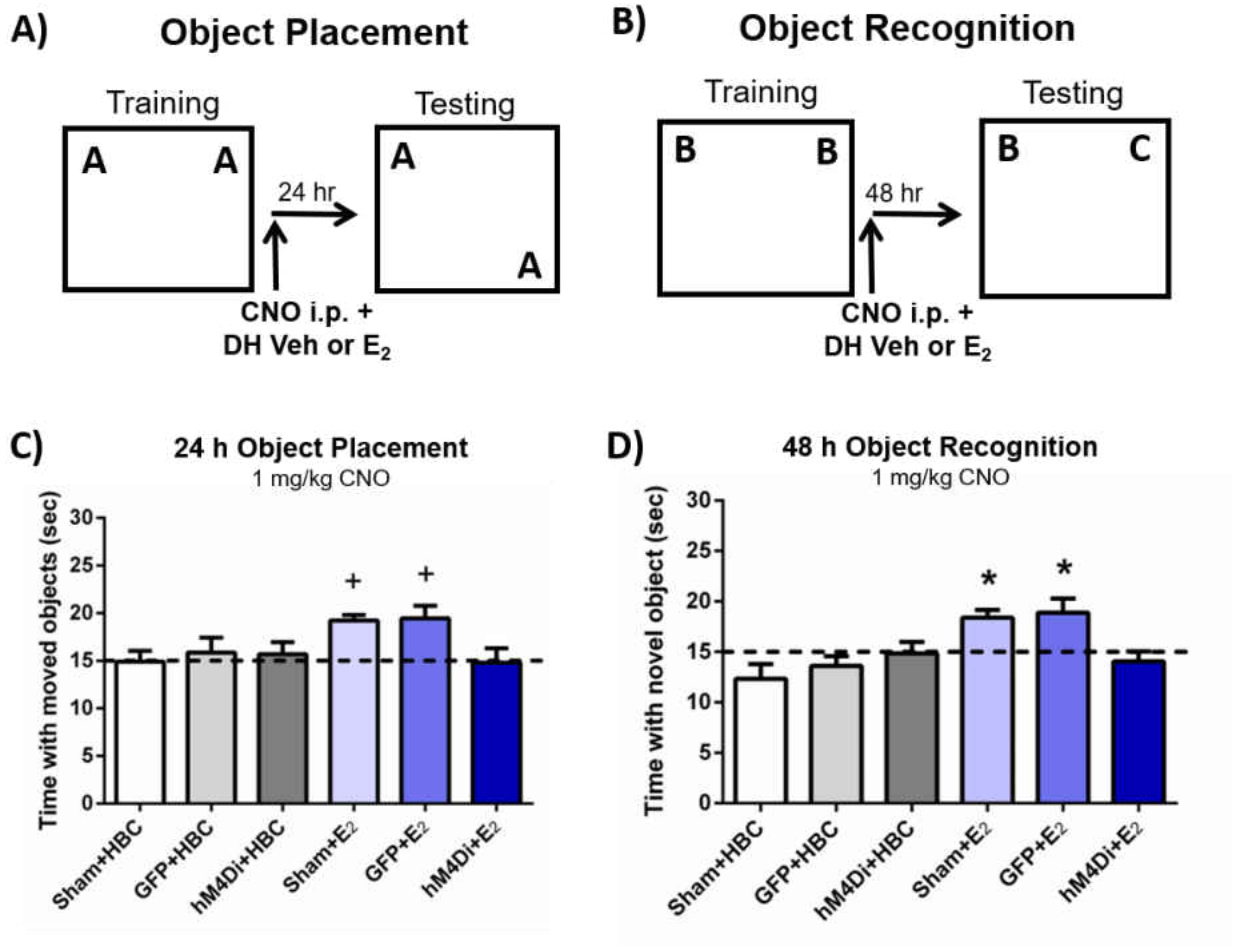


Fig. 17: Sham, eGFP or hM4Di groups receiving CNO+HBC did not spend significantly more time than chance (15 s) with the moved object when tested 24 hours after OP training **(A & C)** or with the novel object **(B & D)** 24 hours after training. In contrast, Sham or eGFP mice receiving CNO+E_2 immediately after training did spent significantly more time than chance with the moved and novel objects, displaying intact OP and OR memory. However, hM4Di mice treated with CNO+E_2 immediately after training did not demonstrate intact memory, suggesting that DREADD-mediated suppression of the mPFC blocks the beneficial mnemonic effects of DH-infused E_2. Bars represent the mean ± SEM, *p < 0.05 relative to chance, all HBC-infused groups, and the hM4Di-E_2 group (n=9-11/group). +p < 0.05 relative to chance and the Sham+HBC-infused and hM4Di-E_2 groups (n=9-11/group).

DISCUSSION

The hippocampus has been the focal point of much neuroendocrinology research examining how E_2 mediates memory formation and neuroplasticity. More recently, studies have begun to address how E_2 might impact other brain regions important for regulating cognitive function in females, including the mPFC, amygdala, striatum and perirhinal cortex (Maeng et al., 2017; Zurkovsky et al., 2007; Gervais et al., 2013). In the present study, we found that delivering E_2 directly into the mPFC significantly increased mPFC apical spine density and enhanced object recognition and spatial memory consolidation in ovariectomized mice. These data are the first to demonstrate that E_2 can act directly within the mPFC to facilitate memory consolidation and spinogenesis in female mice. Such findings are consistent with previous work from our laboratory demonstrating that infusion of E_2 into the DH can also enhance object recognition and spatial memory consolidation and increase dendritic spine density in the DH and mPFC within 2 hours (Fernandez et al., 2008; Tuscher et al., 2016, Kim et al., 2016, Boulware et al., 2013). The present data also align well with previous work reporting concomitant increases in mPFC dendritic spine density and memory enhancement in OR and OP after i.p. injection of E_2 (Inagaki et al., 2012, Luine, 2015). Although the increase in mPFC spine density in these studies occurred during a timeframe consistent with enhanced consolidation (i.e., 30 min and 4 hours after training), the memory-enhancing effects of E_2 could not be directly attributed to actions within the mPFC due to the systemic nature of E_2 delivery. Here, we found that direct delivery of E_2 to the mPFC of ovariectomized mice significantly enhanced

memory consolidation and mPFC apical spine density, suggesting that the mPFC plays a critical role in E_2-mediated enhancement of memory consolidation.

In contrast to our previous observation that DH infusion of E_2 increased *basal* spine density in the mPFC, here, we found direct infusion into the mPFC resulted in an increase in *apical* mPFC spine density. One potential reason for this discrepancy could be that input from the DH after E_2 infusion may preferentially synapse onto basal dendrites in the mPFC, while our direct infusions in the present study may have had a greater impact on apical tufts near the midline, in close proximity to where the infusions occurred. Another interesting observation is that despite DH infusion of E_2 significantly increasing mPFC spine density 2 hours after infusion, mPFC E_2 infusion did not appear to have reciprocal effects in CA1, at least at the 2 hour time point. These data suggest that hippocampal input may exert greater control over the mPFC than vice versa, or that mPFC input to the DH may take longer to occur. However, additional time points would need to be evaluated before concluding neural input from the mPFC has no effect on spine density changes in the DH.

The specific cellular and molecular mechanisms through which E_2 may regulate memory and spinogenesis in the mPFC are currently unclear. However, given the necessity of ERK activation for E_2-mediated spine changes in cortical neuron cultures (Srivastava et al., 2008), and the requirement of ERK and mTOR activation in the DH for E_2-mediated memory enhancement and spinogenesis (Fernandez et al., 2008, Fortress et al., 2014, Tuscher et al., 2016), we suspect that these pathways are also critical for the memory-enhancing effects and spine density changes observed after direct mPFC E_2 infusion, although this remains to be empirically tested. The specific ERs involved in mediating such effects also remain to be

elucidated. ERα, ERβ, and GPER are all expressed in neurons throughout the mPFC (Almey et al., 2014), so any may play a role. Recent work suggests that the rapid effects of E_2 on hippocampal dendritic spine density in ovariectomized mice are at least in part mediated by ERα and GPER (Phan et al., 2015), therefore, these receptors are prime candidates for further examination in future studies.

In addition to the direct effects of E_2 in the mPFC, the mPFC may also regulate memory by influencing the effects of E_2 infused into the DH. We examined whether excitatory neurotransmission in the mPFC was necessary for DH-infused E_2 to enhance memory by chemogenetically inactivating the mPFC immediately before DH E_2 infusion. Suppressing neurotransmission in the mPFC with the inhibitory hM4Di DREADD prevented DH-infused E_2 from enhancing object recognition and spatial memory consolidation in female mice. These findings demonstrate for the first time that mPFC activation is essential for the memory-enhancing effects of E_2 in the DH and illustrate a novel systems-level relationship between these brain regions is necessary for mediating the mnemonic effects of E_2. This experiment extends our previous work demonstrating that infusion of E_2 into the DH of ovariectomized mice increases dendritic spine density in the mPFC within 2 hours, an effect that depends on activation of ERK and mTOR signaling in the DH (Tuscher et al., 2016). This work, in combination with our present findings, suggests that activation of the mPFC is necessary for the E_2-mediated enhancement of object recognition and spatial memory consolidation previously observed after DH-E_2 infusion (Fernandez et al., 2008, Boulware et al., 2013, Fortress et al., 2013). To ensure that we observed DREADD-mediated suppression of the memory enhancing effects of E_2, rather than DREADD-mediated impairment in our object tasks, we used a dose of

CNO that does not impair memory on its own (Fig. 16). This step was important given recent findings showing that the CNO metabolite clozapine can significantly affect brain and locomotor behavior (Gomez et al., 2017). The fact that chemogenetic disruption of the mPFC prevents the beneficial mnemonic effects of DH-infused E_2 suggests these regions communicate during object memory consolidation, and lend further behavioral relevance to the E_2-mediated spine changes we recently observed in the mPFC after DH E_2 infusion (Tuscher et al., 2016).

How might the hippocampus and mPFC interact to facilitate memory formation? Accumulating evidence from studies using retrograde tracers supports the existence of at least three potential routes of communication between the hippocampus and mPFC: 1) direct unilateral projections originating from dorsal CA1 and subiculum to the mPFC (Ye et al., 2017; Hoover and Vertes, 2007), 2) unilateral projections between the ventral hippocampus and subiculum of the hippocampus to the mPFC (Ferino et al., 1987; Jay et al., 1989; Jay and Witter, 1991; Cenquizca and Swanson, 2007), and 3) indirect reciprocal connections routed through the nucleus reunions of the thalamus or lateral entorhinal cortex (Burwell and Amaral, 1998; Hoover and Vertes, 2007; Vertes et al., 2007). Evidence for the functional relevance of these connections is supported by electrophysiological studies demonstrating that tetanic stimulation in the ventral CA1/subiculum of anesthetized rats results in stable long-term potentiation (LTP) in prefrontal neurons (Laroche et al., 1990; Jay et al., 1992). Further, LTP between hippocampal and prefrontal synapses leads to a persistent (several day) increase in synaptic strength in awake behaving rats (Jay et al., 1996), suggesting direct excitatory input from the hippocampus to the mPFC. Accumulating research has also reported temporally-coordinated neuronal activity occurs between the hippocampus and mPFC during periods of wakefulness and sleep in

rodents, and that this synchronous activity between hippocampal-prefrontal circuitry is essential for systems memory consolidation (Jones and Wilson, 2005; Hyman et al., 2010; Sigurdsson et al., 2010; Schwindel and McNaughton, 2011). Given that hippocampal-prefrontal neural synchrony appears to be important for memory consolidation in the aforementioned studies, it follows that chemogenetic suppression of the mPFC in the present study may have disrupted functional connectivity between the DH and mPFC, resulting in impaired consolidation of OR and OP memories.

In congruence with studies noting the importance of coordinated neural activity between the DH and mPFC for memory formation, our present findings also indicate that systems-level interactions between the DH and mPFC are necessary for DH-infused E_2 to enhance memory in ovariectomized mice. Although the specific mechanisms through which these circuit-level changes occur are unclear at present, it is possible that DH-infusion of E_2 leads to the activation of cell-signaling cascades and the downstream transcription of genes beneficial for memory in projection regions such as the mPFC, and that DREADD-mediated suppression of this brain region prevents this activation from occurring. The idea that neuronal activity in the DH can lead to changes in gene expression in projection regions that are essential for memory formation is supported by at least two recent studies. One recent report demonstrated that disruption of neural input from the DH to the mPFC during contextual fear conditioning prevents later reactivation of mPFC engram cells and training-induced increases in spine density in the mPFC (Kitamura et al., 2017). Another study found that injection of Arc anti-sense oligonucleotides directly into the DH blocks reactivation-induced increases in markers of neural activity (e.g., Arc, pCREB, and pCofilin protein) in both the DH and in the

mPFC, and prevents reactivation-induced enhancement of fear memory expression (Ye et al., 2017). Further, DREADD-mediated inhibition of DH projection terminals in the mPFC prior to reactivation sessions also prevented reactivation-induced increases in fear memory expression and memory-associated proteins in the mPFC (Ye et al., 2017). Both studies provide evidence that inhibiting either neurotransmission or translation of a neural activity marker within the DH alters cellular activity and spine density in the mPFC, and that this disruption of cellular processes during memory formation impairs memory. These findings align with our observation that E_2-induced enhancements in memory and mPFC spinogenesis that occur after DH-E_2 infusion are prevented by ERK or mTOR inhibition in the DH (Tuscher et al., 2016, Fernandez et al., 2008, Fortress et al., 2013). Together, these findings provide evidence that cellular and molecular processes in the DH (e.g., cell-signaling activation, protein translation) influence the mPFC. The present findings expand on this work by demonstrating that disrupting interactions between the mPFC and DH after DH E_2 infusion prevents estrogenic facilitation of memory consolidation. These findings collectively support a model whereby DH infusion of E_2 leads to increased excitatory input to or activation of signal transduction pathways in the mPFC, which ultimately drives changes in gene expression and/or protein translation essential for spinogenesis and memory. Ongoing studies are evaluating the extent to which DREADD-mediated inactivation of the mPFC also prevents DH-infused E_2 from increasing spine density in the DH and mPFC, which may provide additional support for this putative model.

SUMMARY AND CONCLUSIONS

Several lines of converging neuroendocrinology research have supported a role for E_2 modulation of hippocampal physiology, morphology, and synaptic plasticity, as well as hippocampus-dependent memory. However, ERs are expressed in numerous brain regions, including the mPFC, that act in concert with the hippocampus to modulate cognitive function. Here, we provide evidence that E_2 within the mPFC can also regulate mPFC spine density and facilitate memory consolidation in female mice, and that E_2-mediated enhancement of memory requires communication between the DH and mPFC. Future studies should address whether disruption of neurotransmission in the mPFC has reciprocal consequences on protein translation and spinogenesis in the DH at additional time points, as bidirectional communication is likely necessary between these structures. Given that disruption of normal hippocampal-prefrontal communication has been implicated in a number of psychiatric and neurodegenerative disorders (Godsil et al., 2013, Sampath et al., 2017), some of which females are at greater risk for developing (i.e., depression, PTSD, AD) (Albert et al., 2015, Tolin and Foa, 2006, Solomon and Herman, 2009, Zandi et al., 2002, Dye et al., 2012), gaining a better understanding of how the hippocampus interacts with other brain regions to support the estrogenic regulation of memory will be essential for elucidating the systems-level basis of mental disorders, and for developing potential circuit-based therapeutic interventions.

CHAPTER FOUR: Summary, future directions and concluding remarks

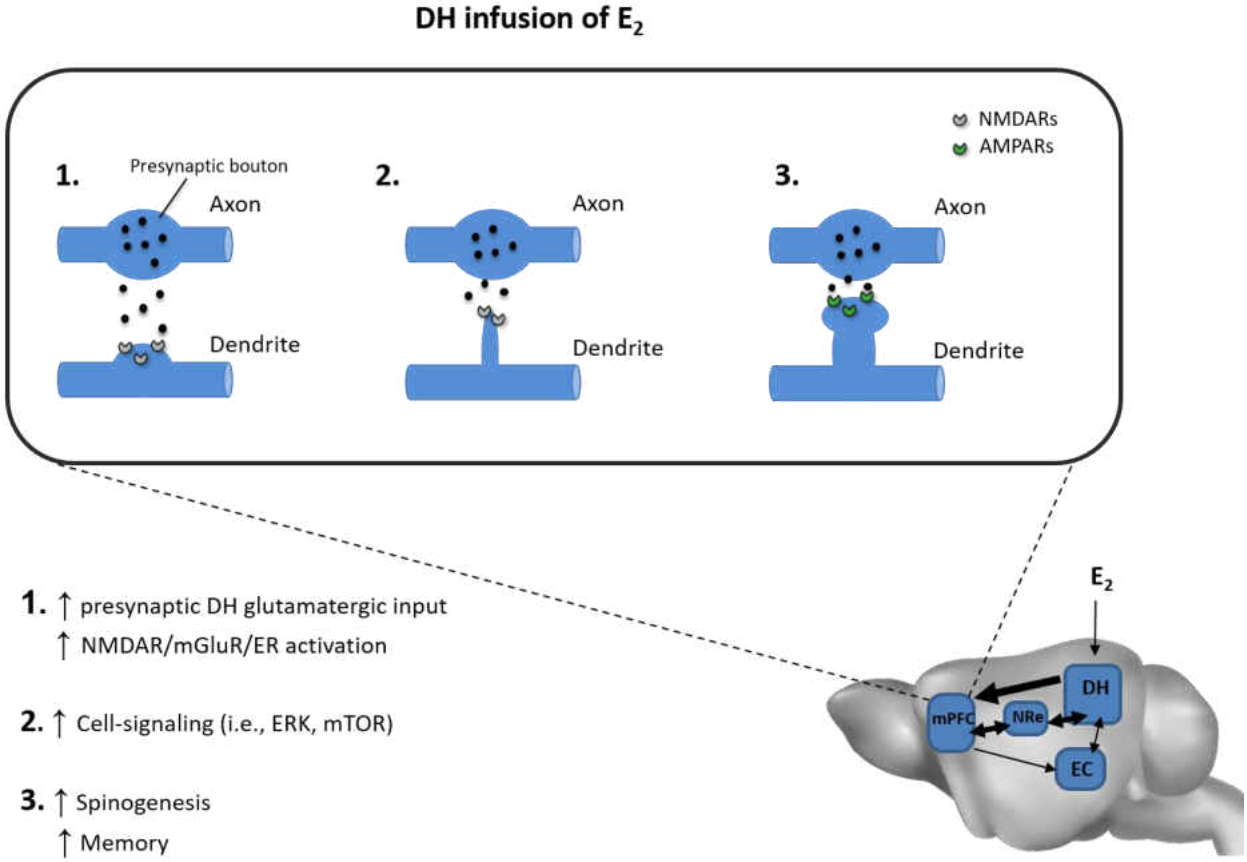
In summary, we found that the DH and the mPFC play an integral role in both object recognition and spatial memory, and likely act in concert for the successful consolidation of episodic-like memories. We also found that infusion of E_2 directly into the mPFC can enhance memory consolidation and increase mPFC apical spine density, an effect that likely contributes to the beneficial mnemonic effects observed. At present, it does not appear that mPFC infusion of E_2 can impact spine density in the DH, perhaps suggesting unidirectional regulation of the mPFC from afferent DH projections. However, additional time points will need to be evaluated prior to drawing any final conclusions regarding mPFC regulation of the DH. Finally, we demonstrated that the mPFC is necessary for DH E_2 infusion to facilitate object recognition and spatial memory consolidation, further supporting the notion that these brain regions interact during E_2-mediated memory enhancement. Although the specific cellular and molecular mechanisms that support DH-mPFC interactions during estrogenic memory formation remain to be elucidated, a putative mechanism of action is described below.

Proposed mechanism for mPFC-DH interactions that contribute to the estrogenic regulation of memory

E_2 has been shown to increase intrinsic excitability and presynaptic glutamate release in hippocampal neurons (Woolley, 2006, Oberlander et al., 2016). Therefore, E_2 infusion into the DH may lead to increased direct or indirect excitatory glutamatergic input into the mPFC,

activating ionotropic (i.e., NMDARs, AMPARs) and/or metabotropic (i.e., mGluRs) glutamate receptors expressed in the mPFC (Fig. 18A). Previous work has demonstrated that NMDAR activation is necessary for E_2-mediated changes in hippocampal spinogenesis and LTP (Smith et al., 2009), and both NMDARs and mGluRs have been directly linked to E_2-mediated enhancement of object recognition memory (Vedder et al., 2013; Lewis et al., 2008; Boulware et al., 2013). NMDARs, mGluRs, and ERs are all expressed throughout the mPFC, providing a potential mechanism through which E_2 could directly interact with ERs (after infusion into mPFC) or exert indirect effects by increasing presynaptic glutamate release from the DH to activate NMDARs and/or mGluRs in the mPFC. Activation of any of these receptors may then lead to downstream initiation of cell-signaling cascades such as ERK or mTOR within mPFC neurons, which are necessary for the increase in spine density and E_2-mediated memory enhancement after DH infusion of E_2. This mechanism of action would also be consistent with research demonstrating E_2 treatment increases ERK phosphorylation in cortical neuron cultures, an effect that is required for E_2 to increase cortical spine density in vitro. Our present findings suggest that chemogenetic suppression of neurotransmission in the mPFC may then blunt the E_2-induced increase in excitatory input and prevent synaptic connections from being strengthened after E_2 infusion, thereby reducing potential synaptic connections formed between the DH and mPFC after E_2 infusion, and preventing enhanced memory consolidation (Fig. 18B).

Fig. 18: Proposed mechanism for mPFC-DH interactions that contribute to the estrogenic regulation of memory



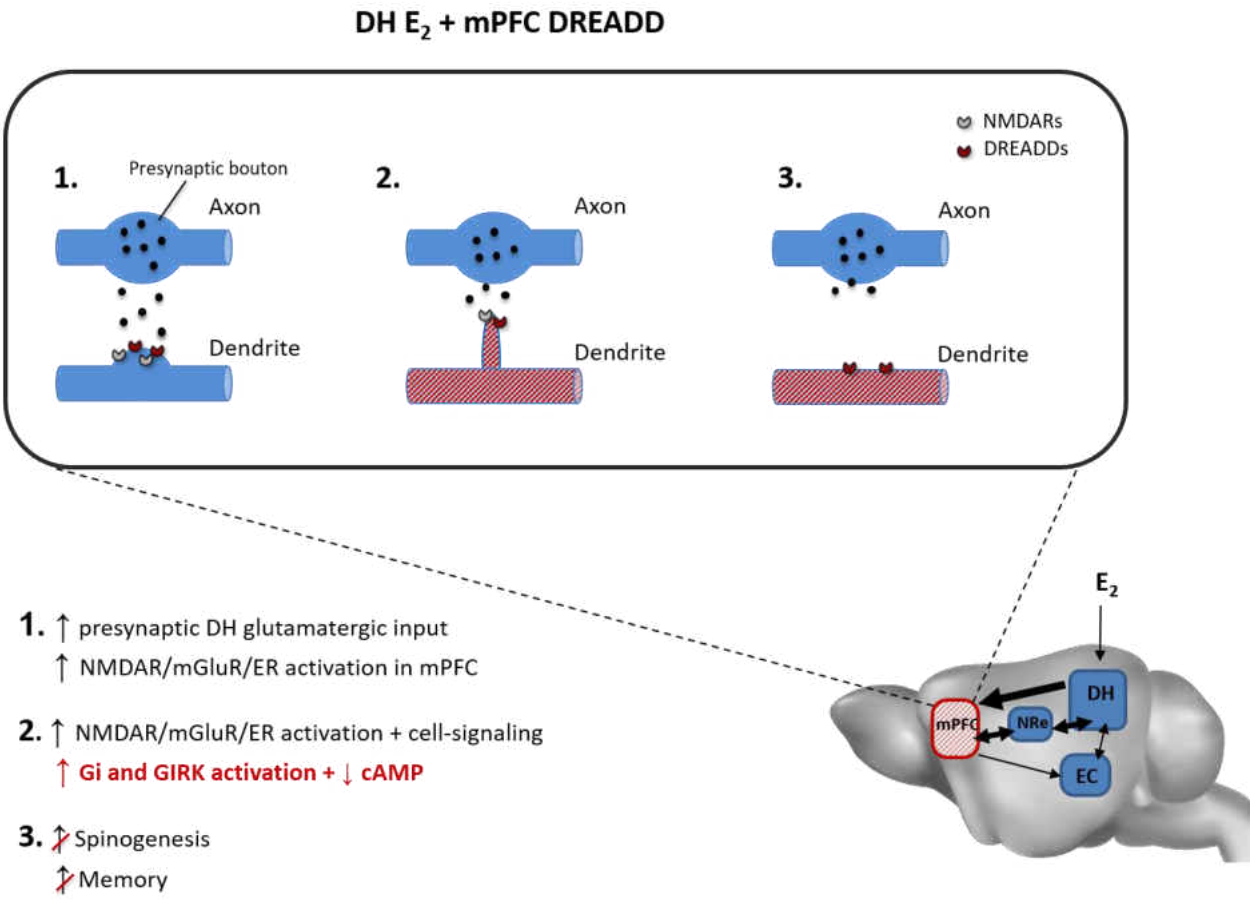


Fig. 18: Schematic for putative interactions between the DH and mPFC that contribute to the estrogenic regulation of memory. **(A)** E_2 infusion into the DH may lead to increased direct or indirect excitatory glutamatergic input into the mPFC, activating ionotropic (i.e., NMDARs, AMPARs) and/or metabotropic (i.e., mGluRs) glutamate receptors expressed in the mPFC. Activation of any of these receptors, as well as co-activation of mPFC ERs, may then lead to downstream initiation of cell-signaling cascades such as ERK or mTOR within mPFC neurons, which are necessary for the increase in spine density and E_2-mediated memory enhancement after DH infusion of E_2. **(B)** Chemogenetic suppression of neurotransmission in the mPFC may then blunt the E_2-induced increase in excitatory input by exerting opposing effects on neuronal excitability (via cAMP reduction and GIRK activation). This may ultimately prevent synaptic connections between the DH and mPFC from being strengthened after E_2 infusion, and thus prevent enhanced memory consolidation.

FUTURE DIRECTIONS

Examination of direct DH-mPFC projections and intermediary structures through which the DH and mPFC may communicate

Data presented in this dissertation suggest that the mPFC and DH collaborate during object memory consolidation, however this work does not definitively establish direct monosynaptic communication between the two structures is necessary for episodic memory formation. To more directly address this question in future studies, one might inject inhibitory DREADDs (e.g., hM4Di, KORD) into the DH and implant guide cannulae into the mPFC to deliver CNO directly to DH projection terminals in the mPFC. This would allow for selective chemogenetic inactivation of DH terminals projecting to the mPFC without affecting receptors expressed in neuronal populations directly within the hippocampus. Similarly, an optogenetic approach could be utilized wherein inhibitory opsins (e.g., ArchT, Halorhodopsin) would be injected into the DH, combined with optic fiber implantation in the mPFC to optogenetically silence DH projection terminals in the mPFC. Although the present study focused on the necessity of neurotransmission in the DH and mPFC for normal episodic-like memory consolidation, and therefore used inhibitory DREADDs to evaluate memory impairment after inactivation of the DH or mPFC in the object memory paradigm, excitatory DREADDs (e.g., hM3) or opsins (e.g., ChR2) could also be used to stimulate neurotransmission between these brain regions to examine facilitation of episodic memory consolidation. Using such an approach would allow one to ask how long the window of intact OR and OP memory can be extended via optogenetic or chemogenetic stimulation of the DH, mPFC, or DH axons terminating in mPFC.

Further, potential intermediary structures through which the DH and mPFC likely communicate could be investigated in a similar fashion. The nucleus reunions of the thalamus and the entorhinal and perirhinal cortices are brain regions identified by tract tracing studies as potential intermediate routes of neural communication between the DH and mPFC, and as such, examining the necessity of these structures in episodic memory formation is another area ripe for further investigation.

Bidirectional effects on the cellular and molecular mechanisms that support memory formation between DH-mPFC projections

Although most tract tracing studies suggest the monosynaptic projections between the DH and mPFC are not bidirectional, and are instead predominantely unidirectional from the DH to mPFC, whether the mPFC exerts control over the DH either directly or indirectly has not been addressed. Despite the fact that we did not see any spine density changes in the DH 2 hours after mPFC-E_2 infusion, this does not rule out the possibility that other important molecular changes may occur in the form of cell-signaling activation or changes in gene transcription. Further, spine changes were only examined at a single time point in our present experiments (2 hours), which does not eliminate the possibility that downstream or feedback-type changes occur from mPFC input back to DH at later time points. As such, it may be worth analyzing additional time points beyond 2 hours, or cell-signaling events in the DH earlier than 2 hours after mPFC infusion of E_2.

Elucidation of the molecular mechanisms through which E_2 mediates spinogenesis and memory enhancement in the mPFC

The specific signal transduction cascades through which E_2 facilitates memory formation in the mPFC also remain to be elucidated. Given the requirement of DH ERK and mTOR activation for E_2-mediated enhancement of memory and spine density changes in CA1 and the mPFC after DH infusion of E_2 (Fernandez et al., 2008, Fortress et al., 2013, Tuscher et al., 2016), these pathways are likely also involved in E_2's beneficial mnemonic effects when delivered directly into the mPFC. The likelihood that mPFC spinogenesis is mediated in part by the ERK pathway is also supported by in vitro work identifying the necessity of ERK activation for E_2-mediated spine density increases in cortical neuron cultures (Srivastava et al., 2008). However, such questions remain to be empirically tested in vivo.

Epigenetic regulation of E_2 sensitive genes in the mPFC

Although not the focus of this dissertation, E_2 can modify the expression of epigenetic enzymes (e.g., DNA methyltransferases, histone deacetylases; Zhao et al., 2010, 2012) which likely impact the expression of proteins important for spinogenesis and memory formation. At least one study has demonstrated that E_2 can epigenetically regulate the expression of the Bdnf gene in the DH of young and middle-aged female mice (Fortress et al., 2014). However, whether E_2 epigenetically regulates gene expression in the mPFC is yet to be addressed. Future studies should investigate E_2-mediated epigenetic regulation of memory-associated genes in the mPFC and other brain regions important for cognitive function, including the amygdala, entorhinal and perirhinal cortices and nucleus reunions of the thalamus.

The potential contribution of brain-synthesized E_2 in the mPFC

One other relatively novel area of neuroendocrine research is the contribution of E_2 synthesized locally in the brain to memory formation. Recent work from our lab has provided evidence that hippocampal E_2 levels are elevated in an experience-induced manner after novel object training, and that blocking E_2 synthesis directly in the DH of ovariectomized mice impairs object recognition and spatial memory consolidation. However, whether local E_2 synthesis is also important in the mPFC for object memory consolidation remains an open question. This area of research may be increasingly important given that emerging evidence from clinical studies suggests that aromatase inhibition, which suppresses endogenous E_2 synthesis, may negatively impact cognitive function in human females. For example, aromatase inhibitors, which are used to treat hormone-receptor positive forms of breast cancer (Geisler et al., 2002, Puddefoot et al., 2002), are reportedly associated with impaired working memory, concentration, and performance in verbal and visual memory tasks (Collins et al., 2009, Bender et al., 2007, Bender et al., 2015). Therefore, gaining a better understanding of how aromatase inhibition impacts E_2 synthesized locally in the brain, and what the consequences of such inhibition are on the circuitry that supports cognitive function, will be particularly important for future studies to address.

CONCLUDING REMARKS

The hippocampus has been the focal point of much neuroendocrinology research the past 25 years. This is in part due to seminal work published in the early 1990s by Bruce

McEwen that expanded the scope of neuroendocrinology research in rodents beyond the confines of brain regions involved in regulating reproduction and sexual maturation. This work, demonstrated that brain regions important for learning and memory such as the hippocampus were also responsive to estrogens, and ignited an entire new subfield of research dedicated to the effects of estrogens on cognition. Only more recently has research on E_2-mediated effects on neuroplasticity and memory been extended to other brain regions important for regulating cognitive function in females, including the mPFC, amygdala, striatum, and perirhinal cortex (Maeng et al., 2017; Zurkovsky et al., 2007; Gervais et al., 2013). This expanded perspective, including hippocampal interactions with other brain regions that support a variety of cognitive processes, will be critical for gaining a more comprehensive, circuit-level understanding of the estrogenic regulation of memory. Although this dissertation used a model of episodic-like memory without aversive or appetitive components to address questions about the role of E_2 in memory, the DH and mPFC collaborate to create and store other types of memoriesthat have important implications for neuropsychiatric disorders like addiction, depression, and PTSD. Given that several psychiatric disorders including depression, anxiety, and PTSD disproportionately affect more females relative to males (Albert et al., 2015, Tolin and Foa, 2006, Solomon and Herman, 2009), and that loss of E_2 during middle age can increase susceptibility to neurodegernative diseases like AD (Alzheimer Association, Zandi et al., 2002, Dye et al., 2012), gaining a better understanding of systems-level interactions between the hippocampus and other brain regions regulated by E_2 will be particularly important for the future of neuroendocrinology research.

REFERENCES

Acosta JI, Mayer LP, Braden BB, Nonnenmacher S, Mennenga SE, Bimonte-Nelson HA (2010) The cognitive effects of conjugated equine estrogens depend on whether menopause etiology is transitional or surgical. Endocrinology 151:3795-3804.

Acosta JI, Mayer L, Talboom JS, Tsang CW, Smith CJ, Enders CK, Bimonte-Nelson HA (2009) Transitional versus surgical menopause in a rodent model: etiology of ovarian hormone loss impacts memory and the acetylcholine system. Endocrinology 150:4248-4259.

Akirav I, Maroun M (2006) Ventromedial prefrontal cortex is obligatory for consolidation and reconsolidation of object recognition memory. Cereb Cortex 16:1759-1765.

Almey A, Cannell E, Bertram K, Filardo E, Milner TA, Brake WG (2014) Medial prefrontal cortical estradiol rapidly alters memory system bias in female rats: ultrastructural analysis reveals membrane-associated estrogen receptors as potential mediators. Endocrinology 155:4422-4432.

Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL (2007) Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc Natl Acad Sci U S A 104:5163-5168.

Atkins CM, Selcher JC, Petraitis JJ, Trzaskos JM, Sweatt JD (1998) The MAPK cascade is required for mammalian associative learning. Nat Neurosci 1:602-609.

Baker KB, Kim JJ (2002) Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. Learn Mem 9:58-65.

Barha CK, Dalton GL, Galea LA (2010) Low doses of 17alpha-estradiol and 17beta-estradiol facilitate, whereas higher doses of estrone and 17alpha- and 17beta-estradiol impair, contextual fear conditioning in adult female rats. Neuropsychopharmacology 35:547-559.

Barker GR, Bird F, Alexander V, Warburton EC (2007) Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. J Neurosci 27:2948-2957.

Barker GR, Banks PJ, Scott H, Ralph GS, Mitrophanous KA, Wong LF, Bashir ZI, Uney JB, Warburton EC (2017) Separate elements of episodic memory subserved by distinct hippocampal-prefrontal connections. Nat Neurosci 20:242-250.

Bimonte HA, Granholm A-CE, Seo H, Isacson O (2002) Spatial memory testing decreases hippocampal amyloid precursor protein in young, but not aged, female rats. Neurosci Lett 298:50-54.

Bimonte-Nelson HA, Acosta JI, Talboom JS (2010) Neuroscientists as cartographers: mapping the crossroads of gonadal hormones, memory and age using animal models. Molecules 15:6050-6105.

Blum S, Moore AN, Adams F, Dash PK (1999) A mitogen-activated protein kinase cascade in the CA1/CA2 subfield of the dorsal hippocampus is essential for long-term spatial memory. J Neurosci 19:3535-3544.

Boulware MI, Heisler JD, Frick KM (2013) The memory-enhancing effects of hippocampal estrogen receptor activation involve metabotropic glutamate receptor signaling. J Neurosci 33:15184-15194.

Boulware MI, Weick JP, Becklund BR, Kuo SP, Groth RD, Mermelstein PG (2005) Estradiol activates group I and II metabotropic glutamate receptor signaling, leading to opposing influences on cAMP response element-binding protein. J Neurosci 25:5066-5078.

Bowman RE, Ferguson D, Luine VN (2002) Effects of chronic restraint stress and estradiol on open field activity, spatial memory, and monoaminergic neurotransmitters in ovariectomized rats. Neuroscience 113:401-410.

Broadbent NJ, Squire LR, Clark RE (2004) Spatial memory, recognition memory, and the hippocampus. Proc Natl Acad Sci U S A 101:14515-14520.

Broadbent NJ, Gaskin S, Squire LR, Clark RE (2010) Object recognition memory and the rodent hippocampus. Learn Mem 17:5-11.

Burwell RD, Amaral DG (1998) Perirhinal and postrhinal cortices of the rat: interconnectivity and connections with the entorhinal cortex. J Comp Neurol 391:293-321.

Cenquizca LA, Swanson LW (2007) Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. Brain Res Rev 56:1-26.

Chang YJ, Yang CH, Liang YC, Yeh CM, Huang CC, Hsu KS (2009) Estrogen modulates sexually dimorphic contextual fear extinction in rats through estrogen receptor beta. Hippocampus 19:1142-1150.

Choleris E, Clipperton-Allen AE, Phan A, Valsecchi P, Kavaliers M (2012) Estrogenic involvement in social learning, social recognition and pathogen avoidance. Front Neuroendocrinol 33:140-159.

Churchwell JC, Kesner RP (2011) Hippocampal-prefrontal dynamics in spatial working memory: interactions and independent parallel processing. Behav Brain Res 225:389-395.

Cohen SJ, Munchow AH, Rios LM, Zhang G, Asgeirsdottir HN, Stackman RW, Jr. (2013) The rodent hippocampus is essential for nonspatial object memory. Curr Biol 23:1685-1690.

Corcoran KA, Quirk GJ (2007) Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. J Neurosci 27:840-844.

Daniel JM (2006) Effects of oestrogen on cognition: What have we learned from basic research? J Neuroendocrinol 18:787-795.

Daniel JM, Bohacek J (2010) The critical period hypothesis of estrogen effects on cognition: Insights from basic research. Biochim Biophys Acta 1800:1068-1076.

Daniel JM, Roberts SL, Dohanich GP (1999) Effects of ovarian hormones and environment on radial maze and water maze performance of female rats. Physiology and Behavior 66:11-20.

Daniel JM, Hulst JL, Berbling JL (2006) Estradiol replacement enhances working memory in middle-aged rats when initiated immediately after ovariectomy but not after a long-term period of ovarian hormone deprivation. Endocrinology 147:607-614.

Daniel JM, Fader AJ, Spencer AL, Dohanich GP (1997) Estrogen enhances performance of female rats during acquisition of a radial arm maze. Horm Behav 32:217-225.

Dere E, Huston JP, De Souza Silva MA (2005) Integrated memory for objects, places, and temporal order: evidence for episodic-like memory in mice. Neurobiol Learn Mem 84:214-221.

DeVito LM, Eichenbaum H (2010) Distinct contributions of the hippocampus and medial prefrontal cortex to the "what-where-when" components of episodic-like memory in mice. Behav Brain Res 215:318-325.

Eichenbaum H (2017) Prefrontal-hippocampal interactions in episodic memory. Nat Rev Neurosci.

Ennaceur A (2010) One-trial object recognition in rats and mice: methodological and theoretical issues. Behav Brain Res 215:244-254.

Fader AJ, Hendricson AW, Dohanich GP (1998) Estrogen improves performance of reinforced T-maze alternation and prevents the amnestic effects of scopolamine administered systemically or intrahippocampally. Neurobiol Learn Mem 69:225-240.

Fader AJ, Johnson PEM, Dohanich GP (1999) Estrogen improves working but not reference memory and prevents amnestic effects of scopolamine on a radial-arm maze. Pharm Biochem Behav 62:711-717.

Fan L, Zhao Z, Orr PT, Chambers CH, Lewis MC, Frick KM (2010) Estradiol-induced object memory consolidation in middle-aged female mice requires dorsal hippocampal extracellular signal-regulated kinase and phosphatidylinositol 3-kinase activation. J Neurosci 30:4390-4400.

Farrell MS, Roth BL (2013) Pharmacosynthetics: Reimagining the pharmacogenetic approach. Brain Res 1511:6-20.

Ferino F, Thierry AM, Glowinski J (1987) Anatomical and electrophysiological evidence for a direct projection from Ammon's horn to the medial prefrontal cortex in the rat. Exp Brain Res 65:421-426.

Fernandez SM, Lewis MC, Pechenino AS, Harburger LL, Orr PT, Gresack JE, Schafe GE, Frick KM (2008) Estradiol-induced enhancement of object memory consolidation involves hippocampal ERK activation and membrane-bound estrogen receptors. J Neurosci 28:8660-8667.

Floresco SB, Seamans JK, Phillips AG (1997) Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. J Neurosci 17:1880-1890.

Fortress AM, Fan L, Orr PT, Zhao Z, Frick KM (2013) Estradiol-induced object recognition memory consolidation is dependent on activation of mTOR signaling in dorsal hippocampus. Learn Mem 20:147-155.

Forwood SE, Winters BD, Bussey TJ (2005) Hippocampal lesions that abolish spatial maze performance spare object recognition memory at delays of up to 48 hours. Hippocampus 15:347-355.

Foster TC, Sharrow KM, Kumar A, Masse J (2003) Interaction of age and chronic estradiol replacement on memory and markers of brain aging. Neurobiol Aging 24:839-852.

Frankfurt M, Salas-Ramirez K, Friedman E, Luine V (2011) Cocaine alters dendritic spine density in cortical and subcortical brain regions of the postpartum and virgin female rat. Synapse 65:955-961.

Frick KM (2009) Estrogens and age-related memory decline in rodents: what have we learned and where do we go from here? Horm Behav 55:2-23.

Frick KM (2012) Building a better hormone therapy? How understanding the rapid effects of sex steroid hormones could lead to new therapeutics for age-related memory decline. Behav Neurosci 126:29-53.

Frick KM, Berger-Sweeney J (2001) Spatial reference memory and neocortical neurochemistry vary with the estrous cycle in C57BL/6 mice. Behav Neurosci 115:229-237.

Frick KM, Gresack JE (2003) Sex differences in the behavioral response to spatial and object novelty in adult C57BL/6 mice. Behav Neurosci 117:1283-1291.

Gabbott PL, Warner TA, Jays PR, Salway P, Busby SJ (2005) Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers. J Comp Neurol 492:145-177.

Galea LA, Uban KA, Epp JR, Brummelte S, Barha CK, Wilson WL, Lieblich SE, Pawluski JL (2008) Endocrine regulation of cognition and neuroplasticity: our pursuit to unveil the complex interaction between hormones, the brain, and behaviour. Can J Exp Psychol 62:247-260.

Gibbs RB (2010) Estrogen therapy and cognition: A review of the cholinergic hypothesis. Endocr Rev 31:224-253.

Gillies GE, McArthur S (2010) Estrogen actions in the brain and the basis for differential action in men and women: a case for sex-specific medicines. Pharmacol Rev 62:155-198.

Gomez JL, Bonaventura J, Lesniak W, Mathews WB, Sysa-Shah P, Rodriguez LA, Ellis RJ, Richie CT, Harvey BK, Dannals RF, Pomper MG, Bonci A, Michaelides M (2017) Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. Science 357:503-507.

Goto Y, Grace AA (2008) Dopamine modulation of hippocampal-prefrontal cortical interaction drives memory-guided behavior. Cereb Cortex 18:1407-1414.

Gould E, Woolley CS, Frankfurt M, McEwen BS (1990) Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. J Neurosci 10:1286-1291.

Gresack JE, Frick KM (2006) Post-training estrogen enhances spatial and object memory consolidation in female mice. Pharm Biochem Behav 84:112-119.

Hammond R, Gibbs RB (2011) GPR30 is positioned to mediate estrogen effects on basal forebrain cholinergic neurons and cognitive performance. Brain Res 1379:53-60.

Hammond RS, Tull LE, Stackman RW (2004) On the delay-dependent involvement of the hippocampus in object recognition memory. Neurobiol Learn Mem 82:26-34.

Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Strom A, Treuter E, Warner M, Gustafsson JA (2007) Estrogen receptors: how do they signal and what are their targets. Physiol Rev 87:905-931.

Hoeffer CA, Klann E (2010) mTOR signaling: at the crossroads of plasticity, memory and disease. Trends Neurosci 33:67-75.

Holloway CM, McIntyre CK (2011) Post-training disruption of Arc protein expression in the anterior cingulate cortex impairs long-term memory for inhibitory avoidance training. Neurobiol Learn Mem 95:425-432.

Hoover WB, Vertes RP (2007) Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct 212:149-179.

Hyman JM, Zilli EA, Paley AM, Hasselmo ME (2010) Working Memory Performance Correlates with Prefrontal-Hippocampal Theta Interactions but not with Prefrontal Neuron Firing Rates. Front Integr Neurosci 4:2.

Inagaki T, Frankfurt M, Luine V (2012) Estrogen-induced memory enhancements are blocked by acute bisphenol A in adult female rats: role of dendritic spines. Endocrinology 153:3357-3367.

Itoi K, Sugimoto N (2010) The brainstem noradrenergic systems in stress, anxiety and depression. J Neuroendocrinol 22:355-361.

Izaki Y, Takita M, Akema T (2008) Specific role of the posterior dorsal hippocampus-prefrontal cortex in short-term working memory. Eur J Neurosci 27:3029-3034.

Jay TM, Witter MP (1991) Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. J Comp Neurol 313:574-586.

Jay TM, Glowinski J, Thierry AM (1989) Selectivity of the hippocampal projection to the prelimbic area of the prefrontal cortex in the rat. Brain Res 505:337-340.

Jay TM, Burette F, Laroche S (1996) Plasticity of the hippocampal-prefrontal cortex synapses. J Physiol Paris 90:361-366.

Jay TM, Thierry AM, Wiklund L, Glowinski J (1992) Excitatory Amino Acid Pathway from the Hippocampus to the Prefrontal Cortex. Contribution of AMPA Receptors in Hippocampo-prefrontal Cortex Transmission. Eur J Neurosci 4:1285-1295.

Jensen EV (1962a) On the mechanism of estrogen action. Perspect Biol Med 6:47-59.

Jensen EV, and Jacobson, H.I. (1962b) Basic guides to the mechanism of estrogen action. Recent Prog Horm Res 18:387–414.

Jones MW, Wilson MA (2005) Theta rhythms coordinate hippocampal-prefrontal interactions in a spatial memory task. PLoS Biol 3:e402.

Kim J, Szinte, J.S., Boulware, M.I., Frick, K.M. (2016) 17β-estradiol and agonism of G-protein Coupled Estrogen Receptor (GPER) enhance hippocampal memory via different cell-signaling mechanisms. JNeurosci:in press.

Kitamura T, Ogawa SK, Roy DS, Okuyama T, Morrissey MD, Smith LM, Redondo RL, Tonegawa S (2017) Engrams and circuits crucial for systems consolidation of a memory. Science 356:73-78.

Kranz GS, Kasper S, Lanzenberger R (2010) Reward and the serotonergic system. Neuroscience 166:1023-1035.

Kuiper GGJM, Carlsson B, Grandien K, Enmark E, Häggblad J, Nilsson S, Gustafsson JA (1997) Comparison of the ligand and binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138:863-870.

LaLumiere RT, Niehoff KE, Kalivas PW (2010) The infralimbic cortex regulates the consolidation of extinction after cocaine self-administration. Learn Mem 17:168-175.

Laroche S, Jay TM, Thierry AM (1990) Long-term potentiation in the prefrontal cortex following stimulation of the hippocampal CA1/subicular region. Neurosci Lett 114:184-190.

Lebron-Milad K, Milad MR (2012) Sex differences, gonadal hormones and the fear extinction network: implications for anxiety disorders. Biol Mood Anxiety Disord 2:3.

Leon WC, Bruno MA, Allard S, Nader K, Cuello AC (2010) Engagement of the PFC in consolidation and recall of recent spatial memory. Learn Mem 17:297-305.

Leuner B, Mendolia-Loffredo S, Shors TJ (2004) High levels of estrogen enhance associative memory formation in ovariectomized females. Psychoneuroendocrinology 29:883-890.

Lewis MC, Kerr KM, Orr PT, Frick KM (2008) Estradiol-induced enhancement of object memory consolidation involves NMDA receptors and protein kinase A in the dorsal hippocampus of female C57BL/6 mice. Behav Neurosci 122:716-721.

Li C, Brake WG, Romeo RD, Dunlop JC, Gordon M, Buzescu R, Magarinos AM, Allen PB, Greengard P, Luine V, McEwen BS (2004) Estrogen alters hippocampal dendritic spine shape and enhances synaptic protein immunoreactivity and spatial memory in female mice. Proceedings of the National Academy of Sciences, USA 101:2185-2190.

Li R, Cui J, Shen Y (2014) Brain sex matters: estrogen in cognition and Alzheimer's disease. Mol Cell Endocrinol 389:13-21.

Li R, He P, Cui J, Staufenbiel M, Harada N, Shen Y (2012) Brain Endogenous Estrogen Levels Determine Responses to Estrogen Replacement Therapy via Regulation of BACE1 and NEP in Female Alzheimer's Transgenic Mice. Mol Neurobiol.

Long J, He P, Shen Y, Li R (2012) New evidence of mitochondria dysfunction in the female Alzheimer's disease brain: deficiency of estrogen receptor-beta. J Alzheimers Dis 30:545-558.

Long JA, Evans HM (1922) The oestrous cycle in the rat and its associated phenomena. Berkeley, CA: University of California Press.

Lopez AJ, Kramar E, Matheos DP, White AO, Kwapis J, Vogel-Ciernia A, Sakata K, Espinoza M, Wood MA (2016) Promoter-Specific Effects of DREADD Modulation on Hippocampal Synaptic Plasticity and Memory Formation. J Neurosci 36:3588-3599.

Luine V (2015) Recognition memory tasks in neuroendocrine research. Behav Brain Res 285:158-164.

Luine V, Rodriguez M (1994) Effects of estradiol on radial arm maze performance of young and aged rats. Behav Neural Biol 62:230-236.

Luine VN (2014) Estradiol and cognitive function: Past, present and future. Horm Behav 66:602-618.

Luine VN, Frankfurt M (2012) Estrogens facilitate memory processing through membrane mediated mechanisms and alterations in spine density. Front Neuroendocrinol 33:388-402.

Luine VN, Jacome LF, Maclusky NJ (2003) Rapid enhancement of visual and place memory by estrogens in rats. Endocrinology 144:2836-2844.

Luine VN, Richards ST, Wu VY, Beck KD (1998) Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. Horm Behav 34:149-162.

MacLusky NJ, Luine VN, Hajszan T, Leranth C (2005) The 17alpha and 17beta isomers of estradiol both induce rapid spine synapse formation in the CA1 hippocampal subfield of ovariectomized female rats. Endocrinology 146:287-293.

Maier SF, Watkins LR (2005) Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. Neurosci Biobehav Rev 29:829-841.

Maki PM (2012) Minireview: effects of different HT formulations on cognition. Endocrinology 153:3564-3570.

Manns JR, Eichenbaum H (2006) Evolution of declarative memory. Hippocampus 16:795-808.

Maviel T, Durkin TP, Menzaghi F, Bontempi B (2004) Sites of neocortical reorganization critical for remote spatial memory. Science 305:96-99.

McEwen BS (2001) Estrogens effects on the brain: Multiple sites and molecular mechanisms. Journal of Applied Physiology 91:2785-2801.

McGaugh JL (1989) Dissociating learning and performance: Drug and hormone enhancement of memory storage. Brain Res Bull 23:339-345.

Milad MR, Zeidan MA, Contero A, Pitman RK, Klibanski A, Rauch SL, Goldstein JM (2010) The influence of gonadal hormones on conditioned fear extinction in healthy humans. Neuroscience 168:652-658.

Milner TA, McEwen BS, Hayashi S, Li CJ, Reagan LP, Alves SE (2001) Ultrastructural evidence that hippocampal alpha estrogen receptors are located at extranuclear sites. J Comp Neurol 429:355-371.

Milner TA, Ayoola K, Drake CT, Herrick SP, Tabori NE, McEwen BS, Warrier S, Alves SE (2005) Ultrastructural localization of estrogen receptor beta immunoreactivity in the rat hippocampal formation. J Comp Neurol 491:81-95.

Mumby DG (2001) Perspectives on object-recognition memory following hippocampal damage: Lessons from studies in rats. Behav Brain Res 127:159-181.

Nelson RJ (2000) An Introduction to Behavioral Endocrinology. Sunderland, MA: Sinauer Associates.

Osterlund MK, Keller E, Hurd YL (2000) The human forebrain has discrete estrogen receptor alpha messenger RNA expression: High levels in the amygdaloid complex. Neuroscience 95:333-342.

Packard MG (1998) Posttraining estrogen and memory modulation. Horm Behav 34:126-139.

Packard MG, Teather LA (1997) Intra-hippocampal estradiol infusion enhances memory in ovariectomized rats. Neuroreport 8:3009-3013.

Peters J, LaLumiere RT, Kalivas PW (2008) Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. J Neurosci 28:6046-6053.

Phan A, Lancaster KE, Armstrong JN, MacLusky NJ, Choleris E (2011) Rapid effects of estrogen receptor alpha and beta selective agonists on learning and dendritic spines in female mice. Endocrinology 152:1492-1502.

Phan A, Gabor CS, Favaro KJ, Kaschack S, Armstrong JN, MacLusky NJ, Choleris E (2012) Low doses of 17beta-estradiol rapidly improve learning and increase hippocampal dendritic spines. Neuropsychopharmacology 37:2299-2309.

Phan A, Suschkov S, Molinaro L, Reynolds K, Lymer JM, Bailey CD, Kow LM, MacLusky NJ, Pfaff DW, Choleris E (2015) Rapid increases in immature synapses parallel estrogen-induced hippocampal learning enhancements. Proc Natl Acad Sci U S A 112:16018-16023.

Phillips SM, Sherwin BB (1992) Effects of estrogen on memory function in surgically menopausal women. Psychoneuroendocrinology 17:485-495.

Rhodes ME, Frye CA (2004) Estrogen has mnemonic-enhancing effects in the inhibitory avoidance task. Pharmacol Biochem Behav 78:551-558.

Rodgers SP, Bohacek J, Daniel JM (2010) Transient estradiol exposure during middle age in ovariectomized rats exerts lasting effects on cognitive function and the hippocampus. Endocrinology 151:1194-1203.

Runyan JD, Moore AN, Dash PK (2004) A role for prefrontal cortex in memory storage for trace fear conditioning. J Neurosci 24:1288-1295.

Saavedra MA, Abarca N, Arancibia P, Salinas V (1990) Sex differences in aversive and appetitive conditioning in two strains of rats. Physiology and Behavior 47:107-112.

Sandstrom NJ, Williams CL (2004) Spatial memory retention is enhanced by acute and continuous estradiol replacement. Horm Behav 45:128-135.

Schlinger BA, Remage-Healey L (2012) Neurosteroidogenesis: insights from studies of songbirds. J Neuroendocrinol 24:16-21.

Schmolck H, Kensinger EA, Corkin S, Squire LR (2002) Semantic knowledge in patient H.M. and other patients with bilateral medial and lateral temporal lobe lesions. Hippocampus 12:520-533.

Schultz W (2001) Reward signaling by dopamine neurons. Neuroscientist 7:293-302.

Schwarz JM, McCarthy MM (2008) Steroid-induced sexual differentiation of the developing brain: multiple pathways, one goal. J Neurochem 105:1561-1572.

Schwindel CD, McNaughton BL (2011) Hippocampal-cortical interactions and the dynamics of memory trace reactivation. Prog Brain Res 193:163-177.

Segal M, Murphy D (2001) Estradiol induces formation of dendritic spines in hippocampal neurons: functional correlates. Horm Behav 40:156-159.

Shughrue PJ, Merchenthaler I (2000) Evidence for novel estrogen binding sites in the rat hippocampus. Neuroscience 99:605-612.

Shughrue PJ, Lane MV, Merchenthaler I (1997) Comparative distribution of estrogen receptor-α and -ß mRNA in the rat central nervous system. J Comp Neurol 388:507-525.

Shughrue PJ, Scrimo PJ, Merchenthaler I (2000) Estrogen binding and estrogen receptor characterization (ERα and ERß) in the cholinergic neurons of the rat basal forebrain. Neuroscience 96:41-49.

Sigurdsson T, Stark KL, Karayiorgou M, Gogos JA, Gordon JA (2010) Impaired hippocampal-prefrontal synchrony in a genetic mouse model of schizophrenia. Nature 464:763-767.

Singh M, Meyer EM, Millard WJ, Simpkins JW (1994) Ovarian steroid deprivation results in a reversible learning impairment and compromised cholinergic function in female Sprague-Dawley rats. Brain Res 644:305-312.

Smith CC, Vedder LC, Nelson AR, Bredemann TM, McMahon LL (2010) Duration of estrogen deprivation, not chronological age, prevents estrogen's ability to enhance hippocampal synaptic physiology. Proc Natl Acad Sci U S A 107:19543-19548.

Squire LR (2009) The legacy of patient H.M. for neuroscience. Neuron 61:6-9.

Squire LR, Wixted JT (2011) The cognitive neuroscience of human memory since H.M. Annu Rev Neurosci 34:259-288.

Squire LR, Wixted JT, Clark RE (2007) Recognition memory and the medial temporal lobe: a new perspective. Nat Rev Neurosci 8:872-883.

Squire LR, Berg, D., Bloom, F.E., du Lac, S., Ghosh, A., Spitzer, N.C. (2008) Fundamental Neuroscience, 3rd Edition. Burlington, MA: Elsevier, Inc.

Srivastava DP, Evans PD (2013) G-protein oestrogen receptor 1: trials and tribulations of a membrane oestrogen receptor. J Neuroendocrinol 25:1219-1230.

Tee MK, Rogatsky I, Tzagarakis-Foster C, Cvoro A, An J, Christy RJ, Yamamoto KR, Leitman DC (2004) Estradiol and selective estrogen receptor modulators differentially regulate target genes with estrogen receptors alpha and beta. Mol Biol Cell 15:1262-1272.

Tuscher JJ, Fortress AM, Kim J, Frick KM (2014) Regulation of object recognition and object placement by ovarian sex steroid hormones. Behav Brain Res.

Tuscher JJ, Luine V, Frankfurt M, Frick KM (2016a) Estradiol-Mediated Spine Changes in the Dorsal Hippocampus and Medial Prefrontal Cortex of Ovariectomized Female Mice Depend on ERK and mTOR Activation in the Dorsal Hippocampus. J Neurosci 36:1483-1489.

Tuscher JJ, Szinte JS, Starrett JR, Krentzel AA, Fortress AM, Remage-Healey L, Frick KM (2016b) Inhibition of local estrogen synthesis in the hippocampus impairs hippocampal memory consolidation in ovariectomized female mice. Horm Behav 83:60-67.

Twining RC, Tuscher JJ, Doncheck EM, Frick KM, Mueller D (2013) 17beta-estradiol is necessary for extinction of cocaine seeking in female rats. Learn Mem 20:300-306.

Vardy E et al. (2015) A New DREADD Facilitates the Multiplexed Chemogenetic Interrogation of Behavior. Neuron 86:936-946.

Vertes RP, Hoover WB, Szigeti-Buck K, Leranth C (2007) Nucleus reuniens of the midline thalamus: link between the medial prefrontal cortex and the hippocampus. Brain Res Bull 71:601-609.

Wade CB, Dorsa DM (2003) Estrogen activation of cyclic adenosine 5'-monophosphate response element-mediated transcription requires the extracellularly regulated kinase/mitogen-activated protein kinase pathway. Endocrinology 144:832-838.

Walf AA, Koonce CJ, Frye CA (2008) Estradiol or diarylpropionitrile administration to wild type, but not estrogen receptor beta knockout, mice enhances performance in the object recognition and object placement tasks. Neurobiol Learn Mem 89:513-521.

Wang GW, Cai JX (2006) Disconnection of the hippocampal-prefrontal cortical circuits impairs spatial working memory performance in rats. Behav Brain Res 175:329-336.

Wang GW, Cai JX (2008) Reversible disconnection of the hippocampal-prelimbic cortical circuit impairs spatial learning but not passive avoidance learning in rats. Neurobiol Learn Mem 90:365-373.

Warburton EC, Brown MW (2010) Findings from animals concerning when interactions between perirhinal cortex, hippocampus and medial prefrontal cortex are necessary for recognition memory. Neuropsychologia 48:2262-2272.

Warburton EC, Brown MW (2015) Neural circuitry for rat recognition memory. Behav Brain Res 285:131-139.

Wilson DI, Langston RF, Schlesiger MI, Wagner M, Watanabe S, Ainge JA (2013) Lateral entorhinal cortex is critical for novel object-context recognition. Hippocampus 23:352-366.

Winters BD, Forwood SE, Cowell RA, Saksida LM, Bussey TJ (2004) Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. J Neurosci 24:5901-5908.

Woolley CS, McEwen BS (1992) Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J Neurosci 12:2549-2554.

Woolley CS, McEwen BS (1993) Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat. J Comp Neurol 336:293-306.

Woolley CS, Gould E, Frankfurt M, McEwen BS (1990) Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. J Neurosci 10:4035-4039.

Ye X, Kapeller-Libermann D, Travaglia A, Inda MC, Alberini CM (2017) Direct dorsal hippocampal-prelimbic cortex connections strengthen fear memories. Nat Neurosci 20:52-61.

Zandi PP, Carlson MC, Plassman BL, Welsh-Bohmer KA, Mayer LS, Steffens DC, Breitner JCS (2002) Hormone replacement therapy and incidence of Alzheimer disease in older women. J Am Med Assoc 288:2123-2129.

Zeidan MA, Igoe SA, Linnman C, Vitalo A, Levine JB, Klibanski A, Goldstein JM, Milad MR (2011) Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. Biol Psychiatry 70:920-927.

Zhang Y, Fukushima H, Kida S (2011) Induction and requirement of gene expression in the anterior cingulate cortex and medial prefrontal cortex for the consolidation of inhibitory avoidance memory. Mol Brain 4:4.

Zhao MG, Toyoda H, Lee YS, Wu LJ, Ko SW, Zhang XH, Jia Y, Shum F, Xu H, Li BM, Kaang BK, Zhuo M (2005) Roles of NMDA NR2B subtype receptor in prefrontal long-term potentiation and contextual fear memory. Neuron 47:859-872.

Zhao Z, Fan L, Frick KM (2010) Epigenetic alterations regulate the estradiol-induced enhancement of memory consolidation. Proc Natl Acad Sci U S A 107:5605-5610.

Zhao Z, Fan L, Fortress AM, Boulware MI, Frick KM (2012) Hippocampal histone acetylation regulates object recognition and the estradiol-induced enhancement of object recognition. J Neurosci 32:2344-2351.

Zhu H, Pleil KE, Urban DJ, Moy SS, Kash TL, Roth BL (2014) Chemogenetic inactivation of ventral hippocampal glutamatergic neurons disrupts consolidation of contextual fear memory. Neuropsychopharmacology 39:1880-1892.

Jennifer Jacqueline Tuscher

Curriculum Vitae

EDUCATION

Fall 2011 – present	**UW Milwaukee** Milwaukee, WI M.S. in Psychology, December 2014 Ph.D. in Psychology, anticipated August 2017
Fall 2004 – Spring 2007	**UW Madison** Madison, WI B.S. in Biology, August 2007
Spring 2004	**Deakin University** Melbourne, Australia Concentration in Biological studies
Fall 2002 – Fall 2003	**UW Whitewater** Whitewater, WI

RESEARCH EXPERIENCE

06/11 – present **UW-Milwaukee Neuromnemonics of Aging Lab**
Advisor: Dr. Karyn Frick

Graduate Research Assistant

- Master's Thesis: The role of brain-synthesized E_2 in hippocampal learning and memory (2014)
- Ph.D. Thesis: The role of hippocampal and medial prefrontal interactions in the estrogenic regulation of memory (in progress)

08/08 – 08/10 **UW Madison AIDS Vaccine Lab – WNPRC**
Supervisors: Dr. David O'Connor and Dr. Roger Wiseman

Associate Research Specialist, Genetic Services

- My primary responsibility involved Major Histocompatibility Complex (MHC) genotyping primate species to better understand of how specific sets of genes or alleles impact susceptibility and progression of infectious diseases

- Our lab focused on how certain MHC alleles conferred protection or susceptibility to SIV (Simian Immunodeficiency Virus), although I also conducted MHC genotyping for other investigators nation-wide to guide other types of biomedical research (e.g., transplantation studies, pedigree determination, and organization of genetically defined breeding colonies)

11/07 – 08/08 **USGS National Wildlife Health Center**
Supervisor: Dr. Hon Ip

Avian Influenza Molecular Research Technician

- Worked with virologist Hon Ip on surveillance for the introduction of H5N1 by migratory birds to North America
- In addition to H5N1 surveillance, we were also interested in observing the amount and type of recombinant influenza strains passed amongst the avian species collected by and sent to our agency
- Specific duties entailed isolating, amplifying, and purifying viral genome segments of interest, followed by identification of the resulting sequences using BLAST, and recording recombination events between North American and non-North American strains of the virus

5/07 – 7/07 **Chulalongkorn University, Thailand**
Advisor: Dr. Sirirat Kokpol

Microbiology Research Internship

- Studied the antibacterial properties of Thai root extracts (e.g., pomelo, sugar cane) under the supervision of Dr. Sirirat Kokpol

PEER REVIEWED PUBLICATIONS

Doncheck, E.M., Urbanik, L.A., DeBaker, M.C., Barron, L.M., Liddiard, G.T., **Tuscher, J.J.,** Frick, K.M., Hillard, C.J., Mantsch, J.R. 2017. Estradiol potentiates the reinstatement of cocaine seeking in females: role of the prelimbic prefrontal cortex and cannabinoid type-1 receptors. In press, *Neuropsychopharmacology*.

Frick, K.M., **Tuscher, J.J.**, Koss, W.A., Kim, J.K., Taxier, L.R. 2017. Estrogenic regulation of memory consolidation: A look beyond the hippocampus, ovaries, and females. In press, *Physiology and Behavior*.

Tuscher, J.J., Szinte, J.S., Starrett, J.R., Krentzel, A.A., Fortress, A.M., Remage-Healey, L., Frick, K., 2016. Inhibition of local estrogen synthesis in the hippocampus impairs hippocampal memory consolidation in ovariectomized female mice. *Hormones and Behavior* 83: 60-7.

Tuscher, J.J., Luine, V., Frankfurt, M., Frick, K.M., 2016. Estradiol-mediated spine changes in the dorsal hippocampus and medial prefrontal cortex of ovariectomized female mice depend on ERK and mTOR activation in the dorsal hippocampus. *The Journal of Neuroscience* 36(5):1483-9. Featured in the April 2016 *International Behavioral Neuroscience Society (IBNS)* newsletter: http://www.ibnsconnect.org/newsletter-vol20-2 - article-1

Warren, W.C., Jasinska, A.J., García-Pérez, R., Svardal, H., Tomlinson, C., Rocchi, M., Archidiacono, N., Capozzi, O., Minx, P., Montague, M.J., Kim, K., Hillier, L.W., Graser, M., Graves, T., Hughes, J., Tran, N., Huang, Y., Ramensky, V., Juretic, N., Wasserscheid, J., Marques-Bonet, T., Turner, T.R., Wiseman, R.W., **Tuscher, J.J.**, Karl, J.A., Schmitz, J.E., O'Connor, D.H, Schmidt, C., Kaplan, J.R., Jorgensen, M.J, Nordborg, M., Tomas, G.W., Hahn, M.W., Raney, B.J., Aken, B., Schmitz, J., Churakov, G., Noll, A., Stanyon, R., Webb, D., Thibaud-Nissen, F., Dewar, K., Weinstock, G.M., Wilson, R.K., Freimer, N.B., 2015. The genome of the vervet (*Chlorocebus aethiops sabaeus*). *Genome Research* 25(12):1921-33.

Frick, K.M., Kim, J.K., **Tuscher, J.J.**, and Fortress, A.M., 2013. Sex steroid hormones matter for learning and memory: estrogenic regulation of hippocampal function in male and female rodents. *Learning and Memory* 22(9):472-93.

Tuscher, J.J., Fortress, A.M., Kim, J.K., and Frick, K.M., 2013. Regulation of object recognition and object placement by ovarian sex steroid hormones. *Behavioral Brain Research* 285:140-57.

Hafenbreidel, M, Rafa Todd, C, Twining, R.C., **Tuscher, J.J.**, Mueller, D.M., 2014. Bidirectional effects of inhibiting or activating NMDA receptors on extinction after cocaine self-administration in rats. *Psychopharmacology* 231(24): 4585-94.

Fortress, A.M., Schram, S.L., **Tuscher, J.J.**, and Frick, K.M., 2013. Canonical Wnt signaling is necessary for object recognition memory consolidation. *The Journal of Neuroscience* 33(31):12619-26. Highlighted in *Nature Reviews Neuroscience*: http://www.nature.com/nrn/journal/v14/n9/full/nrn3583.html

Twining, R.C., **Tuscher, J.J.**, Doncheck, E.D., Frick, K.M., and Mueller, D.M., 2013. Estradiol enhances extinction of cocaine seeking. *Learning and Memory* 20(6):300-6.

O'Connor, S.L., Lhost, J.J, Becker, E.A., Detmer, A.M., Johnson, R.C., MacNair, C.E., Wiseman, R.W., Karl, J.A., Greene, J.M., Burwitz, B.J., Bimber, B.N., Lank, S.M., **Tuscher, J.J.**, Mee, E.T., Rose, N.J., Desrosiers, R.C., Friedrich, T.C., Carrington, M., and O'Connor, D.H., 2010. MHC heterozygote advantage in simian immunodeficiency virus-infected Mauritian cynomolgus macaques. *Science Translational Medicine* 2(22): 22ra18.

Nadazdin, O, Boskovic, S, Murakami, T, O'Connor, D.H., Wiseman, R.W., Karl, J.A, **Tuscher, J.J.**, Sachs, D.H., Madsen, J.C., Tocco, G, Kawai, T, Cosimi, A.B., and Benichou, G., 2010. Phenotype, distribution and alloreactive properties of memory T cells from cynomolgus monkeys. *American Journal of Transplantation* 10(6):1375-84.

Bolton, D.L., Minang, J.T., Trivett, M.T., Song, K., **Tuscher, J.J.**, Li, Y., Piatak, M., O'Connor, D., Lifson, J.D., Roederer, M., and Ohlen, C., 2009. Trafficking, persistence, and activation state of adoptively transferred allogeneic and autologous simian immunodeficiency virus-specific CD8+ T cell clones during acute and chronic infection of rhesus macaques. *Journal of Immunology* 184(1):303-14.

O'Leary, C. E., Wiseman, R.W., Karl, J.A., Bimber, B.N., Lank, S.M., **Tuscher J.J.**, and O'Connor, D.H., 2009. Identification of novel MHC class I sequences in pig-tailed macaques by amplicon pyrosequencing and full-length cDNA cloning and sequencing. *Immunogenetics* (10):689-701.

Wiseman, R. W., Karl, J.A., Bimber, B.N., O'Leary, C.E., Lank, S.M., **Tuscher, J.J.**, Detmer A.M., Bouffard, P., Levenkova, N., Turcotte, C.L., Szekeres, E.J., Wright, C., Harkins T., and O'Connor, D.H., 2009. Major histocompatibility complex genotyping with massively parallel pyrosequencing. *Nature Medicine* 15:1322-1326.

PUBLICATIONS IN PREP

Tuscher, J.J., Frick, K.M. Medial prefrontal E_2 infusion enhances object memory consolidation in female mice. *In prep*.

Tuscher, J.J., Taxier, L.T., Fortress, A.M., Frick, K.M. E_2-mediated memory enhancement of object memory consolidation requires interactions between the dorsal hippocampus and medial prefrontal cortex. *In prep*.

AWARDS

2017	Travel Fellowship, Alzheimer's Association International Conference
2016	Trainee Professional Development Award, Society for Neuroscience
2016	American Psychological Foundation/Council of Graduate Departments of Psychology Ruth G. and Joseph D. Matarazzo Scholarship
2014 - 2017	Advanced Opportunity Program Fellowship, UW-Milwaukee Graduate School
2014	Young Investigator Scholarship, Alzheimer's Drug Discovery Foundation
2014	Graduate Student Travel Award, UW-Milwaukee Graduate School
2014	Summer Research Fellowship, UW-Milwaukee Psychology Department
2014	2nd Place Graduate Presentations, UW-Milwaukee AGSIP Symposium
2013	Graduate Student Travel Award, UW-Milwaukee Graduate School
2012	(Spring) 3rd Place Graduate Student Poster Presentations, Wisconsin Psychological Association

CONFERENCE PRESENTATIONS

Tuscher, J.J., Frick, K.M. The role of the dorsal hippocampus and medial prefrontal cortex in estradiol-mediated enhancement of memory formation. Alzheimer's Association International Conference, July, 16, 2017.

Debaker, M.C., Doncheck, E.M., **Tuscher, J.J.**, Urbanik, L.A., Barron, L.M., Schuh, L.J., Liddiard, G.T., Herdeman, E.E., Frick, K. M., Mantsch, J. R. Proestrus-level 17β-estradiol potentiates the reinstatement of cocaine seeking. Society for Neuroscience, November, 14, 2016.

Doncheck, E.M., **Tuscher, J.J.**, Urbanik, L.A., Debaker, M.C., Barron, L.M., Frick, K. M., Liu, Q.S., Hillard, C.J., Mantsch, J. R. Localization and mechanisms underlying 17β-estradiol-potentiated reinstatement of cocaine seeking behavior in female rats. Society for Neuroscience, November, 14, 2016.

Tuscher, J.J., Fortress, A.M., Frick, K.M. The role of the dorsal hippocampus and medial prefrontal cortex in estradiol-mediated enhancement of object memory consolidation in female mice. Society for Neuroscience, November, 13, 2016.

Tuscher, J.J., Luine, V., Frankfurt, M., Frick, K.M. Estradiol-mediated spine changes in the dorsal hippocampus and medial prefrontal cortex depend on ERK and mTOR activation in the dorsal hippocampus of ovariectomized female mice. Society for Neuroscience, October, 20, 2015.

Doncheck, E.M., **Tuscher, J.J.**, Debaker, M.C., Urbanik, L.A., McCartan, L.E., Herdeman, E.E., Frick, K.M., Mantsch, J.R. Estrogen-potentiated reinstatement of cocaine seeking. Society for Neuroscience, October, 19, 2015.

Tuscher, J.J., Luine, V., Frankfurt, M., Frick, K. Estradiol-mediated spine changes in the dorsal hippocampus and medial prefrontal cortex depend on ERK and mTOR activation in the dorsal hippocampus of ovariectomized female mice. Molecular and Cellular Cognition Society Conference, October, 15, 2015.

Tuscher, J.J., Frankfurt, M., Luine, V., Frick, K. Dorsal hippocampal infusion of 17β-estradiol increases dendritic spine density in the CA1 subfield of the hippocampus in ovariectomized female mice. Society for Neuroscience, November, 17, 2014.

Tuscher, J.J., Frankfurt, M., Luine, V., Frick, K. Dorsal hippocampal infusion of 17β-estradiol increases dendritic spine density in the CA1 subfield of the hippocampus in ovariectomized female mice. Molecular and Cellular Cognition Society Conference, November, 13, 2014.

Tuscher, J.J., Szinte, J.S., Starrett, J.R., Krentzel, A.A., Remage-Healey, L. and Frick, K.M. Hippocampally-synthesized estrogens are essential for hippocampal memory consolidation in female mice. 15th International Conference on Alzheimer's Drug Discovery, September 8, 2014.

Fortress, A.M., Schram, S.L., **Tuscher, J.J.**, and Frick, K.M. Canonical Wnt Signaling is Necessary for Object Recognition Memory Consolidation. Society for Neuroscience, November 11, 2013.

Tuscher, J.J., Szinte, J.S., Starrett, J.R., Krentzel, A.A., Remage-Healey, L. and Frick, K.M. Hippocampally-synthesized estrogens are essential for spatial memory consolidation in female mice. Society for Neuroscience, November 11, 2013.

Szinte, J.S., **Tuscher, J.J.**, Frick, K.M. Hippocampally-synthesized estradiol is necessary for spatial memory consolidation in female mice. Association of Graduate Students in Psychology, April 5, 2013.

Twining, R.C., **Tuscher, J.J.**, Doncheck, E.D., Frick, K.M., and Mueller, D.M. Estradiol enhances extinction of cocaine seeking. Society for Behavioral Neuroendocrinology, June 15, 2012. Society for Neuroscience, October 17, 2012.

Tuscher, J.J., Twining, R.C., Doncheck, E.D., Frick, K.M., and Mueller, D.M. Estradiol enhances extinction of cocaine seeking. Society for Behavioral Neuroendocrinology, June 15, 2012.

Rafa Todd, C.S., **Tuscher, J.J.**, Twining, R.C., Doncheck, E.D., Frick, K.M., and Mueller, D.M. Estradiol enhances extinction of cocaine seeking. UWM Undergraduate Research Symposium, April 20, 2012.

Rafa Todd, C.S., **Tuscher, J.J.**, Twining, R.C., Doncheck, E.D., Frick, K.M., and Mueller, D.M. Estradiol enhances extinction of cocaine seeking. Association of Graduate Students in Psychology, April 20, 2012.

Tuscher, J.J., Twining, R.C., Doncheck, E.D., Frick, K.M., and Mueller, D.M. Estradiol enhances extinction of cocaine seeking. Wisconsin Psychological Association, April 12, 2012.

ORAL PRESENTATIONS

"Estrogenic regulation of spinogenesis in the dorsal hippocampus and medial prefrontal cortex." UW-Milwaukee AGSIP Symposium, April, 8, 2016.

"Estrogenic regulation of spinogenesis in the dorsal hippocampus and medial prefrontal cortex." UW-Milwaukee Neuroscience Seminar, February, 27, 2015.

"The role of local estradiol synthesis in hippocampal learning and memory." UW-Milwaukee AGSIP Symposium, April, 4, 2014.

"The role of local estradiol synthesis in hippocampal learning and memory." UW-Milwaukee Neuroscience Seminar, March, 4, 2014.

TECHNIQUES UTILIZED

Behavioral testing: object recognition, object placement, Morris water maze, cocaine-induced conditioned place preference, cocaine self-administration

Surgical and procedural: stereotaxic surgery (implantation of guide cannulae), ovariectomy, intracranial drug infusion, intracranial injection for virally-mediated gene transfer (DREADDs), intraperitoneal injection, vaginal lavage, brain dissection, intracardiac perfusion, histology

Molecular: western blotting, golgi staining, confocal imaging, ELISA, protein and DNA gel electrophoresis, cDNA synthesis, real time PCR, RNA/DNA/protein extraction, gel extraction, ChIP, plasmid isolation, cloning, gram stain, bacterial cultures/isolation, aseptic technique, 454 multiplexed pyrosequencing, STR analysis, phylogenetic analysis, primer design, target gene and whole (viral) genome sequencing

Programs: Bioedit, Sequencher, 4Peaks, DAx, CodonCode Aligner, Excel, Power point, ANY-Maze, Gen5, ImageLab, Prism

TEACHING EXPERIENCE

Fall 2016 - Spring 2017	Associate Lecturer, Advanced Physiological Psychology, UW-Milwaukee
Spring 2014	Teaching assistant, Cognition and Perception, UW-Milwaukee
Fall 2013	Teaching assistant, Intro to Psychology, UW-Milwaukee

IMPACT

Works by Jennifer Tuscher have been cited 260 times in the scientific literature (source: ISI Web of Science, 2/19/17). *H-index* = 8.

SOCIETY MEMBERSHIPS

05/12 – present	**Society for Neuroscience**
10/14 – present	**Molecular and Cellular Cognition Society**
09/12 – present	**Association of Graduate Students in Neuroscience**
09/12 – present	**Association of Graduate Students in Psychology**

SERVICE

01/17– present	**AMERICAN ASSOCIATION OF UNIVERSITY WOMEN UW-MILWAUKEE CHAPTER** Vice president, charter member
01/17– present	**ENGAGING GIRLS IN STEM (EgGS)** Mentor/role model
09/14 – present	**ASSOCIATION OF GRADUATE STUDENTS IN NEUROSCIENCE** President
09/12 – 09/14	**ASSOCIATION OF GRADUATE STUDENTS IN PSYCHOLOGY** Treasurer