Effects of estradiol on central dopamine function and dopamine-mediated behaviors in female rats

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ABSTRACT

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Estrogen plays an important role in the modulation of cognitive performance in both women and female rodents. These effects may be attributed to the estrogenic modulation of neuroanatomical structures and neurotransmitter systems in the brain, including dopamine. Here, the influence of estrogen on dopamine-mediated behaviors as well as dopamine release and synaptic plasticity were examined.

Study 1 showed that latent inhibition is exhibited by females in proestrus, a time of high estrogen, but not by females in estrus or metestrus, periods of low estrogen. Study 2 examined the effects of chronic estradiol (E2) in ovariectomized rats on cognitive strategy. Rats with high levels of E2 predominantly use a place strategy while rats with low levels of E2 predominantly use a response strategy. Systemic administration of dopamine D1 receptor (D1R) and dopamine D2 receptor (D2R) antagonists caused a switch of strategy use in low E2 rats. To determine where in the brain this effect was occurring, Study 3 utilized intracranial infusions of D1R and D2R antagonists in the dorsal striatum (DS) or ventral striatum. D1R, but not D2R, antagonists in the DS caused a switch in strategy use by low E2 females. This suggests that strategy use in low E2 rats is altered by D2R antagonism in another brain region. To this end, in Study 4 D1R and

D2R antagonists were infused into the medial prefrontal cortex (mPFC). Both D1R and D2R antagonism resulted in a switch of strategy use by low E2 rats.

In Study 5, *in vivo* microdialysis was utilized in anaesthetized rats to demonstrate that local infusion of E2 into the DS rapidly increases dopamine transmission. Dual-probe *in vivo* microdialysis was also used in Study 6 to investigate the effects of E2 on dopamine transmission in the mPFC. Additionally, Western immunoblotting was conducted to evaluate the effects of E2 on synaptic protein levels. Study 7 examined the role of E2 on performance in a mPFC-mediated working memory task. Together, these studies suggest that estrogen influences cognitive performance in the female rat and that this effect is mediated, in part, by its action in the DS and mPFC.

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17-β estradiol.....E2
β-estradiol receptor knockout.....βERKO
Attention deficit hyperactivity disorder..... ADHD
Analysis of variance.....ANOVA
Dopamine.....DA
Dopamine D1 receptors.....D1Rs
Dopamine D2 receptors.....D2Rs
Dopamine D3 receptors.....D3Rs
Dopamine D4 receptors.....D4Rs
Dopamine D5 receptors.....D5Rs
Dopamine transporter.....DAT
Dorsal striatum.....DS
Estrogen receptors.....ERs
Gamma-aminobutyric-acid.....GABA
Hippocampus.....HPC
Latent inhibition.....LI
Long-term potentiation.....LTP
Medial prefrontal cortex.....mPFC
Non-preexposed.....NPE
Nucleus accumbens.....NA
Ovariectomized.....OVX
Parkinson's disease.....PD
Pre-exposed.....PE
Prepulse inhibition.....PPI
Postnatal day.....PND
Protein kinase A.....PKA
Substantia nigra.....SN
Tyrosine hydroxylase.....TH
Ventral tegmental area.....VTA

CONTRIBUTIONS OF AUTHORS

Study 1: This study was conceived by Matthew Quinlan in conjunction with Wayne Brake based on work done by Barbara Nofrey and Wayne Brake at University of California, Santa Barbara. Matthew Quinlan conducted these experiments under the supervision of Wayne Brake in collaboration with Andrew Duncan, Catherine Loiselle, and Nicole Graffe.

Study 2: This study was conceived by Matthew Quinlan in conjunction with Wayne Brake. Matthew Quinlan conducted this experiment under the supervision of Wayne Brake in collaboration with Dema Hussain.

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Study 5: This study was conceived by Matthew Quinlan in conjunction with Wayne Brake. Matthew Quinlan conducted this experiment under the supervision of Wayne Brake in collaboration with Marie-Pierre Cossette.

Study 6: These studies were conceived by Matthew Quinlan in conjunction with Wayne Brake. Matthew Quinlan conducted these experiments under the supervision of Wayne Brake in collaboration with Marie-Pierre Cossette. Western immunoblotting was conducted in collaboration with Natalina Salmaso under the co-supervision of Wayne Brake and Barbara Woodside.

Study 7: This study was conceived by Matthew Quinlan in conjunction with Wayne Brake and Dave Mumby. Matthew Quinlan conducted this experiment under the supervision of Wayne Brake in collaboration with Marie-Pierre Cossette, Varuni Bentotage, and Dema Hussain. Dave Mumby contributed to the editing of the manuscript.

CHAPTER 1:

GENERAL INTRODUCTION

1. Frame of Reference

Traditionally, research investigating the neurobiological effects of steroid hormones on behavior has focused on the control of reproductive processes. In the last 25 years, however, an increased emphasis has been placed on the role of hormones, especially estrogen, on the performance of behavioral tasks. A host of studies have established that estrogen significantly influences the performance of both women (e.g. Duff and Hampson 2000; Sherwin 2003) and female rats (e.g. Luine and Rodriguez 1994; Gibbs 1999) in studies that evaluate task acquisition (Gibbs 1999; Gibbs 2000), working memory (Bimonte and Denenberg 1999; Holmes, Wide et al. 2002), and response inhibition (Wang, Sable et al. 2008). Despite strong evidence demonstrating the modulatory effects of estrogen on learning and memory behaviors a consensus regarding the direction and magnitude of its effects has not yet been reached. Estrogen has been shown to enhance (Gibbs 1999; Gibbs 2000; Asthana, Baker et al. 2001; Keenan, Ezzat et al. 2001), decrease (Holmes, Wide et al. 2002; Rapp, Espeland et al. 2003; Wide, Hanratty et al. 2004), or have no effect (Resnick, Maki et al. 1998; Galea, Wide et al. 2001; Gibbs 2002; Gabor, Nagle et al. 2003) on performance in several types of behavioral tasks including place and response learning, delayed alternation, and working memory.

17-β estradiol (E2) is the most abundant and psychoactive estrogen in humans (Morissette, Le Saux et al. 2008) as well as the estrogen most commonly used in experimental learning and memory research. E2 has been shown to modulate the neural function of glutamate (Cyr, Ghribi et al. 2000), GABA (Schultz, von Esenwein et al.

2009), acetylcholine (Gibbs and Aggarwal 1998), serotonin (Fink, Sumner et al. 1998), norepinephrine (Conde, Bicknell et al. 1995), and dopamine (DA; Becker 1990). High levels of E2 abate DA release in the medial prefrontal cortex (mPFC; Dazzi, Seu et al. 2007) and enhance DA release in the dorsal striatum (DS; Xiao and Becker 1994); changes which can be correlated with alterations in learning and memory behavior (e.g. Castner, Xiao et al. 1993). High levels of E2 influence several types of DA-mediated behavior including the attenuation of response learning (Korol and Kolo 2002; Daniel, Hulst et al. 2006), the improvement of working memory (Daniel, Hulst et al. 2006), an enhancement of spontaneous alternation (Walf, Koonce et al. 2009), and biasing the use of a place strategy (Davis, Jacobson et al. 2005). Additionally, previous work from our lab shows that high E2 abolishes latent inhibition (LI; Nofrey, Ben-Shahar et al. 2008); a task dependent on dorsal striatal DA function (Lubow 1997).

It is clear that E2 is a strong modulator of performance in a number of behavioral tasks, including those which are mediated by DA transmission in the brain. However, relatively little research concerning the behavioral consequences of the estrogenic modulation of DA has been conducted and further examination is necessary in order to fully understand its effect on performance in learning and memory tasks. Thus, the experiments described within this thesis were conducted with the goal of examining the role of high and low levels of E2 on the performance of behavioral tasks mediated by DA. In addition, the influence of E2 on the structure and neurotransmission of dopaminergic target regions in the brain was also investigated in order to determine the neurobiological foundation for these behavioral changes. A characterization of this

modulation may, in part, contribute to the resolution of inconsistencies seen among studies investigating the role of E2 on performance in behavioral tasks.

1.1. The role of E2 on performance in behavioral tasks

1.1.1. E2 action in the brain

All steroid hormones, including estrogens, are produced within an organism from native cholesterol molecules and are distributed throughout the body via the bloodstream (Nelson 2005). E2, like other steroid hormones, is lipid soluble and passes through the membranes of cells and neurons in order to bind to intracellular estrogen receptors (ERs) which then regulate gene expression (McEwen 2002). Although initially only identified and studied in the hypothalamus and pituitary gland (Pfaff and Keiner 1973), ERs were subsequently identified in varied brain regions including the hippocampus (HPC), diverse cerebral cortices, the midbrain, and the brain stem of rats (McEwen 2002). All estrogens, including E2, are converted from testosterone via the enzyme aromatase (Naftolin, Ryan et al. 1972; Nelson 2005). This may occur in the ovaries, adrenal glands, or brain of several species including rats and mice (Roselli, Horton et al. 1985; Shinoda, Nagano et al. 1994), monkeys (Flores, Naftolin et al. 1973), and humans (Stoffel-Wagner, Watzka et al. 1998; Stoffel-Wagner, Watzka et al. 1999).

1.1.2. The role of E2 on cognition

Initial research on steroid hormones emphasized the estrogenic mediation of regulatory, growth, and reproductive processes (Luine 2008). However, anecdotal evidence from medical doctors describing the disruptive cognitive symptoms associated with the decrease in ovarian hormones at menopause (as cited in: McEwen and Alves 1999) suggested that E2 may be involved in learning and memory behaviors as well. One of the first experimental studies to support this influence of E2 on cognition demonstrated that the neonatal gonadectomy of male rats attenuates spatial working memory in adulthood but the neonatal administration of E2 to female rats improves performance in this task (Williams, Barnett et al. 1990). This, and similar findings, led to clinical trials which indicated that E2 could be an effective treatment for the cognitive deficiencies associated with Alzheimer's Disease (Simpkins, Green et al. 1997).

Further research in this area has confirmed the role of E2 in the mediation of performance in tasks evaluating learning and memory. The administration of E2 to ovariectomized (OVX) female rats enhances task acquisition and working memory performance in a radial arm maze (Daniel, Fader et al. 1997; Luine, Richards et al. 1998; Fader, Johnson et al. 1999), a T-maze (Fader, Hendricson et al. 1998), and a water escape task (O'Neal, Means et al. 1996). E2 appears to be particularly important as task difficulty increases (Bimonte and Denenberg 1999) although these benefits are task- and dose-dependent. In an 8-arm radial maze high E2 impairs, while low E2 enhances, performance in a spatial working memory task (Holmes, Wide et al. 2002). High E2 also impairs performance in a delayed alternation task in a T-maze (Wide, Hanratty et al. 2004) but has no effect in a delayed matching-to-place task (Gibbs 1999; Gibbs 2002). In

contrast, similarly high levels of E2 delay the acquisition of a water maze task in both intact (Warren, Humphreys et al. 1995) and OVX (Chesler and Juraska 2000) females. Still other studies find no effect of E2 on task acquisition (Holmes, Wide et al. 2002; Wide, Hanratty et al. 2004). Despite these inconsistent findings, it is apparent that E2 is an important modulatory factor in several types of behavior involving learning and memory.

The majority of behavioral effects associated with E2 have been observed subsequent to chronic hormone administration and are thought to be mediated by the genomic activity of classic ERs. However, E2 can also act rapidly, in a time course of hours rather than days, to affect learning and memory behavior. For example, E2 administered within four hours of a testing period has been shown to enhance visual and place memory (Luine, Jacome et al. 2003), spatial learning (Frye, Duffy et al. 2007), and object recognition (Walf, Rhodes et al. 2006).

1.1.3. Mechanisms of E2 action on cognition

The effects of E2 on performance in tasks evaluating learning and memory are thought to be primarily mediated through the ER-β system. The relatively recent identification of this ER subtype, with significant population densities in the dentate gyrus and frontal and parietal cortices, provided a neurobiological basis for the effects of E2 on cognition (Kuiper, Enmark et al. 1996; Kuiper, Shughrue et al. 1998). ER-β is now known to play a major role in the regulation of mood, fear, and anxiety (Weiser, Foradori et al. 2008) and the modulation of performance in behavioral tasks (Osterlund

and Hurd 2001). For example, OVX female β-estrogen receptor knockout (βERKO) mice have impairments in spatial memory tasks that cannot be restored by E2 administration (Rissman, Heck et al. 2002). In wild-type mice, learning is enhanced by ER-β, but rarely by ER-α, agonists (Rhodes and Frye 2006; Liu, Day et al. 2008; Walf, Koonce et al. 2008).

At least some of the effects of E2 that benefit behavioral performance may be attributed to the enhancement of cellular functions. Dendritic spine density in the CA1 area of the HPC is augmented by high E2 levels in both OVX (Gould, Woolley et al. 1990) and cycling female rats (Woolley and McEwen 1992). Long-term potentiation (LTP) in the hippocampal neurons of wild-type mice is enhanced by ER-β agonists (Rhodes and Frye 2006; Liu, Day et al. 2008; Walf, Koonce et al. 2008) and is attenuated in βERKO mice (Day, Sung et al. 2005). LTP fluctuates across the estrous cycle and is associated with improved learning and memory performance (Warren, Humphreys et al. 1995).

Much of the estrogenic influence on learning and memory behaviors is attributed to the classic genomic effects of ER-α and ER-β, however, recent studies have described a rapid action of E2 in the brain, even in regions lacking classic ERs (for a review see: McEwen 2002). This non-genomic regulation of neurons includes alterations of cell excitability, activation of second messenger pathways, modulation of G-protein coupling, and changes in calcium currents. It is possible that these actions of E2 are mediated by membrane-bound classic ERs (Levin 2002; Toran-Allerand 2004) but another possible candidate is an orphan receptor, GPR-30, which has been found in high concentrations in the substantia nigra (SN) and HPC (Brailoiu, Dun et al. 2007) and in lower

concentrations in the mPFC and DS (O'Dowd, Nguyen et al. 1998). Whatever the mechanism, E2 can alter neuronal activity within minutes, or even seconds. For example, the application of physiological concentrations of E2 to cultured striatal neurons rapidly reduces calcium currents through effects at the cell membrane (Mermelstein, Becker et al. 1996). Similar mechanisms potentiate cell excitability through protein kinase A (PKA) activation in hippocampal cell populations of wild-type (Gu and Moss 1998) and αERKO, but not βERKO, mice (Gu, Korach et al. 1999). When administered with classic ER antagonists or bound to bovine serum albumin (thereby making it impermeable to the neuronal membrane), E2 can still benefit performance in an inhibitory avoidance task suggesting non-genomic action (Frye and Rhodes 2002).

Although there is little doubt that E2 influences performance in learning and memory tasks, controversy still remains as to the exact nature of its modulatory effects. Recent research strongly supports the role of ER-β in cognition and suggests that membrane-associated E2 activity may be relevant as well, yet relatively little is known concerning the consequences of these actions on particular neurotransmitter systems. Thus far, the estrogenic modulation of cholinergic systems important to learning and memory and the subsequent behavioral consequences have been the primary focus of research in this area. However, DA, best known for its influence on reward-related and goal-seeking behaviors (Wise 1996; Berridge and Robinson 1998), is another neurotransmitter with a principal role in learning and memory tasks.

1.2. The role of DA on performance in learning and memory tasks

Much like E2, the influence of DA on cognition was not a primary focus of initial research. Following the identification of DA in the 1950's (as cited in: O'Donnell 2003), research centered around dopaminergic control of voluntary movement and the role of DA in Parkinson's Disease (PD; Bernheimer, Birkmayer et al. 1973). Other major areas of interest included the contribution of DA to the behavioral symptoms associated with pathologies such as schizophrenia, depression, and attention deficit hyperactivity disorder (ADHD; Benkert, Grunder et al. 1992; Wilens 2008) as well as its critical role in motivation and the onset and maintenance of addictive behaviors (Wise and Bozarth 1985; Berridge and Robinson 1998; Koob 2006). As these avenues of study identified DA as a major factor in the regulation of motor control, mood, and motivation, it also became apparent that DA plays an important role in the cognitive deficits that occur concomitant with psychiatric disorders and drug addiction. For example, it was noted that PD patients exhibit impairments in spatial planning and working memory tasks (Owen, James et al. 1992) which can be temporarily relieved by L-Dopa treatment (Lange, Robbins et al. 1992). Age-related cognitive deficits in monkeys (Arnsten 1993; Murphy, Arnsten et al. 1996) and rats (Lee, Ross et al. 1994) are also correlated with dopaminergic dysfunction. Based on these and other findings, DA has been implicated in the performance of two main functions; the incentive salience of motivational stimuli in reward-related seeking behaviors (Berridge and Robinson 1998; Arias-Carrion and Poppel 2007) and working memory (Sawaguchi and Goldman-Rakic 1991).

1.2.1. DA action in the brain

There are two main DA pathways in the brain; the mesocorticolimbic system and the nigrostriatal system. The nigrostriatal system originates in the SN and primarily projects to the caudate-putamen, otherwise known as the DS (Arias-Carrion and Poppel 2007). Nigrostriatal DA is principally involved in the initiation and maintenance of voluntary motor movement (Bernheimer, Birkmayer et al. 1973; Mackin 2000) although it has also been implicated in the regulation of drug reward (Quinlan, Sharf et al. 2004). In addition, a number of studies have shown the target region of this system, the DS, to be especially important in response selection, habit learning, and procedural memory (Eagle, Humby et al. 1999; Myhrer 2003). The mesocorticolimbic system originates in the ventral tegmental area (VTA) and has two main components; the mesolimbic pathway which primarily innervates the nucleus accumbens (NA) and the mesocortical pathway which mainly projects to the mPFC (Arias-Carrion and Poppel 2007). In general, this system governs the motivational and reinforcing properties of environmental stimuli, such as food, sexual cues, or drugs, involved in the learning and expression of appetitive behaviors (Fields, Hjelmstad et al. 2007). In addition, optimal dopaminergic function in the mPFC is thought to be essential for peak performance in working memory tasks for rodents, monkeys, and humans (Sawaguchi and Goldman-Rakic 1991; Goldman-Rakic, Muly et al. 2000).

Dopaminergic neurotransmission is mediated by two distinct families of G-protein-coupled receptors which do not directly influence the membrane potentials of target neurons but rather modify the responses of incoming afferent signals to these neurons (Greengard 2001; O'Donnell 2003). The D1-like family consists of DA D1

receptors (D1Rs) and DA D5 receptors (D5Rs) which, when activated, generally stimulate second messenger cascades through adenylate cyclase (O'Donnell 2003; Seamans and Yang 2004). The D2-like family consists of DA D2 receptors (D2Rs), DA D3 receptors (D3Rs), and DA D4 receptors (D4Rs) which, when activated, tend to decrease intracellular second messenger activity through negative regulation of cAMP activity (Thompson, Moore et al. 2000). Of the five subtypes, D1Rs and D2Rs are the most densely expressed and mediate the majority of dopaminergic activity in the brain (Sesack, Deutch et al. 1989). The remaining three subtypes have been implicated in several types of behaviors and psychiatric disorders but less is known concerning their behavioral consequences (Tarazi, Campbell et al. 1998; Floresco and Magyar 2006).

Studies in humans, non-human primates, and rodents have identified several brain regions in which D1Rs and D2Rs are expressed, including the mPFC, DS, NA, VTA, and SN. In the mPFC of rodents and non-human primates, D1Rs and D2Rs are primarily located on post-synaptic terminals in cortical layers IV and V (the cortical layers most heavily innervated by the VTA) with D1Rs being most dense (Goldman-Rakic, Lidow et al. 1992; Gaspar, Bloch et al. 1995; Tzschentke 2001). Additionally, both D1Rs and D2Rs have been identified on the dendrites of GABA interneurons (Gaspar, Bloch et al. 1995; Tzschentke 2001), pre-synaptic terminal boutons, and extra-synaptic sites (Lidow, Goldman-Rakic et al. 1991; Smiley, Levey et al. 1994). Within the NA and DS, D1Rs are more densely populated than D2Rs but both tend to be found on intrinsic postsynaptic neurons and not meso-or cortico-striatal projection neurons (Boyson, McGonigle et al. 1986; Tarazi, Campbell et al. 1998). Similarly in the SN (pars reticulata and pars compacta) and VTA, D1R populations are less densely populated than in target regions

but are denser than D2Rs (Boyson, McGonigle et al. 1986; Sesack, Aoki et al. 1994; Djouma and Lawrence 2002).

1.2.2. The role of DA on cognition

While it is likely that all five DA receptor subtypes contribute to performance in DA-mediated behavioral tasks, the majority of studies identify D1Rs to be of primary importance and D2Rs to play a smaller, but still significant, role in spatial learning and memory, incentive learning, and reversal learning (El-Ghundi, Fletcher et al. 1999). It has been suggested that DA in the brain is sensitive to changes in the environment and serves as a neural signal to adapt one's behavior, especially in reward-related situations (Nieoullon 2002). Hence, a number of studies indicate that DA transmission is essential for effective performance in goal-oriented tasks that require approach or appetitive behaviors (Ikemoto and Panksepp 1999) and when the online maintenance of salient information is required, such as during working memory tasks (Dalley, Cardinal et al. 2004).

A number of studies, especially those that utilize lesions and receptor antagonism, have demonstrated the integral role of DA in reward-related seeking behaviors and spatial learning tasks. For example, the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA), often used in conjunction with a noradrenergic reuptake inhibitor (Glinka, Gassen et al. 1997), impairs spatial learning and memory after local administration into the HPC (Gasbarri, Sulli et al. 1996), DS (Hagan, Alpert et al. 1983), NA (Grigoryan, Hodges et al. 1996), and mPFC (Rezvani, Eddins et al. 2008). In a mouse model of PD,

6-OHDA lesions to the DS impair performance in spatial learning tasks (De Leonibus, Pascucci et al. 2007). Disruption of DA transmission also results in cognitive impairments. Spatial learning and memory performance is attenuated in D1R knockout mice (El-Ghundi, Fletcher et al. 1999) and with the administration of the D2R antagonists to intact rats (Ploeger, Spruijt et al. 1992; Ploeger, Spruijt et al. 1994; Setlow and McGaugh 1998). D1R and D2R antagonism also impair responding in a serial reaction task (Domenger and Schwarting 2006) and the acquisition of inhibitory avoidance (Manago, Castellano et al. 2009). These studies illustrate the integral role of DA in tasks that have a goal in mind but interference with DA transmission can also affect the ability to perform tasks with no specific reward.

This is evident in tasks that evaluate attention, such as prepulse inhibition (PPI; Koch and Bubser 1994) and LI (Lubow 1997), as well as based on the role of DA in clinical disorders such as ADHD (Heilman, Voeller et al. 1991). PPI is disrupted by 6-OHDA lesions (Bubser and Koch 1994) and D1R and D2R antagonism in the mPFC (Ellenbroek, Budde et al. 1996). Systemic and intra-accumbal administration of DA agonists as well as classic neuroleptics also disrupt PPI performance (Swerdlow, Braff et al. 1990; Swerdlow, Caine et al. 1992; Caine, Geyer et al. 1995; Johansson, Jackson et al. 1995). Similarly, LI is attenuated by intra-accumbal (Weiner, Lubow et al. 1988) and intra-striatal (Konstandi and Kafetzopoulos 1993; Ellenbroek, Knobbout et al. 1997; Jeanblanc, Hoeltzel et al. 2003) infusion of the indirect dopaminergic agonist amphetamine. These effects can be reversed by the D2R antagonist haloperidol (Weiner, Gal et al. 1996). Haloperidol alone enhances LI and augments learning of the task (Weiner and Feldon 1987). Performance in another attentional task, the 5-choice serial

reaction time task, is improved in low baseline performers and attenuated in high baseline performers after D1R antagonism (Granon, Passetti et al. 2000). This suggests that an optimal level of DA function is necessary for effective performance in certain types of behavioral tasks, an idea that is more commonly identified with paradigms involving a delay (Sawaguchi and Goldman-Rakic 1994; Williams and Castner 2006).

In fact, one of the first studies to implicate DA in learning and memory performance demonstrated that depletion of DA in the mPFC leads to impairments in a delayed response task (Brozoski, Brown et al. 1979). Fittingly, it had been previously shown that neurons in the mPFC remain active during such a delay (Fuster 1973). More recently, Floresco and Phillips (2001) have demonstrated that D1R agonists infused into the mPFC improve spatial memory during long delays but impair memory during short delays. In the mPFC, an increase in extracellular DA from basal levels is correlated with accurate performance in a working memory task (Watanabe, Kodama et al. 1997) while excessive DA turnover in this region is associated with an attenuation of spatial working memory in both rats and monkeys (Murphy, Arnsten et al. 1996). Goldman-Rakic and Sawaguchi (1991; 1994) found that local administration of D1R antagonists, but not D2R antagonists, into the mPFC of monkeys disrupts performance in delayed response tasks. Moreover, 6-OHDA lesions in the mPFC impair the acquisition (Bubser and Schmidt 1990) and expression (Simon, Scatton et al. 1980) of behavior in delayed response, but not uninterrupted, tasks.

Other types of memory tasks, which also involve aspects of motivation and working memory, that are affected by interference with central DA transmission include spontaneous alteration (Oades, Taghzouti et al. 1985; Taghzouti, Louilot et al. 1985;

Holter, Tzschentke et al. 1996) and passive avoidance (Taghzouti, Louilot et al. 1985; Lazarova-Bakarova, Petkova et al. 1991). DA, primarily in the mPFC, is also implicated in cognitive set-shifting, also referred to as behavioral flexibility. Sustained levels of DA efflux in the mPFC are associated with effective performance during the shift between discrimination rules (Stefani and Moghaddam 2006) while only brief enhancements of DA release were seen in the NA and DS. Local injections of both D1R (Ragozzino, Ragozzino et al. 2002) and D2R (Floresco, Magyar et al. 2006) antagonists into the mPFC disrupt effective switching of strategy but have no effect on acquisition of the task. When the ability to switch task strategies is impaired by DA receptor antagonism the ability to detect changes in reward value is not affected (Winter, Dieckmann et al. 2009).

In conclusion, while the neurotransmitters acetylcholine (Hasselmo 2006) and glutamate (Brown, Chapman et al. 1988) have been shown to directly regulate performance in learning and memory tasks it is thought that DA modulates the response of neurons to incoming signals from the environment (e.g. Nieoullon 2002; Girault and Greengard 2004). Electrophysiological studies suggest this is accomplished by DA through the mediation of membrane potentials in learning-relevant neurons (O'Donnell 2003). It has been shown that midbrain DA efflux increases in response to unpredicted rewards early in learning and decreases in the absence of predicted rewards later in learning (Hollerman and Schultz 1998). Hence, the antagonism of mesolimbic DA during performance of a task may negate the motivational significance of an environmental stimulus due to a lack of modulatory influence on neurons directly involved in the learning process. Behavior tends to occur based on the availability, salience, and location

of a reward in the environment (Phillips, Vacca et al. 2008) and it appears that a DA plays an indirect, but significant, role in this process.

Similar alterations of DA function within the mesocortical pathway will disrupt performance in working memory tasks; those requiring the online maintenance of salient information. It has been suggested that, within the mPFC, D1Rs mediate the tonic firing of DA neurons (Cohen, Braver et al. 2002). This may increase the signal-to-noise ratio of previously learned information in the face of competing stimuli. On the other hand, D2Rs may be involved in the coding and maintenance of meaningful novel inputs (Cohen, Braver et al. 2002). However indirect the effect may be, it is apparent that the influence of mesolimbic and mesocortical DA is an important factor on performance in learning and memory tasks.

1.3. The effects of E2 on DA function

It is clear that, independently, E2 and DA each have a significant role in the performance of tasks evaluating learning and memory. However, little work has been done investigating the influence of E2 on DA neurotransmission and how this may affect such tasks. Several studies have shown that baseline and stimulant-induced DA transmission are differentially affected by high and low levels of E2 (e.g. Becker and Beer 1986; e.g. Becker 1990) but very few studies have examined the behavioral consequences of this action in the brain. Those which do demonstrate that alterations of dopaminergic activity in the brain can be an influential factor during performance in behavioral tasks (e.g. Daniel, Sulzer et al. 2006).

1.3.1. The effects of E2 on DA-mediated behaviors

Some of the first studies to identify the estrogenic modulation of DA-mediated behavior can be found in studies investigating sex differences in response to stimulants. For example, female rats are not only more likely to self-administer higher doses of stimulant drugs but to also display exaggerated locomotor responses after comparable doses of acute and chronic administration as compared to males (Becker 1990; Lynch, Arizzi et al. 2000). Gonadectomy significantly reduces amphetamine-induced rotational behavior in female, but not male, rats (Becker, Beer et al. 1984; Camp, Becker et al. 1986). It has also been shown that E2 in females potentiates DA agonist-induced stereotypic behaviors regulated by the SN (Chiodo, Caggiula et al. 1981; Chiodo and Caggiula 1983) and that ovariectomy attenuates the magnitude of CPP for cocaine (Russo, Festa et al. 2003). These differences in stimulant-induced behavior led to investigations of the effect of E2 on DA-mediated learning and memory behaviors.

Subsequent to gonadectomy, female rats exhibit greater deficits in overall accuracy and working memory than males in a maze task (Gibbs and Johnson 2008). OVX rats (Wallace, Luine et al. 2006) and menopausal women (Keenan, Ezzat et al. 2001) exhibit deficits in mPFC-mediated memory tasks which can be ameliorated by E2 administration or hormone replacement therapy, respectively. With aging, female rats display a faster rate of decline in a DA-mediated passive avoidance task when compared to males (Benice, Rizk et al. 2006). Conversely, a lack of, or low levels of, E2 may assist in the performance of some tasks. During training in a striatum-dependent response learning task, OVX females receiving vehicle needed fewer trials to acquire the task than OVX females receiving E2 replacement (Davis, Jacobson et al. 2005). In a similar task

also mediated by dorsal striatal DA, the administration of E2 enhances the disruptive effects of a D1R, but not a D2R, antagonist (Daniel, Sulzer et al. 2006). These studies are not only part of a growing literature which demonstrates that E2 exerts a strong influence on the performance of DA-mediated behavioral tasks but also suggest that E2 may alter DA function in the brain to enact these changes. The estrogenic alteration of DA function can include changes in transmission, uptake, and receptor levels.

1.3.2. Localization of ERs in dopaminergic brain regions

There are a number of dopaminergic brain regions in which ERs, especially ER-β, have been co-localized with tyrosine hydroxylase (TH) staining; these include the VTA, NA, SN, DS, and mPFC (Kuiper, Shughrue et al. 1998; Creutz and Kritzer 2002; Yamaguchi-Shima and Yuri 2007). In addition, ER-β has been identified in several structures which are adjacent to and communicate with the SN such as the subthalamic nucleus and zona incerta (Kritzer 1997). In the VTA and SN, ER-β was found to be more abundant than ER-α (Shughrue and Merchenthaler 2001) although ER-remains a critical component for the regulation of the nigrostriatal DA system (Kuppers, Krust et al. 2008). Retrograde labelling studies in the DS have identified connections with DA neurons located in projection regions in the dorsal SN and VTA, both of which are dense in ER-β (Kritzer 1997; Creutz and Kritzer 2004). Significant labelling of ER-β-dense regions in the VTA was also found after infusion of retrograde tracers into the NA.

ER-β tends to be similarly distributed in the brains of adult male and female rats although in dopaminergic brain regions these receptors are twice as dense in males than

females (Creutz and Kritzer 2002). Moreover, ER-β density is 50% greater in proestrus females than in diestrus females (Creutz and Kritzer 2002).

1.3.3. Influence of E2 on DA transmission

E2 alters DA transmission in several regions of the brain and has primarily been studied in the DS. For example, E2 has been shown to stimulate the synthesis of DA in this region, most likely through a non-genomic modulation of this process (Pasqualini, Olivier et al. 1995). Dopaminergic neurons in the SN are a direct target of E2 which stimulates neurite branching and the expression of TH (Kuppers, Ivanova et al. 2000). In addition, the administration of E2 to OVX females rapidly induces the expression of the immediate early gene c-*jun* in both the DS and NA (Zhou and Dorsa 1994).

E2 also alters extracellular levels of DA. Female rats in proestrus display higher baseline levels of dorsal striatal DA than female rats in diestrus or OVX females (Xiao and Becker 1994). This is true in both young and aged females (McDermott 1993). Likewise, the administration of E2 enhances accumbal DA release in OVX rats (Thompson and Moss 1997). Castration of male rats has no effect on DA levels in the DS (Xiao and Becker 1994). Agonist-induced DA release is also augmented in the presence of E2 subsequent to intra-striatal infusions of amphetamine in gonadectomized female, but not male, rats (Castner, Xiao et al. 1993). This also holds true for cycling rats in proestrus (Becker, Robinson et al. 1982; Becker and Cha 1989) and for *in vitro* cultures of striatal neurons (Becker and Beer 1986). A number of studies have characterized the

estrogenic modulation of dorsal striatal DA but fewer studies have investigated these changes in other brain regions.

One study to do so found that ovariectomy depletes DA levels in the VTA and NA of female rats (Russo, Festa et al. 2003). Subsequent administration of E2 results in a recovery of DA levels within the VTA but not the NA, which requires the co-administration of E2 and progesterone. In cycling female rats, the basal and burst firing of DA neurons in the VTA is highest in estrus, a time of low E2, and lowest in proestrus when E2 levels are at their peak (Zhang, Yang et al. 2008). In addition, OVX rats have significantly higher firing rates than proestrus rats. This is in contrast to studies reporting an enhancement of DA release in the DS with high E2 but studies of extracellular DA in the mPFC, a primary target region of the VTA, agree with this evidence. Baseline levels of DA in the mPFC are highest during estrus and lowest during proestrus (Dazzi, Seu et al. 2007). Likewise, chronic E2 reduces DA levels in the mPFC of OVX rats (Luine, Richards et al. 1998). While its effects on DA transmission may vary from region to region, it is clear that E2 is an important influence on DA synthesis and function.

E2 may also indirectly influence DA transmission by altering DA transporter (DAT) function. In cultured NA neurons, the application of E2 does not alter basal DA uptake but does attenuate uptake after DA agonist-induced activity (Thompson, Moore et al. 2000). In striatal cultures, this attenuation occurs through a decrease in affinity of DAT for DA in a dose-dependent manner (Disshon, Boja et al. 1998). This may take place due to a change in the association of D2Rs with their G-protein (i/o) which indirectly affects DAT function (Thompson and Certain 2005). On the other hand, during proestrus in cycling females, DA uptake is enhanced in the NA (Thompson and Moss

1997) and dorsal striatal DA uptake sites also increase in number, coincident with the highest levels of DA release in this region (Morissette and Di Paolo 1993). It has also been shown that ovariectomy results in an upregulation of striatal DAT which is prevented by E2 administration; there was no effect in gonadectomized males (Attali, Weizman et al. 1997). These studies provide evidence of yet another avenue by which E2 can alter DA function.

1.3.4. Changes in DA receptor function with changes in E2.

Several studies have demonstrated that E2 alters the quantity and function of D1Rs and D2Rs. Progressive reductions in the density, but not affinity, of D1Rs and D2Rs are seen in the DS with time after ovariectomy (Bosse and DiPaolo 1996). In addition, D2Rs have a greater decline in density thereby upsetting the normal D1R:D2R ratio. Chronic E2 treatment for two weeks restores the D2R, but not the D1R, population density (Bosse and DiPaolo 1996). Striatal D1Rs may be enhanced in OVX females but only if E2 administration is initiated the day after surgery (Levesque and Di Paolo 1989). Chronic E2 administration in OVX female rats results in decreased D2R, but not D1R, mRNA in the DS (Lammers, D'Souza et al. 1999). In monkeys, striatal D2R availability is increased during the luteal phase, a time of high E2 (Czoty, Riddick et al. 2009). In cycling female rats, striatal D2R antagonist binding remains constant but D2R agonist binding sites increase during proestrus (Di Paolo, Falardeau et al. 1988). In OVX female rats, D2R binding is reduced within 30 minutes of E2 administration (Bazzett and Becker 1994).

Changes in DA receptor function have also been demonstrated in other dopaminergic brain regions. E2 administration produces a supersensitivity of DA autoreceptors (Chiodo, Caggiula et al. 1981; Chiodo and Caggiula 1983) and increased D2R mRNA (Zhou, Cunningham et al. 2002) in the midbrain of OVX female rats. In the NA, ovariectomy decreases agonist and antagonist binding sites on D2Rs (Le Saux, Morissette et al. 2006). This is prevented by E2 and ER-β agonist treatment. In the mPFC, D1R density decreased over time with no change in affinity subsequent to OVX (Bosse and DiPaolo 1996). These studies illustrate several ways by which E2 can alter DA transmission as well as describe the necessary neurobiological mechanisms by which this influence can affect behavior. However, further investigation is necessary to fully understand the influence of E2 on DA transmission and DA-mediated behavior.

1.4. Rationale and Hypotheses

While studies investigating the respective roles of E2 and DA in learning and memory tasks remain largely individual pursuits, research examining the estrogenic modulation of DA transmission has shown this, too, is critical for performance in learning and memory tasks. This research has not only identified the necessary co-localization of estrogenic and dopaminergic anatomy but has shown that the influence of E2 on DA transmission in the brain leads to alterations of performance in behavioral tasks. Further examination of the neurobiological and behavioral consequences of the estrogenic modulation of E2 will assist in elucidating the conflicting findings of previous studies and direct future work in this area. The following studies were designed with this

in mind. Importantly, in the interest of studying E2 within a rat model of healthy, intact adult females, these studies utilized either intact, cycling adult female rats or OVX female rats in which hormone replacement closely mimicked natural circulating levels of E2 during the proestrus (high E2) and estrus (low E2) stages of the rat estrous cycle. Thus, the experiments contained in this thesis were conducted in order to further investigate the consequences of differential levels of physiological E2 on DA-mediated behaviors and on neuroanatomical structure in brain regions which are targets of dopaminergic pathways and which underlie these behaviors.

Previous studies from our laboratory (Nofrey, Ben-Shahar et al. 2008) show that the administration of high levels of E2 to OVX females interrupts performance in a dorsal striatal DA-dependent LI paradigm. Study 1 evaluates the role of ovarian hormones on the performance of intact, naturally-cycling, adult female rats in this task. Additionally, pre-pubertal male and female rats were tested in order to identify whether it is the perinatal (organizational) or adolescent (activational) surge of hormones which alters performance. It was hypothesized that rats in proestrus, a period of high E2 levels, during the conditioning phase would have attenuated LI when compared to rats in estrus during the conditioning phase. It was also hypothesized that the expression of LI would depend on the pubertal surge of hormones and, thus, juvenile female rats would exhibit LI behavior on the testing day.

Based on previous research investigating the effect of E2 on dorsal striatal DA-mediated tasks (McDonald and White 1994; Packard and McGaugh 1996; Korol and Kolo 2002; Korol, Malin et al. 2004), Study 2 examines the effect of high and low levels of physiological E2 on the use of response or place strategies by OVX adult female rats

in a modified plus-maze. It was hypothesized that rats with high levels of physiological E2 would tend to use a place strategy while rats with low physiological E2 would be biased towards use of a response strategy. In addition, because DS-dependent response learning has been shown to be disrupted by D2R antagonism (Daniel, Sulzer et al. 2006) the role of the D1R and D2R systems in the use of cognitive strategy was evaluated using systemic administration of the D1R antagonist SKF 83566 and the D2R antagonist raclopride. It was hypothesized that both D1R and D2R antagonism would alter the tendency to use a response strategy in low E2 rats due to interference with dorsal striatal DA transmission.

In order to identify the specific brain regions in which D1R and D2R antagonism were acting to alter strategy use in low E2 rats, Study 3 tests OVX adult female rats in a modified plus-maze. Here, the D1R antagonist SCH 23390 and the D2R antagonist raclopride were locally infused into either the DS or ventral striatum (NA). It was hypothesized that high E2 rats would use a place strategy and that low E2 rats would use a response strategy. It was also hypothesized that D1R and D2R antagonism within the DS, but not the NA, would alter the use of response strategy in low E2 rats. Based on the previous findings that only D1R antagonists in the DS will alter strategy use in low E2 rats, Study 4 evaluates the role of the mPFC in strategy use. Here, either D1R or D2R antagonists were locally infused into the mPFC. It was hypothesized that high E2 rats would use a place strategy, low E2 rats would use a response strategy, and medial prefrontal cortical D2R antagonism would alter the strategy used by low E2 rats.

These behavioral studies from our lab demonstrate that E2 can alter performance in dorsal striatal DA-mediated behavioral tasks. E2 has been shown to affect dorsal

striatal DA release in vitro (Becker and Ramirez 1981), in OVX females (Becker 1990), and across the estrous cycle in cycling females (Xiao and Becker 1994). These alterations often have rapid effects on DA transmission (Bazzett and Becker 1994; Becker and Rudick 1999) and can change dorsal-striatal mediated behaviors (Daniel, Sulzer et al. 2006). Utilizing single-probe *in vivo* microdialysis in anaesthetized OVX female rats with low levels of E2, Study 5 investigates the effects of the local application of E2 on DA transmission in the DS in response to systemic injections of vehicle and amphetamine. It was hypothesized that local administration of E2 to the DS would rapidly enhance DA activity. Furthermore, it was hypothesized that local E2 infusion would enhance amphetamine-induced DA activity in the DS.

Previous findings provide evidence that E2 modulates neuroanatomical structure and synaptic plasticity (Gould, Woolley et al. 1990; Woolley and McEwen 1992) as well as catecholamine neurotransmission (Luine, Richards et al. 1998) in several regions of the brain. In Study 6, Western immunoblotting is used to examine the effects of E2 on pre- and postsynaptic proteins in the target regions of dopaminergic pathways in the brains of OVX female rats; these include the mPFC, the DS, and the HPC. It was hypothesized that high levels of E2 would increase the quantities of synaptic proteins in all three areas when compared to low levels of E2. In addition, both high and low levels of physiological E2 would result in enhanced quantities of synaptic proteins when compared to OVX rats. To investigate the effects of E2 on neurotransmission in the mPFC, DA is measured using dual probe *in vivo* microdialysis in OVX female rats receiving vehicle injections or high or low E2 replacement. DA was independently evaluated in the left and right hemispheres of the mPFC after single systemic injections

of both saline and amphetamine. It was hypothesized that high levels of E2 would attenuate DA neurotransmission while low levels of E2 would enhance DA neurotransmission after saline administration. It was also hypothesized that high levels of E2 would augment medial prefrontal cortical DA activity after amphetamine injection.

Study 7 investigates the influence of physiological levels of E2 on the working memory performance of OVX female rats in a T-Maze; a task which is dependent on medial prefrontal cortical DA in rats (Jones 2002), monkeys, (Sawaguchi and Goldman-Rakic 1991) and humans (Keenan, Ezzat et al. 2001). In this study, an OVX group receiving vehicle injections was also included as a point of comparison with other studies. It was hypothesized that rats with physiologically high levels of E2 would make fewer errors than rats with physiologically low levels of E2. Moreover, it was expected that both high and low E2 rats would make fewer errors than rats receiving only vehicle injections.

CHAPTER 2:

THE ATTENUATION OF LATENT INHIBITION BY HIGH LEVELS OF OVARIAN HORMONES DURING PROESTRUS IS AN ACTIVATIONAL EFFECT

Matthew G. Quinlan, Andrew Duncan, Catherine Loiselle, Nicole Graffe, and Wayne G. Brake

Preface:

Previous work from our lab at the University of California, Santa Barbara conducted by Barbara Nofrey demonstrated that high levels of E2 abolish latent inhibition in OVX female rats (Nofrey, Ben-Shahar et al. 2008). Performance in this task has been shown to be dependent on dorsal striatal DA function (Lubow 1997; Jeanblanc, Hoeltzel et al. 2003). Here, cycling females were tested in latent inhibition paradigm to investigate if the high levels of ovarian hormones present during proestrus interfere with this dorsal striatal DA-mediated task.

Abstract:

Estrogen has been shown to have a strong modulatory influence on several types of learning and memory behaviors in both women and female rodents. Latent inhibition is a task in which pre-exposure to a neutral stimulus, such as a tone, later impedes the association of that stimulus with a particular consequence, such as a shock. Previous work from our lab demonstrates that high levels of estradiol (E2) administered to ovariectomized (OVX) female rats abolishes latent inhibition when compared to female rats with low levels of E2 or male rats. To determine if this E2-induced impairment also occurs with the natural variations of ovarian hormones during the estrous cycle this behavior was investigated in cycling female rats. In addition, prepubertal male and female rats were also tested in this paradigm to determine if the previously described sex differences are activational or organizational in nature. In a latent inhibition paradigm using a tone and a shock, adult rats were conditioned during different points of the estrous cycle. Rats conditioned during proestrus, a period of high E2 levels, exhibit attenuated latent inhibition when compared to rats conditioned during estrus or metestrus, periods associated with low levels of E2. Moreover, this effect is not seen until puberty indicating it is dependent on the surge of hormones at puberty. This study confirms recent findings that high E2 interferes with latent inhibition and is the first to show this is based in the activational actions of hormones.

1. Introduction

Estrogen is known to play a significant role in the modulation of performance in behavioral tasks in both women (e.g. Duff and Hampson 2000; Sherwin 2003) and female rats (e.g. Luine and Rodriguez 1994; Bimonte and Denenberg 1999). However, there is no clear consensus as to the direction or extent of these effects. Estrogen has been shown to either enhance (Gibbs 1999; Gibbs 2000; Asthana, Baker et al. 2001; Keenan, Ezzat et al. 2001), decrease (Holmes, Wide et al. 2002; Rapp, Espeland et al. 2003; Wide, Hanratty et al. 2004), or have no effect (Resnick, Maki et al. 1998; Galea, Wide et al. 2001; Gibbs 2002) on several types of behavioral tasks. There are a number of factors which may contribute to such discrepancies in the literature; these include the type of task used to evaluate the effects of estrogen (Korol and Kolo 2002), the dose and duration of estrogen replacement (Gibbs 1997; Sandstrom and Williams 2004), the particular brain region where estrogen is acting (Sinopoli, Floresco et al. 2006), and the effect of estrogen on cognitive strategy (Korol, Malin et al. 2004; Quinlan, Hussain et al. 2008).

Another factor known to play an important role during performance in behavioral tasks is attention. Estrogen modulates attention in both humans (Portin, Polo-Kantola et al. 1999) and rats (McGaughy and Sarter 1999; Barnes, Staal et al. 2006). For example, pre-pulse inhibition (PPI), a measure of sensorimotor gating, is reduced in women during the luteal phase of the menstrual cycle when estrogen levels are high (Jovanovic, Szilagyi et al. 2004). There is a similar attenuation of PPI in female rats during proestrus, a period of high estradiol (E2) levels, when compared to males or females in the estrus stage of the estrous cycle, a phase associated with low levels of E2 (Koch 1998). These studies suggest that higher levels of E2 interfere with the ability to effectively attend to relevant

stimuli or disregard irrelevant stimuli. This has been previously tested in our lab using a latent inhibition (LI) paradigm (Nofrey, Ben-Shahar et al. 2008). In this LI task, subjects receive a pre-exposure phase in which they are repeatedly exposed to a neutral stimulus, the tone, with no consequences. This pre-exposure will later impede the association of that neutral stimulus (tone) with an unconditioned stimulus, a shock, during the conditioning phase. It has been suggested that LI is based on a learned inattentional response in which the pre-exposed stimulus has been rendered irrelevant due to repeated presentations with no consequences (Lubow 1997). It is also possible that LI is a result of competing associations that are made during the pre-exposure and conditioning phases (Escobar, Oberling et al. 2002). Regardless, when E2 levels are high during the conditioning phase, the LI effect is abated (Nofrey, Ben-Shahar et al. 2008). This paradigm is uniquely suited to exploring the effects of E2 on learning. E2 need only be present during the conditioning phase of the task and, thus, cannot modulate any other aspect of behavior during the testing phase. As a result, any differences in latent inhibition during testing can be attributed to the effects of E2.

To determine if this impairment of LI in OVX females with high E2 observed in our lab also occurs under natural fluctuations of ovarian hormones during the estrous cycle this behavior was investigated in intact adult female rats. In a LI paradigm, cycling females were conditioned during different phases of the estrous cycle. It was hypothesized that if an intact adult female rat was conditioned during proestrus, a period of high E2 levels, the ability to exhibit LI on the testing day would be reduced. More specifically, rats which have been pre-exposed to a tone and then conditioned to a tone-shock pairing while in proestrus will have an attenuated ability to ignore the tone on the

testing day when compared to pre-exposed rats which were conditioned during estrus, a time of low E2. It was also expected that rats not pre-exposed to the tone will not be able to ignore the tone during the testing phase and will exhibit freezing behavior.

In a second experiment, intact pre-pubertal male and female rats were tested in a LI paradigm to determine if the sex differences previously observed (Nofrey, Ben-Shahar et al. 2008) are organizational or activational in nature. Previous studies show that sex-related cognitive differences in place learning tasks (Kanit, Taskiran et al. 2000) and spatial learning tasks (Galea, Ossenkopp et al. 1994) in rodents do not emerge until adulthood. Thus, it was hypothesized that both male and female pre-pubertal rats would exhibit LI on the testing day.

2. Methods

Subjects. Experiment 1 included 42 young adult, female, Sprague-Dawley rats (Charles River, St. Constant, Quebec) aged three months and weighing approximately 225-250 grams at the time of arrival. Upon arriving, all animals were allowed three weeks to habituate to the animal facility and were handled daily from time of arrival until completion of the experiments. Experiment 2 included 34 pre-pubertal, juvenile, Sprague-Dawley rats, 17 males and 17 females, aged post-natal day (PND 24) and weighing approximately 75-100 grams. All juvenile rats used in Experiment 2 were birthed by dams, raised, and weaned at PND 21 in the Concordia University Animal Care Facility.

For both experiments, prior to the commencement of behavioral training, animals were group-housed in same-sex polyurethane shoebox cages, maintained on a reverse

12h:12h light/dark cycle with lights off from 0900-2100h, and were allowed standard lab chow and water *ad libitum.* 48 hours prior to the commencement of behavioral training all animals were water-deprived and only allowed access to water for 30 minutes each day at approximately 1300h. All training and testing was performed during the dark phase of the light/dark cycle beginning at 1300h. All behavioral testing and procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Concordia University Animal Research Ethics Committee.

Determination of Estrous Cycle. Experiment 1 included only intact females exhibiting regular 4-5 day estrous cycles for two weeks prior to the study and for its duration. The phase of estrous cycle was determined by vaginal cytology characterization using a cotton swab dampened with saline to collect epithelial cells from the vaginal wall. All samples were collected daily from approximately 1200-1300h and immediately examined under a microscope with 10x magnification. Rats with a majority of cornified epithelial cells were considered to be in estrus; rats with a mix of cornified epithelial cells, nucleated epithelial cells, and leukocytes were considered to be in metestrus; rats with a majority of leukocytes were considered to be in diestrus; and, rats with a majority of nucleated epithelial cells were considered to be in proestrus. Rats exhibiting irregular estrous cycles were excluded from the experiment. The phase of the cycle on Day 7, Conditioning Day, determined which experimental group each rat was included in.

Apparatus. All testing took place inside six separate modular isolation cubicles (Coulbourn Instruments; Whitehall, PA) each insulated with foam walls which absorbed

ambient light and noise. Each cubicle is approximately 31"W x 21"D x 21"H, held an inner testing chamber measuring approximately 28"W x 18"D x 19"H, that was equipped with a fan. Each inner chamber was identically outfitted with a house light, a cue light, a tone box and speaker, a water bottle accessible from inside the chamber, and a water lickometer placed outside the chamber. In addition, the grid floor was connected to a precision adjustable shock module which was located outside the cubicle. The cubicles, inner cages, shock modules, and data collection were controlled by a personal computer running *Graphic State Notation* software (Coulbourn Instruments).

Behavior. In Experiment 1, rats were randomly divided into two groups; pre-exposed (PE), n=17, and non-pre-exposed (NPE), n=23. Rats were further grouped according to their phase of estrous cycle as determined on Day 7, Conditioning Day. For the PE group; 6 rats were in estrus, 3 rats were in metestrus, 3 rats were in diestrus, and 5 rats were in proestrus. For the NPE group; 7 rats were in estrus, 6 rats were in metestrus, 3 rats were in diestrus, and 7 rats were in proestrus. For Experiment 2, juvenile males and females were also randomly divided into PE, n=17, and NPE, n=17, groups. In the PE group; there were 8 males and 8 females. In the NPE group; there were 9 males and 9 females. Because all female rats in Experiment 2 were pre-pubertal, they were not swabbed for vaginal cytology characterization. All rats in both Experiments 1 and 2 underwent the same behavioral procedure, which was based on Nofrey et al. (2008).

Days 1-3 were referred to as Baseline Days. Each rat spent 20 minutes in the same respective testing chamber each day with free access to water and only the chamber house light on. Baseline licking behavior was recorded by the *Graphic State Notation*

software every time an infrared beam was broken as a lick was made by the rat. Days 4-6 were referred to as Pre-exposure Days. Each rat spent 23.5 minutes in its testing chamber without access to water and only the house light on. During this period, each rat in the PE group was exposed to 40 presentations of a 5-second, 2.5 kHZ tone at a variable 30-second schedule. Rats in the NPE group spent time in the chamber but were not exposed to the tone presentations. During this phase, water was made available for 30 minutes after the testing session. Day 7 was the Conditioning Day. All rats in both PE and NPE groups spent 20.5 minutes in the testing chambers without access to water and were pseudorandomly exposed 4 times to a 5-second, 2.5 kHz tone coinciding with a cue light that was immediately followed by a 1-second, 1 mA foot shock (juvenile rats in Experiment 2 received shocks of 0.5mA). The pseudorandom tone/light-shock pairings were spaced approximately 5 minutes apart. Day 8 was a re-Baseline Day. Each rat spent 20 minutes in the testing chamber with access to water and only the house light on.

Day 9 was Testing Day. Each rat was placed in the testing chamber with access to water and allowed to make 100 licks; the time to make licks 81-100 was recorded (Bin A). Upon the completion of 100 licks, the tone was presented continuously until the rat made 120 licks; the time to make licks 101-120 was recorded (Bin B). If any rat failed to complete 120 licks within a 5-minute period the testing phase was over and the time to complete Bin B was recorded as 5 minutes. A suppression ratio was calculated by dividing Bin A (the time to complete licks 81-100) by the sum of Bin A and Bin B (the time to complete licks 101-120); or A/A+B. Thus, a 0.5 suppression ratio indicates no suppression of licking behavior during the tone, or no conditioned response, and a suppression ratio of 0.025 demonstrates complete suppression of licking behavior during

the tone, or exhibition of a conditioned response. In other words, if a rat has a high suppression ratio, then it exhibited LI in that was more effective in ignoring the tone and continued licking. If a rat has a low suppression ratio, then it did not exhibit LI as it could not ignore the tone and stopped its licking behavior, most probably due to a freezing response.

Statistical Analyses. For both experiments, one-tailed t-tests were employed to test for significant differences between PE and NPE groups within each larger experimental group. For Experiment 1, this was phase of the estrous cycle (estrus, metestrus, or proestrus) and for Experiment 2 this was sex (male or female).

3. Results

Behavior, Experiment 1. In the estrus group, PE rats had a significantly higher suppression ratio than the NPE group; $t(11)=1.92$, $p=0.04$ (Figure 1). In the metestrus group, the PE group also had a significantly higher suppression ratio than NPE group; $t(7)=2.07$, $p=0.038$ (Figure 1). There were no other significant differences, including the proestrus group. Statistical analysis of the diestrus group was not done due to the low number of total subjects which were tested in this phase of the estrous cycle resulting in insufficient statistical power. In Experiment 2, both the male ($t(11)=-1.953$, $p=0.038$) and female ($t(15)=-2.192$, $p=0.022$) PE groups had a significantly higher suppression ratios than the NPE groups (Figure 2). One-tailed t-tests were used in both experiments because it was hypothesized that the PE groups would exhibit higher suppression ratios than the NPE groups.

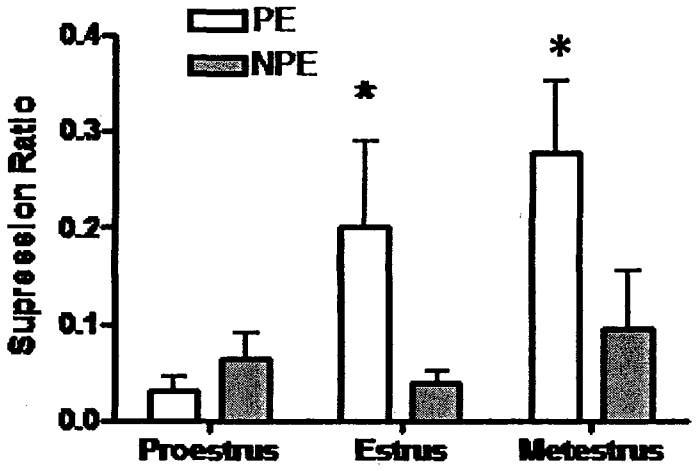

Figure 1. Suppression ratios for cycling adult female rats. PE rats in the estrus (p=0.04) and metestrus (p=0.038) phases of the estrous cycle, periods of low E2 levels, exhibited high suppression ratios indicating latent LI. PE rats in the proestrus phase, a period of high E2 levels, exhibited low suppression ratios indicating a lack of LI. This suggests high levels of E2 interfere with LI. The diestrus group was excluded from analysis due to a small number of subjects.

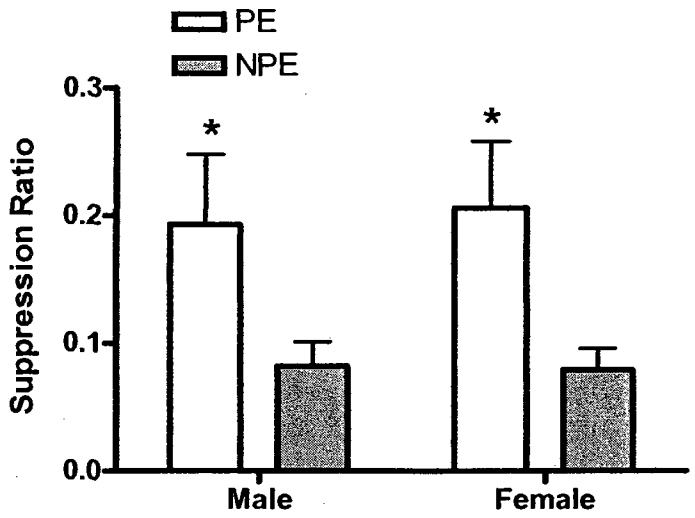

Figure 2. Suppression ratios for prepubertal male and female rats. Both male (p=0.038) and female (p=0.022) PE groups exhibited high suppression ratios indicating latent inhibition. This suggests that the interfering effect of E2 on latent inhibition is activational in nature.

4. Discussion

These studies investigated the effects of ovarian hormones in a LI paradigm utilizing a conditioned response to a tone. Cycling females that were conditioned during proestrus, a time of high E2 levels, exhibited disrupted LI when compared to cycling females conditioned during either estrus or metestrus, periods of low levels of E2. More specifically, while PE rats in the estrus and metestrus groups displayed significantly higher suppression ratios during the testing phase than NPE rats in the same groups, the suppression ratios of PE rats in the proestrus group did not significantly differ from that of the NPE rats in these groups. These data suggest that, in the LI paradigm employed here, high levels of estrogen during the conditioning phase interfere with the ability to ignore an irrelevant stimulus during the testing phase. This confirms recent data indicating that cycling females conditioned during periods of low estrogen exhibit LI while those conditioned during periods of high estrogen do not (Arad and Weiner 2008). The present findings are the first to demonstrate that this impairment is based in the activational (pubertal), rather than the organizational (perinatal), effects of ovarian hormones as pre-pubertal males and females in the PE groups had significantly higher suppression ratios than those of the male and female NPE groups.

The LI paradigm begins with a pre-exposure phase in which the subject receives repeated exposure to a neutral stimulus, such as a tone, that has no consequences. It is thought that this pre-exposure will later impede the association of that neutral stimulus (tone) with an unconditioned stimulus, such as a shock, during the conditioning phase. This association, or lack thereof, is then evaluated in the testing phase. Some attentional theorists posit that the non-reinforced presentation of the tone during the pre-exposure

phase will result in a learned inattentional response to the tone-shock pairing during the conditioning phase (Lubow 1997). The subject has learned that the stimulus is irrelevant as there are no consequences to the tone during pre-exposure and will ignore it in the testing phase. An alternative explanation suggests that LI is a product of the competitive expression of different conditioned stimulus-unconditioned stimulus associations; these may include tone-shock, tone-no shock, and tone-context (Escobar, Oberling et al. 2002). Accordingly, if a subject exhibits LI during the testing phase, then the tone-no shock pairing is thought to be the strongest association. While the LI is likely a product of both learned inattention and associative learning, the detrimental effects of high E2 on this behavior appear to be based in an impairment of the ability to effectively ignore irrelevant stimuli.

E2 has been shown to negatively impact attention in adult females in a variety of paradigms, including LI. High levels of E2 administered to OVX female rats results in the abolition of LI when compared to males and OVX females receiving only vehicle (Nofrey, Ben-Shahar et al. 2008). Arad and Weiner (2008) found a similar attenuation of LI in intact females conditioned during phases of the estrous cycle associated with high levels of E2. Conversely, that group later reported that ovariectomy diminishes LI but the behavior is restored by supraphysiological levels of E2 (Arad and Weiner 2009). On the other hand, high levels of E2 administered to OVX female rats attenuate performance in a task of sustained attention (McGaughy and Sarter 1999; Barnes, Staal et al. 2006) and female rats in proestrus show diminished PPI compared to males or females in estrus (Koch 1998). In addition, a study of cycling women found that PPI is disrupted during the luteal phase of the menstrual cycle, a time of high estrogen (Swerdlow, Hartman et al.

1997; Jovanovic, Szilagyi et al. 2004). This type of estrogenic modulation in attentional tasks is generally seen only in adult females. While some pre-pubertal cognitive sex differences have been identified in humans (McGivern, Huston et al. 1997; Everhart, Shucard et al. 2004), the effects of estrogen in rodents do not typically manifest until after the onset of puberty (Galea, Ossenkopp et al. 1994; Kanit, Taskiran et al. 2000; Hodes and Shors 2005). In accordance with such findings, the present data do not support an effect of estrogen in the LI behavior of pre-pubertal rats, only in adult females.

The mechanisms by which E2 alters LI performance have not yet been characterized. One possibility is that high levels of estrogen interfere with neurotransmitter function in brain regions which support attentional processes. For instance, it is thought that the medial prefrontal cortex (mPFC) plays an important role in attention-related tasks (e.g. Pezze, Dalley et al. 2009). Baseline dopamine (DA) levels in the mPFC significantly change with fluctuating estrogen levels across the estrous cycle (Dazzi, Seu et al. 2007). DA transmission in the dorsal striatum (DS), another brain region central to tasks which evaluate attention, is also affected by E2. DA baseline levels and release are enhanced during proestrus in this region (Xiao and Becker 1994). Although E2 has no effect on DA in male rats, DA release and dorsal striatal DA-mediated behaviors are enhanced during proestrus in cycling females (Becker 1999). Likewise, the administration of E2 to OVX females restores depleted basal DA levels and enhances amphetamine-induced DA release in the DS (Becker and Rudick 1999). DA neurons in the DS are activated during behavior in a LI paradigm (Jeanblanc, Hoeltzel et al. 2003) and both LI and PPI are sensitive to altered DA transmission in this region (Koch and Bubser 1994; Lubow 1997; Swerdlow, Braff et al. 2000). Dorsal striatal

administration of amphetamine (Konstandi and Kafetzopoulos 1993; Ellenbroek, Knobbout et al. 1997) or neuroleptics (Solomon, Crider et al. 1981) alter performance in these tasks. Although it has not been investigated directly, it is possible that the modulation of DA transmission in the DS by high levels of E2 may contribute, at least in part, to the concomitant attenuation of LI behavior.

These data provide further evidence that high levels of E2 alter performance in a dorsal striatal DA-mediated LI task. Moreover, these effects are dependent on the activational, rather than the organizational, effects of estrogen. At this time, it is not known if the estrogenic modulation of DA in the DS is a major factor in the impairment of LI but these effects have been shown to alter behavior in several other types of DA-mediated behavioral tasks. Further investigation is required in order to evaluate these effects as serotonin also plays a key role in attentional tasks (Kehne, Padich et al. 1996; Padich, McCloskey et al. 1996; Molodtsova 2003) and is also affected by E2 (Gabor, Nagle et al. 2003; Pongrac, Gibbs et al. 2004). The relative contribution of attention and associative learning in the LI paradigm also necessitates additional study.

CHAPTER 3:

USE OF COGNITIVE STRATEGIES IN RATS: THE ROLE OF ESTRADIOL AND ITS INTERACTION WITH DOPAMINE.

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Preface:

The experiments in the previous chapter demonstrated that high levels of ovarian hormones during proestrus, which include E2, interfere with the expression of latent inhibition, a dorsal striatal DA-mediated task. Performance in another dorsal striatal DA-mediated task, response learning in a maze (Packard and McGaugh 1996), can also be modulated by E2 (Korol and Kolo 2002; Korol, Malin et al. 2004; Daniel, Sulzer et al. 2006). Thus, the study presented in this chapter was conducted to confirm this alteration of a DA-dependent behavior by E2 in a plus maze. In addition, this study investigated the effect of DA D1 receptor and DA D2 receptor antagonism in low E2 and high E2 OVX female rats.

Abstract:

Accumulating evidence suggests a role for estrogen in the use of a particular cognitive strategy when solving a maze task. In order to confirm the role of estrogen in this phenomenon, ovariectomized (OVX) female rats receiving either high (~ 90 pg/ml) or low (~ 32 pg/ml) circulating levels of 17β-estradiol (E2) performed a plus maze task for a reward. Consistent with previous research, OVX rats receiving low levels of E2 utilized a striatum-mediated response strategy while OVX rats administered high levels of E2 employed a hippocampus-mediated place strategy. Furthermore, following a systemic injection of a moderate dose of either a dopamine D1 (SKF 83566, 0.1 mg/kg IP) or D2 (raclopride, 0.5 mg/kg IP) receptor antagonist, low E2 rats reverted to use of the opposite strategy and exercised a hippocampus-mediated place strategy in order to obtain the reward. At the same doses, high E2 rats did not change from using a place strategy. At a lower dose of these drugs, high E2 rats did not exhibit a preference for either strategy while low E2 rats predominantly used a place strategy in response to D2 receptor antagonism. These results corroborate previous findings that E2 plays a significant role in the use of either a response or place strategy when solving a maze for a reward. In addition, the shift in strategy after dopamine receptor blockade implies the importance of central dopamine function in selecting a cognitive strategy to solve such tasks. It is suggested that estrogen alters cognitive strategy not only by improving hippocampal function, but also by altering dopamine-regulated striatal function.

1. Introduction

There is extensive evidence demonstrating the effects of estrogen (E) on performance in behavioral tasks (for reviews see Hampson 1995; for reviews see Sherwin 2003). These effects have been confirmed in varied learning and memory paradigms and across several species. For instance, a review of these benefits in a clinical population (e.g. Sherwin 2003) reports that high levels of naturally circulating ovarian hormones enhance performance in female-typical tasks such as verbal memory and fine-motor skills. In addition, E replacement therapy in menopausal and hysterectomized women maintains or re-invigorates the same cognitive abilities. Although the reported effects of E on performance in a cognitive task are well established, E may also have additional effects including an influence on the utilization of a particular strategy necessary to solve a task than solely the level of performance.

In fact, recent evidence in rats points to an effect of E on the relative efficacy of employing a hippocampus-mediated 'place' strategy versus a striatum-mediated 'response' strategy when solving a cognitive task (Korol and Kolo 2002; Korol, Malin et al. 2004). Evidence for multiple learning and memory systems primarily based in these brain areas comes from numerous studies.

It was originally demonstrated in male rats that selective inactivation of the hippocampus affects the expression of a place strategy while damage to the caudate nucleus results in deficient response learning in a cross-maze task (Packard and McGaugh 1996) and in the Morris water maze (McDonald and White 1994). White and McDonald (2002) suggest that interference with hippocampal processing impaired the

ability of rats to learn the location of a food reward in an 8-arm radial maze during a win-shift task but had no effect on learning in a win-stay task (White and McDonald 2002). Conversely, lesions of the dorsal striatum attenuated performance in the win-stay, but not the win-shift, condition. In an 8-arm radial maze, retrieval of a reward in win-shift tasks is primarily dependent on the development of a spatial map of the maze and its immediate environment, or a place strategy. Conversely, a win-stay task requires the formation of a stimulus-response relationship without the involvement of any extra-maze cues, or a response strategy (White and McDonald 2002). Moreover, spatial working memory is impaired in rats with hippocampal damage but not striatal damage (Kesner 1990). However, response memory of a directional turn is attenuated in rats with striatal lesions but not hippocampal lesions (Cook and Kesner 1988). During response tasks in which striatal lesions significantly impair performance, rats with damaged hippocampi, but intact striatal function, exhibit an enhanced performance compared to normal rats (Matthews, Simson et al. 1995). Studies such as these suggest a dissociation, yet potential interaction, of the neural bases for particular cognitive strategies. In addition, the effectiveness of a particular strategy in solving a task is enhanced if relevant information from the alternative structure is made unavailable. Investigations of neurotransmitter release have also provided evidence for multiple memory systems. McIntyre et al. (2003) showed that acetylcholine release during acquisition of a dual solution T-maze task is increased in both the hippocampus and striatum of rats. However, this release was greater in the hippocampus of those rats employing a place strategy whereas in rats utilizing a response strategy striatal acetylcholine release was greater.

Importantly, evidence indicates that these distinct learning and memory systems are differentially regulated by the presence of E. An early study by Williams and colleagues (1990) established that neonatal hormonal manipulations alter the strategy by which rats solve a task in adulthood. In addition, OVX rats receiving E replacement acquired a place task more quickly than those receiving the vehicle which, in turn, performed better in a response task (Korol and Kolo 2002). The removal of a static cue signalling the location of a platform in the Morris water maze interfered with the ability of animals receiving vehicle to complete the task but did not affect E treated animals (Daniel and Lee 2004). Most convincingly, Korol et al. (2004) have shown that when naturally cycling rats in a plus maze are free to use a place or response strategy with which to solve the task, rats in the proestrus phase (high E) were biased towards utilization of a place strategy whereas rats in the estrus phase (low E) were more likely to employ a response strategy. A large body of research has been conducted examining the E-mediated enhancement of hippocampal function and its effects on performance in learning and memory tasks and strategy solution. Yet, little research has investigated the role of striatal dopamine (DA) and its interaction with differential internal hormonal states during the selection of a cognitive strategy.

There is converging evidence implicating striatal DA in response-based learning. Electrophysiological studies indicate that circuits within the basal ganglia promote the acquisition of a task through trial and error learning in which a particular behavioral response is shaped by reward-related contingencies (Graybiel 2005). Moreover the striatum contains response- and reward-related neural representations (Mizumori, Yeshenko et al. 2004). Chemical and structural lesions to the dorsal striatum interfere

with response learning tasks (McDonald and White 1994; Packard and McGaugh 1996; Compton 2004) and similar damage impaired acquisition of plus maze and T-maze tasks in cue-deficient environments (Chang and Gold 2004). Anatomical evidence shows that the striatum contains the highest density of DA D1 receptors (D1R) and DA D2 receptors (D2R) in the brain, both within the caudate-putamen and the nucleus accumbens (Boyson, McGonigle et al. 1986). A recent study by Daniel et al. (2006) showed that E interacts with D2R but not D1R to affect performance in a response learning task.

Although such results support a role for striatal DA in the performance of response-based behavioral tasks, there is a lack of evidence describing the estrogenic influence on DA in the selection of a cognitive strategy by which these tasks are solved. In order to investigate this interaction, here rats were trained in a dual solution T maze task that allows for the utilization of either a place or response strategy. Adult female rats were ovariectomized and administered levels of either low or high 17β-estradiol (E2). Based on findings by Korol et al. (2004) using the same task, it was expected that during a no-drug probe trial most low E2 rats would solve the maze using a response strategy and most high E2 rats would employ a place strategy. Furthermore, in order to determine the influence of E2 on DAergic mediation of strategy selection, both groups of animals received systemic injections of either a D1R or D2R antagonist prior to subsequent probe trials. Considering that performance in a response learning task is reportedly sensitive to D2R but not D1R antagonism (Daniel, Sulzer et al. 2006), both were examined here to determine whether the same pattern is seen in use of strategy.

These effects were investigated with a hormone administration regimen that was intended to mimic the levels of E during the estrous cycle. That is, the low E2 group received constant levels of E2 similar to that seen during the estrus phase and the high E2 group received constant low E2 plus daily injections resulting in peaks of E2 similar to that seen during the proestrus phase.

2. Materials and Methods

Subjects and Surgery:

Subjects included 30 female Sprague-Dawley rats (Charles River, St. Constant, QC, Canada), approximately three months of age. All rats weighed between 225-250 grams. Before training began, animals were housed in pairs in polyurethane shoebox cages and maintained on a reverse 12h:12h light/dark cycle with lights off from 0900-2100h. Standard lab chow and water were available *ad libitum* until training began. The rats were handled daily from time of arrival until completion of the experiment.

Approximately one week after arrival, all rats were anaesthetized using a mixture of ketamine (50 mg/ml) and xylazine (4mg/ml) in a 4:3 ratio (1 ml/kg, IP) and bilaterally ovariectomized using a standard aseptic procedure through a dorsal incision. Post-surgical care included administration of the antibiotic Baytril (0.03 ml/animal, SC), the analgesic banamine (0.03 ml/animal, SC), and 0.9% saline (3ml/animal, SC). Subsequent to surgery, all animals were allowed to recover in their home cages for several days until surgery for implantation of Silastic tubes. All animal protocols were in accordance with

guidelines established by the Canadian Council on Animal Care and were approved by the Concordia University Animal Research Ethics Committee.

Hormone administration and measurement:

Animals were randomly assigned to one of two groups; low E2 (n=15) or high E2 (n=15). Three days after the ovariectomy surgeries, all animals were anaesthetized using Halothane gas (4% for induction and 2% for maintenance) and a Silastic tube (1cm long; i.d. 1.47 mm; o.d. 1.96 mm) containing 5% 17β-E2 (Sigma Chemical Co., St Louis, MO) in cholesterol (Sigma) was subcutaneously implanted in the nape of the neck. This has been reported to produce a serum concentration of approximately 20 pg/ml consistent with naturally circulating low levels of E such as those seen during the estrus phase of the rat estrous cycle (Mannino, South et al. 2005). For the high E2 group, in addition to the subcutaneous implants, daily subcutaneous injections were given of 17β-estradiol benzoate (10μg/kg) dissolved in sesame oil (Sigma) designed to achieve levels seen during the proestrus phase of the estrous cycle (75-90 pg/ml). During the same period all animals in the low E2 group received daily subcutaneous injections of sesame oil as a control (1ml/kg). All injections began two days before habituation training and occurred between 1200-1400h each day.

Approximately 30 days following the implantation of the Silastic tubes, i.e. at the completion of behavioral testing, blood was collected from the tail vein at 1200h. Blood samples were immediately centrifuged and plasma was collected and stored at -20 °C until assayed. E2 was measured using a commercially available ELISA kit (Immuno-

Biological Laboratories Inc., Minneapolis, MI). The assay antibodies have 100% cross-reactivity with E2 and 0.2% and 0.05% cross-reactivity with estrone and estriol respectively. The range of the assay is between 0 – 2000 pg/ml and the reported inter-assay variation is 7-9%.

DA receptor antagonist administration:

Animals received systemic injections of DA antagonists on separate days. The selective D1R antagonist SKF 83566 (Sigma) was administered in a moderate dose of 0.1 mg/kg and a lower dose of 0.01 mg/kg. Raclopride, a selective D2R antagonist, was administered in a moderate dose of 0.5 mg/kg and lower dose of 0.1 mg/kg. These doses were selected because they are in the mid and low ranges for modifying behaviors in attention and learning tasks for both SKF 83566 (Serafim and Felicio 2001; Salamone, Arizzi et al. 2002; Domenger and Schwarting 2006) and raclopride (Wise and Carlezon 1994; Shaham and Stewart 1996). Both drugs were dissolved in 0.9% saline at room temperature and stored no longer than four days at 4°C. All drugs were administered intraperitoneally (IP) 30 minutes prior to the onset of behavioral testing and animals were allowed a washout period for a minimum of 24 h between each drug probe trial.

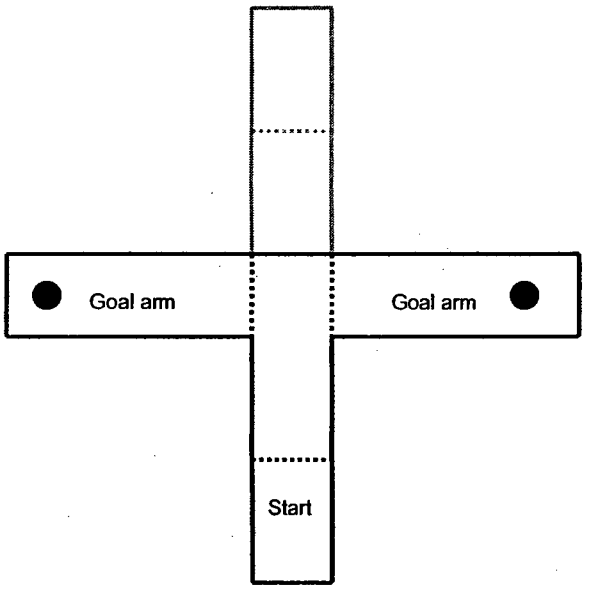
Apparatus, Modified Plus Maze:

All training was carried out in a polyurethane plus maze placed on a table one meter above the floor. The maze was constructed with black walls extending 23cm above

a wire grid floor that is 10.5cm wide and enclosed with removable clear polyurethane roof panels. The start and probe arms do not have roof panels on them. The modified plus maze had four arms arranged at 90° angles around a 14x14cm central chamber; two goal arms, a training start arm, and a probe start arm, all of which were 75cm in length (figure 1). Entrance from the central chamber to all arms could be occluded by black polyurethane guillotine gates which could be lifted by the experimenter using a string from a remote location.

Throughout the training trials the probe start arm was blocked off from the central chamber creating a T-shaped maze (figure 1A). At the commencement of the probe trial, the probe arm was unblocked and the original start arm was blocked creating an alternative T-shaped maze exactly 180° in orientation (figure 1B). Each start arm contained a start-box 30cm in length which blocked access to the central chamber by a black polyurethane guillotine gate halfway down the arm. Each goal arm contained a white ceramic bowl in which a food reward (Kellogg's Froot Loops®) could be placed. Froot Loop crumbs were placed underneath both goal arms of the maze during all trials to prevent any confounds due to odor cues. Extra-maze cues were very obvious and included a large dark poster on a plain white wall opposite blue metal cupboards on the other wall. The experimenter in a white lab coat stood in the same position during all trials. For all trials, the maze was kept stationary in a position relative to all extra-maze cues throughout testing. All testing took place under dim red light illumination.

A.



B.

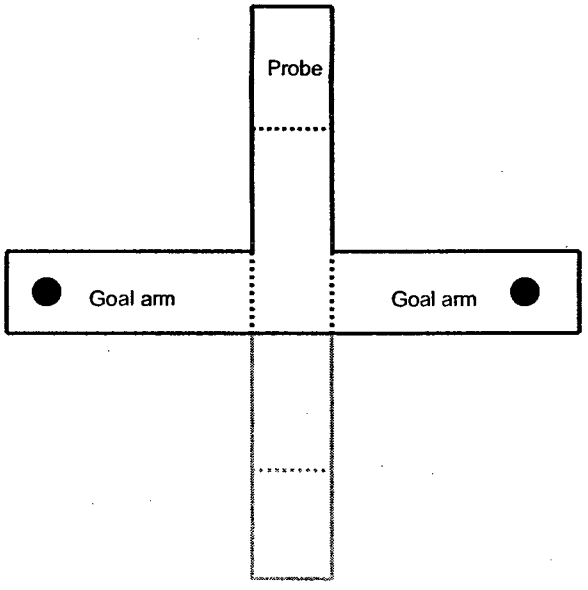


Figure 1: The modified plus maze used in this experiment as adapted from Korol et al. (2004). A) Rats were initially trained to receive a reward in either the left or right goal arm. Upon reaching criterion, rats were then placed in the opposite arm B) for the start of the probe trial.

Procedure:

Habituation. Approximately five days after ovariectomy, all animals were placed on food restriction which lasted until completion of the experiment and maintained body weight at 90% of free-feeding weight. All training was performed at the beginning of the dark phase of the light/dark cycle commencing at 0900h. Beginning seven days after implantation of the Silastic tubes, all animals were given three days of habituation to the modified plus maze with the probe trial start arm blocked off creating a T-shaped maze (see figure 1A). Habituation consisted of 15-min sessions in the maze with Froot Loops scattered throughout the apparatus.

Training. Following habituation, all rats began acquisition training. Each animal was assigned to receive a food reward, half of a Froot Loop, in either the right or left goal arm. The baited goal arm was counterbalanced across rats such that half the rats in each group received the reward in the right arm and half in the left arm. For each particular rat the Froot Loop was always in the same goal arm. Training consisted of 10 daily trials per rat beginning in the start box of the training arm. Rats were placed in the start-box behind the guillotine gate and were allowed to rear and look and sniff around the room while waiting to start.. Once the gate panels of both goal arms were raised the animal was released from the start-box and allowed a free choice to enter either the right or left goal arm. A trial was ended when all four limbs of a rat crossed into a goal arm and the gate could be closed, or when a 2-min time limit was reached. Rats that chose correctly were allowed to eat the food reward in the arm before being returned to their home cage. Rats that chose incorrectly were allowed to investigate the empty food bowl before being

taken out of the arm and placed into the home cage. Inter-trial intervals were 10-30 sec during which time another rat completed its trial. Rats were considered to have reached criterion after performing 8/10 correct trials for three days in a row.

Probe testing. On the third day of criterion performance the rat was given a probe trial starting from the probe arm (figure 1B). The rat was placed in the probe start arm, 180° opposite the blocked training start arm, and the experimenter returned to the standard location. From this position, the goal arm gates were lifted and the animal was released and allowed to enter either the right or left arm. If an animal made the same directional turn in its choice of an arm for which it had been rewarded in the training phase, then it was scored as a response strategy. If an animal made an opposite directional turn in its choice of an arm towards the same spatial location of its reward during the training phase, then it was scored as a place strategy.

D1R and D2R Antagonism. Each rat was injected with a particular type and dose of drug in a counterbalanced manner. All drug trials were given subsequent to achievement of criterion and completion of the initial probe trial. One day after the no-drug probe trial each rat was injected with a drug and trained again for10 trials then given the drug probe trial. No subsequent drug injections were given for at least 24 h. Every rat maintained its performance of 8/10 correct trials during all drug trials.

Statistical Analyses:

This was a mixed design with E2 level as a between subjects factor and drug as a within subjects factor. Because the dependent variable was percent of rats using a type of cognitive strategy, probe and drug trial data were analyzed using non-parametric statistics. A Chi-square (χ^2) analysis was conducted to compare high and low E rats in their use of strategy in the no-drug probe trial. Multiple χ^2 tests were also conducted for these two groups for each of the drug probe trials at both doses of SKF 83566 and raclopride to test whether there was a significant probability of using one strategy over another in each individual case. In order to test the effects of DA antagonists, McNemar tests were conducted using a binomial for small frequencies to compare no-drug probe trials with each of the respective doses of SKF 83566 and raclopride.

To control for the effects, if any, of repeated testing in this task, a Cochrane Q test was conducted on percent of rats using either strategy across all 5 days of probe and drug trials. Finally, to determine if there were any differences in learning this task, a two-tailed t-test was conducted on the number of days to reach criterion for low and high E2 groups.

3. Results

Plasma E2 levels, task acquisition and no-drug probe test:

The mean (± S.E.M.) plasma E2 level for the low E2 rats was 32.62 pg/ml (± 7.05) and for the high E2 rats it was 90.44 pg/ml (± 19.70). These levels are within range for estrus and proestrus respectively.

By the time the first criterion day was reached the entire testing session for each rat lasted no more than 15 min and trials were completed in 20 sec or less, but most often within 5 sec. There were no significant differences ($t = -0.259$, $p = 0.79$) between the high and low E2 groups in the number of days to reach criterion. The means (± S.E.M) were 4.68 (± 0.32) for Low E2 and 4.81 (± 0.37) for High E2. In addition, there was no effect of trial test day on prevalence of using either a place or a response strategy ($Q = 4.857$, $p = 0.302$).

Figure 2 illustrates that on the no-drug probe trial, high E2 rats were significantly more likely to employ a place strategy (80%, $\chi^2 = 0.02$) than a response strategy (20%). In contrast, Low E2 rats showed a non-significant but strong trend to utilize a response strategy (73%, $\chi^2 = 0.071$) over a place strategy (27%).

D1R antagonist probe trials (SKF 83566):

As can be seen in figure 3 (top) D1R blockade by a 0.1 mg/kg dose of SKF 83566 in the low E2 group resulted in a significant shift (McNemar, $p = 0.039$) in the method used to solve the probe trial from a majority of rats using a response strategy in the no drug probe to using a place strategy (80%, $\chi^2 = 0.02$). Conversely, the low dose of SKF 83566 had no effect in comparison to the no drug probe trial as there was a significantly higher probability that these rats would employ a response strategy (87%, $\chi^2 = 0.005$) over a place strategy.

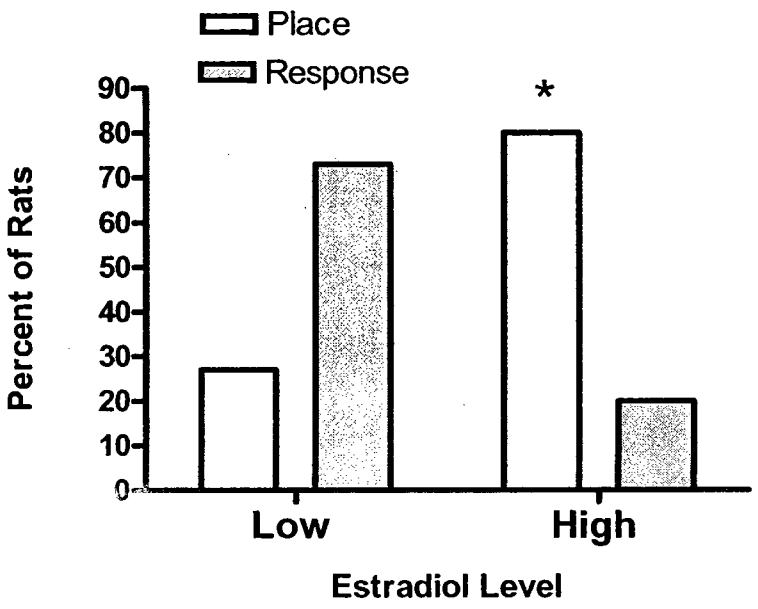


Figure 2: Strategy use in ovariectomized rats administered either high or low estradiol. A significantly greater proportion of High estradiol rats employed a place strategy *(χ^2 = 0.02, Chi-Square test) over a response strategy.

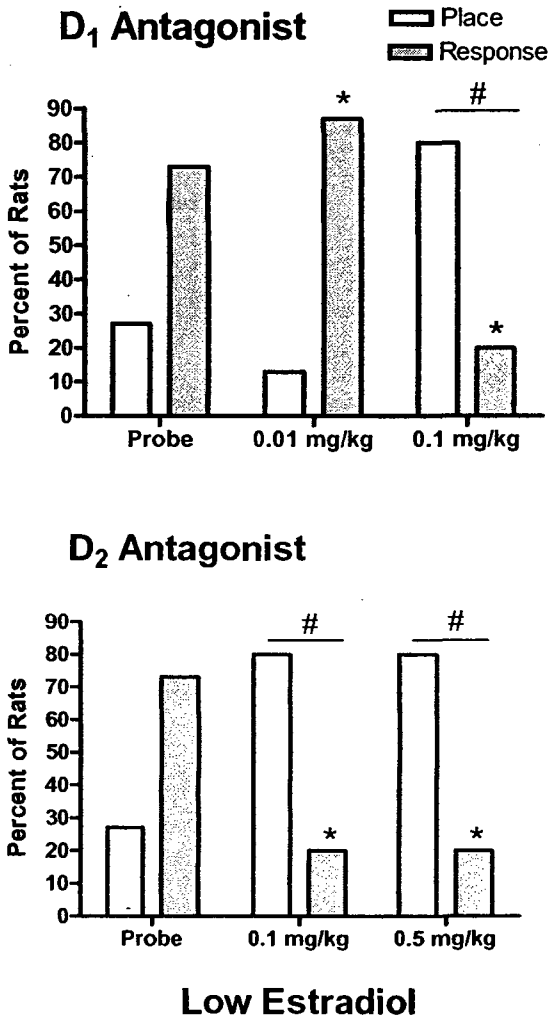


Figure 3: Strategy use in ovariectomized rats administered low estradiol during the no-drug probe trial and in response to doses of a dopamine D_1 receptor antagonist (SKF 83566) and a dopamine D_2 receptor antagonist (raclopride). * significant ($p < 0.05$) difference in percent of rats using response strategy versus place strategy (Chi Square). # significant switch in strategy compared to probe trial (McNemar).

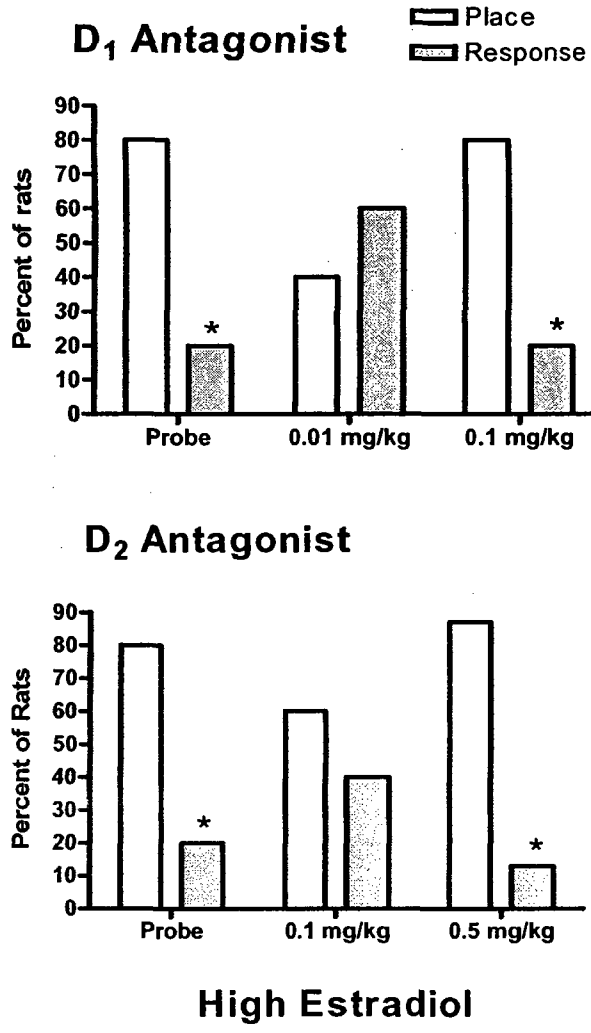


Figure 4: Strategy use in ovariectomized rats administered high estradiol during the no-drug probe trial and in response to doses of a dopamine D_1 receptor antagonist (SKF 83566) and a dopamine D_2 receptor antagonist (raclopride). * significant ($p < 0.05$) difference in percent of rats using response strategy versus place strategy (Chi Square).

As can be seen in figure 4 (top), after systemic administration of a 0.1 mg/kg dose of the selective D1R antagonist SKF 83566, high E2 animals maintained use of their no-drug strategy and were significantly more likely to employ a place strategy (80%, χ^2 = 0.02) than a response strategy (20%). However, administration of a 0.01 mg/kg dose resulted in these animals being equally likely to use either a place strategy (40%) or a response strategy (60%) in order to solve the task.

D2R antagonist probe trials (Raclopride):

Figure 3 (bottom) illustrates that comparable to intermediate D1R antagonism, systemic administration of 0.5 mg/kg raclopride in low E2 animals resulted in a significant shift of cognitive strategy (McNemar, p = 0.008) as significantly fewer animals exercised a response strategy (13%, χ^2 = 0.005) compared to a place strategy (87%). In contrast to the effect of a low dose D1R antagonism in low E2 animals, low dose (0.1 mg/kg) D2R antagonism with raclopride resulted in a significant shift of cognitive strategy (McNemar, p = 0.021) as significantly more low E2 animals utilized a place strategy (80%, χ^2 = 0.02) over a response strategy (20%).

As can be seen in figure 4 (bottom), systemic injection of a 0.5 mg/kg dose of the D2R antagonist raclopride did not effect of strategy selection in high E2 animals as the majority employed a place strategy (80%, χ^2 = 0.005) over a response strategy (20%) which is how these rats responded in the no drug condition. After administration of 0.1mg/kg of raclopride, however, rats were equally likely to utilize either a place strategy

(40%) or a response strategy (60%), similar to the effects subsequent to low dose D1R antagonism.

4. Discussion

The present results confirm previous findings (Korol, Malin et al. 2004) that demonstrate variations in the levels of E2 in female rats strongly influence the probable use of a particular cognitive strategy when solving a maze for a reward. The data show that chronic low levels of E2 will bias an animal towards the utilization of a striatum-mediated 'response' strategy and repeated high levels of E2 will bias a rat towards use of a hippocampus-mediated 'place' strategy.

The most dramatic effects of DA receptor antagonism in the current study were observed in the low E2 rats. Both doses of raclopride and the higher dose of SKF 83566 caused the majority of low E2 rats that employed a response strategy in the no-drug trial, to employ a place strategy. The only exception was in response to the lower dose of the D1R antagonist, SKF 83566, which did not change the strategy used by most low E2 rats.

The effects of DA receptor antagonism on high E2 rats were the same for both the D1R antagonist, SKF 83566, and the D2R antagonist, raclopride. Neither of these drugs had any effect on strategy selection at the higher doses. That is, high E2 rats maintained a greater probability of using a place strategy when administered the higher doses of these drugs. On the other hand, high E2 rats responded to the lower doses of both drugs by being equally likely to use a place or a response strategy, showing no probability of

using one strategy over another. It has been shown that high levels of E when acutely administered will increase DA release in the striatum (Becker 1990) and E2 will increase both D1R and D2R binding (Di Paolo, Poyet et al. 1981; Levesque and Di Paolo 1989).

Strategy use was strongly influenced by systemic administration of either a D1R or D2R antagonist and these effects were different depending on whether rats were administered high or low E2. Although it has been shown that E2 increases binding of both D1R and D2R in the striatum (Di Paolo, Poyet et al. 1981; Levesque and Di Paolo 1989), a recent behavioral study found that E2 interacted with D2R but not D1R antagonism. In examining the effects of E2 on performance in a response task, D2R antagonism disrupted performance to a greater extent in the high E2 OVX rats than in no E2 OVX controls. Conversely, there was no difference in high E2 or no E2 in rats in response to D1R antagonism (Daniel, Sulzer et al. 2006). This is not in agreement with the results reported here insofar as antagonism of both receptor subtypes had an effect on strategy selection. On the other hand, in the study by Daniel and colleagues (2006) the measured outcome was performance in a response task, whereas here the measure was the use of either a place or a response strategy to solve a task that could be completed using either strategy.

These results suggest that a hippocampus-dependent place strategy is more likely to be used by rats receiving high E2. The strategy use of High E2 rats was not altered following administration of a moderate dose of either a D1R or D2R antagonist. In addition, Low E2 rats administered either a moderate dose of D1R or both doses of D2R antagonist were biased to use a place strategy. Numerous studies demonstrate a beneficial

influence of high E on hippocampal function and hippocampus-dependent behaviors across several species (e.g. McEwen, Akama et al. 2001). For instance, higher levels of acute E enhance hippocampal dendritic spine density (Woolley and McEwen 1992) and synaptic proteins in rats (Brake, Alves et al. 2001) as well as in rhesus macaques (Choi, Romeo et al. 2003). The potential influence of acute E on learning and memory has also been demonstrated insofar as it facilitates long term potentiation (Warren, Humphreys et al. 1995; Cordoba Montoya and Carrer 1997) and attenuates long term depression (Day and Good 2005) in the hippocampus. These studies suggest that E increases hippocampal function. In addition, the data presented here suggest that the bias toward hippocampus-dependent strategy is not only influenced by E but also by its interaction with central DA function.

Low E2 rats show a bias toward use of a striatum-dependent response strategy. In addition, following administration of a low dose D1R antagonist, low E2 rats were more likely to maintain use of a response strategy. Although less is known concerning estrogenic effects on striatum-based learning, evidence suggests that high E levels impair learning and performance in tasks dependent on striatal function. For example, high E2 administration to OVX female rats impaired acquisition of tasks dependent upon response learning such as a response variant of the T-maze (Davis, Jacobson et al. 2005) and a cue-based Morris water maze subsequent to removal of the cue (Daniel and Lee 2004). E2 infusion into the striatum also impaired a cue-deficient version of a Y-maze (Zurkovsky, Brown et al. 2007). Moreover, low levels of E2 have been shown to improve learning and performance in tasks that depend on striatal functioning such as non-spatial

working memory in an alternating T-maze task (Wide, Hanratty et al. 2004) and in a radial arm maze (Holmes, Wide et al. 2002).

Studies in this lab using gonadally-intact female rats have shown that during proestrus, a period of high E, performance in a latent inhibition task is interrupted (Quinlan, Graffe et al. 2006) and high E replacement in OVX rats disrupts latent inhibition (Nofrey, Ben-Shahar et al. 2007). Lubow (1997) has reviewed evidence strongly suggesting latent inhibition is a task dependent on striatal DA function. Additionally, the effects of E on pre-pulse inhibition (PPI), another striatal DA-dependent task, are evident in both clinical and experimental populations. Investigations of PPI in human subjects indicate that sensorimotor gating performance is reduced during the luteal phase of the menstrual cycle when circulating levels of E are high (Jovanovic, Szilagyi et al. 2004). Studies in rats also show a significant reduction of PPI during high E proestrus females as compared to males or to females in the diestrus or estrus phases of the estrous cycle when E levels are low (Koch 1998).

There are also physiological data to support the idea that E may affect striatal function. For example, E is known to affect DA function in the striatum (for review see, Becker 1999) and recent evidence suggests that E2 inhibits gamma- aminobutyric acid (GABA) release in the striatum (Hu, Watson et al. 2006).

In summary, the present results are a confirmation of previous findings demonstrating the important influence of E2 levels in the use of a strategy during performance of a cognitive task. In addition, a comparably relevant role of DA in this process is implicated by the shift in strategy selection subsequent to modulation of D1R

and D2R. However, this is not merely an isolated effect of hormonal influence or neurotransmitter activity but rather an interaction between these two systems that significantly impacts the behavior of a rat in a learning and memory task. Dependent on the levels of E2 in the brain, there are differential modulations of the DAergic activity which significantly alter the approach by which a rat will solve a task for a reward.

CHAPTER 4:

DORSAL BUT NOT VENTRAL DOPAMINE D1 RECEPTOR ANTAGONISM INTERACTS WITH CHRONIC ESTRADIOL TO INFLUENCE COGNITIVE STRATEGY IN FEMALE RATS

Matthew G. Quinlan, Meghen Caissie, Ivonne LaChappelle, George Radiotis, and Wayne G. Brake

Preface:

The findings from the studies in the previous chapter show that the systemic injection of DA D1 and DA D2 receptor antagonists differently affects the cognitive strategy use of low E2 rats and high E2 rats. To determine the brain regions in which DA receptor antagonism is acting to alter the strategy use of low E2 rats, DA D1 receptor and DA D2 receptor antagonists were infused into the dorsal striatum and the ventral striatum (nucleus accumbens). The dorsal striatum was chosen as a likely area to begin the investigation as high levels of E2 attenuate performance in dorsal striatal DA-mediated response learning tasks (Korol and Kolo 2002; Daniel, Sulzer et al. 2006). The nucleus accumbens was chosen as a control for the dorsal striatum.

Abstract:

Estradiol (E2) has been shown to modulate the cognitive strategy used by female rats to find a reward. For example, rats with high levels of E2 tend to use an allocentric (place) strategy while rats with low levels of E2 use an egocentric (response) strategy. It has been shown that systemic dopamine receptor antagonism interacts with E2 to affect strategy use. Here, dopamine antagonists were administered directly into the dorsal striatum or nucleus accumbens to determine where in the brain this interaction takes place. Seventy-four young adult, female, Sprague-Dawley rats were trained and tested in a modified plus maze. All rats were ovariectomized, received a subcutaneous E2 implant, and were implanted with bilateral cannulae into either the dorsal striatum or the nucleus accumbens. In addition, high E2 subjects received daily injections of E2 in a sesame oil solution while low E2 subjects received daily injections of vehicle only. After reaching criterion levels of performance in a T-Maze task, subjects were administered microinjections of either a dopamine D1 receptor (SCH 23390; 0.1μg/ml and 0.01μg/ml) or D2 receptor (raclopride; 2μg/ml and 0.5 μg/ml) antagonist or a vehicle control (saline) in a counterbalanced manner. Dorsal striatal administration of a D1, but not D2, receptor antagonist caused a shift in strategy in both high and low E rats. There was no significant effect of dopamine antagonism in the nucleus accumbens group. Thus, E2 biases the cognitive strategy used in a maze when searching for a reward. This effect is modulated by dopamine D1R antagonism in the dorsal but not ventral striatum suggesting that cognitive strategy is in part mediated by an interaction between E2 and dopamine in this region.

1. Introduction

There is extensive evidence, albeit controversial, implicating estrogen in the performance of behavioral tasks by women and other female mammals. Higher levels of estrogen increase performance in some behavioral tasks while reducing performance in others (e.g. Hampson 1995; e.g. Sherwin 2003). Such conflicting findings have led to the suggestion that the effect of estrogen on task performance may be underpinned by its effect on cognitive strategy (Korol, Malin et al. 2004; Daniel 2006). Although alternative strategies may be used to solve a given cognitive task correctly, one particular strategy may allow the task to be completed more quickly and/or with fewer errors than other strategies.

Rats learning to complete a maze can use one of several cognitive strategies (Tolman, Ritchie et al. 1946; Blodgett and McCutchan 1947). For example, they may use environmental, or allocentric, cues surrounding the maze to develop a cognitive map and use this to find the reward. This approach is referred to as a place learning strategy and is mediated predominantly by the hippocampus (Packard and McGaugh 1996). Another approach involves the use of internal, or egocentric, cues to find the reward. In this case, rats learn and employ a series of habitual motor responses such as 'turn left' or 'turn right.' This approach is referred to as a response learning strategy and is mediated predominately by the dorsal striatum (DS), also known as the caudate/putamen (Packard and McGaugh 1996; Chang and Gold 2004). As would be expected, rats use a combination of these strategies under normal circumstances (Chang and Gold 2003). However, when the brain region responsible for one strategy is made unavailable (e.g. via

lesion or lidocaine) the other strategy predominates (e.g. Packard and White 1989; Packard and McGaugh 1996; e.g. White and McDonald 2002). Accordingly, the use of either a place or response strategy can be influenced by altering the function of either the hippocampus or the DS. For example, Packard and White (1991) showed that both D1 and D2 receptor agonists injected directly into the DS improve performance on a response task with no effect on a place task. However, when these agonists are injected into the hippocampus, they improve performance on a place task with no effect on the response task.

There is a preponderance of evidence that estradiol (E2) affects hippocampal function (for review see McEwen, Akama et al. 2001) and, thus, it should not be surprising that high E2 levels improve hippocampus-based place learning either in a plus maze (Korol and Kolo 2002) or a radial arm maze (Davis, Jacobson et al. 2005). A study by Korol et al. (2004) showed that when cycling female rats were allowed to solve a modified plus maze using either strategy, the majority of proestrus (high E2) rats used a place strategy while most estrus (low E2) rats used a response strategy. These findings were replicated in ovariectomized (OVX) rats administered high or low E2 replacement (Quinlan, Hussain et al. 2008).

While higher levels of E2 improve performance on tasks requiring the hippocampus they may impair performance on tasks regulated by other brain regions, including the DS. For example, direct infusion of E2 into the DS impairs response learning (Zurkovsky, Brown et al. 2007). Higher levels of E2 also impair performance in other behavioral tasks regulated by the DS, viz. response learning in a radial arm maze

(Davis, Jacobson et al. 2005), latent inhibition (Nofrey, Ben-Shahar et al. 2008) and pre-pulse inhibition (Koch 1998). Tasks such as these are particularly sensitive to levels of dopamine in the DS (Lubow 1997; Swerdlow, Braff et al. 2000) and it has been also shown that E2 alters DS dopamine function (Di Paolo, Poyet et al. 1981; Levesque and Di Paolo 1989; Becker 1990).

Recent evidence demonstrates that E2 interacts with dopamine to influence cognitive strategy in female rats. Previous work in this lab (Quinlan, Hussain et al. 2008) has shown that dopamine D1 receptor (D1R) and D2 receptor (D2R) antagonists are able to shift the strategy used by OVX rats administered low but not high E2. That is, D1R and D2R antagonists shifted the cognitive strategy used by low E2 rats from a response to a place strategy. It is hypothesized that higher E2 levels may not only bias strategy toward hippocampal-dependent place learning but may also attenuate DS-dependent response learning, specifically by altering DS dopamine transmission.

Although these data are consistent with this hypothesis, it cannot yet be concluded that E2 is specifically interacting with dopamine in the DS to produce these behavioral effects because the antagonists were administered systemically in our previous study. Other systems including the mesolimbic dopamine system that innervates the ventral striatum also play a role in solving a maze for a reward. The current study was designed to examine the interaction between chronic E2 and dopamine within the DS and ventral striatum (nucleus accumbens; NA). It was hypothesized that D1R and D2R antagonist microinjections into the dorsal, but not ventral, striatum would alter the cognitive strategy used by low but not high E2 rats.

2. Materials and Methods

Subjects and Surgery: Eighty-six female, Sprague-Dawley rats (Charles River, St. Constant, QC, Canada), approximately three months of age and weighing between 225-250 grams were initially included in this study. Before training began, rats were housed in pairs in polyurethane shoebox cages and maintained on a reverse 12h:12h light/dark cycle with lights off from 0900-2100h. Standard lab chow and water were available *ad libitum* until training began.

Approximately one week after arrival all rats were anaesthetized using Halothane gas (4% for induction; 2% for maintenance) and bilaterally ovariectomized using a standard aseptic procedure through a dorsal incision. During the ovariectomy procedure all rats were implanted with a Silastic tube (1cm long; i.d. 1.47 mm; o.d. 1.96 mm) containing 5% 17β-estradiol benzoate (Sigma Chemical Co., St Louis, MO) in cholesterol (Sigma) which was subcutaneously implanted in the nape of the neck. This has been reported to produce a serum concentration of approximately 20-25 pg/ml. This is consistent with naturally circulating levels of E2 typical of those seen during the estrus phase of the rat estrous cycle (Butcher, Collins et al. 1974; Mannino, South et al. 2005). Post-surgical care included administration of the antibiotic Baytril (0.03 ml/animal, SC; CDMV, St. Hyacinthe, Quebec), the analgesic banamine (0.03 ml/animal, SC; CDMV), and 0.9% saline (3ml/animal, SC). Rats were allowed to recover in their home cages for several days prior to implantation of cannulae.

Rats were randomly assigned to be implanted with cannulae directed at either the DS (DS group) or NA (NA group). Rats were anaesthetized using Halothane gas and

received stereotaxic bilateral implantations of 21g stainless steel tubing guide cannulae (Plastics One, Roanoke, VA). Cannulae were secured with dental cement and skull screws. Stereotaxic co-ordinates from bregma for the DS were AP -0.3, ML ± 4.0, DV -5.0; and for NA were AP +1.4, ML ± 2.8 at 10°, DV -6.8 (Paxinos and Watson 1998). Cannulae were blocked with 26g obdurators (Plastics One) which extended 1mm below the tip of the guide cannula. Rats received the same post-surgical care as after the ovariectomy surgery and were allowed several days to recover in their home cages. All animal protocols were in accordance with guidelines established by the Canadian Council on Animal Care and approved by the Concordia University Animal Research Ethics Committee.

Hormone administration: The DS and NA groups were further randomly divided in half and assigned to either low or high E2 conditions. In addition to the low constant levels of E2 supplied by the Silastic implants to all rats, rats in the high E2 condition received additional daily subcutaneous injections of 17β-estradiol (20μg/kg) dissolved in sesame oil (Sigma) designed to achieve E2 levels seen during the proestrus phase of the estrous cycle (75-90 pg.ml; Mannino, South et al. 2005; Quinlan, Hussain et al. 2008). To control for the daily injection procedure, rats in the low E2 condition received daily subcutaneous injections of sesame oil (1ml/kg). All rats received the first injection two days before habituation training. Injections were given between 1200-1400h each day. This hormone administration regimen was intended to mimic E2 levels seen during the estrus and proestrus phases of the natural estrous cycle. Subcutaneous implants maintain a steady

low baseline level of E2 in both E2 groups while daily injections of an E2 solution imitates the intermittent peaks of E2 associated with proestrus. Testing began approximately 10 days following ovariectomy.

Approximately 30 days following the implantation of the Silastic tubes, blood was collected from the tail vein 22 hrs following the previous E2 or oil injection. Blood samples were immediately centrifuged and plasma was collected and stored at -20 °C until assayed. E2 was measured using a commercially available ELISA kit (Immuno-Biological Laboratories Inc., Minneapolis, MI). The assay antibodies have 100% cross-reactivity with E2 and 0.2% and 0.05% cross-reactivity with estrone and estriol respectively. The reported inter-assay variation is 7-9%.

Dopamine Receptor Antagonist Administration: Animals received bilateral intracerebral injections of DA antagonists on separate days; all drugs were administered five minutes immediately prior to the start of testing at a rate of 0.5µl/min/side using a Harvard Apparatus Model 22 automatic dual pump, 26g stainless steel injectors which extended 1mm below the tip of the cannulae, and two Hamilton 10µl syringes. Injections lasted for one minute after which the injectors were left in place for an additional minute to allow for drug diffusion. Drugs were only infused on drug probe testing days and not on training days.

The D1R antagonist SCH 23390 (Sigma) was administered in a moderate dose of 0.1µg/ml and a low dose of 0.01µg/ml. Raclopride (Sigma), a selective D2R antagonist, was administered in a moderate dose of 2.0µg/ml and a low dose of 0.5µg/ml. These

doses were selected because they are in the mid and low ranges for modifying behaviors in attention and learning tasks for both SCH 23390 and raclopride (Fernandez-Ruiz, Hernandez et al. 1991; Callahan, De La Garza et al. 1997; Seamans, Floresco et al. 1998; Zavitsanou, Cranney et al. 1999). Both drugs were dissolved in 0.9% saline at room temperature and stored at -20°C until used. Each rat was injected with a particular type and dose of antagonist or saline in a counterbalanced schedule using a Balanced Latin Square design to rule out sequence and order effects within each group. A washout period of at least 24 hrs was given between each drug probe trial.

Apparatus, Modified Plus Maze: All training was carried out in a polyurethane plus maze placed on a table one meter above the floor. The maze was constructed with black walls extending 23cm above a wire grid floor that is 10.5cm and enclosed with a removable clear polyurethane roof panels. The modified plus maze had four arms arranged at 90° angles around a 14x14cm central chamber; two goal arms, a training start arm, and a probe start arm, all of which were 75cm in length. Entrance from the central chamber to all arms could be occluded by black polyurethane guillotine gates which could be lifted by the experimenter using a string from a remote location.

Throughout the training trials the probe start arm was blocked off from the central chamber creating a T-shaped maze (see Quinlan, Hussain et al. 2008). At the commencement of the probe trial, the probe arm was unblocked and the original start arm was blocked creating an alternative T-shaped maze 180° in orientation. Each goal arm contained a white ceramic bowl in which a food reward (Kellogg's Froot Loops®) could

be placed. Froot Loop crumbs were placed underneath both goal arms of the maze during all trials to mask confounding effects of odor cues. Extra-maze cues included a large dark poster on a plain white wall opposite a series of blue metal cupboards on the other wall. The experimenter in a white lab coat stood in the same position during all trials. For all trials, the maze was kept stationary relative to all extra-maze cues throughout testing. All testing took place with overhead red lights with an additional 15 W light for illumination. The room was lit only moderately to avoid evoking anxiety in the rats during the task.

Procedure, Modified Plus Maze: Testing in the modified plus maze was the same as that employed previously (Quinlan, Hussain et al. 2008). Briefly, five days after the final surgery all animals were placed on food restriction and maintained at a body weight of 90% of free-feeding weight. All training was performed at the beginning of the dark phase of the light/dark cycle commencing at 0900h. All rats were habituated to the modified plus maze. Habituation consisted of three, once-daily 15-min sessions in the maze with Froot Loops scattered throughout the apparatus.

Following habituation, each rat was assigned to receive a food reward, half of a Froot Loop, in either the right or left goal arm. The baited goal arm was counterbalanced across rats such that half the rats in each group received the reward in the right arm and half in the left arm. For each particular rat the Froot Loop was always in the same goal arm. Training consisted of 10 daily trials per rat beginning in the start box of the training arm. The doors to both goal arms were opened and the rat was released from the start-box and allowed a free choice to enter either the right or left goal arm. A trial was ended

when all four limbs of a rat crossed into a goal arm and the door could be closed, or when a 2-min time limit was reached. Rats that chose correctly were allowed to eat the food reward in the arm before being returned to their home cage. Rats that chose incorrectly were allowed to investigate the empty food bowl before being placed back into the home cage. Rats were considered to have reached criterion after performing 8/10 correct trials for three consecutive days.

On the day following the third day of criterion performance probe testing began. Each rat ran 10 trials and was then placed in the probe start arm, 180° opposite the blocked training start arm, and the experimenter returned to the standard location. From this position, the goal arm gates were lifted and the rat was allowed to enter either the right or left arm. If a rat made the same directional turn in its choice of an arm for which it had been rewarded in the training phase, then it was scored as a 'response' strategy. If a rat made an opposite directional turn in its choice of an arm towards the same spatial location, then it was scored as a 'place' strategy.

Histology: At the completion of the experiment rats were deeply anaesthetized with Euthanyl (65 mg/kg; CDMV, St. Hyacinthe, QC, Canada) and transcardially perfused with 0.9% saline followed by 4% formalin. Brains were removed and post-fixed in 4% formalin at 4°C overnight and then cryoprotected in 30% sucrose in 1M phosphate buffer for additional 4 days at 4°C. Brains were then frozen and stored at -80°C until sectioned.

Brains were sectioned on a cryostat along the coronal plane at 40μm thickness. Sections were mounted on ColorFrost plus slides (Fisher Scientific, Ottawa, ON, Canada)

and Nissl stained with Cresyl Violet. Injector tip placements were verified using a rat brain stereotaxic atlas (Paxinos and Watson 1998). Rats that did not have cannula placements in the correct brain area were omitted from the behaviour analysis.

Statistical Analyses: To examine strategy use in rats for each the DS and NA groups, a mixed design with E2 level as a between subjects factor and drug as a within subjects factor was used. Because the dependent variable was number of rats using a type of cognitive strategy, data were analyzed using non-parametric statistics. To examine whether E2 had an effect on strategy use in general, a chi square analysis was employed to test whether there was a significant use of one strategy over another in the saline control condition. In order to test the effects of DA antagonists on strategy use, McNemar tests were conducted using a binomial for small frequencies to compare saline trials with each of the respective doses of SCH23390 and raclopride.

To determine if there were any differences in learning this task, a two-tailed t-test was conducted on the number of days to reach criterion for low and high E2 conditions in both the DS and NA groups. Finally, to determine whether there were any differences in the time to complete the task following each drug, a two-way mixed analysis of variance (ANOVA) was conducted for each the DS and NA groups. The independent variables for the two-way ANOVAs were E2 condition as a between factor and drug as a within factor.

3. Results

Histological Confirmation and Plasma Estradiol: Histological confirmation of cannula placements into the DS and NA are shown in figures 1 and 2. For the DS group, all cannula were distributed between -.26 mm and -.80 mm from bregma (fig. 1). For the NA group, cannulae were located between 1.60 mm and 1.00 mm from bregma (fig. 2). This placed the cannula injector tips within the core of the NA. One rat in the NA group was removed from the analysis because the cannulae were not located in this area.

Mean plasma levels (± SEM) of E2 for the DS group were 68.79 (± 16.00) for the high E2 rats and 18.97 (± 4.24) for the low E2 rats. For the NA group, mean plasma levels were 55.68 (± 10.09) for the high E2 rats and 20.24 (± 6.71) for the low E2 rats. These levels are within the range of E2 observed in proestrus and estrus respectively.

Task Acquisition and Completion Times: During the early stages of testing, inter-trial intervals lasted approximately 10-30 seconds but were closer to 5 seconds at criterion. This is because, as testing progressed, the rats became more adept at the task and the time to complete the task decreased. All rats achieved criterion performance in the T-Maze but eleven rats from the NA group (five low E2 and six high E2) did not complete the probe trials after infusion of the dopamine antagonists and were thus removed from the data analysis. Of these subjects, most did not approach the goal arms and, if they did, would not eat the reward. More rats were assigned to the NA group to make up for the higher attrition rate. Thus, the final number of rats included in both studies was seventy-four, with 38 rats in the DS group and 36 in the NA group. There were no significant differences between low and high E2 groups in the number of days to

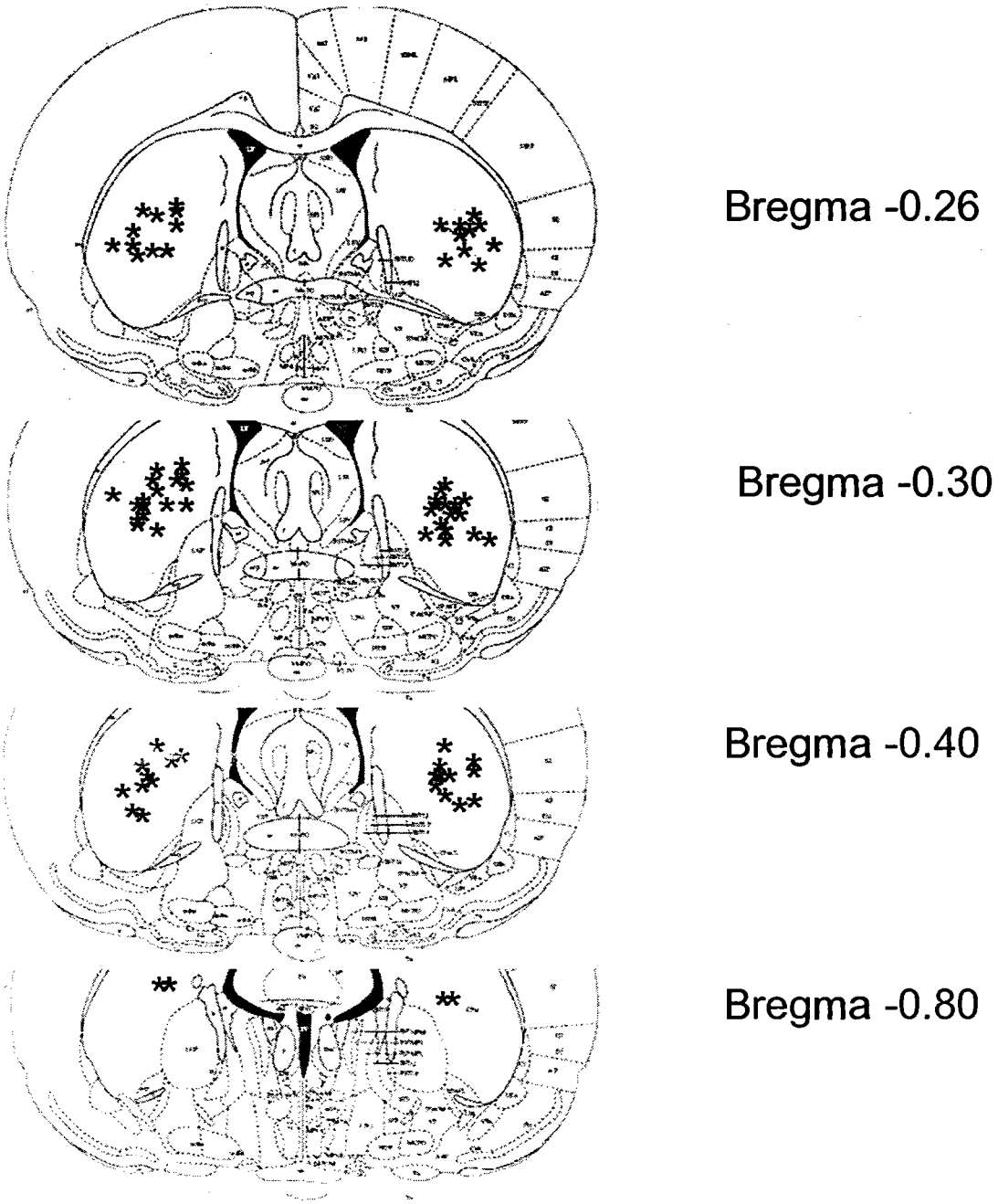


Figure 1. Locations, indicated with asterisks, of the lowest point of penetration of cannula injectors within the dorsal striatum for all animals included in the DS group. Images from Paxinos and Watson (1998).

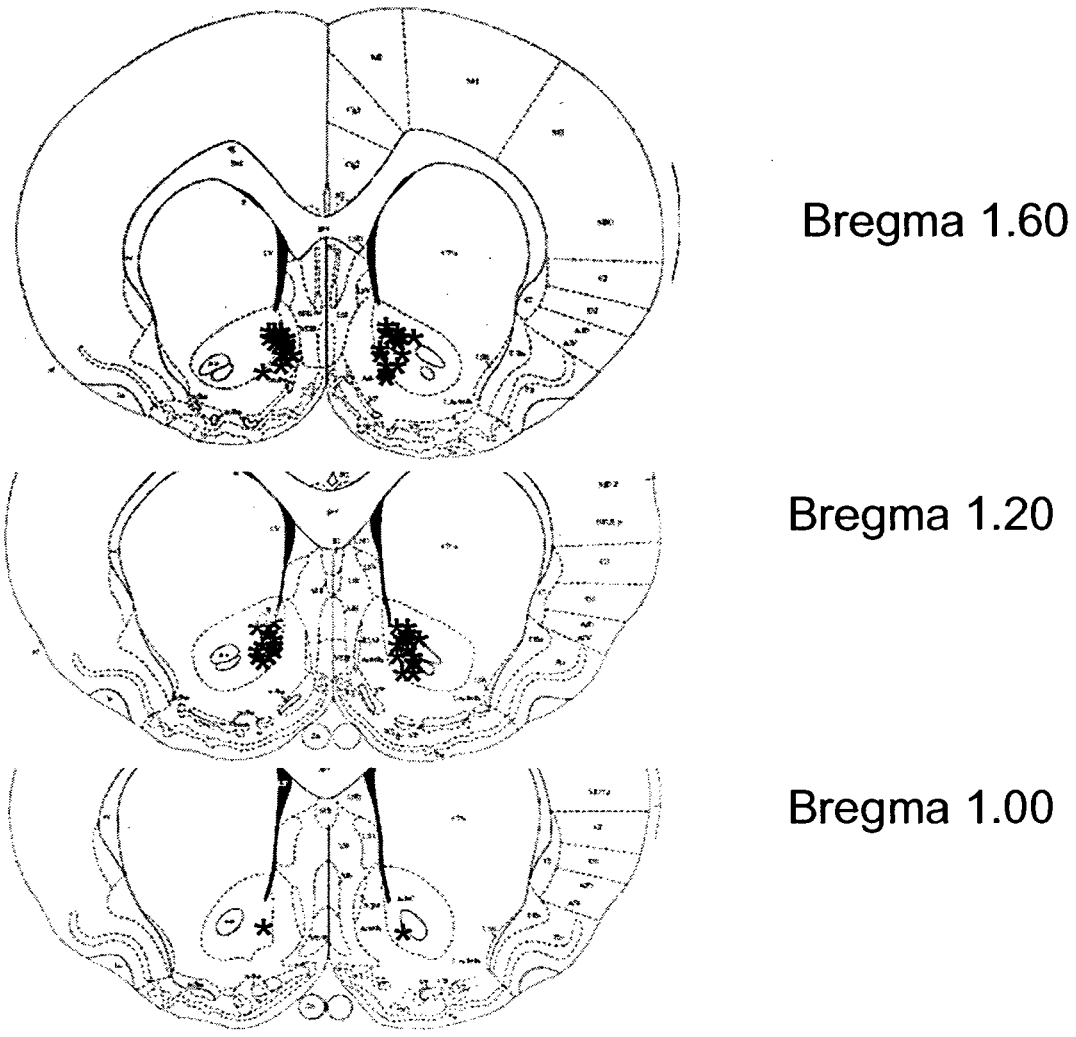


Figure 2. Locations, indicated with asterisks, of the lowest point of penetration of cannula injectors within the nucleus accumbens for all animals included in the NA group. Images from Paxinos and Watson (1998).

criterion. The mean number of days (± S.E.M) required to reach criterion in the DS group was 7.19 (± 0.49) for low E2 rats and 6.78 (± 0.63) for high E2 rats. The days to criterion for the NA groups were 7.47 (± 0.60) for the low E2 rats and 6.58 (± 0.76) for the high E2 rats.

The mean times it took rats to complete the probe trials in response to saline and each drug are provided in table 1. A two-way mixed ANOVA revealed a main effect for E2 level for the NA group ($F_{(1,11)} = 13.801$, $p = 0.003$). That is, within the NA rats, those that received low E2 took significantly longer to complete the task than those that received high E2. No other significant effects were observed.

Table 1. Effects of saline vehicle or dopamine antagonism on time to complete probe trials.

Dorsal Striatum	Saline	Low SCH23390	Moderate SCH23390	Low Raclopride	Moderate Raclopride
Low E2	5.88 (±1.33)	3.85 (±0.61)	4.12 (±0.79)	4.33 (±0.82)	5.44 (±0.75)
High E2	3.23 (±0.37)	4.31 (±0.66)	3.21 (±0.46)	3.27 (±0.42)	3.01 (±0.44)
Nucleus Accumbens					
Low E2**	20.96 (±7.51)	20.60 (±8.73)	38.43 (±14.0)	12.82 (±3.72)	19.71 (±6.39)
High E2	5.85 (±1.27)	5.10 (±0.65)	7.72 (±3.36)	5.73 (±1.97)	8.35 (±3.35)

Mean (± SEM) times in seconds to complete probe trials in response to bilateral saline control or dopamine D1 receptor (SCH23390; 0.01 & 0.1 μg/ml) and D2 receptor (raclopride; 0.5 & 2.0 μg/ml) antagonist injections into the dorsal striatum and nucleus accumbens in ovariectomized rats administered low or high estradiol (E2) replacement.** Indicates a significant main effect of Low E2 rats versus high E2 rats ($p = 0.003$).

Saline and Dopamine Receptor Antagonist Effects on Strategy: When administered saline, rats showed the same pattern of behavior that has been reported previously (Korol, Malin et al. 2004; Quinlan, Hussain et al. 2008). That is, low E2 rats were significantly more likely to use a response strategy ($\chi^2 = 7.811$, $p = 0.005$; fig. 3) while high E2 rats showed a tendency to use a place strategy, although this did not reach statistical significance.

McNemar tests revealed that low E2 rats showed a significant ($p = 0.004$) switch of strategy use in response to a moderate dose (0.1 μg/ml) of the dopamine D1R antagonist, SCH23390, when it was infused into the DS (fig. 4A). The high E2 rats in this group also showed a switch in strategy use in response to the moderate dose of SCH23390 ($p = 0.016$; fig. 5A). The low dose (0.01 μg/ml) of SCH23390 did not have any effect on strategy use. The dopamine D2R antagonist, raclopride, had no effect on strategy use when injected into the DS in either low or high E2 rats (figs. 4B & 5B respectively). There were no significant effects of either dopamine D1R or D2R antagonists on strategy use when injected into the NA in either low or high E2 rats (figs. 6 & 7).

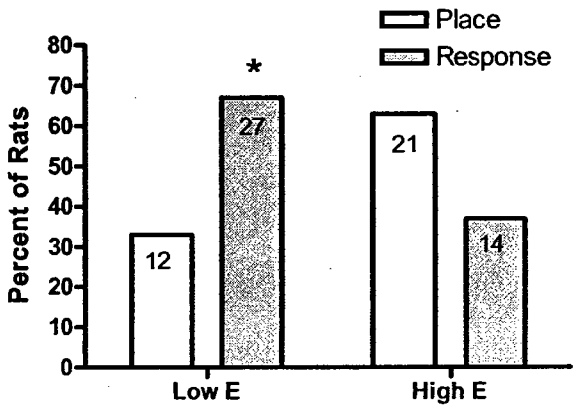


Figure 3. Strategy use in all ovariectomized rats included in this study in response to a saline vehicle. Values in the bars represent the number of rats within each group exhibiting a particular strategy. Those with low estradiol replacement showed a significantly greater probability of using a response strategy over a place strategy (* p = 0.005, Chi Square).

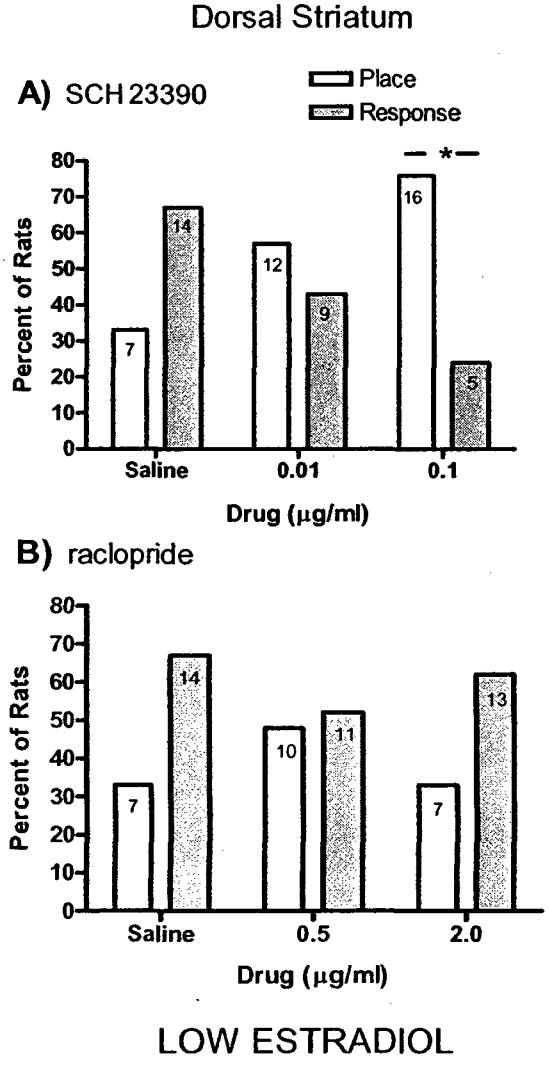


Figure 4. Strategy use in ovariectomized rats with low estradiol replacement in response to intra-dorsal striatal injections of either saline vehicle or a low and moderate dose of A) the dopamine D1 receptor antagonist, SCH 23390 or B) the dopamine D2 receptor antagonist, raclopride. Values in the bars represent the number of rats within each group exhibiting a particular strategy. Asterisk indicates a significant ($p = 0.004$, McNemar) difference in strategy use when compared to saline vehicle.

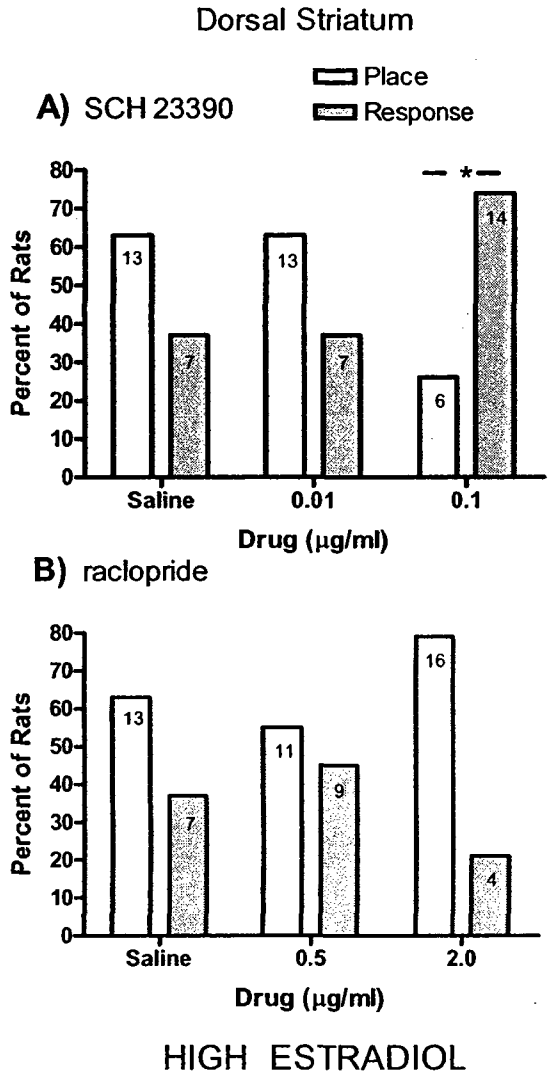


Figure 5. Strategy use in ovariectomized rats with high estradiol replacement in response to intra-dorsal striatal injections of either saline vehicle or a low and moderate dose of A) the dopamine D1 receptor antagonist, SCH 23390 or B) the dopamine D2 receptor antagonist, raclopride. Values in the bars represent the number of rats within each group exhibiting a particular strategy. * Significant ($p = 0.016$, McNemar) difference in strategy use when compared to saline vehicle.

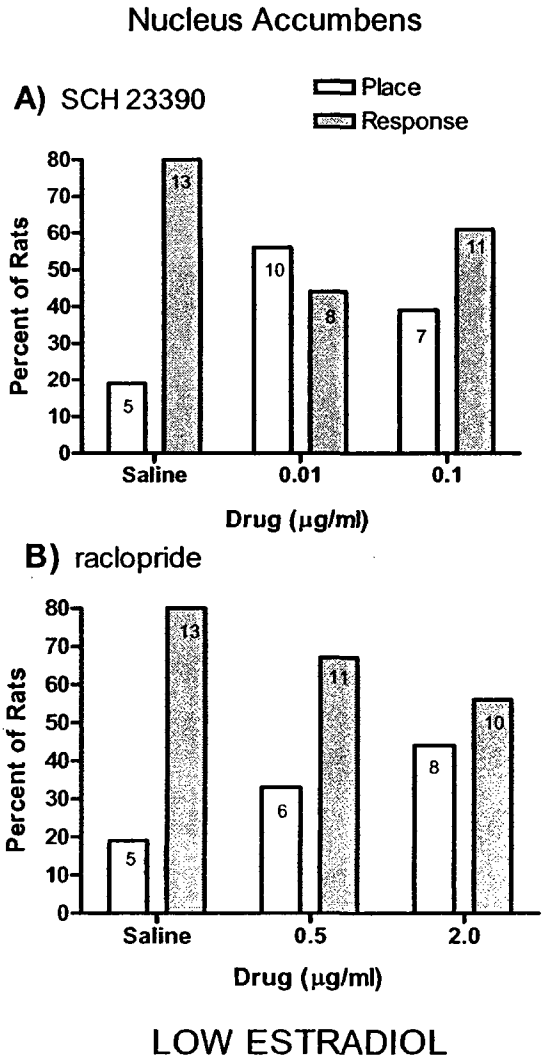


Figure 6. Strategy use in ovariectomized rats with low estradiol replacement in response to intra-nucleus accumbens injections of either saline vehicle or a low and moderate dose of A) the dopamine D1 receptor antagonist, SCH 23390 or B) the dopamine D2 receptor antagonist, raclopride. Values in the bars represent the number of rats within each group exhibiting a particular strategy. No significant effects were observed.

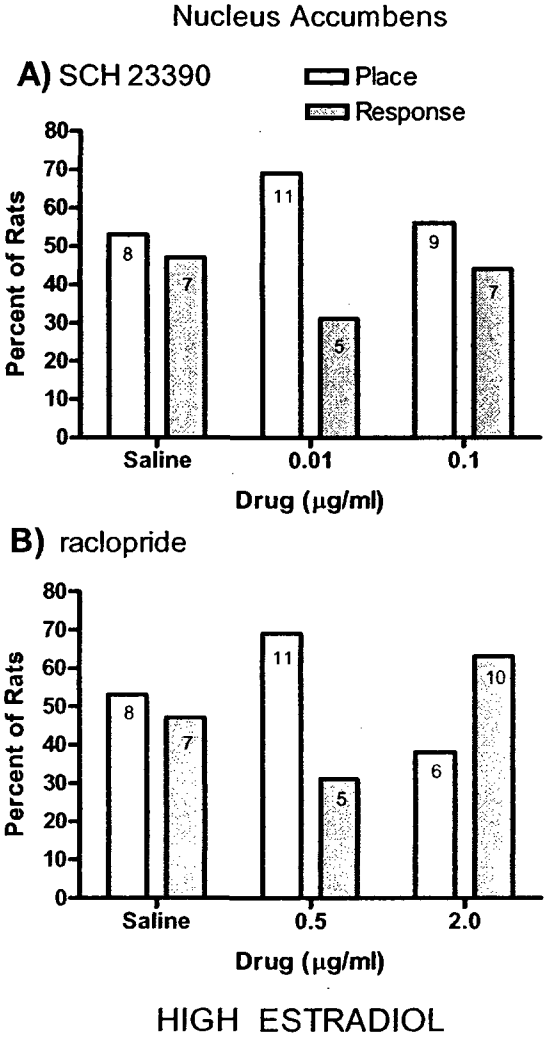


Figure 7. Strategy use in ovariectomized rats with high estradiol replacement in response to intra-nucleus accumbens injections of either saline vehicle or a low and moderate dose of A) the dopamine D1 receptor antagonist, SCH 23390 or B) the dopamine D2 receptor antagonist, raclopride. Values in the bars represent the number of rats within each group exhibiting a particular strategy. No significant effects were observed.

4. Discussion

These findings confirm that OVX rats receiving physiologically low levels of E2 are significantly more likely to use a response strategy to solve a maze for a reward while rats with physiologically high levels of E2 predominantly use a place strategy. These results are in agreement with others. Korol et al. (2004) initially showed that intact rats in proestrus with high E2 levels are more likely to use a place strategy and rats in estrus with low E2 levels predominantly use a response strategy. Previous research in this lab replicated these findings in OVX rats with chronic E2 replacement (Quinlan, Hussain et al. 2008). In the same study, systemic administration of dopamine antagonists prior to probe trials indicated that that strategy use is dependent upon the interaction between E2 and dopamine. The current results demonstrate that the interactive effects of D1R antagonists and E2 are specific to the DS and not the NA. Moreover, there was no effect of D2R antagonists on cognitive strategy when administered to either the DS or the nucleus accumbens in high or low E2 rats.

It was previously shown that a systemic injection of the D1R antagonist, SKF83566, is able to switch the strategy used by low E2 but not high E2 rats (Quinlan, Hussain et al. 2008), that is low E2 rats switch to using a place strategy while high E2 rats maintain the use of a place strategy. Likewise, the current findings show that a microinjection of the D1R antagonist, SCH23390, into the DS also causes low E2 rats to switch from using a response strategy to using a place strategy (fig. 3A). As with systemic injections, only a moderate, but not a low, dose of the D1R antagonist was

effective in this manner. These data suggest that, as hypothesized, the interaction between E2 and D1R antagonism on strategy use is centered in the DS.

Unexpectedly, when the D2R antagonist, raclopride, was infused into the DS of low E2 subjects they do not switch but maintained using a response strategy (fig. 3B). This suggests that the effects of D2R antagonism observed following systemic administration are mediated by DA transmission in a different brain region. Indeed, studies from our lab have demonstrated that the prefrontal cortex is a site of interaction between E2 and a D2R antagonist (Quinlan et al., submitted). Conversely, Daniel et al. (2006) found that E2, when compared to an OVX + vehicle group, increased sensitivity to the impairing effects of a D2R antagonist, eticlopride, on a striatal-dependent response learning task. However, in that study the dopamine antagonists were administered systemically and it cannot be concluded that D2R antagonists are working directly within the DS. Thus, perhaps the interaction between E2 and D2R antagonists is occurring at another dopaminergic innervation site such as the prefrontal cortex to affect striatal-dependent behaviours (Quinlan et al., submitted).

Another unexpected finding was that high E2 rats also switched to using a response strategy in response to a dorsal striatal infusion of the D1R antagonist, SCH23390 (fig. 4A). This was not observed when the drug was administered systemically (Quinlan, Hussain et al. 2008). There could be a number of reasons for this discrepant result. Obviously, a different D1R antagonist and dose was used in each case. There are differences between SKF83566 and SCH23390, for example SCH23390 also binds with high affinity to serotonin 5-HT_{2C} receptors (McMillan, Singer et al. 1996).

SCH23390 was used in this experiment because more information on intracerebral doses of SCH23390 was available in the literature. Another possible explanation for this discrepancy is that after systemic administration of the drug, it is acting at all sites of dopaminergic innervation which could mask the effect it may have at any single site.

As hypothesized, neither dopamine D1R nor D2R antagonists affected strategy use when infused into the NA. Although these rats acquired criterion in the same number of days as the dorsal striatal group, ~25% did not complete the probe trials after administration of the dopamine antagonists. More rats were tested in this group to make up for this shortfall so that both NA and DS groups had roughly the same number. Dopaminergic projections to the NA are critically important for motivation when solving a task for a reward (e.g. Grace, Floresco et al. 2007) and disruption of dopamine transmission in this area may have played a role in the higher attrition rate. Although the doses of dopamine antagonists employed here are lower than what is normally used to reduce motivation for a reward in reinforcement studies, low E2 rats within the NA group took significantly longer to complete the probe trials when compared to a saline control (table 1). At this point, it is unclear why high E2 rats did not behave in a similar manner. Finally, it should be noted that one limitation inherent to microinjections into the NA is that the drug solution can diffuse back along the track of the cannulae and into the DS. As can be seen in fig 5, the injectors were placed well within the core of the NA but combined dorsal striatal effects cannot be ruled out.

The established finding that high E2 will bias rats toward using a place strategy is somewhat perplexing. It has been shown that male rats, when extensively trained on a

similar task, will switch from the initial use of a place strategy to predominant use of a response strategy (Chang and Gold 2003). That is, while most males initially used a place strategy to solve the task, after only 40 training trials most males switched to using a response strategy. After 100 trials, all the male rats used a response strategy. In this and other studies, high E2 female rats continue to use a place strategy even after extensive training. Female rats might be considered over-trained insofar as once they have reached criterion they have found the reward in the same arm for approximately 60-100 trials. This begs the question, why do high E2 females, even after extensive training, not switch to using a response strategy when males and low E2 females do? While it is acknowledged that high E2 increases hippocampus-dependent place learning (Zurkovsky, Brown et al. 2007), this does not explain why these animals do not eventually use a response strategy.

It is proposed here that high E2 impairs DS-dependent tasks and does so by altering dopamine transmission in this region. Dopaminergic inputs to the DS consist of nigrostriatal projections that originate in the substantia nigra pars compacta. Dopamine seems to have a role in plasticity within the DS as it is shown to modulate both long-term potentiation and long-term depression (Walsh 1993; Centonze, Saulle et al. 2001). These neurons tend to change firing pattern not during movement per se, but specifically in response to reward-related contingencies (Romo and Schultz 1990; Schultz and Romo 1990). It has been suggested that these neurons play a role in striatal information processing determining when striatal synapses should be strengthened or weakened (Alexander 1994).

High E2 impairs response learning while improving place learning; the opposite is true for low E2 (Korol and Kolo 2002). High E2 also impairs performance on other dorsal striatal-dependent tasks such as latent inhibition (Nofrey, Ben-Shahar et al. 2008) and prepulse inhibition (Koch 1998). Thus, low E2, rather than high E2, seems to be advantageous for dorsal striatal dependent tasks. Moreover, tasks such as latent inhibition and prepulse inhibiton are not only dependent upon the DS, but are particularly sensitive to dopamine transmission in this structure. It has been shown that high E2 increases dopamine transmission in the DS (Becker 1990; Becker and Rudick 1999; Becker 2000). In E2-primed rats, a decrease in dopamine uptake rate was accompanied by an increase in dopamine clearance time in the DS when compared to OVX rats receiving vehicle (Thompson 1999). Furthermore, E2 has been shown to decrease the affinity of the dopamine transporter for dopamine (Disshon, Boja et al. 1998). It is thought that the decrease of dopamine removal from the synapse is due to alterations between the D2R and its associated second messenger system (Thompson, Bridges et al. 2001; Dluzen 2005).

Recently the subdivisions of the DS have been dissociated based upon their roles in different types of learning and memory (for review see Yin, Ostlund et al. 2008). It has been shown that the dorsolateral striatum is predominantly involved in habit formation whereas the dorsomedial striatum is responsible for goal-directed actions (Yin, Knowlton et al. 2004). Both of these subdivisions most likely play a role in the expression of the response strategy and future studies might examine the potential differential effects of the interaction of E2 and dopamine within each subregion.

In summary, E2 biases the cognitive strategy used in a maze when searching for a reward. This effect is modulated by dopamine D1R antagonism in the dorsal but not ventral striatum suggesting that cognitive strategy is in part mediated by an interaction between E2 and dopamine in this region. However, no effect of D2R antagonism was observed, suggesting that the effects of D2R antagonism observed following systemic administration are mediated by DA transmission in a different brain region.

CHAPTER 5:

MEDIAL PREFRONTAL CORTICAL DOPAMINE ANTAGONISM ALTERS *COGNITIVE STRATEGY IN FEMALE RATS*

Matthew G. Quinlan, George Radiotis, Ivonne LaChappelle, Meghen Caissie, and Wayne G. Brake

Preface:

The study in the previous chapter demonstrated that, in a task thought to be mediated by dorsal striatal DA (Packard and McGaugh 1996), DA D1 receptor antagonism acts in the dorsal striatum to affect strategy use in low E2 rats. DA D2 receptor antagonism had no effect on strategy use and there was no change in high E2 rats. To determine where in the brain DA D2 receptor antagonists are acting to cause the change in strategy use in low E2 rats seen after systemic injection, the study in this chapter investigated the effects of DA D1 and DA D2 receptor antagonism in the medial prefrontal cortex of low E2 and high E2 rats.

Abstract:

The strategy used to solve a maze for a reward is influenced by estradiol (E2) in female rats. Previous work from this lab has demonstrated that systemically administered dopamine D1 receptor (D1R) and dopamine D2 receptor (D2R) antagonists alter the strategy used by low E2 rats. Subsequent findings show that a D1R, but not a D2R, antagonist locally infused into the dorsal striatum alters the strategy use of low E2 females. Here, D1R and D2R antagonists were administered directly into the medial prefrontal cortex (mPFC) to determine if E2 and D2R antagonists are acting in this brain region to influence cognitive strategy. Forty-five Sprague-Dawley young adult female rats were implanted with bilateral cannulae into the mPFC. All rats were ovariectomized and received subcutaneous implants of 5% 17β-estradiol benzoate that produce low serum E2 levels. Half the rats received a daily injection of 10 ug/kg 17β-estradiol benzoate (high E2 condition) and half received sesame oil vehicle (low E2 condition). Upon reaching criterion performance in a T-maze task, rats received microinjections of either a D1R (SCH 23390; 0.1μg/μl and 0.01μg/μl) or a D2R (raclopride; 2μg/μl and 0.5 0μg/μl) antagonist or a vehicle control (saline) in a counterbalanced manner. The higher concentrations of both the D1 and D2 receptor antagonists reversed the strategy used by most of the low E2 rats. High E2 rats did not alter their strategy in response to either drug. These data confirm that E2 biases strategy use in female rats and show that this is partially mediated by its effects on both the D1 and D2 receptors in the mPFC.

1. Introduction

There are several potential strategies a rat can use when searching for a reward in a maze. Among these are a place strategy which utilizes extra maze cues and a response strategy that relies on habitual motor responses (Tolman, Ritchie et al. 1946). A number of studies indicate that place strategy is primarily mediated by the hippocampus (HPC) while response strategy is associated with the dorsal striatum (DS; Packard and White 1989; Packard and White 1991; DS; Packard and McGaugh 1996; White and McDonald 2002). For example, temporary inactivation of the HPC led rats to display response learning in a maze while inactivation of the DS biased rats towards use of place learning (Packard and McGaugh 1996). In addition, inactivation of the DS negatively affects the acquisition of a response task but only in a cue-deficient environment (Chang and Gold 2004). These and other studies suggest that when one type of learning and memory system is made unavailable the other will be engaged.

It has been demonstrated that 17β-estradiol (E2) may render one type of memory system ineffective or unavailable when solving a maze task for a reward. In a plus maze, ovariectomized (OVX) rats learn a response task more readily than rats receiving E2 injections (Korol and Kolo 2002). Conversely, OVX rats receiving E2 injections learn a place task more quickly than rats receiving vehicle injections. In cycling rats, those in proestrus (high E2) tend to use a place strategy to solve a maze while estrus (low E2) rats are more likely to use a response strategy (Korol, Malin et al. 2004). Direct infusion of E2 into the HPC of OVX rats has been shown to enhance place learning while having no effect on response learning (Zurkovsky, Brown et al. 2007). On the other hand, E2

infusion into the DS impairs response learning without affecting place learning (Zurkovsky, Brown et al. 2007).

E2 is not the only influence which may bias the use of a particular cognitive strategy when solving a maze task. Previous work from this lab shows that systemic administration of both dopamine D1 receptor (D1R) and dopamine D2 receptor (D2R) antagonists alter the cognitive strategy used (Quinlan, Hussain et al. 2008). OVX rats administered low levels of E2 switch from a predominant use of response strategy to predominant use of place strategy while high E2 rats maintain their use of a place strategy. A subsequent study found that intracranial infusions of D1R, but not D2R, antagonists into the DS of low E2 rats also resulted in a switch from use of a response strategy to use of a place strategy (Quinlan et al., submitted). D1R and D2R antagonism in the nucleus accumbens (NA) had no effect on strategy use in either low E2 or high E2 rats. Thus, there must be another region where D2R antagonism affects strategy use in low E2 females. Here, in order to help identify the brain region where D2R antagonism is affecting cognitive strategy in low E2 rats, D1R and D2R antagonists were infused into the medial prefrontal cortex (mPFC) of OVX female rats receiving either low E2 or high E2.

The mPFC is a brain region where E2 may affect dopamine neurotransmission and contribute to the biased use of a particular cognitive strategy. While no studies have investigated whether the mPFC is directly involved in the expression of cognitive strategy, this structure is an important component of fronto-striatal loops which are associated with the basal ganglia and motor response learning (Alexander, DeLong et al.

1986; Winstanley, Chudasama et al. 2003). In addition, there are connections between the mPFC and the HPC (Jay and Witter 1991). Thus, the mPFC is in a position to influence both the DS and HPC and indirectly affect the use of cognitive strategy. Few studies have directly examined the effects of E2 on prefrontal cortical dopamine levels. Chronic E2 has been shown to reduce dopamine in frontal cortical homogenates of OVX rats (Dupont, Di Paolo et al. 1981; Luine, Richards et al. 1998) and basal medial prefrontal cortical dopamine release is reduced during proestrus when estrogen levels are highest (Dazzi, Seu et al. 2007). Considering that the mPFC is heavily innervated by dopamine afferents from the ventral tegmental area (VTA), further examination of the effects of E2 on dopamine transmission in this area is merited. Thus, the present study investigated the role of D1R and D2R antagonism in the mPFC on strategy use in OVX rats receiving chronic high or low E2.

These effects were investigated with a hormone administration regimen that was intended to mimic the levels of E2 during the estrous cycle. That is, the low E2 group received constant levels of E2 similar to that seen during the estrus phase and the high E2 group received constant low E2 plus daily injections resulting in peaks of E2 similar to that seen during the proestrus phase.

2. Materials and Methods

Subjects, Surgery and Drug Administration: This study employed forty five female Sprague-Dawley rats (Charles River, St. Constant, QC, Canada), approximately three months of age weighing between 225-250 grams. Rats were housed in pairs in

polyurethane shoebox cages and maintained on a reverse 12h:12h light/dark cycle with lights off from 0900-2100h. Standard lab chow and water were available *ad libitum* until training began.

All rats were anaesthetized using Halothane gas (4% for induction; 2% for maintenance) and bilaterally ovariectomized using a standard aseptic procedure and implanted with a Silastic tube (1cm long; i.d. 1.47 mm; o.d. 1.96 mm) containing 5% 17β-estradiol (Sigma Chemical Co., St Louis, MO) in cholesterol (Sigma). Post-surgical care included administration of the antibiotic Baytril (0.03 ml/rat, SC; CDMV, St. Hyacinthe, QC, Canada), the analgesic banamine (0.03 ml/rat, SC; CDMV), and 0.9% saline (3ml/rat, SC). Rats were allowed to recover in their home cages for several days prior to implantation of cannulae.

Rats were again anaesthetized using Halothane gas and received stereotaxic bilateral implantations of 21g stainless steel tubing guide cannulae (Plastics One, Roanoke, VA). Stereotaxic co-ordinates from bregma for the mPFC were AP +3.2, ML ± 1.8 at 10°, DV -3.0 (Paxinos and Watson 1998). Cannulae were blocked with 26g obdurators (Plastics One) which extended 1mm below the tip of the guide cannula. All rat protocols were in accordance with guidelines established by the Canadian Council on Animal Care and approved by the Concordia University Animal Research Ethics Committee.

For the high E2 group, in addition to the subcutaneous implants, daily subcutaneous injections were given of 17β-E2 (10μg/kg) dissolved in sesame oil (Sigma) designed to achieve levels seen during the proestrus phase of the estrous cycle (75-90

pg/ml). During the same period all rats in the low E2 group received daily subcutaneous injections of sesame oil as a control (1ml/kg). All injections began two days before habituation training and occurred between 1200-1400h each day.

Approximately 30 days following the implantation of the Silastic tubes, blood was collected from the tail vein 22 hrs following the previous E2 or oil injection. Blood samples were immediately centrifuged and plasma was collected and stored at -20°C until assayed. E2 was measured using a commercially available ELISA kit (Immuno-Biological Laboratories Inc., Minneapolis, MI). The assay antibodies have 100% cross-reactivity with E2 and 0.2% and 0.05% cross-reactivity with estrone and estriol respectively. The reported inter-assay variation is 7-9%.

The procedure for administration of dopamine antagonists were the same as described elsewhere (Quinlan et al., submitted). Briefly, rats received bilateral intracerebral injections of DA antagonists on separate days at a rate of 0.5µl/min/side using injectors which extended 1mm below the tip of the cannulae. Injections lasted for one minute after which the injectors were left in place for an additional minute to allow for drug diffusion. The D1R antagonist SCH 23390 (Sigma) was administered in a moderate concentrations of 0.1µg/µl and a low concentration of 0.01µg/µl. Raclopride (Sigma), a selective D2R antagonist, was administered in a moderate concentration of 2.0µg/µl and a low concentration of 0.5µg/µl. These concentrations were selected because they are in the moderate and low ranges for modifying behaviors in attention and learning tasks for both SCH 23390 and raclopride (Fernandez-Ruiz, Hernandez et al. 1991; Callahan, De La Garza et al. 1997; Seamans, Floresco et al. 1998; Zavitsanou,

Cranney et al. 1999). Both drugs were dissolved in 0.9% saline at room temperature and stored at -20 °C until used. Rats were allowed a washout period for a minimum of 24 hrs between each drug probe trial.

Modified Plus Maze: Training and apparatus were identical to that described elsewhere (Quinlan et al., submitted). All training was carried out in a polyurethane plus maze placed on a table one meter above the floor. Throughout the training trials the probe start arm was blocked off from the central chamber creating a T-shaped maze (see Quinlan, Hussain et al. 2008). At the commencement of the probe trial, the probe arm was unblocked and the original start arm was blocked creating an alternative T-shaped maze exactly 180° in orientation. Each start arm contained a start-box 30cm in length which blocked access to the central chamber by a black polyurethane guillotine gate halfway down the arm. Each goal arm contained a white ceramic bowl in which a food reward (Kellogg's Froot Loops®) could be placed. Froot Loop crumbs were placed underneath both goal arms of the maze during all trials to prevent any confounds due to odor cues. All testing took place with overhead red lights with an additional 15 W light for illumination. The room was lit only moderately to avoid evoking anxiety in the rats while completing the maze.

Five days after the final surgery all rats were placed on food restriction and maintained at a body weight of 90% of free-feeding weight. Training was performed at the beginning of the dark phase of the light/dark cycle commencing at 0900h. Rats were

habituated to the maze during three, once-daily 15-min sessions with Froot Loops scattered throughout the apparatus.

Following habituation, each rat was assigned to receive a food reward, half of a Froot Loop, in either the right or left goal arm. The baited goal arm was counterbalanced across rats such that half the rats in each group received the reward in the right arm and half in the left arm. For each particular rat the Froot Loop was always in the same goal arm. Rats were released from the start arm and allowed to enter either the left or right goal arm, once they entered the arm, a door was closed behind them. Training consisted of 10 daily trials per rat beginning in the start box of the training arm. Rats were considered to have reached criterion after performing 8/10 correct trials for three days in a row.

After reaching criterion, each rat was placed in the probe start arm (180° opposite the blocked training start arm) and was released and allowed to enter either the right or left goal arm. If a rat made the same directional turn in its choice of an arm for which it had been rewarded in the training phase, then it was scored as a 'response' strategy. If a rat made an opposite directional turn in its choice of an arm towards the same spatial location of its reward during the training phase, then it was scored as a 'place' strategy.

Each rat was injected with a particular type and dose of antagonist or saline in a counterbalanced schedule using a Balanced Latin Square design to rule out sequence and order effects within each group. All drug trials began on the testing day subsequent to achievement of criterion performance. Each rat was injected with a drug, placed in its home cage for five minutes, and trained again for 10 trials. After the 10 trials it was

given the probe trial where the start arm was oriented 180° from the training arm. No subsequent drug injections were given for at least 24 hrs. At the completion of the experiment brains were sectioned and stained to histologically confirm cannula placements.

Statistical Analyses: Data on strategy use were analyzed using non-parametric statistics because the dependent variable was percent of rats using a type of cognitive strategy. To examine whether E2 had an effect on strategy use in general, a chi square analysis was employed to test whether there was a significant use of one strategy over another in the saline control condition. In order to test the effects of DA antagonists, McNemar tests were conducted using a binomial for small frequencies to compare saline trials with each of the concentrations of SCH 23390 and raclopride.

To determine if there were any differences in learning this task, a two-tailed t-test was conducted on the number of days to reach criterion for low and high E2 groups for each brain region. Finally, to determine whether there were any differences in the time to complete the task following each drug, a two-way mixed analysis of variance (ANOVA) was conducted. The independent variables for the two-way ANOVAs were E2 group as a between factor and drug as a within factor.

3. Results

Histological Confirmation and Plasma Estradiol Analysis: Histological confirmation of cannula placements into the mPFC are shown in figure 1. All cannula were distributed between +3.7 mm and +2.7 mm from bregma. This placed the cannula injector tips within the prelimbic and infralimbic areas of the medial prefrontal cortex. No rats were discarded from the analysis due to cannula placements. Mean plasma hormone levels (± SEM) for the high E2 rats were 97.42 (± 29.40) and for the low E2 rats they were 18.14 (± 4.58). These are the within the range of plasma E2 observed during proestrus and estrus respectively.

Behavioral Results:

There were no significant differences between low and high E2 groups in the number of trials to criterion. The mean number of trials (± S.E.M) required to reach criterion was 6.68 (± 0.69) for low E2 rats and 7.05 (± 0.47) for high E2 rats. The mean times it took rats to complete the probe trials in response to saline and each drug are provided in table 1. No significant differences were observed.

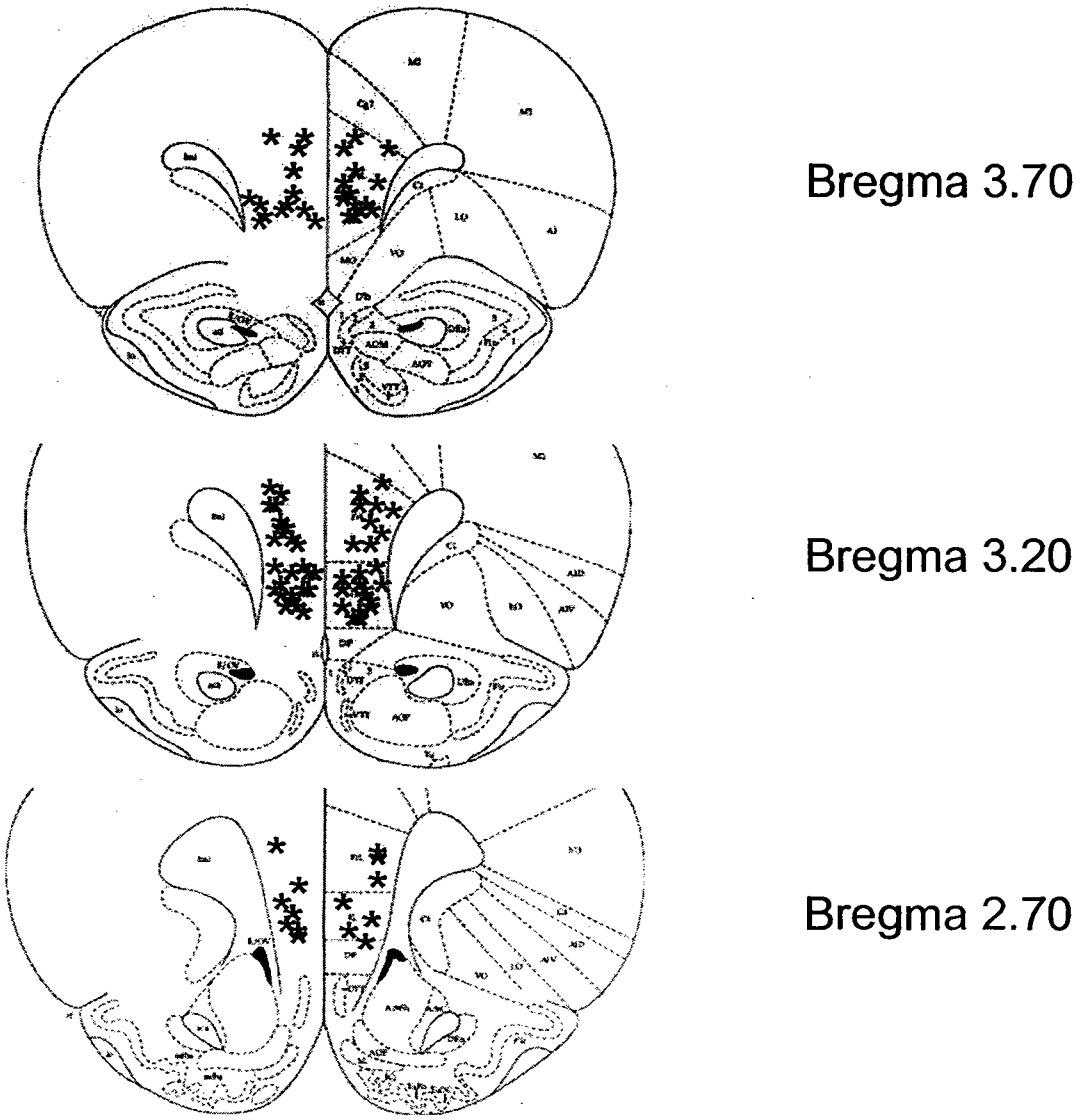


Fig. 1. Locations, indicated with asterisks, of the lowest point of penetration of cannula injectors within the medial prefrontal cortex for all animals included in the study. Images from Paxinos and Watson (1998).

Table 1. Effects of saline vehicle or dopamine antagonism on time to complete probe trials.

Prefrontal Cortex	Saline	Low SCH23390	Moderate SCH23390	Low Raclopride	Moderate Raclopride
Low E2	4.48 (±0.68)	5.00 (±0.88)	6.45 (±1.50)	5.49 (±1.14)	7.03 (±1.57)
High E2	8.43 (±3.19)	18.11 (±13.1)	8.83 (±3.73)	5.67 (±2.57)	7.66 (±2.27)

Mean (± SEM) times in seconds to complete probe trials in response to bilateral saline control or dopamine D1 receptor (SCH23390; 0.01 & 0.1 μg/ml) and D2 receptor (raclopride; 0.5 & 2.0 μg/ml) antagonist injections into the medial prefrontal cortex in ovariectomized rats administered low or high estradiol (E2) replacement. There were no significant effects.

When administered saline, rats showed the same pattern of behaviour that has been shown previously (Korol, Malin et al. 2004; Quinlan, Hussain et al. 2008). That is, low E2 rats predominantly used a response strategy and high E2 rats predominantly used a place strategy (fig. 2). Although this did not reach statistical significance there was a strong trend for both the low ($\chi^2 = 2.91$, $p = 0.08$) and high ($\chi^2 = 3.20$, $p = 0.07$) E2 rats.

McNemar tests revealed that low E2 rats showed a significant ($p = 0.002$) switch in strategy use in response to a moderate dose (0.1 μg/μl) of the dopamine D1R antagonist, SCH23390 (fig. 3). Furthermore, low E2 rats administered a moderate dose of the D2R antagonist, raclopride also showed a significant switch in strategy ($p = 0.016$; fig. 3). There was no effect of either SCH 23390 or raclopride in the high E2 rats (fig. 4).

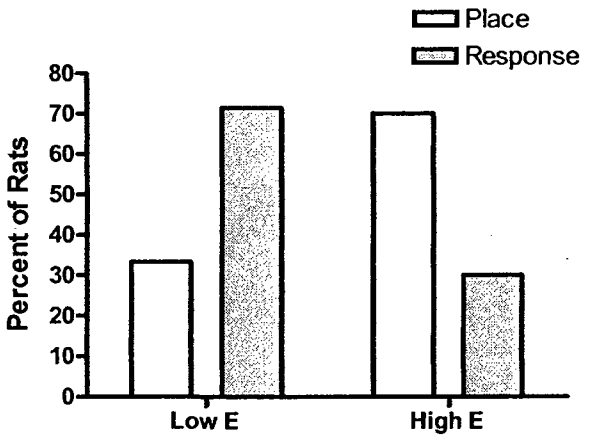


Figure 2. Strategy use in ovariectomized rats in response to an intra-prefrontal cortical saline (control) injection. There was a higher tendency for low estradiol rats to use a response strategy and a greater tendency for high estradiol rats to use a place strategy.

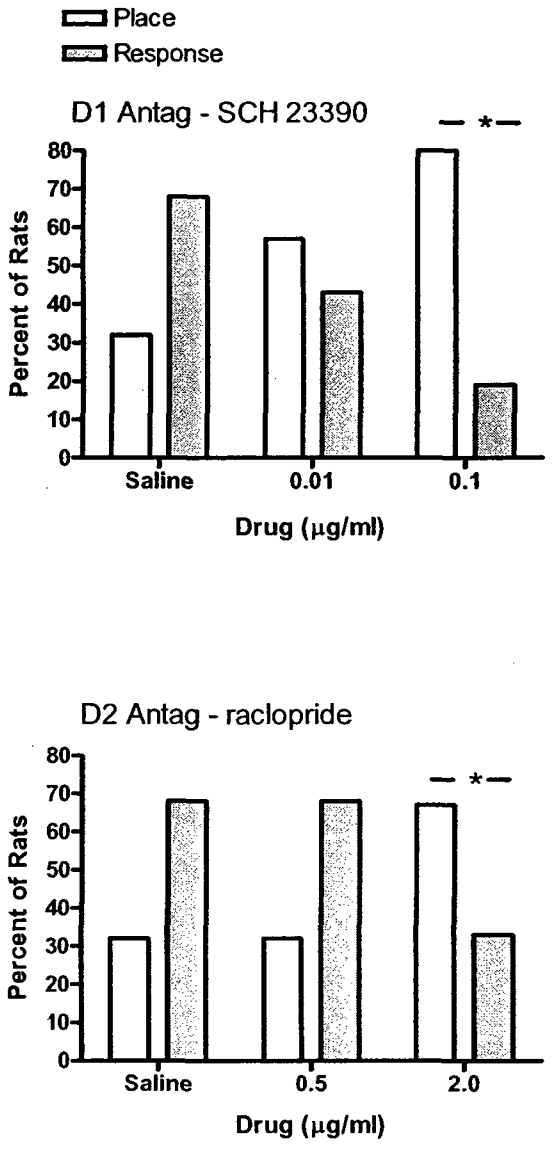


Figure 3. Strategy use in ovariectomized rats with low estradiol replacement in response to intra-prefrontal cortical injections of either saline vehicle or a low and moderate dose of the dopamine D1 receptor antagonist, SCH 23390 or the dopamine D2 receptor antagonist, raclopride. Asterisk indicates a significant ($p < 0.01$, McNemar) difference in strategy use when compared to saline vehicle.

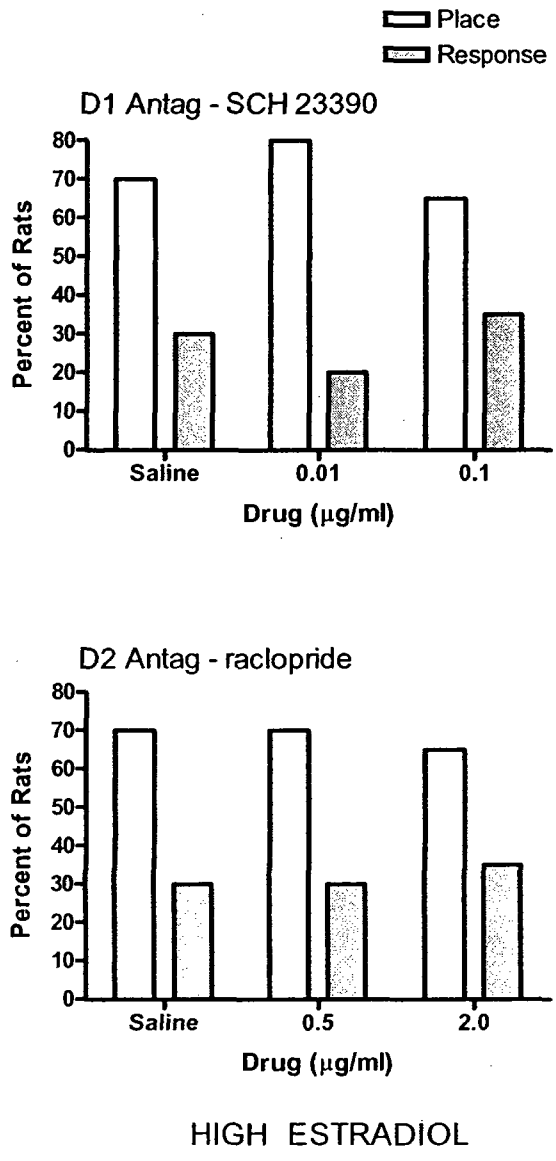


Fig. 4. Strategy use in ovariectomized rats with high estradiol replacement in response to intra-prefrontal cortical injections of either saline vehicle or a low and moderate dose of the dopamine D1 receptor antagonist, SCH 23390 or the dopamine D2 receptor antagonist, raclopride. No significant effects were observed.

4. Discussion

These data confirm previous findings that cognitive strategy use in the female rat is influenced by E2 levels. That is, high E2 rats tend to use a place strategy while low E2 rats predominantly use a response strategy. Although the findings did not reach statistical significance here, there is a strong trend which is congruent with previous reports (Korol and Kolo 2002; Korol, Malin et al. 2004; Quinlan, Hussain et al. 2008) (Quinlan et al., submitted). In addition, a direct infusion of moderate doses of both D1R and D2R antagonists into the mPFC induced a switch in low E2 rats from predominant use of a response strategy to use of a place strategy. High E2 rats were not affected by prefrontal cortical dopamine antagonism. It has been shown that systemic administration of both D1R and D2R antagonists will affect strategy use in low, but not high, E2 rats (Quinlan, Hussain et al. 2008). In addition, administration of D1R, but not D2R, antagonists directly into the DS of low E2 subjects also alters strategy use (Quinlan et al, submitted). These data demonstrate that the previously shown systemic effects of D2R antagonism on strategy use are located, at least in part, in the mPFC. Previously, investigations of place and response learning have only evaluated the roles of the HPC and the DS. These findings are the first to demonstrate that the mPFC also mediates cognitive strategy use.

A number of studies provide evidence suggesting that the DS and the HPC are, respectively, the main neural substrates underlying response and place learning. For example, when the HPC is inactivated place learning is disrupted in both a plus maze (Packard and McGaugh 1996) and a Morris water maze (McDonald and White 1994; Compton 2004). Similar inactivation of the DS results in an impairment of response

learning. Conversely, post-training intracranial injections of dopamine agonists into the HPC facilitate performance in a win-shift task but have no effect on a win-stay task (Packard and White 1991). Intracranial infusions into the DS improved performance in a win-stay, but not a win-shift, task. Measurements of neurotransmitter release in the HPC and DS support these behavioral data. Acetylcholine release is enhanced in the HPC during early training, when a place strategy is used, but is higher in the DS during later training, when a response strategy is predominantly used (Chang and Gold 2003). These findings are the first to establish a role for the mPFC in cognitive strategy use, but only in low E2 rats. At this time it is unknown why the antagonism of D1R and D2R in the mPFC does not affect strategy use in rats with high levels of E2. It is possible that the well-documented enhancement of hippocampal function by E2 (for a review see; McEwen, Akama et al. 2001) may play a role in the continued use of a place strategy by high E2 rats.

It is unclear whether this influence on strategy use in low E2 rats is a direct effect of medial prefrontal cortical dopamine or if it is a result of indirect modulation of dorsal striatal or hippocampal function by the mPFC. There are at least three possible means by which the mPFC may affect cognitive strategy in this task; through connections with the HPC, through direct projections to the DS, and/or through indirect pathways to the DS via midbrain structures, primarily the VTA. While a significant portion of the pathway between the HPC and the mPFC consists of hippocampal efferents (Jay and Witter 1991; Conde, Bicknell et al. 1995; Carr and Sesack 1996) there is also evidence of reciprocal communication consisting of mPFC efferents to the HPC (Goldman-Rakic, Selemon et al. 1984; Wall and Messier 2001) and its related cortices (Sesack, Deutch et al. 1989;

Takagishi and Chiba 1991). Considering the central role of the HPC in the use of a place strategy in this task, these reciprocal fronto-hippocampal connections could exert a considerable influence on the tendency to use a particular cognitive strategy. It is also possible that direct projections from the mPFC to the DS (Alexander, DeLong et al. 1986; Sesack, Deutch et al. 1989; Takagishi and Chiba 1991; Berendse, Galis-de Graaf et al. 1992) may affect the use of response strategy. Electrical stimulation of the mPFC has been shown to enhance dopamine release in this brain region (Taber and Fibiger 1993) and interference with dorsal striatal dopamine transmission has been shown to alter response learning (Daniel, Sulzer et al. 2006) and cognitive strategy use (Quinlan et al., submitted). A third possibility is that the medial prefrontal cortical influence on the DS could occur through a more indirect route, possibly via reciprocal connections from the mPFC through the VTA (Swanson 1982; Oades and Halliday 1987; Carr and Sesack 2000) or the substantia nigra (SN; Ferreira, Del-Fava et al. 2008).

It is also possible that the estrogenic modulation of dopamine transmission in the DS (Becker 2000) or mPFC (Dazzi, Seu et al. 2007) alters the ability of the DS to support the use of a response strategy. White and McDonald (2002) suggest that parallel memory systems supported by the DS and HPC may function in a competitive manner. One factor that has been shown to play a role in this competition is E2 (Mizumori, Yeshenko et al. 2004). The fact that high and low levels of E2 differentially bias strategy use in female rats is becoming an established phenomenon (Korol and Kolo 2002; Daniel and Lee 2004; Korol, Malin et al. 2004; Davis, Jacobson et al. 2005; Quinlan, Hussain et al. 2008). It has also been suggested that it is the effects of E2 on dopamine function in the DS which play a role in mediating the respective contributions of the DS and HPC on

learning strategy (Mizumori, Yeshenko et al. 2004). High levels of E2 have been shown to enhance dopaminergic activity in the DS in vitro (Becker and Ramirez 1981), in OVX females (Castner, Xiao et al. 1993), and in cycling females (Xiao and Becker 1994). This alteration of dopaminergic activity in the DS by E2 has been shown to affect DS-mediated behaviors as well (Chiodo, Caggiula et al. 1981; Becker, Robinson et al. 1982; Chiodo and Caggiula 1983; Becker and Cha 1989). E2 has also been shown to affect dopamine activity in the mPFC. Post-mortem studies have shown that E2 reduces dopamine in the mPFC of OVX rats (Dupont, Di Paolo et al. 1981; Luine, Richards et al. 1998). In addition, basal medial prefrontal cortical dopamine release, measured using microdialysis, is reduced during proestrus as compared to estrus (Dazzi, Seu et al. 2007).

These and previous findings suggest that, while the tendency to use a particular strategy is primarily mediated by the DS and HPC, the mPFC also plays a role. It appears that the mPFC is important in the ability of an animal to effectively regulate the switch between two competing strategies. This competition has been shown to be directly mediated by dopamine levels in the DS and the enhancing effects of E2 in the HPC but little is known concerning the influence of E2 on dopamine neurotransmission and its behavioral consequences concerning the use of a cognitive strategy. Further research is required to ascertain whether the influence of these factors in the mPFC exerts a direct or indirect effect on the use of strategy when solving a maze for a reward.

CHAPTER 6:

INTRA-STRIATAL ESTRADIOL RAPIDLY INCREASES DOPAMINE RELEASE IN FEMALE RATS

Matthew G. Quinlan, Marie-Pierre Cossette, Wayne G. Brake

Preface:

Rats with low levels of E2 predominantly use a response strategy when solving a maze task (Korol and Kolo 2002; Korol, Malin et al. 2004). DA in the dorsal striatum supports response learning in a maze (Packard and McGaugh 1996; Daniel, Sulzer et al. 2006). Studies from the previous chapters have demonstrated that interference with DA function in the dorsal striatum through receptor antagonism alters cognitive strategy use in female rats with low E2. The studies in this chapter will focus on the manner in which E2 alters DA transmission in the dorsal striatum. A number of *in vivo* studies have demonstrated that chronic high levels of systemically administered E2 enhance baseline and amphetamine-induced DA levels in the dorsal striatum (e.g. Becker 1990; e.g. Xiao and Becker 1994). The rapid effects of E2 have been evaluated in cultured striatal neurons (Mermelstein, Becker et al. 1996). The present study is the first to investigate the *in vivo* rapid effects of locally infused E2 in the dorsal striatum of anaesthetized rats during baseline and in response to systemic injections of amphetamine.

Abstract:

Estradiol (E2) has been shown to modulate the function of different neurotransmitter systems in several areas of the brain. A number of studies demonstrate that the estrogenic modulation of dopamine (DA) results in alterations of behavior. The majority of investigations into the effects of E2 on DA have focused on dopaminergic activity within the dorsal striatum (DS) and have utilized ovariectomized (OVX) females receiving chronic systemic administration of high levels of E2. Hence, the resulting effects of E2 on DA neurotransmission can only be attributed to the genomic activity of classic intracellular estrogen receptors. The rapid effects of E2 have mainly been investigated in cultured striatal neurons. To determine if locally infused E2 can rapidly affect DA neurotransmission *in vivo,* single probe microdialysis was used to measure extracellular DA levels in the DS of anaesthetized OVX female rats with chronic levels of low E2. In addition, DA levels were measured subsequent to systemic injections of the indirect DA agonist amphetamine administered one hour after E2 or its vehicle, cyclodextrin. Locally infused E2, but not cyclodextrin, resulted in a rapid and transient increase of DA levels in the DS. DA levels returned to baseline before the injection of amphetamine and had no potentiating effect on the amphetamine-induced increase in DA. These results are the first to indicate that locally infused E2 rapidly enhances dorsal striatal DA neurotransmission *in vivo* although this does not affect amphetamine-induced DA increases.

1. Introduction

A growing number of studies have provided evidence that estradiol (E2) exerts a strong influence on synaptic density and plasticity (e.g. Woolley and McEwen 1992), neurotransmission (e.g. Gabor, Nagle et al. 2003), and learning and memory (e.g. Daniel, Fader et al. 1997). E2 has been shown to modulate several different neurotransmitter systems, including dopaminergic neurons in the nigrostriatal pathway (for a review see: Kuppers, Ivanova et al. 2000). Initial reports suggest that the estrogenic modulation of dopamine (DA) might be accomplished through classic estrogen receptors (ERs) as most behavioral changes in ovariectomized (OVX) females require E2 to be administered at least 72 hours prior to testing (Dohanich, Fader et al. 1994; Sandstrom and Williams 2001).

Systemic administration of high levels of E2, such as those observed during proestrus, enhance baseline levels of extracellular DA in the dorsal striatum (DS), the primary target region of the nigrostriatal pathway (Xiao and Becker 1994). In OVX female rats, basal levels of DA in the DS are diminished but can be restored by the administration of systemic E2 (Ohtani, Nomoto et al. 2001). Similarly, agonist-induced release of dorsal striatal DA is augmented by systemically administered E2 (Becker, Beer et al. 1984; Becker and Cha 1989; Becker 1990). In addition, high levels of systemic E2 administration increase DA turnover (Russo, Festa et al. 2003) and uptake sites (Morissette, Biron et al. 1990). Studies such as these demonstrate the impact of E2 on nigrostriatal DA function. However, the relative contribution of E2 via genomic activity mediated by classic nuclear ERs versus rapid, non-genomic effects acting locally through putative membrane-bound ERs or other G-protein-coupled receptors is still unknown. It

is known that the genomic actions of E2 through nuclear steroid receptors take one to two hours to produce an effect whereas more rapid E2 effects occur through non-genomic, presumably membrane receptor, mechanisms.

Recent findings support a role for a non-genomic influence of E2 on dopaminergic function and behavior. For example, the application of E2 to cultured striatal neurons results in a reduction of calcium currents within seconds (Mermelstein, Becker et al. 1996) and rapidly amplifies amphetamine-induced DA, but not norepinephrine, release (Becker and Ramirez 1981; Becker and Beer 1986). In the DS of OVX females, increases in DA turnover develop within 30 minutes of systemic E2 administration (Di Paolo, Rouillard et al. 1985) as does the enhancement of amphetamine-induced DA release (Becker 1990). These rapid effects can induce changes in certain types of behaviour as well. If administered to OVX females within four hours of testing, E2 improves performance in visual and place learning (Luine, Jacome et al. 2003), spatial learning (Frye, Duffy et al. 2007), and object recognition (Walf, Rhodes et al. 2006).

While it is becoming established that E2 may alter DA function within the nigrostriatal system through both long-term and rapid mechanisms, most studies have utilized in vivo, systemic injections of E2 or in vitro application of E2 to cultured neurons. At this point, it is unclear whether the rapid, and presumably non-genomic, effects of E2 on DA release in the DS in vivo occur through local dorsal striatal mechanisms, via another mechanism in DA cell bodies in the substantia nigra, or elsewhere in the brain. Thus, the present study utilized *in vivo* microdialysis to

investigate the acute effects of locally applied E2 on baseline levels of DA release in the DS of anaesthetized OVX rats receiving chronic levels of physiologically low E2. In addition, an amphetamine challenge was systemically administered one hour after the local E2 infusion in order to evaluate the ability of the putative rapid estrogenic effects to influence stimulant-induced dopamine release. In Group 1, water soluble E2 encapsulated in cyclodextrin was administered into the DS one hour prior to systemic amphetamine to determine if locally applied E2 would affect amphetamine-induced DA release in this area. The effect of the vehicle, cyclodextrin, on DA levels when locally administered into the DS one hour prior to amphetamine was investigated in Group 2. Finally, in Group 3, both cyclodextrin and E2 were independently locally administered without amphetamine to examine their effects on baseline DA levels in the DS. Based upon previous findings, it was hypothesized that local infusions of E2 would rapidly increase extracellular DA levels in the DS. It was also hypothesized that the effects of locally applied E2 would potentiate amphetamine-induced DA release in the DS.

2. Materials and Methods

Subjects. This experiment included 12 young adult, female, Sprague-Dawley rats (Charles River, St. Constant, Quebec) aged four months and weighing approximately 300-350 grams. Before the experiment began, all rats were allowed to habituate to the animal facility and were handled daily from time of arrival until completion of the experiment. All rats were pair-housed in polyurethane shoebox cages, maintained on a reverse 12h:12h light/dark cycle with lights off from 0900-2100h, and were allowed standard lab chow and water *ad libitum.* All animal handing and testing procedures were

conducted in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Concordia University Animal Research Ethics Committee.

Surgical Procedures, Hormone Administration, and Drugs. Approximately one week after arrival, all rats were anaesthetized using Halothane gas (4% for induction; 2% for maintenance), bilaterally ovariectomized using a standard aseptic procedure through a dorsal incision, and implanted with a Silastic tube (1cm long; i.d. 1.47 mm; o.d. 1.96 mm) containing 5% 17β-E2 (Sigma Chemical Co., St Louis, MO) in cholesterol (Sigma). This has been reported to produce a serum concentration of approximately 20-25 pg/ml which is consistent with naturally circulating low levels of E2 such as those seen during the estrus phase of the rat estrous cycle (Hurn and Macrae 2000; Mannino, South et al. 2005). Previous studies from this lab have shown this implant to produce serum E2 levels of 18-32 pg/ml (Quinlan et al., submitted; Quinlan et al., submitted; Quinlan, Hussain et al. 2008). This experiment included only rats with chronic low levels of E2 as it has been repeatedly shown that chronic high levels of E2 administered systemically to OVX rats results in an enhancement of DA release in the DS (e.g. Becker and Rudick 1999). Post-surgical care included administration of the antibiotic Baytril (0.03 ml/rat, SC; CDMV, St. Hyacinthe, QC, Canada), the analgesic banamine (0.03 ml/rat, SC; CDMV), and 0.9% saline (3ml/rat, SC). Following ovariectomy surgery, rats were allowed to recover in their home cages for one week.

Within 1-2 weeks of ovariectomy surgery, each rat was again anaesthetized using Halothane gas (4%) and placed in stereotaxic equipment. A three-pronged cannula was

implanted into the left or right DS with the center cannula at AP -0.3, ML ±4.0, and DV -3.0. Each arm of the three-pronged cannula was 21g stainless steel (Plastics One, Roanoke, VA), 40mm in length, and permanently fixed in a parallel manner approximately 0.5mm apart using solder. The long length of the arms was necessary for the secure fixation of the three-pronged cannula in the stereotaxic arm and accurate placement of the microdialysis probe due to the solder fixing the arms together. The center cannula housed the microdialysis probe while the outer two cannulae were used to hold microinjectors when appropriate. Each rat remained under Halothane gas anaesthesia (2%) for the duration of the experiment at which time it was decapitated and the brain was removed.

Rats were pseudorandomly assigned to one of the three groups; Group 1 (baseline-E2-drug; n=3), Group 2 (baseline-cyclodextrin-drug; n=5), or Group 3 (baseline-cyclodextrin-E2; n=4). Rats received local intracranial injections of cyclodextrin (519.6μg/μl; Sigma) or E2-cyclodextrin complex (544μg/μl; Sigma). Cyclodextrin was utilized due to its rapid dissolution and action. Concentrations of E2-cyclodextrin and cyclodextrin were approximated from previous experiments using intracranial injections in which E2 infusion had a beneficial effect on memory behavior (Packard and Teather 1997; Zurkovsky, Brown et al. 2007). Based on the molecular weight of E2 (Sigma), its concentration in E2-cyclodextrin is 0.045% (45mg/1g), or 24.4μg. Therefore, the vehicle dose of cyclodextrin was made using 95.5% of the E-cyclodextrin complex (544μg – 24.4μg = 519.6μg). This results in infusions with a total concentration of 1nmol/μl which is approximately 2x higher than doses used previously (Packard and Teather 1997; Zurkovsky, Brown et al. 2007). This is the first study to

investigate the rapid effects of E2 on DA levels in response to acute local administration and, thus, this dose was chosen to maximize the likelihood of achieving an observable effect. Rats receiving the drug, amphetamine (0.5mg/kg), were administered intraperitoneal injections in a saline vehicle.

Microdialysis Apparatus and Equipment. All testing took place under anaesthesia in the stereotaxic equipment (David Kopf Instruments; Tujunga, CA). Sample collection was achieved using a dialysis probe with a semi-permeable membrane (Spectra/Por, Spectrum Laboratories, Rancho Dominguez, CA, USA) which has a molecular cut-off weight of 13,000 kDa. The total length of the tip extending from the center cannula was 4mm. Dialysate was collected from the probe outlet silica (Polymicro Technologies; Phoenix, AZ) into a 0.5 mL Eppendorf (Sigma) tube. Artificial cerebrospinal fluid (aCSF), 150 mm Cl^-, 145 mm Na^+, 2.7 mm K^+, 2 mm Na_2HPO_4, 1.22 mm Ca^{2+}, 1.0 mm Mg^{2+}, 0.2 mm ascorbate, pH 7.4 ± 0.1, was pushed through the probe using a pump (KD Scientific, Model 780100; Holliston, MA) at a rate of 1.0 µl/min. Once testing began, samples were collected every 10 minutes; In addition to 6 baseline samples collected from each rat, 6 samples were collected after infusions of E2-cyclodextrin or cyclodextrin and 12 samples were collected after amphetamine injection. Intracranial infusions of E2-cyclodextrin and cyclodextrin through the outer two cannulae were given with two 10µl Hamilton syringes using 26g injectors. The tips of the injectors extended 4mm beyond the end of the cannulae. Injections were manually infused over 1 minute in a total volume of 1.5µl and the injectors were left in for 1 additional minute to allow for diffusion of the liquid.

Approximately 10µl of each sample was analyzed for DA using high performance liquid chromatography (HPLC) with electrochemical detection. Samples were injected into a 15cm C_{18} column (Higgins Analytical Co.) through manual injection ports (Rheodyne 7125, Rheodyne, Rohnert Park, CA, 20µl loop). The separated samples passed through a dual channel electrochemical detector (ESA Biosciences; Chelmsford, MA) and compounds within each sample were detected with a Coulochem III detector (Model 5100A analytical cell, ESA, Inc.). The detectors were set to provide the reduction and oxidation currents for DA and its metabolites. The system was calibrated using estimates from peak height by comparison with injections of known amounts of standard DA concentrations (Sigma). Mobile phase consisted of 20% acetonitrile 40mg, 0.076 M sodium dodecyl sulphate, 0.1 M EDTA, 0.058 M $NaPO_4$, and 0.27 M citric acid with a pH of 3.35 and circulated at 1.0ml/min by Waters 515 HPLC pumps (Lachine, QC, Canada). EZChrom Chromatography Software Data System (Scientific Software, San Ramon, CA) was used to analyze and integrate the data.

Microdialysis Procedure. On the testing day, each rat was brought into the testing room at approximately 1000h. The rat was anaesthetized using Halothane gas (4%) while the three-pronged cannula was inserted into the DS; the location of implantation was counterbalanced between the left and right hemispheres. Using digital stereotaxic controls, the center cannula with the microdialysis probe already inserted was manually lowered at the appropriate coordinates; the bottom of the microdialysis probe was at -7.0 DV. This was done at a rate of approximately 1mm per minute in order to minimize shock and damage to the brain tissue. Once lowered, the rat was given 1 hour to allow for

equilibrium of liquid flow to and from the probe tip. Testing began at approximately 1100h. Samples were collected every 10 minutes and immediately placed in dry ice. After collection of 6 baseline samples, the injections began according to the assigned group. E2-cyclodextrin and cyclodextrin were always given intracranially while amphetamine was administered intraperitoneally. All injections were done at the start of each 10-minute sample bin. After sample collection was complete, the rat was immediately decapitated, the brain was flash frozen using 2-methylbutane in dry ice, and the brain was stored at -80°C until being coronally sectioned at 40μm for confirmation of cannula placement.

Statistical Analyses. Levels of analyzed compounds were expressed as concentrations (pg/ml) and basal values were estimated as the mean of three samples preceding the first local infusion. The effects of hormone, drug, or vehicle administration were analyzed using a two-way mixed analysis of variance (ANOVA). Time and drug condition were the independent variables and concentration of DA was the dependent variable. Post hoc tests were done using a paired two sample t-tests.

3. Results

In Group 1, there was a significant main effect of time such that DA levels in the DS were significantly higher after systemic administration of amphetamine when compared to baseline DA levels; $F(11,44)=2.271$, $p=0.027$ (Figure 1A). There was also a significant interaction between time and drug condition; $F(11,44)=2.297$, $p=0.025$. Post hoc analysis did not reveal any significant differences between individual time points

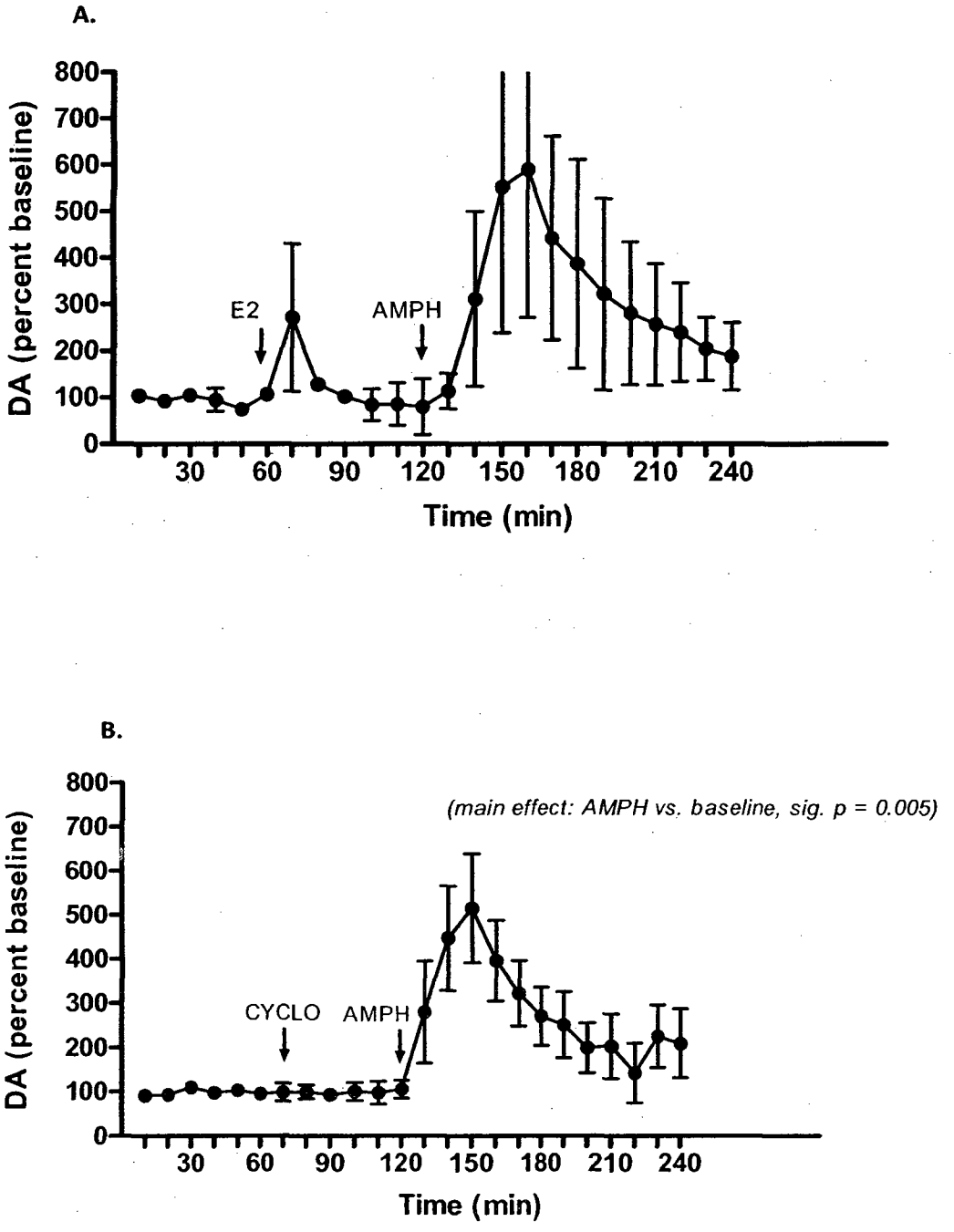


Figure 1. Effects of systemic amphetamine injection on dorsal striatal DA levels. A) No effect of E2-cyclodextrin infusion. There was a significant interaction of time and drug, p=0.025, but post hoc analysis did not reveal significant differences at individual time points. B) No effect of vehicle infusion. There was a significant interaction of time and drug, p=0.002. Post hoc analysis revealed a significant difference at the third time point after amphetamine injection.

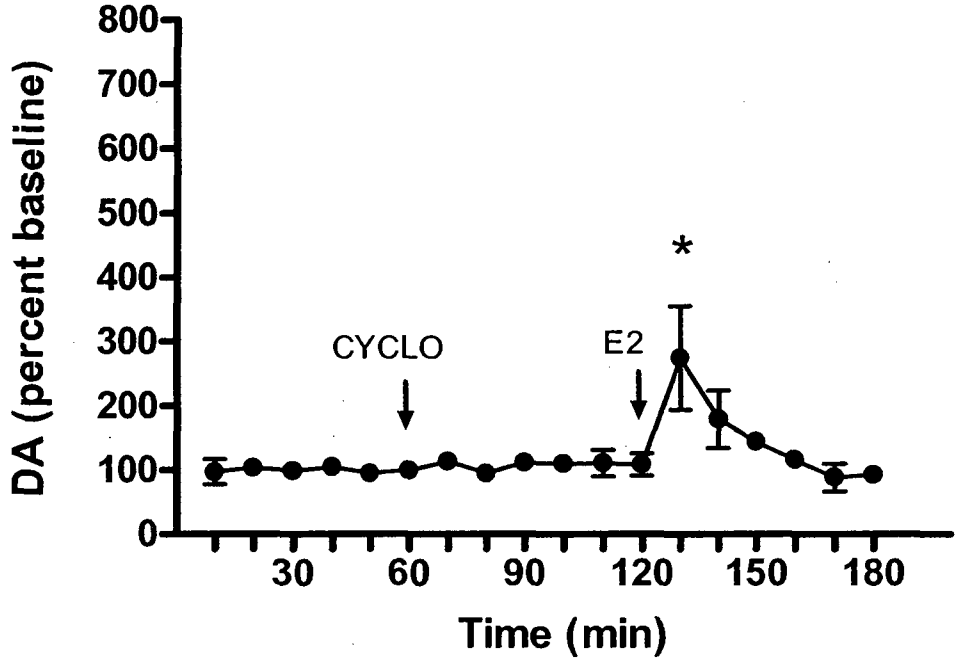


Figure 2. Effects of cyclodextrin and E2-cyclodextrin on DA levels in the dorsal striatum. There was no effect of the vehicle, cyclodextrin, on DA levels. There was a significant effect of E2 at the first time point after E2 infusion, p=0.05.

after systemic amphetamine injections suggesting that local infusion of E2-cyclodextrin did not increase extracellular DA levels while amphetamine injection did.

In Group 2, there was a significant main effect of time such that DA levels in the DS were significantly higher after systemic administration of amphetamine when compared to baseline DA levels; $F(9, 63)=3.284$, $p=0.002$ (Figure 1B). There was also a significant interaction; $F(9,63)=3.357$, $p=0.002$. Post hoc analysis revealed that DA levels at the third time point after systemic injection of amphetamine was significantly higher than baseline DA levels; $t(3)=-2.337$, $p=0.05$. In addition, there was a significant drug effect; $F(1,7)=16.82$, $p=0.005$. Thus, while the vehicle cyclodextrin did not significantly increase extracellular DA levels, amphetamine did.

Analysis comparing the elevation in DA levels after systemic administration of amphetamine following local infusion of E2 or its vehicle cyclodextrin revealed a significant main effect of time such that DA levels in the DS were significantly higher after amphetamine injection when compared to baseline; $F(11,55)=4.576$, $p=0.000$. However, there was neither a group effect nor an interaction. This indicates that local E2 infusion did not enhance amphetamine-induced DA release in the DS relative to its vehicle cyclodextrin.

In Group 3, there was a main effect of time such that DA levels were significantly higher immediately after a local infusion of E2-cyclodextrin into the DS; $F(5,30)=3.299$, $p=0.017$ (Figure 2). There was also a significant interaction between time and group; $F(5,30)=3.288$, $p=0.017$. Post hoc analysis revealed DA levels in the first time point after

E2-cyclodextrin infusion to be significantly higher than baseline levels of DA; t(3)=-2.329, p=0.05. Thus, E2 enhanced DA release while its vehicle cyclodextrin did not.

4. Discussion

The present findings confirm the well-documented enhancement of extracellular dorsal striatal DA levels in response to systemic administration of amphetamine (e.g. Becker 1990). In addition, this study is the first to demonstrate that *in vivo* local infusion of E2 in anaesthetized rats rapidly augments baseline extracellular DA release in the DS. This effect occurs within minutes and DA returns to baseline levels within 40-50 minutes. However, this rapid estrogenic modulation of DA release did not have a potentiating effect on the amphetamine-induced enhancement of DA in the DS one hour later. Local infusion of the cyclodextrin vehicle had no effect on DA release.

The majority of studies investigating the effects of E2 administer chronic high levels of hormone beginning 72 hours prior to testing (e.g. Sandstrom and Williams 2001) suggesting that it is the genomic activity of classic intracellular ERs which enacts changes in the brain and in behavior. For example, in a dorsal striatal DA-mediated response learning task, chronic E2 augments the disruptive effects of a DA D2 receptor antagonist (Daniel, Sulzer et al. 2006). However, several studies have identified relatively quick alterations of behavior after systemic E2 administration. Several types of learning (Luine, Jacome et al. 2003; Walf, Rhodes et al. 2006; Frye, Duffy et al. 2007) as well as DS-mediated rotational behaviors (Becker 1990) are enhanced by acute E2 administration. DS-mediated tasks are often supported by DA activity.

It has been repeatedly demonstrated that E2 augments dorsal striatal extracellular DA levels *in vitro* (Becker and Ramirez 1981; McDermott 1993) and *in vivo* (Xiao and Becker 1994; Ohtani, Nomoto et al. 2001). In general, these studies utilize high levels of chronic E2 replacement in OVX female rats or in cultured cells from OVX rats which are then compared to OVX females receiving a vehicle injection. As a result, the deliberate action of nuclear ERs versus the rapid effects of membrane-bound ERs, or other E2-activated membrane-bound G-protein coupled receptors, in the estrogenic modulation of DA function cannot be extricated from one another. Thus, OVX rats with chronic low levels of E2 were used here to demonstrate that local infusions of E2 into the DS rapidly, and transiently, enhance extracellular levels of DA. However, this increase was only seen after E2 infusion in Group 3 and not in Group 1. This is presumably due to a high attrition rate in Group 1 which led to a large variance that may have obscured the enhancing effects of E2. An examination of DA levels in the second time period following E2 infusion in both Groups 1 and 3 shows a similar increase in average DA levels over baseline. It is likely that with the addition of more subjects in both experiments that the variance will decrease and the rapid augmentation of dorsal striatal DA levels subsequent to local E2 infusions will become more apparent.

Groups 1 and 3 also demonstrated that the rapid modulation of DA by E2 had no effect on extracellular DA levels subsequent to amphetamine administration one hour later. This finding contradicts a number of studies which show that high levels of E2 enhance amphetamine-induced DA levels in the DS (e.g. Becker, Beer et al. 1984; Di Paolo, Rouillard et al. 1985; e.g. Becker 1990; Castner, Xiao et al. 1993; Ohtani, Nomoto et al. 2001). However, the majority of these studies employ chronic systemic

administration of E2 resulting in the constant presence of high E2. Here, an E2-cyclodextrin complex, which is rapidly metabolized, was used. This resulted in a rapid and transient increase in DA which peaked within 20 minutes and returned to baseline within 50 minutes. Thus, by the time amphetamine was systemically administered 60 minutes later, the E2-induced enhancement of DA had already subsided. E2 has also been shown to transiently increase DA levels in cultured cells within two minutes (Thompson and Moss 1994) and stimulate second messenger pathways linked to membrane-bound receptors within 20 minutes (Kelly, Lagrange et al. 1999; Kelly and Levin 2001). Although the primary objective of this study was to investigate the rapid effects of E2 on baseline DA levels in the DS, the time course of this action was also of concern. The results indicate that the action of locally infused E2 diminishes too quickly to affect amphetamine-induced DA levels one hour later. One way to investigate these rapid effects on stimulant-induced DA is to inject amphetamine immediately, or soon, after E2 infusion. Another possibility would be to use several concentrations of E2 to create a dose-response curve that may uncover other enduring effects. It is also possible to use other estrogenic compounds, such as an E2-sulfate complex (Zurkovsky, Brown et al. 2007), which are reported to be more slowly metabolized. Using local infusions of an E2-cyclodextrin complex, the present findings suggest that E2 has a rapid and transient enhancing effect on baseline DA levels in the DS.

These data also demonstrate that E2 acts to directly influence DA activity in the DS. It has been shown that DA levels in the nigrostriatal pathway can be enhanced through the modulation of tyrosine hydroxylase (Kuppers, Ivanova et al. 2000) and DA synthesis (Pasqualini, Olivier et al. 1995) in the substantia nigra which indirectly affect

dorsal striatal DA levels. However, the direct infusion of E2 into the DS indicates that E2 action on dopaminergic cell bodies in the ventral tegmental area is not necessary for enhanced release in target regions. To this end, it is possible that E2 is acting rapidly through ERs on presynaptic DA terminals in the DS. E2 stimulates PKA and PKC activity through G-protein coupled receptors (Kelly, Lagrange et al. 1999) and Akt, ERK, and MAPK pathways through membrane-bound ERs (Kelly and Levin 2001). In addition, low concentrations of an E2-activated membrane-bound receptor, GPR-30, have been found in the DS (O'Dowd, Nguyen et al. 1998; Brailoiu, Dun et al. 2007). It is also possible that E2 is acting on the cell bodies and/or terminals of non-dopaminergic neurons in the DS to alter DA transmission. Although not investigated in the DS, ERs have been identified on GABA interneurons in the prefrontal cortex (Blurton-Jones and Tuszynski 2006) and hippocampus (Hart, Patton et al. 2001). ERs are also located on astrocytes in the substantia nigra (Quesada, Romeo et al. 2007) which results in an increase in the expression of glutamate transporters (Pawlak, Brito et al. 2005) and MAPK activation (Pawlak, Karolczak et al. 2005).

The present findings demonstrate for the first time that E2 rapidly enhances DA release in the DS *in vivo*. Although this rapid estrogenic effect does not enhance amphetamine-induced DA release in this paradigm, future work utilizing different E2 complexes or more contiguous injections of amphetamine may demonstrate similar results to studies using chronic E2 replacement regimens.

CHAPTER 7:

EFFECTS OF CHRONIC ESTRADIOL ON SYNAPTIC PROTEINS AND LEFT AND RIGHT PREFRONTAL CORTICAL DOPAMINE LEVELS IN OVARIECTOMIZED RATS

Matthew G. Quinlan, Marie-Pierre Cossette, Natalina Salmaso, Barbara Woodside, and Wayne G. Brake

Preface:

E2 modulates synaptic structure and density in the hippocampus of cycling (Woolley and McEwen 1992) and ovariectomized (Gould, Woolley et al. 1990) female rats. E2 also alters baseline levels of DA in the medial prefrontal cortex across the estrous cycle in female rats (Dazzi, Seu et al. 2007). Studies from previous chapters have shown that antagonism of DA D1 and DA D2 receptors in the medial prefrontal cortex modulates cognitive strategy use in low E2 rats. Thus, the influence of E2 on the quantity of synaptic proteins in brain areas innervated by DA was investigated in this chapter. In addition, dual-probe *in vivo* microdialysis was used to evaluate the effect of E2 on extracellular DA levels in the medial prefrontal cortex.

Abstract:

Numerous studies have demonstrated that (E2) modulates synaptic structure and density in several target regions of dopaminergic pathways in the brain. The majority of these changes are found in the hippocampus. Relatively few studies have investigated the estrogenic modulation of synaptic proteins in the dorsal striatum (DS) and medial prefrontal cortex (mPFC). Similarly, numerous studies have demonstrated that E2 modulates DA neurotransmission in the DS while fewer studies have investigated these changes in other brain regions containing DA, such as the mPFC. In the present study, the effect of E2 on the quantity of the synaptic proteins synaptophysin and spinophilin was examined in the hippocampus, DS, and mPFC using Western immunoblotting. In addition, the estrogenic modulation of DA levels in the mPFC at baseline and in response to systemic injection of amphetamine was evaluated using *in vivo* dual-probe microdialysis. Because a lateralization of medial prefrontal cortical function is implicated in the regulation of stress as well as in pathologies such as attention deficit hyperactivity disorder, the influence of E2 on synaptic density and neurotransmission was investigated in both the left and right hemispheres. There was no effect of E2 on the quantities of synaptic proteins in any of the three brain regions. While systemic injections of amphetamine significantly increased DA levels in both the left and right mPFC, there was no effect of E2 at baseline or in potentiating amphetamine-induced increases.

1. Introduction

Estrogen has been shown to modulate synaptic structure and neurotransmission in several target regions of the main dopaminergic pathways in the brain. For example, synaptic density in the hippocampus (HPC) fluctuates with the estrous cycle such that it is highest during proestrus, a period of high estradiol (E2) levels (Woolley and McEwen 1992). Ovariectomy results in a dramatic decrease of hippocampal dendritic spine density which can be prevented by the administration of E2 (Gould, Woolley et al. 1990). Similar decreases in synaptic density are seen in the medial prefrontal cortex (mPFC) of ovariectomized (OVX) female rats (Wallace, Luine et al. 2006) and monkeys (Tang, Janssen et al. 2004). In the dorsal striatum (DS), the intra-membranous composition of dendritic spines changes with the estrous cycle (Morissette, Garcia-Segura et al. 1992).

E2 also affects neurotransmission in these brain areas. Although changes in hippocampal dopamine (DA) transmission have not been identified, E2 facilitates acetylcholine release in this region (Gabor, Nagle et al. 2003) as well as in the basal forebrain (Luine, Richards et al. 1998). Luine et al. (1998) also show that levels of norepinephrine, serotonin, and DA decrease in the mPFC following chronic E2 treatment. DA release in the mPFC is lowest during proestrus and highest during estrus, a period of low E2 levels (Dazzi, Seu et al. 2007). In the DS, there is a robust enhancement of DA transmission concomitant with high levels of E2 in both cycling (Xiao and Becker 1994) and OVX (Becker 1990) females.

In vivo microdialysis is a common method utilized in the examination of extracellular neurotransmitter levels. DA levels are often measured using this technique

but few studies have investigated the effect of E2 on extracellular DA levels in this manner. Likewise, the effect of E2 on synaptic structure and morphology is commonly evaluated through quantification of synaptic proteins but there is a relative lack of studies examining the effects of E2 in brain areas receiving dopaminergic projections. Synaptophysin (SYN) is a 38-kDA protein primarily located in the membranes of neurotransmitter vesicles and is associated with the docking of these vesicles to the presynaptic membrane (Navone, Jahn et al. 1986; Calakos, Bennett et al. 1994; Calakos and Scheller 1994). E2 administration in OVX female mice increases hippocampal SYN levels (Frick, Fernandez et al. 2002). Spinophilin (SPI) is a 90-kDA protein that is found in the dendrites of postsynaptic neurons (Allen, Ouimet et al. 1997). It is thought to play a role in cytoskeletal structure, synaptic plasticity, and the creation of new spines (Futter, Uematsu et al. 2005; Terry-Lorenzo, Roadcap et al. 2005; Calhoun, Fletcher et al. 2008). Hippocampal levels of SPI are enhanced after E2 administration to OVX female rats (Lee, Romeo et al. 2004). Because these proteins are primarily found at pre- and postsynaptic sites, they may be used as markers of the increased synaptogenesis associated with high levels of E2. Using radiolabelled immunocytochemistry, it has been demonstrated that the administration of E2 to OVX females produces a significant increase of both SYN and SPI in CA1 neurons in the HPC (Brake, Alves et al. 2001).

To further evaluate the effects of E2 on synaptic density, Study 1 investigated levels of SYN and SPI of the HPC, DS, and mPFC using Western immunoblotting. Study 2 utilized *in vivo* microdialysis to investigate the influence of E2 on DA transmission in the mPFC. Because a lateralization of function in the mPFC has been implicated in stress (Sullivan 2004) as well as the pathophysiology of dopaminergic diseases such as

attention deficit hyperactivity disorder (ADHD; for a review, see: Sullivan and Brake 2003), the effects of E2 on synaptic structure and DA neurotransmission were investigated in both the left and right hemispheres.

2. Materials and Methods

Subjects. All subjects included in Studies 1 and 2 were young adult, female, Sprague-Dawley rats (Charles River, St. Constant, Quebec) aged three months and weighing approximately 225-250 grams at the time of arrival. Upon arriving, all rats were allowed one week to habituate to the animal facility and were handled daily from time of arrival until completion of testing. All rats were pair-housed in same-sex polyurethane shoebox cages, maintained on a reverse 12h:12h light/dark cycle with lights off from 0900-2100h, and were allowed standard lab chow and water *ad libitum.* In Study 2, rats were single-housed subsequent to cannulae implantation surgery for the remainder of the experiment. All animal handing and testing procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Concordia University Animal Research Ethics Committee.

Study 1 included 24 rats; 8 rats in the proestrus phase of the estrous cycle, 8 rats in the estrus phase of the estrous cycle, and 8 rats which were OVX and received no hormone replacement. Study 2 included 20 rats, all of which were ovariectomized; 6 received high levels of E2 replacement (see below for hormone replacement paradigm), 8 rats received low levels of E2 replacement (see below), and 6 rats did not receive E2 replacement.

Study 1, Surgical Procedures. Approximately one week after arrival, 8 of the 24 rats were anaesthetized using Halothane gas (4% for induction; 2% for maintenance) and bilaterally ovariectomized using a standard aseptic procedure through a dorsal incision. Post-surgical care included administration of the antibiotic Baytril (0.03 ml/rat, SC; CDMV, St. Hyacinthe, QC, Canada), the analgesic banamine (0.03 ml/rat, SC; CDMV), and 0.9% saline (3ml/rat, SC). Following surgery, rats were allowed to recover in their home cages for 2-3 weeks prior to tissue collection. All surgical procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Concordia University Animal Research Ethics Committee.

Study 1, Determination of Estrous Cycle. In Study 1, 16 of 24 rats were intact females exhibiting regular 4-5 day estrous cycles for two weeks prior to tissue collection. The phase of estrous cycle was determined daily by vaginal cytology characterization using a cotton swab dampened with saline to collect epithelial cells from the vaginal wall. All samples were collected daily from approximately 1600-1700h and immediately examined under a microscope with 10x magnification. Rats with a majority of cornified epithelial cells were considered to be in estrus; rats with a mix of cornified epithelial cells, nucleated epithelial cells, and leukocytes were considered to be in metestrus; rats with a majority of leukocytes were considered to be in diestrus; and, rats with a majority of nucleated epithelial cells were considered to be in proestrus. Rats exhibiting irregular estrous cycles were excluded from the experiment. In Study 2, all OVX rats were divided into three groups and received hormone or vehicle injections as described above.

Study 1, Procedure: Western Immunoblot Analysis. For the 16 intact rats, tissue collection occurred immediately following the determination of estrous cycle phase. 8 rats were sacrificed during the proestrus phase of the estrous cycle and 8 rats were sacrificed during the estrus phase of the estrous cycle. For the 8 OVX rats, tissue collection occurred approximately 14 days after ovariectomy surgery. For all rats, this took place between approximately 1800-2000h.

All rats were live decapitated after which the brains were removed. Fresh tissue samples were immediately collected from both hemispheres of several dopaminergic target regions; the mPFC including the prelimbic and infralimbic areas, the dorsal aspect of the HPC, and the DS. Brains were placed on a cold plastic tray in a wet ice bucket and samples were manually collected using a standard razor blade. Once collected, samples were immediately placed in 0.5ml Eppendorf tubes (Sigma), flash frozen in dry ice, and stored in at -80°C until analysis. 120µl of lysis buffer was added to each sample and protein was homogenized using a sonicator. Samples were then centrifuged after which the supernatant was collected and stored at -20°C. Protein analysis was conducted using the BCA-200 Protein Assay Kit (Pierce) according to manufacturer's instructions and 15µg of protein was loaded into wells of 12% SDS-PAGE (NuPAGE- Invitrogen) gels along with the sample buffer, reducing agent (Invitrogen) and water (if needed) to a total volume of 14µl/ well. All buffers were obtained from Invitrogen and used with the PowerEase TM 500 from Invitrogen. Gels were run at 110 volts for 15 minutes followed by 150 volts for one hour and then transferred to nitrocellulose membranes (Invitrogen) at 100 volts for 1 hour. Ponceau S (Sigma) was used to verify successful transfer of proteins. Membranes were then washed in a blocking and incubation buffer and

incubated in a primary antibody solution at 4°C overnight (anti-synaptophysin 1:2000, Sigma; anti-spinophilin 1:1000, Sigma).

On the following day, membranes were washed and incubated in appropriate HRP-conjugated secondary antibody for 2 hours, washed, and processed using Chemiluminescence Reagent Plus (Perkin Elmer Life Sciences) detection kit following the manufacturer's directions. Images were captured using Kodak ID Image Station and Software. Analysis of the immunoblots was evaluated using relative optical density. The sum intensity for each blot migrating at the proper weight for each antibody was measured; each lane contained tissue from a single rat.

Study 2, Surgical Procedures, Hormone Administration, and Drugs. Approximately one week after arrival, rats were anaesthetized using Halothane gas (4% for induction; 2% for maintenance) and bilaterally ovariectomized using a standard aseptic procedure through a dorsal incision. 16 of the 24 rats were implanted with a Silastic tube (1cm long; i.d. 1.47 mm; o.d. 1.96 mm) containing 5% 17β-E2 (Sigma Chemical Co., St Louis, MO) in cholesterol (Sigma). This has been reported to produce a serum concentration of approximately 20-25 pg/ml which is consistent with naturally circulating low levels of E2 such as those seen during the estrus phase of the rat estrous cycle (Hurn and Macrae 2000; Mannino, South et al. 2005). Previous studies from our lab have shown that this implant results in serum E2 levels of 18-32pg/ml (Quinlan et al., submitted; Quinlan et al., submitted; Quinlan, Hussain et al. 2008). Post-surgical care included administration of the antibiotic Baytril (0.03 ml/rat, SC; CDMV, St. Hyacinthe, QC, Canada), the

analgesic banamine (0.03 ml/rat, SC; CDMV), and 0.9% saline (3ml/rat, SC). Following ovariectomy surgery, rats were allowed to recover in their home cages for several days prior to cannulae implantation. Rats were anaesthetized using Halothane gas and received stereotaxic bilateral implantations of 21g stainless steel tubing guide cannulae (Plastics One, Roanoke, VA). Stereotaxic co-ordinates from bregma for the mPFC were AP +3.2, ML ± 1.8 at 10°, DV -1.5 (Paxinos and Watson 1998). Cannulae were blocked with 26g obdurators (Plastics One) which extended 1mm below the tip of the guide cannula. All surgical procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Concordia University Animal Research Ethics Committee.

For the high E2 group, in addition to the subcutaneous implants, daily subcutaneous injections of 17β-E2 (10 μg/kg) dissolved in sesame oil (Sigma) were given. These injections were designed to achieve E2 levels seen during the proestrus phase of the estrous cycle (75-90 pg/ml; Hurn and Macrae 2000; Mannino, South et al. 2005). Previous studies from our lab have shown that, in conjunction with the E2 implants, this paradigm results in serum levels of 70-97pg/ml (Quinlan et al., submitted; Quinlan et al., submitted; Quinlan, Hussain et al. 2008). During the same period all rats in the low E2 group received subcutaneous injections of sesame oil as a control (1ml/kg) such that E2 levels from the implant were similar to that of the estrus phase of the estrous cycle (20-30 pg/ml; Hurn and Macrae 2000; Mannino, South et al. 2005). OVX rats also received sesame oil injections. All injections began two days before testing occurred between 0800-0900h each day. During microdialysis testing, all rats received intraperitoneal injections of amphetamine (0.5 mg/kg) dissolved in a saline vehicle.

Study 2, Procedure: Microdialysis Apparatus and Equipment. All testing took place inside four separate modular isolation cubicles (Coulbourn Instruments; Whitehall, PA) each insulated with foam walls which absorbed ambient light and noise. Each cubicle is approximately 31"W x 21"D x 21"H, held an inner testing chamber measuring approximately 28"W x 18"D x 19"H, and was equipped with a fan. Each inner chamber was identically outfitted with a house light and a water bottle accessible from inside the chamber.

Sample collection was achieved using a dialysis probe with a semi-permeable membrane (Spectra/Por, Spectrum Laboratories, Rancho Dominguez, CA, USA) which has a molecular cut-off weight of 13,000 kDa. The total length of the tip extending from the cannula was 4mm. Dialysate was collected from the probe outlet silica (Polymicro Technologies; Phoenix, AZ) into a 0.5ml Eppendorf (Sigma) tube. Artificial cerebrospinal fluid (aCSF), 150 mm Cl^-, 145 mm Na^+, 2.7 mm K^+, 2 mm Na_2HPO_4, 1.22 mm Ca^{2+}, 1.0 mm Mg^{2+}, 0.2 mm ascorbate, pH 7.4 ± 0.1, was pushed through the probe using a pump (CMA Syringe pump, Model 402; Boston, MA) at a rate of 1.0 µl/min. Samples were collected every 20 minutes; 6 baseline samples, 2 vehicle samples, and 6 drug samples. Each sample contained approximately 18-20µl.

15µl of each sample was analyzed for DA using high performance liquid chromatography (HPLC) with electrochemical detection. Samples were injected into a 15cm C_{18} column (Higgins Analytical Co.) through manual injection ports (Rheodyne 7125, Rheodyne, Rohnert Park, CA, 20µl loop). The separated samples passed through a dual channel ESA (Chelmsford, MA) and compounds within each sample were detected

with a Coulochem III detector (Model 5100A analytical cell, ESA, Inc.). The detectors were set to provide the reduction and oxidation currents for DA and its metabolites. The system was calibrated using estimates from peak height by comparison with injections of known amounts of standard DA concentrations (Sigma). Mobile phase consisted of 20% acetonitrile 40mg, 0.076 M sodium dodecyl sulphate, 0.1 M EDTA, 0.058 M $NaPO_4$, and 0.27 M citric acid with a pH of 3.35 and circulated at 1.0ml/min by Waters 515 HPLC pumps (Lachine, QC, Canada). EZChrom Chromatography Software Data System (Scientific Software, San Ramon, CA) was used to analyze and integrate the data.

Study 2, Procedure: Microdialysis. On the testing day, each rat was brought into the testing room at approximately 0900h. The rat was briefly anaesthetized using Halothane gas (4%) while a probe was inserted into the medial prefrontal cortices of both the left and the right hemisphere; all rats were awake and freely moving within 5 minutes of anaesthesia. The rat was then given 5 hours to habituate to the chamber and to allow for equilibrium of liquid flow to and from the probe tip. At approximately 1400-1500h testing began. 6 20-minute baseline samples were collected. Then each rat received an intraperitoneal (IP) vehicle injection of saline and two more 20-minute samples were collected. Then each rat received an IP injection of amphetamine (0.5 mg/kg) and 6 more 20-minute samples were collected. Each sample was immediately placed on dry ice and stored at -80°C until analysis. Throughout the habituation period and during the testing procedure animals were freely moving and had *ad libitum* access to food and water.

After sample collection was completed, each rat was live decapitated and the brains were flash frozen using 2-methylbutane (Sigma) cooled in dry ice. Brains were stored at -80°C until slicing for confirmation of cannulae placement.

Statistical Analyses. For Study 1, protein quantities were analyzed using one-way analysis of variance tests for each antibody within each brain area. For Study 2, levels of analyzed compounds were expressed as concentrations (pg/ml) and basal values were estimated as the mean of three samples preceding the first local infusion. The effects of hormone regimen and amphetamine administration on DA levels were analyzed using two-way analysis of variance (ANOVA) tests. Time and E2 group were the independent variables and concentration of DA was the dependent variable. Post hoc tests were done using a paired two sample t-test with a level of $p<.05$ when appropriate.

3. Results

For Study 1, there were no significant differences among hormone groups in any of three brain areas tested for either protein in either the left (Table 1) or right (Table 2) hemispheres. The SYN antibody was immunoreactive to a single band migrating at 38 kDa consistent with the molecular weight of SYN (Figure 1). Similarly, the antibody used for SPI was immunoreactive for a single band migrating at 90 kDa consistent with the molecular weight of SPI.

For Study 2, there was a significant main effect of time such that DA levels in the left mPFC were higher after systemic administration of amphetamine when compared to baseline DA levels; $F(10, 130)=10.193$, $p=0.000$ (Figure 2A). Similarly, there was a significant main effect of time such that DA levels in the right mPFC were higher after systemic administration of amphetamine when compared to baseline DA levels; $F(13, 195)=11.453$, $p=0.000$ (Figure 2B). There were no significant interactions or between-group effects.

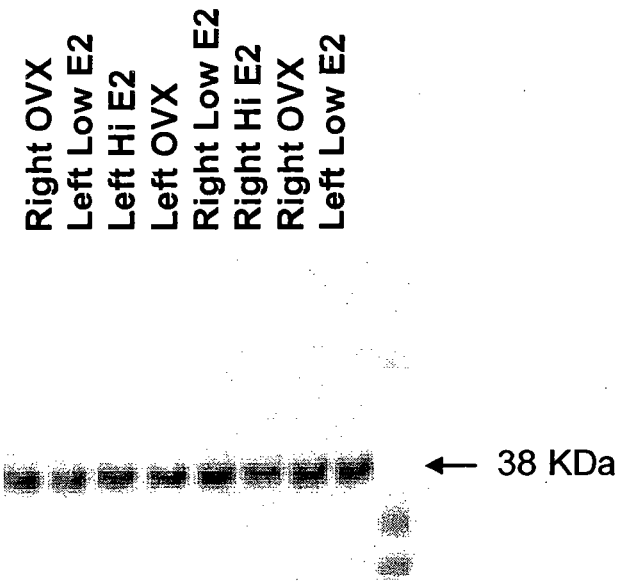


Figure 1. A representative example of an immunoblot stained for synaptophysin in the DS. Both hemispheres and all three hormone groups are represented. There were no significant differences in the mPFC, DS, or HPC or among any of the hormone groups.

Table 1. Effects of OVX, Low E2, or High E2 on quantities of synaptic proteins in the HPC, DS, or mPFC; Left hemisphere

	SYNAPTOPHYSIN			SPINOPHILIN		
LEFT	OVX	Low E2	High E2	OVX	Low E2	High E2
mPFC	64483.33 ±(14372.67)	37610.5 ±(7553.463)	40997.33 ±(8975.343)	5082.6 ±(903.4319)	6910.167 ±(1434.685)	8686.667 ±(1787.885)
DS	45390 ±(6936.542)	34824.5 ±(3350.879)	40457.13 ±(5859.58)	4656.875 ±(2479.659)	3592.125 ±(642.3824)	6525.375 ±(3162.133)
HPC	13059 ±(2173.451)	17946 ±(3138.01)	12546.75 ±(2191.469)	7477.123 ±(1055.794)	8964.513 ±(1581.697)	6985.875 ±(2273.493)

Mean (± SEM) optical density of SYN and SPI in mPFC, DS, and HPC in response to OVX, and systemic administration of low E2 and high E2. There were no significant differences.

Table 2. Effects of OVX, Low E2, or High E2 on quantities of synaptic proteins in the HPC, DS, or mPFC; Right hemisphere

	SYNAPTOPHYSIN			SPINOPHILIN		
RIGHT	OVX	Low E2	High E2	OVX	Low E2	High E2
mPFC	32289.83 ±(7492.641)	65403.8 ±(5960.516)	53138.5 ±(6583.374)	5887.8 ±(1378.133)	6910.167 ±(1544.227)	8686.667 ±(1871.811)
DS	30304.57 ±(4581.033)	39238.71 ±(8164.468)	31939 ±(5418.853)	8476.875 ±(4679.847)	3259.875 ±(1840.437)	5787 ±(2402.254)
HPC	13158.5 ±(2270.795)	13768.38 ±(2185.797)	11270.75 ±(1240.983)	10836.09 ±(1730.324)	11324.1 ±(1418.371)	12018.38 ±(3842.072)

Mean (± SEM) optical density of SYN and SPI in mPFC, DS, and HPC in response to OVX, and systemic administration of low E2 and high E2. There were no significant differences.

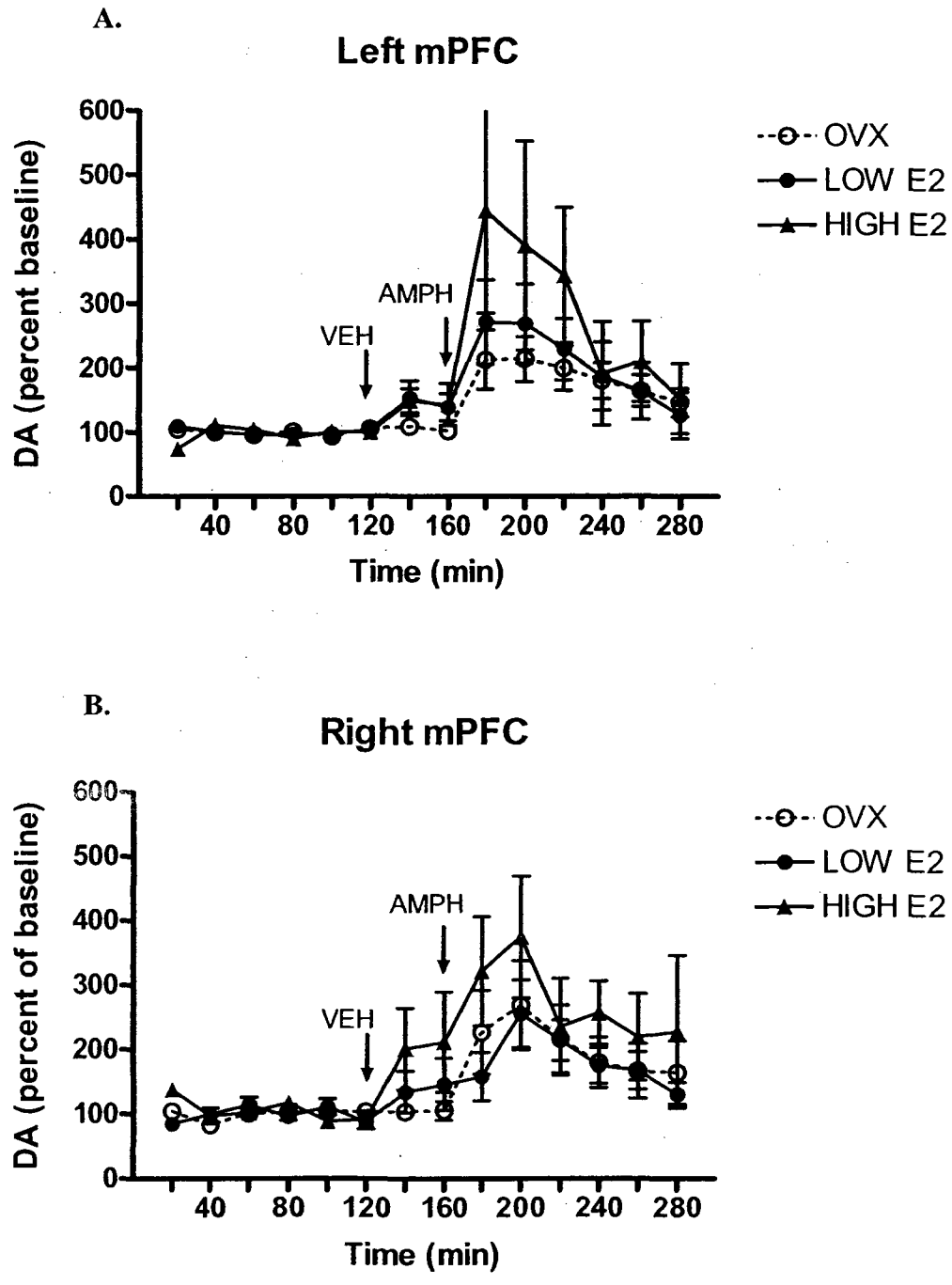


Figure 2. Effects of systemic amphetamine injections on extracellular DA levels in the mPFC. There was a main effect of time such that amphetamine increased DA in both the A) left and B) right hemispheres of the mPFC. There were no significant interactions.

4. Discussion

In Study 1, there was no effect of hormone group on synaptic density in any of the three brain areas studied. Study 2 is one of the first investigations to utilize dual-probe *in vivo* microdialysis to examine DA release simultaneously in the left and right hemispheres of the mPFC. This study confirms previous findings that systemic administration of amphetamine increases extracellular DA levels in the mPFC when compared to baseline (e.g. During, Bean et al. 1992). However, there were no significant differences among the amphetamine-induced DA responses of OVX, low E2, and high E2 rats. Contrary to previous studies (Dazzi, Seu et al. 2007), there was no difference in baseline DA levels among the three hormone groups. These results suggest that DA levels in the left and right hemispheres of the mPFC are similarly increased in response to systemic amphetamine injection. There was no effect of E2 on either baseline or amphetamine-induced DA levels which may be attributed to a high rate of attrition and large variances within groups.

The majority of studies investigating the estrogenic modulation of extracellular DA levels focus on DA in the DS (e.g. Becker 1990). Relatively few studies have examined the mPFC. In this region, high levels of E2 have been shown to reverse deficits in tyrosine hydroxylase concentration subsequent to ovariectomy (Kritzer 2000). Conversely, post-mortem studies in this region show that high levels of E2 decrease DA levels in OVX rats (Luine, Richards et al. 1998). It has also been shown that baseline extracellular DA levels are lowest during proestrus, a period of high E2 levels, and highest during estrus, a time of low E2 levels (Dazzi, Seu et al. 2007). Accordingly, the baseline firing rates of DA neurons in the ventral tegmental area, a dopaminergic

projection region with connections to the mPFC, are highest during estrus and lowest during proestrus. Although the current data did not find a significant difference in baseline DA levels, this is probably due to the high level of attrition in this study. There is a small trend towards significance and with the addition of more subjects, this difference may emerge.

The present findings did not find a potentiation of amphetamine-induced DA release in either the left or right mPFC with any level of E2. The systemic administration of ethanol results in a significant increase in medial prefrontal cortical DA levels during estrus, but not during proestrus (Dazzi, Seu et al. 2007). Similarly, there have been repeated demonstrations of estrogenic modulation of extracellular DA levels in the DS in response to systemic amphetamine (e.g. Becker 1990; e.g. Castner, Xiao et al. 1993). Here, there is an approximately 200-300% increase in DA levels in the mPFC after amphetamine injection for all groups but a large variance within each group at several time points may obscure any differences. Perhaps with the addition of more subjects a difference similar to that seen in the mPFC after ethanol administration or the DS after amphetamine administration will emerge. It should also be noted, although there was no significant difference, that in both the left and right mPFC there appears to be an enhancement in DA levels in response to the systemic injection of a saline vehicle in the low E2 and high E2 groups. This rise in DA levels is not apparent in the OVX group. Here, again, there may be an emerging effect which is obscured by high attrition rates and large variances within groups.

There were no significant differences among the hormone treatment groups in synaptic protein quantities in the mPFC, HPC, or DS. Numerous studies have demonstrated that high levels of E2 in OVX (e.g. Gould, Woolley et al. 1990) and cycling (e.g. Woolley and McEwen 1992) females enhance synaptic density in the HPC. E2 treatment in OVX females augments SYN and SPI in the dorsal HPC (Brake, Alves et al. 2001). Conversely, both SYN and SPI are reduced in hippocampal slices with the application of an aromatase inhibitor (Kretz, Fester et al. 2004) and ovariectomy has been shown to decrease synaptic density in the mPFC (Wallace, Luine et al. 2006). It has been suggested that the Western immunoblotting technique is not sensitive enough to detect changes in protein expression or that long-term exposure to E2 is required in order to quantify these effects (Sharma, Mehra et al. 2007). 4 weeks of chronic E2 administration were necessary to reverse ovariectomy-induced decreases in hippocampal SYN. Likewise, *in vitro* studies demonstrate that 4, but not 2, days of E2 treatment are required to increase SYN in hippocampal primary cell cultures (Chindewa, Lapanantasin et al. 2008). Radiolabelled immunocytochemistry, a more sensitive technique, has been used to identify increases in synaptic proteins after short-term administration of E2 to OVX females (Brake, Alves et al. 2001). It is possible that any changes in synaptic protein numbers or structure require a more sensitive technique than Western immunoblotting or a longer period after ovariectomy in order to be identified.

Differences in left versus right medial prefrontal cortical DA activity have been implicated in the control of stress (Sullivan 2004) and pathologies such as ADHD (Heilman, Voeller et al. 1991; Sullivan and Brake 2003). Specifically, asymmetric function and abnormal activity of DA in the right mPFC are related to these types of

behaviors. Here, there are no differences in baseline DA levels or amphetamine-induced DA levels in either the left or right mPFC among the different hormone groups. However, it appears that there are trends towards differences in amphetamine-induced DA release in the mPFC but more power is necessary to confirm any difference that may exist. Similarly, there is a small trend towards a higher baseline level of DA in low E2 rats. A high rate of attrition and high levels of variance within groups may have contributed to the non-significant differences among groups. Several studies have demonstrated that E2 alters synaptic structure and plasticity in the HPC while fewer studies have found these differences in the DS and mPFC. Future work in this area may require increased periods of time after ovariectomy when utilizing Western immunoblotting or more sensitive techniques. A smaller number of studies have reported significant differences in DA neurotransmission in the mPFC. Here, more subjects may be required to find these differences in the present and future studies.

CHAPTER 8:

CHRONIC LOW LEVELS OF ESTRADIOL IMPAIR WORKING MEMORY IN A DELAYED MATCH-TO-PLACE VERSION OF THE T-MAZE TASK IN FEMALE SPRAGUE-DAWLEY RATS

Matthew G. Quinlan, Varuni Bentotage, Dema Hussain, Marie Pierre Cossette, David G. Mumby and Wayne G. Brake

Preface:

Studies from previous chapters have demonstrated that DA D1 and DA D2 receptor antagonism in the medial prefrontal cortex can alter cognitive strategy use in low E2 rats. It has also been demonstrated E2 modulates DA levels in this brain region across the estrous cycle (Dazzi, Seu et al. 2007). Another type of cognitive task which is mediated by DA in the medial prefrontal cortex is working memory (Sawaguchi and Goldman-Rakic 1991). Thus, the study in this chapter investigates the effect of ovariectomy, low E2, and high E2 on performance in a DA-mediated working memory task in a T-maze.

Abstract:

Estrogen has been found to have varying effects on cognition in female rodents. This is most likely due to differences in behavioural testing procedures and hormone administration regimens from study to study. Here, ovariectomised female rats received either chronic low œstradiol or chronic high œstradiol in a paradigm meant to closely mimic œstradiol levels during the œstrus and pro-œstrus phases of the natural œstrous cycle. In addition, as a point of comparison with other studies, another group of ovariectomised animals received only vehicle injections. All subjects were tested in a match-to-place version of the T-maze. Low œstradiol rats required significantly more trials to acquire the task than the high œstradiol rats. Upon reaching criterion performance in the match-to-place task, a series of extended delays of 60-180 seconds were introduced in order to test working memory. Here, high œstradiol rats made more correct arm choices than low œstradiol rats. Vehicle-treated animals performed comparably to high œstradiol animals in task acquisition and working memory performance. These findings suggest that physiologically low levels of chronic œstradiol replacement to ovariectomised rats may impair performance when compared to ovariectomised rats receiving vehicle administration as well as physiologically high levels of œstradiol replacement.

1. Introduction

An extensive body of literature provides support for a modulatory role of œstrogen (E) during the performance of behavioral tasks (e.g. Gibbs 2002; Sherwin 2003; Korol, Malin et al. 2004); notably working memory (Olton and Samuelson 1976). However, findings in this area may sometimes be contradictory as to the direction and extent of effects. Before firm conclusions concerning the role of E can be made there are a number of factors that must be considered when interpreting the effects of œstradiol (E2) on performance in behavioral tasks in rodents; these include the timing of E2 administration after ovariectomy, the administration regimen (acute, chronic, continuous) and dose of E2, the control group used, age, and the type of task used. While many studies report a beneficial influence of E2 in behavioral tasks the literature as a whole is far from consistent. High levels of E2 have been shown to enhance acquisition of delayed-match-to-place (DMTP) tasks versus ovariectomised (OVX) controls in young adult (Gibbs 1999; Gibbs 2002) and aged female rats (Gibbs 2000). Low levels of E2 improve arm choice accuracy during task acquisition in an 8-arm radial maze (Daniel, Fader et al. 1997; Luine, Richards et al. 1998; Fader, Johnson et al. 1999) and a T-maze (Fader, Hendricson et al. 1998) when compared to vehicle groups. Yet, other studies report no differences between high E2 groups and either low E2 or control groups during acquisition (Holmes, Wide et al. 2002; Wide, Hanratty et al. 2004). Chesler & Juraska (2000) report that treatment with low levels of E plus progesterone, but not E alone, impairs acquisition in a Morris Water maze. Similarly, rats in œstrus performed significantly better in a spatial version of the Morris water maze than rats in pro-œstrus (Warren, Humphreys et al. 1995).

There are also inconsistent findings concerning the effect of E2 on working memory performance. It has been shown that, when compared to a vehicle control, high levels of E2 increase working memory errors while low levels of E2 reduce errors in a win-shift task in an 8-arm radial maze (Holmes, Wide et al. 2002). Subjects administered supraphysiological levels of E2 do not differ from OVX + vehicle controls (Galea, Wide et al. 2001). In a T-Maze, high levels of E2 improve working memory in a delayed alternation task (Wide, Hanratty et al. 2004) but have no effect on a DMTP task (Gibbs 1999; Gibbs 2002). Timing also matters. Acute doses of E2 enhance memory retention in a DMTP task; this effect disappears without continuous administration (Sandstrom and Williams 2001). E2 administration three, but not 10, months after OVX enhances performance in a DMTP task in aged females (Gibbs 2000). Perhaps the conflicting results concerning the effect of E2 on the performance of behavioral tasks by rodents may at least be partially explained by differences in E2 regimens and behavioural testing procedures.

The present study evaluated the effects of E2 on the rate of acquisition in a matching-to-place (MTP) task in a T-Maze using a hormone administration regimen meant to closely mimic circulating E2 levels seen during the œstrus and pro-œstrus phases of the natural œstrous cycle. Post-acquisition working memory performance was also evaluated using a series of extended delays in the same MTP task which, in effect, adapted it into a DMTP procedure. In addition, an OVX + vehicle group was included as a control during both acquisition and working memory testing in order to provide a basis for comparison with other studies using a similar control group. The main purpose of this study was to evaluate working memory independently from acquisition. Many paradigms

test working memory from the inception of training as the rules of the task are still being acquired. Here, the use of extended delays during the DMTP phase only after a predefined set of criterion for acquisition has been reached in the MTP task may allow for a more specific evaluation of œstrogenic effects on working memory. The acquisition of some behavioral tasks, including the presently used MTP task, necessarily requires the use of working memory during acquisition. Likewise, there is a learning component innate to some working memory tasks, including the present DMTP paradigm using extended delays in a MTP task. However, by minimizing the use of working memory during acquisition training through brief inter-trial intervals and depreciating the learning component during the working memory phase by using post-criterion testing it may be possible to assess the influence of E2 on either acquisition or working memory with only a minor influence from the other. In this way, the effects of physiologically high and low E2 levels on the acquisition of a task and on working memory can be investigated relatively independently.

The influence of high E2, low E2, and vehicle administration in young adult OVX female rats on the acquisition of a MTP task and post-criterion working memory performance in a DMTP task in a T-Maze was assessed. It was hypothesized that subjects with physiologically high levels of E2 would require fewer trials to achieve a criterion performance than both OVX + vehicle animals (e.g. Gibbs 1999) and animals administered physiologically low levels of E2 (Holmes, Wide et al. 2002). It was also hypothesized that subjects with low physiological levels of E2 would require fewer trials to acquire the task than OVX + vehicle animals. Based upon previous findings by Bimonte & Denenberg (1999), it was hypothesized that animals with high physiological

levels of E2 would make a greater number of correct arm choices than either animals with low levels of physiological E2 or vehicle animals, particularly as working memory load increased during the longer extended delays. Finally, it was hypothesized that animals with low physiological levels of E2 would make a greater number of correct arm choices at longer extended delays than OVX animals receiving only a vehicle control, as previously demonstrated (Fader, Johnson et al. 1999; Holmes, Wide et al. 2002).

2. Methods

Subjects. 37 young adult, female, Sprague-Dawley rats (Charles River, St. Constant, Quebec) aged three months at the time of arrival and weighing approximately 250-300 grams were used in this experiment. Upon arriving, all animals were allowed one week to habituate to the animal facility. Before behavioural training began, animals were pair-housed in polyurethane shoebox cages, maintained on a reverse 12h:12h light/dark cycle with lights off from 0900-2100h, and were allowed standard lab chow and water *ad libitum.* The rats were handled daily from time of arrival until completion of the experiments.

Five days prior to the commencement of behavioural training all animals were transferred to individual housing, placed on a food-restriction diet, and maintained at 90% of their individual free-feeding weight until completion of testing. All training was performed at the start of the dark phase of the light/dark cycle beginning at 0900h and ending at approximately 1200h. All behavioural testing and surgical procedures were

conducted in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Concordia University Animal Research Ethics Committee.

Surgery and Hormone Administration. Subjects were randomly assigned to one of three groups; high E2 (n=9), low E2 (n=10), or vehicle (n=10). Approximately one week after arrival, all subjects were anesthetised using a mixture of ketamine (50 mg/ml; CDMV, St Hyacinthe, Quebec) and xylazine (4mg/ml; CDMV) in a 4:3 ratio (1 ml/kg, IP) and bilaterally ovariectomised using a standard aseptic procedure through a dorsal incision. Post-surgical care included a single administration of the antibiotic Baytril (0.03 ml/animal, SC; CDMV), the analgesic banamine (0.03 ml/animal, SC; CDMV), and 0.9% saline (3 ml/animal, SC). During OVX surgeries, all animals were subcutaneously implanted with a Silastic tube containing either 100% cholesterol (vehicle group; Sigma Chemical Co., St Louis, MO) or 5% 17β-E2 benzoate (high E2 and low E2 groups; Sigma) in cholesterol in the nape of the neck. This 5% implant produces a serum concentration of approximately 20-25 pg/ml, which is consistent with naturally circulating levels of E2 such as those seen during the œstrus phase of the œstrous cycle (Butcher, Collins et al. 1974; Mannino, South et al. 2005; Quinlan, Hussain et al. 2008) . Animals in the high E2 group received additional daily subcutaneous injections of 17β-E2 (10μg/kg) dissolved in sesame oil (Sigma) designed to achieve circulating E2 levels similar to those seen during the pro-œstrus phase of the œstrous cycle (75-90 pg/ml; Butcher, Collins et al. 1974; Quinlan, Hussain et al. 2008). Animals in the low E2 and vehicle groups received daily subcutaneous injections consisting of sesame oil vehicle

(1ml/kg). E2 replacement and vehicle injections began two days before habituation training and were administered after completion of testing between 1200-1400h each day.

Apparatus. Training for all subjects was carried out in a black polyurethane T-maze placed on a table one meter above the floor with black walls extending 23cm above a wire grid floor 10.5cm wide with removable clear polyurethane roof panels. The T-maze had three arms arranged at 90° angles around a 14x14cm central chamber; two goal arms and a start arm, all of which were 75cm in length. Entrance to the central chamber from the start arm could be occluded by black polyurethane guillotine gate which could be lifted by the experimenter using a string from a remote location. Entrance to the goal arms from the central chamber were occluded in a similar manner.

The start arm contained a start-box 30cm in length which could be blocked off from the rest of the start arm and the central chamber by a black polyurethane guillotine gate halfway down the arm. Each goal arm contained a white ceramic bowl in which a food reward (Kellogg's Froot Loops®) could be placed. Froot Loop crumbs were placed underneath both goal arms of the maze during all trials to prevent any confounds due to odor cues. For all trials, the maze was kept stationary in a position relative to all extra-maze cues throughout testing. All testing took place in a dim, semi-lighted room with extra-maze cues that included a poster on a plain white wall opposite blue cupboards. The experimenter always stood in the same location at the foot of the start box.

Behaviour. Subjects were trained in a MTP task in a T-Maze during both acquisition and working memory testing. A MTP task was used to account for any innate tendencies by the animals to alternate arms from trial to trial (Douglas 1966). All subjects received once-daily 15-min habituation sessions in the T-Maze for three consecutive days. During each session subjects had free access to all components of the T-Maze. Froot Loops were scattered throughout the apparatus on the wire grid floor of the T-Maze and in the food bowls.

The next day following habituation, all subjects began pre-training in the T-maze which consisted of 10 trials per day per rat. Food rewards (half of a Froot Loop) were only available in the food bowls of the assigned goal arms. Pre-training consisted of a series of randomly assigned 'forced choice' runs in which each animal was guided to either the right or left goal arm via occlusion of the opposite goal arm by the polyurethane gate. The open goal arm contained a ceramic bowl which was baited with the food reward. Rats were placed in the start-box behind the guillotine gate. Once the appropriate gate panels of the goal arms were raised by the experimenter the animal was released from the start-box and was free to traverse the maze towards the choice point in the central chamber. The trials were pseudo-randomly assigned each day so that the right and left goal arms were both baited five times. A trial was ended when all four limbs of a rat crossed into a goal arm and the gate could be closed. Each animal was allowed a maximum of two minutes to enter the goal arm after which it was allowed a maximum of one minute to eat the food reward. If an animal did not enter the goal arm or did not eat the reward after entering the goal arm within the time allowed it was removed from the

T-maze and placed in its home cage until the next trial. All subjects received 'forced choice' training for five days.

MTP acquisition testing began the next day. Each trial now consisted of a 'forced choice' run followed immediately by a 'choice' run. Each animal was given a 'forced choice' run with only the assigned goal arm open and baited with a food reward. Immediately after this run, with only a brief delay (~5 seconds) during the transfer from the goal box to the start box, the animal was placed back into the start arm of the T-maze and given a 'choice' run. Here, both goal arms were open but only the arm previously baited during the preceding 'forced choice' run contained a food reward. Thus, if a 'forced choice' run directed an animal to enter the left arm for a reward, only the left arm was baited during the 'choice' run in which both goal arms were available to enter. Rats that chose correctly were allowed to eat the food reward in the arm before being returned to their home cage. Rats that chose incorrectly were allowed to investigate the empty food bowl for 10 seconds before being taken out of the arm and placed into the home cage. Animals were always tested in pairs with one animal performing the task while the other remained in its home cage. Rats were considered to have reached criterion performance and acquired the MTP task after performing a minimum of 8/10 correct trials for three consecutive days.

The day after reaching criterion, rats were subjected to the working memory phase of testing (delayed MTP) in which a series of extended delays were introduced between the 'forced choice' and the 'choice' runs during each of 10 trials in a session. Immediately after the 'forced choice' run had been completed, each animal was placed

into the start-box of the start arm in the T-maze which was occluded from the rest of the maze by a guillotine door. Prior to the door being raised by the experimenter, thereby beginning the 'choice' run, the animal remained in the start box for the duration of a prescribed delay; either 60, 80, 100, 120, 150, or 180 seconds. These extended delay periods were administered to each rat in a random sequence and were considerably longer than the brief delay experienced during the transfer of the animal from the goal box to the start box during the latter parts of acquisition training. As during acquisition testing, if an animal entered the goal arm that had been baited on the immediately preceding 'forced choice' run, a correct choice was scored. If it entered the opposite arm, an incorrect choice was scored. Here, a working memory error was defined as an incorrect choice made during the 'choice' run.

Statistical Analyses. Rate of acquisition in the MTP task in a T-Maze was analyzed using a one-way analysis of variance (ANOVA) by E2 level. The first day of criterion performance achievement (8/10 correct arm choices) was used as the point of comparison. Significant differences were further analyzed using Tukey's honestly significant difference (HSD) post-hoc tests. Differences were considered significant when $p<.05$.

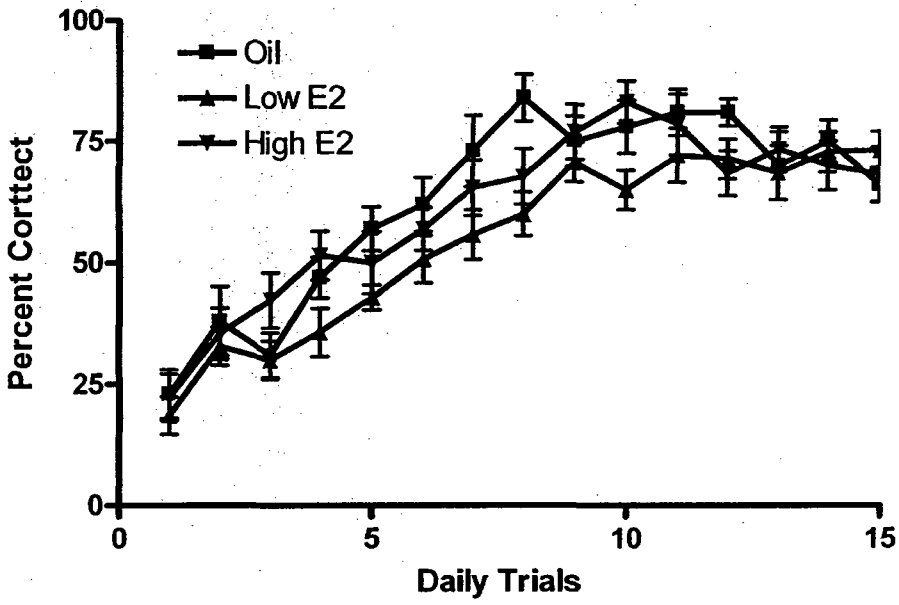
In order to test working memory performance subsequent to achievement of criterion performance, a mixed ANOVA was used to compare E2 groups (between subjects factor) and time delays (within subjects factor). Additionally, the number of correct arm choices after a delay was analyzed using t-tests. For all three groups within

each of the six delay conditions one sample t-tests were conducted comparing the mean performance of the group to a chance level of performance, or 50% (5/10) correct choices. In order to account for the possibility of false positives resulting from multiple comparisons, a Bonferroni correction was employed (.05/18). As a result, differences were considered significant when $p<.0027$.

3. Results

Acquisition. During the early stages of acquisition testing, inter-trial intervals lasted approximately 10-60 seconds but no longer than ~5 seconds by the time criterion levels of performance were being achieved. There was a significant effect of E2 level on rate of acquisition ($F(2, 33) =4.17$, $p=.02$; fig. 1A). Post hoc analysis using Tukey's HSD revealed the high E2 group required significantly fewer testing sessions to reach criterion than the low E2 group ($p=.02$). The mean (± SEM) number of testing sessions required in order to achieve criterion performance (fig. 1B) for the high E2 group was 8.31 (± 0.91), for the low E2 group 11.38 (± 0.96), and for the vehicle group 9.0 (± 0.60).

A.



B.

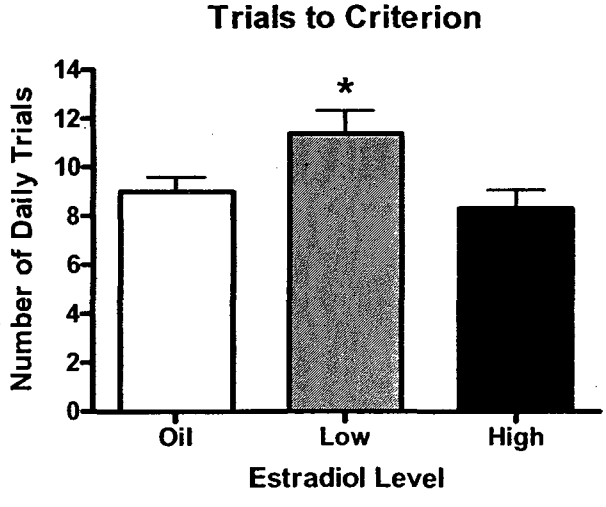


Figure 1. A) Mean (± SEM) number of corrects trials in a T maze task across training days for OVX rats that received chronic low E2, chronic high E2 or oil vehicle. B) Mean (+ SEM) number of trials to reach criterion. Low E2 rats took significantly more days to reach criterion than high E2 rats, *$p < 0.05$.

Working Memory. When comparing mean scores of correct arm choices to chance levels of performance, there were several differences within time delays that were statistically significant (fig. 2). However, a performance limit was reached during the delays of 150 and 180 seconds where none of the groups made more correct arm choices than could be expected by chance. In the 60 second delay, vehicle ($t(9)=5.62$, $p<.001$) and high E2 ($t(8)=4.74$, $p<.02$) groups performed significantly different from chance levels but low E2 animals did not. In the 80 second delay, all three groups performed significantly better than chance; vehicle ($t(9)=.6.09$, $p<.001$), low E2 ($t(9)=6.47$, $p<.001$), high E2 ($t(8)=4.36$, $p<.002$). In the 100 second delay, vehicle ($t(9)=5.66$, $p<.001$) and high E2 ($t(8)=6.49$, $p<.001$) groups performed significantly better than chance levels but low E2 animals did not. In the 120 second delay, low E2 ($t(9)=5.01$, $p<.001$) and high E2 ($t(8)=7.56$, $p<.001$) groups performed significantly better than chance levels but vehicle animals did not.

Because none of the three groups performed better than chance during the two longest delays, a mixed ANOVA was performed on the first four delays only. There was a significant interaction between time delay and E2 group ($F(6, 78) = 2.406$, $p = 0.035$). Tukey's HSD showed that the high E2 group made more correct arm choices than the low E2 group ($p = 0.04$). No other significant differences were observed.

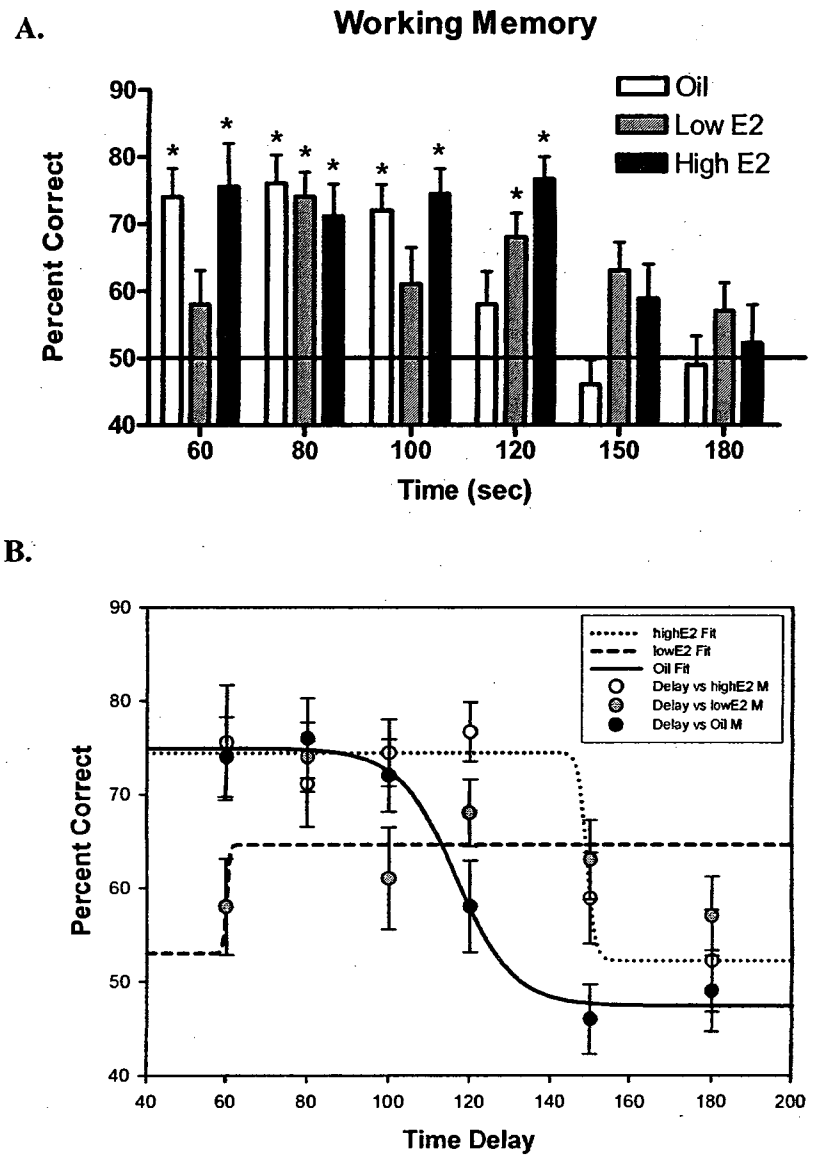


Figure 2. A) Mean (+ SEM) percent correct trials of OVX rats that received chronic high œstradiol (E2), chronic low E2, or oil vehicle in a delayed match-to-place (DMTP) working memory version of the T maze task which utilized six extended inter-trial delays (60-180 seconds). Low E2 rats performed significantly worse than the high E2 groups. *p < 0.0027 (with Bonferroni correction) indicates significantly higher than chance performance (50%). B) Percent of correct trials represented in a sigmoidal curve fit to illustrate the relative performance of each group across time trials.

4. Discussion

OVX female rats receiving physiologically high levels of E2 required significantly fewer trials to acquire a MTP task in a T-Maze than rats receiving physiologically low levels of E2. In addition, during a post-criterion working memory DMTP task in the T-Maze, high E2 rats made significantly more correct arm choices than low E2 rats. OVX + vehicle rats performed comparably to high E2 rats during both acquisition and working memory testing. These findings suggest that physiologically low levels of E2 impair performance in OVX female rats.

Effects of Physiological Levels of E2 on Acquisition of a MTP Task

A majority of studies demonstrate the modulatory nature of E2 but do not necessarily agree on its precise role. The present results indicate that low E2 impairs task acquisition when compared to subjects with high levels of E2 and OVX + vehicle subjects. High levels of E2 (~50-90 pg/ml) have been shown to improve task acquisition relative to OVX controls in a similar DMTP task, however, these studies do not include a low E2 group (Gibbs 1999; Gibbs 2002). Had the present study included only high E2 and OVX + vehicle subjects the results would demonstrate a slight, but not significant, enhancement of task acquisition by E2. This interpretation would be limited, however. When a low E2 group is included, as is the case here, the relatively poor performance of these subjects as compared to both a high E2 group and an OVX + vehicle group suggests that low E2 attenuates task acquisition. It has also been shown that low E2 enhances reinforced T-Maze alterations, however, a high E2 group was not included

(Fader, Hendricson et al. 1998). In a delayed alternation task in a T-Maze comparing OVX + vehicle, low E2, and two high E2 groups, Wide et al., (2004) found no differences in task acquisition (7, 27, 103, and 197 pg/ml, respectively). However, this paradigm included inter-trial delays meant to test working memory from the inception of training. It is possible that procedures which test working memory during acquisition training introduce additional cognitive load that affects learning. The use of a post-criterion testing paradigm along with two physiologically-relevant E2 groups shows that low E2 may impair task acquisition rather than high E2 enhancing it.

In contrast to the present findings, low levels of E2 (~15-30 pg/ml) enhance task acquisition in an 8-arm radial maze when compared to OVX + vehicle subjects (Daniel, Fader et al. 1997; Luine, Richards et al. 1998; Fader, Johnson et al. 1999). Similarly, studies utilizing a Morris water maze (Warren, Humphreys et al. 1995; Chesler and Juraska 2000) demonstrate that low levels of E2 improve task acquisition and performance. High E2 groups were not included in these studies and each involved a working memory component during acquisition. In addition, perhaps the relatively complex apparatuses and behavioral tasks are more sensitive to the influence of E2. Although it is clear E2 plays a modulatory role during task acquisition it is also apparent that the interpretation of this role is dependent upon several factors including dose of E2, task environment, cognitive task, and pre- vs. post-criterion testing.

Effects of Physiological Levels of E2 on Working Memory in a DMTP Task

According to Dudchenko (2004), working memory in rats can be defined as information concerning an object, stimulus, or location that is acquired, maintained for a short period of time, and used within a discrete testing session. This information can be retrieved and manipulated such that it may influence future behaviour (Olton and Samuelson 1976). During post-criterion testing in a DMTP task in a T-Maze, OVX + vehicle subjects and subjects with high physiological levels of E2 made more correct arm choices than animals with low physiological levels of E2. However, as working memory load increased a performance limit was reached and none of the groups make more correct arm choices than can be expected by chance. These findings do not agree with other studies using a post-criterion task in a T-Maze which demonstrate a beneficial effect of high E2 (~50-90 pg/ml; Gibbs 1999; ~50-90 pg/ml; Gibbs 2002). However, these studies do not include a low E2 group. Additionally, the series of extended delays employed in these studies was somewhat shorter (10-90 seconds) and may not have been robust enough to demonstrate the effect of E2. Using the present paradigm with delays of 10, 30, 45, and 60 seconds, high E2 and low E2 groups do not differ in performance until the longest delay (Quinlan et al., unpublished data). Similarly, in a water maze, only when working memory load increased did higher levels of administered E2 (50 pg/ml) benefit performance (Bimonte and Denenberg 1999). Furthermore, this effect of E2 was not evident until the latter stages of testing after extensive training.

It has been shown that, relative to OVX + vehicle controls, subjects with high E2 (103 and 197 pg/ml) make more errors in a T-Maze while subjects with low E2 (27

pg/ml) make fewer errors (Wide, Hanratty et al. 2004). Here, working memory was evaluated from the inception of training prior to achievement of criterion performance. In pre-criterion testing paradigms, cognitive load seems to be ample enough to necessitate the beneficial effects of E2. Perhaps the nature of post-criterion paradigms in a T-Maze, with only two possible goal sites from the choice point, require longer extended delays with which to increase working memory load and demonstrate an effect. Thus, the differing effects of E2 may be explained by differences in behavioural procedures from study to study. For example, in a T-Maze, high E2 subjects in a pre-criterion testing paradigm make more errors than an OVX + vehicle control (Wide, Hanratty et al. 2004) while during post-criterion testing high E2 levels enhanced performance (Gibbs 1999; Gibbs 2002). It is also possible the use of different apparatuses may play a role in these differences. Acute administration of E2 improved performance in a place task in a plus maze (Korol and Kolo 2002) but attenuated performance in a Morris water maze (Chesler and Juraska 2000; Snihur, Hampson et al. 2008).

Studies which utilize relatively complex paradigms may more readily differentiate the effects of E2 on working memory. Performance in the Morris water maze is improved during estrus in naturally-cycling females (Warren, Humphreys et al. 1995) and in subjects administered low levels of E2 (Chesler and Juraska 2000). Likewise, studies employing the 8-arm radial maze have shown that low levels of E2 (15-30 pg/ml) are sufficient to improve working memory performance (Daniel, Fader et al. 1997; Luine, Richards et al. 1998; Fader, Johnson et al. 1999). Holmes et al. (2002) report that in an 8-arm radial maze low E2 levels (23 pg/ml) decrease working memory errors relative to an OVX control but moderate (38 pg/ml) and high (102 pg/ml) levels of E2 increase

working memory errors. Supraphysiological levels of E2 (225 pg/ml), on the other hand, do not have an effect on working memory performance (Luine and Rodriguez 1994; Galea, Wide et al. 2001). It has also been shown that E2 has varied effects in different brain regions. In an 8-arm radial maze, working memory performance is enhanced by high levels of E2 injected into the prefrontal cortex but only by low levels of E2 when injected into the dorsal hippocampus (Sinopoli, Floresco et al. 2006). Similar to the differential effects of high E2 and low E2 seen in the T-Maze (Wide, Hanratty et al. 2004), perhaps these results can be explained by differences in behavioural procedures, such as pre- vs. post-criterion testing, and the differential effects of E2 in specific regions of the brain.

Effect of E2 on Performance

It is clear that E2 plays an important modulatory role in task acquisition and working memory performance. What is not so evident, however, is the exact nature of this role and the factors that influence it. The basis for many of the differences in the literature may lie in varied hormone administration procedures and dissimilar behavioural testing paradigms as well as the differential effects of E2 in various brain regions and alternative memory systems required for specific tasks.

There are a number of methods which have been utilized to study the effects of E2 on learning and memory performance. Some studies use naturally-cycling subjects (Warren, Humphreys et al. 1995; Bimonte and Denenberg 1999) while those utilizing OVX subjects administer exogenous E2 through either subcutaneous implants (Daniel,

Fader et al. 1997; Luine, Richards et al. 1998; Bimonte and Denenberg 1999; Fader, Johnson et al. 1999; Gibbs 1999; Gibbs 2002) or daily subcutaneous injections of E2 in oil (Fader, Hendricson et al. 1998; Chesler and Juraska 2000; Galea, Wide et al. 2001; Holmes, Wide et al. 2002; Wide, Hanratty et al. 2004; Sinopoli, Floresco et al. 2006). In an effort to mimic natural œstrous cycle conditions, the current study employed both implants and injections. Use of E2 implants maintains a steady low baseline level of E2 in both high and low groups throughout testing while daily injections to the high E2 group imitate the pulsatile nature of E2 seen in the pro-œstrus phase (see Quinlan, Hussain et al. 2008). Differing methods of administration from study to study often produce differing levels of circulating E2 and may contribute to discrepancies when comparing results. Interpretation of results may also be complicated due to the steady, chronic levels achieved with E2 implants versus daily spikes of E2 resulting from daily injections. Additionally, recent evidence has shown that increased handling, such as during daily injections, may obscure the enhancing effects of E2 in behavioral tasks (Bohacek and Daniel 2007). A similar effect may be associated with the daily swabs necessary when utilizing naturally-cycling subjects.

The timing of E2 administration is important as well. Initial studies demonstrate that E2-based changes in the hippocampus of OVX subjects require administration 24 and 48 hours prior to testing (Woolley and McEwen 1992). Memory retention is enhanced 24, but not 8, hours after the second administration (Sandstrom and Williams 2004). E2 administration in this manner also improved performance in a place task (Korol and Kolo 2002). The timing of E2 replacement relative to OVX also matters. In aged subjects, E2 implanted immediately, but not five months, after OVX improved task

acquisition and memory performance (Daniel, Hulst et al. 2006). Similarly, Gibbs (2000) has shown that E2 implantation during or three months, but not 10 months, after OVX enhances working memory. Disparate regimens of hormone administration among studies in which E2 is given through different routes and at different times relative to OVX may account for some of the differences in E2 effects.

It is also important to consider the control group, or point of comparison, when interpreting the effects of E2. As with the current findings, high E2 levels may appear to enhance learning and memory when compared to a single OVX + vehicle group. With the inclusion of an additional comparison group, however, it becomes apparent that low E2 impairs performance. The OVX + vehicle control reliably serves as a model for the effects of menopause and has been repeatedly utilized to illustrate a beneficial effect of E2 on cognition. However, when utilizing young adult females, this paradigm does not accurately reflect circulating E2 in naturally-cycling subjects. OVX studies using hormone regimens which administer physiological levels of high and low E2 may provide a more accurate representation of estrogenic effects on performance in behavioral tasks. When comparing the performance of high vs. low E2 subjects the effects of hormone administration may not be as robust as when comparing an E2 group to an OVX + vehicle group. However, the alternative use of an OVX + vehicle control or two E2 groups in different studies may explain some of the discrepancies in findings when using similar behavioural procedures and testing apparatuses but dissimilar control groups.

Perhaps the least considered aspect of E2 studies on cognition is the interdependent nature of the learning and working memory phases of the procedure. The

acquisition of the rules of a task sometimes requires the use of working memory while performance during the working memory component of a task often demands an element of learning. The majority of studies do not differentiate a learning phase from a working memory phase and, in fact, have need to as they are investigating the effect of E2 on the acquisition of a working memory task. However, it is possible that the cognitive load associated with maintaining a piece of information may interfere with learning the rules of the task. Likewise, attempting to learn the rules of the task may interfere with the ability to maintain and use that piece of information. Hence, the use of a post-criterion testing paradigm may provide a more unambiguous evaluation of the acquisition of the rules of the task prior to achievement of criterion by minimizing any working memory components. Similarly, this paradigm may also provide an opportunity to more clearly interpret the effects of E2 on working memory after criterion achievement by ensuring the rules of the task are well-learned and extensively practiced.

The idea that E2 may differentially affect task acquisition and working memory performance not only implies the availability of multiple memory systems but the suitability of different systems for different tasks. This is not a new concept (e.g. Tolman, Ritchie et al. 1946). Packard & McGaugh (1996) have demonstrated that inactivation of the hippocampus leads to an enhancement of response learning while inactivation of the striatum leads to augmented place learning. E2 has been shown to differentially modulate performance in striatum-dependent response learning tasks and hippocampus-dependent place learning tasks (Korol and Kolo 2002; Daniel and Lee 2004) as well as cognitive strategies used to solve tasks after learning (Korol, Malin et al. 2004; Quinlan, Hussain et al. 2008). It is not only possible, but likely, that specific memory systems will be

involved in solving particular tasks and that physiologically high and low levels of E2 will have different effects in different systems (e.g. Sinopoli, Floresco et al. 2006). It is also likely that more than one memory system may be involved in solving a task. For example, it has been shown that male rats initially use a hippocampus-dependent place strategy in a plus-maze but use a striatum-dependent response strategy after several days of training (Chang and Gold 2003).

The effects of differing E2 levels in various memory systems may be especially pertinent to several types of behavioral tasks in which dopamine is essential, including working memory (see Williams and Castner 2006). Different levels of E2 have been shown to affect dopamine transmission in several brain regions including the striatum (Xiao and Becker 1994; Thompson and Moss 1997) and the prefrontal cortex (Dazzi, Seu et al. 2007). Dopamine antagonists have been shown to interact with E2 to affect cognitive strategy use (Quinlan, Hussain et al. 2008), an effect that is dependent upon dopamine D1 receptors in the striatum and dopamine D2 receptors in the prefrontal cortex (Quinlan et al., submitted). The prefrontal cortex is important for working memory in both rats (Sawaguchi and Goldman-Rakic 1991) and humans (Keenan, Ezzat et al. 2001). In addition, E2 modulates acetylcholine function in the basal forebrain (Pongrac, Gibbs et al. 2004) and hippocampus (Daniel and Dohanich 2001). Inactivation of either the hippocampus or the prefrontal cortex impairs working memory performance (Lee and Kesner 2003) as does the disruption of communication between these two areas (Floresco, Seamans et al. 1997; Wang and Cai 2006). Perhaps high and low levels of E2 differentially modulate specific memory systems which will affect performance based on the behavioural procedures and cognitive requirements of a task.

The findings of this and many studies clearly demonstrate a robust, albeit complex, influence of E2 on performance during task acquisition and working memory performance. A close comparison of these findings suggests that this complexity may be based in a number of differences that exist among E2 studies including the testing apparatus, type of cognitive task, timing, delivery, and dose of E2, the control group used, and pre- vs. post-criterion testing. For instance, factors contributing to conflicting results concerning the role of E2 have been seen in studies using varied pre- vs. post-criterion testing paradigms (Gibbs 1999; Gibbs 2002; Wide, Hanratty et al. 2004), different types of apparatuses (Chesler and Juraska 2000; Korol and Kolo 2002), and dissimilar control groups (current results, Gibbs 1999; current results, Gibbs 2002). An interpretation of estrogenic results on cognition must take these factors into consideration when comparing various studies. The use of E2 implants in conjunction with daily injections may provide a more ecologically-relevant basis of comparison for these effects. In addition, the use of post-criterion testing may be a valid method with which to separate the influence of E2 on task acquisition and working memory.

Previous studies have demonstrated in rats (Jones 2002; Kritzer, Brewer et al. 2007), non-human primates (Sawaguchi and Goldman-Rakic 1991; Watanabe, Kodama et al. 1997), and humans (Keenan, Ezzat et al. 2001) that effective performance in several types of working memory tasks requires optimal function of DA in the prefrontal cortex. Although it is clear that E2 modulates performance in this paradigm, further investigation is necessary to validate this task as one which is dependent on DA efflux in the prefrontal cortex. This may be accomplished through utilization of intracranial cannulation into the mPFC and infusion of DA D1 and DA D2 receptor antagonists. In addition, the inclusion

of additional subjects with further testing may reduce high levels of variance in some of the hormone groups and address what can be seen as aberrant results, such as the low E2 group in the 80-minute delay.

CHAPTER 9:

GENERAL DISCUSSION

9. General Discussion

Findings from both human (e.g. Duff and Hampson 2000) and animal (e.g. McEwen 2002) studies have established a modulatory role for E2 in the performance of behavioral tasks. However, a consensus concerning the direction and magnitude of these effects has yet to be reached. There are a number of potential factors that may contribute to these inconsistent findings, including how E2 affects the function of neurotransmitter systems in the brain. DA is a neurotransmitter that is central to several types of behavioral tasks including response learning (Daniel, Sulzer et al. 2006), LI (Lubow 1997), and working memory (Sawaguchi and Goldman-Rakic 1991). In addition, DA activity in brain regions that support behavior in these tasks is sensitive to differential levels of circulating E2 (Becker, Robinson et al. 1982; Dazzi, Seu et al. 2007). Although several studies have described the robust effect of high levels of E2 on DA transmission in the brain (e.g. Xiao and Becker 1994; Dazzi, Seu et al. 2007), there are relatively few studies that investigate the behavioral consequences of this neurobiological action, especially in behavioral tasks. Hence, the studies contained in this thesis were conducted to further investigate the behavioral consequences of high and low levels of E2 on performance in DA-mediated behavioral tasks. In addition, the effect of E2 on DA transmission and quantity of synaptic proteins in the DS, mPFC, and HPC, brain regions which support behavior in these tasks, was examined to determine the role of structural and/or functional changes in these areas which may contribute to the modulation of behavior.

9.1. Review of Findings

In general, the findings from the present studies suggest that E2 exerts a modulatory influence on DA transmission in the mPFC and the DS which alters performance in behavioral tasks supported by these brain regions. Specifically, Study 1 demonstrated that rats conditioned during proestrus, a period of high E2 levels, do not display LI during the testing phase. LI is thought to primarily be mediated by dorsal striatal DA (Lubow 1997). This attenuation is only found in adult animals indicating that it is the activational (pubertal) effects of E2 which interfere with LI. Response learning (Packard and White 1991) and response strategy (Compton 2004) have also been shown to be dependent on dorsal striatal DA. Study 2 confirmed that differential levels of E2 bias the use of a particular cognitive strategy when solving a maze for a reward. Furthermore, the systemic administration of D1R and D2R antagonists alters the strategy use of low E2 animals such that they switch from predominant use of a response strategy to use of a place strategy. Utilizing intracranial injections, Study 3 established the DS as a brain region in which D1R antagonists act to cause the switch in strategy seen by low E2 animals. Study 4 found that the change in strategy use by low E2 rats after systemic injection of D2R antagonists is regulated, at least partially, by the mPFC. D1R antagonists also act in the mPFC to cause a switch of strategy by low E2 rats.

To determine the effect of the acute administration of E2 in the DS, Study 5 utilized *in vivo* microdialysis to measure DA. Here, the local infusion of E2 results in a rapid and transient increase of extracellular DA levels. Similarly, Study 6 found a non-significant trend towards the modulation of baseline DA transmission in the mPFC by different levels of E2. However, there were no differences in synaptic protein quantities

in the DS, mPFC, or HPC among low E2, high E2, or OVX rats. Based on the idea that E2 may modulate medial prefrontal cortical DA, Study 7 investigated the effects of E2 on working memory performance, thought to be primarily mediated by medial prefrontal cortical DA (Sawaguchi and Goldman-Rakic 1994; Dalley, Cardinal et al. 2004). In this task, low levels of E2 impair performance when compared to high E2 rats.

9.2. Possible modes of action for estrogenic changes in DA-mediated behaviors

A number of studies have shown that E2 modulates performance in behavioral tasks which are mediated by DA, including LI. There are two primary theories concerning how the LI effect develops; that it is a learned inattentional response due to repeated presentations of a neutral stimulus (Lubow 1997), or that it is the result of competing associations formed during the conditioning phase (Escobar, Oberling et al. 2002). LI is dependent on dorsal striatal DA (Lubow 1997) and interference with DA transmission (agonistic or antagonistic) attenuates performance in this task (Solomon, Crider et al. 1981; Konstandi and Kafetzopoulos 1993; Ellenbroek, Knobbout et al. 1997; Jeanblanc, Hoeltzel et al. 2003). Similarly, previous studies have found that high levels of E2 present during conditioning abolish LI in OVX females (Nofrey, Ben-Shahar et al. 2008). The current findings show that this is also true for rats conditioned during proestrus (Study 1). It has been consistently shown that high levels of E2 result in enhanced levels of baseline and stimulant-induced DA release in the DS (Becker and Beer 1986; Xiao and Becker 1994; Becker 1999). It is possible that when high levels of E2 are present during the conditioning phase and DA transmission is augmented that this

interferes with the ability to ignore an irrelevant stimulus or to form the necessary associations for effective LI during the testing phase. Thus, high levels of E2 amplify dorsal striatal DA transmission to such a degree that it has a negative impact on the acquisition of the LI task. Accordingly, rats in estrus or metestrus, phases of the estrous cycle associated with low levels of E2, exhibit LI during the testing phase. These results provide evidence that high levels of E2 may adversely affect the expression of a dorsal striatal DA-mediated behavior. However, further testing is necessary to confirm that the estrogenic modulation of DA in the DS is the primary cause of attenuated LI behavior. Future work might utilize *in vivo* microdialysis during both the conditioning and testing phases of the LI paradigm so that extracellular DA levels may be correlated to behavior in this paradigm.

Performance in other types of behavioral tasks dependent on dorsal striatal DA, such as response learning in a maze (Packard and White 1991; Packard and McGaugh 1996; Mizumori, Yeshenko et al. 2004), are also sensitive to different levels of E2. Both OVX female rats (Korol and Kolo 2002) and cycling females with low levels of E2 (Korol, Malin et al. 2004) acquire a response learning task more quickly compared to rats with high levels of E2. Likewise, rats with low levels of E2 perform more accurately in a response variant of the T-maze (Davis, Jacobson et al. 2005). Conversely, low levels of E2 impair performance in a cue-deficient version of the Y-maze (Zurkovsky, Brown et al. 2007) and a cue-based water maze after removal of the cue (Daniel and Lee 2004). High E2 rats perform more accurately in place learning tasks mediated by the HPC (Packard and McGaugh 1996; White and McDonald 2002; Mizumori, Yeshenko et al. 2004).

The present findings (Studies 2, 3, and 4) confirm the tendency to use a response strategy by low E2 rats and the predominant use of a place strategy by high E2 rats. Moreover, the antagonism of D1Rs and D2Rs results in a switch of strategy use by low E2 rats but has no effect in high E2 rats. It is possible that the enhancement of dorsal striatal DA by high levels of E2 (e.g. Becker 1990) affects the ability of a rat to effectively use a response strategy to solve a maze. Although both response and place strategies are available at all times, it appears that one or the other may be more advantageous in certain situations (Chang and Gold 2003). The present findings, along with electrophysiological studies (Mizumori, Yeshenko et al. 2004), suggest that E2 may alter the effectiveness of one or both of these strategies and bias the use of the other. In this manner, high levels of E2 may create a situation in which hippocampal function is enhanced (for a review, see: McEwen 2002), dorsal striatal DA release is altered (Becker 1990), and a HPC-mediated place strategy is the more efficacious strategy. On the other hand, low E2 may create a situation in which dorsal striatal DA function is at a lower baseline level and the use of a response strategy is biased. Here again, these findings suggest that E2 alters DA activity in the brain and that this modulation can affect the expression of DA-mediated behaviors.

The strategy use of low E2 rats, but not high E2 rats, is also affected by D1R and D2R antagonism in the mPFC suggesting that differential levels of E2 may also mediate other types of behavioral tasks. Working memory is a behavior for which medial prefrontal cortical DA has been shown to be important in rats (Jones 2002), monkeys (Sawaguchi and Goldman-Rakic 1991), and humans (Keenan, Ezzat et al. 2001). DA in this brain region is modulated by E2 such that high levels of E2 decrease extracellular

DA while low levels of E2 augment DA release (Luine, Richards et al. 1998; Dazzi, Seu et al. 2007). The findings from Study 7 indicate that E2 affects both the acquisition of a working memory task and performance during a post-criterion working memory phase. Here, rather than high levels of E2 improving task performance, low levels of E2 seem to impair accuracy. During both acquisition and working memory testing, OVX rats receiving a vehicle performed comparably to high E2 rats. Although these differences did not reach significance versus low E2 rats, there was a strong trend towards greater accuracy by OVX rats during acquisition and working memory testing.

Several previous studies have demonstrated that any level of E2 improves performance in working memory tasks when compared to OVX subjects (Gibbs 1999; Gibbs 2000; Holmes, Wide et al. 2002; Wide, Hanratty et al. 2004), however, this disparity in results may be founded in procedural differences such as the dose of E2 used, the cognitive task employed, and the use of pre- versus post-criterion testing of working memory. Nonetheless, these present study agrees with previous findings in that E2 modulates performance in working memory tasks. The current results further suggest that it is the estrogenic modulation of DA in the brain that can affect the performance in behavioral tasks. Additional testing is necessary to confirm the role of DA in this particular version of the working memory task, especially as this study is one of only a few (e.g. Bimonte and Denenberg 1999) to utilize a post-criterion testing paradigm. Future studies might employ intracranial infusions of DA receptor antagonists into the mPFC during both the acquisition and working memory phases of testing to evaluate the role of DA. Other studies that may be helpful could include the testing of male rats as a point of comparison versus OVX females receiving vehicle, low E2, or high E2 as well as

a comparison of pre-criterion versus post-criterion testing for working memory performance.

It is likely that the behavioral changes in LI, strategy use, and working memory mediated by E2 are based in the alteration of structure and function in dopaminergic target regions of the brain, including the HPC, DS, and mPFC. Several studies have demonstrated that E2 results in alterations of synaptic density and protein quantities in the brain, especially the HPC (Gould, Woolley et al. 1990; Woolley and McEwen 1992; Brake, Alves et al. 2001). The present findings did not agree with these studies although it is most likely due to methodological concerns stemming from a lack of sensitivity of Western immunoblotting. Future studies might utilize a more sensitive technique, such as radiolabelled immunocytochemistry (Brake, Alves et al. 2001), or a more extensive hormone treatment regimen employing chronic administration of E2 (Sharma, Mehra et al. 2007).

While the present findings did not reveal any differences in synaptic structure related to the presence of E2, they do provide evidence for changes in DA transmission in response to different levels of E2. Study 5 found a rapid and transient increase in baseline DA levels after a local infusion of E2. These effects were short-lived and had no effect on amphetamine-induced DA release one hour later. Similarly, chronic administration of E2 had no effect on amphetamine-induced DA levels in the mPFC (Study 6). Additionally, there was no significant difference among OVX, low E2, and high E2 rats in medial prefrontal cortical baseline DA levels. The few studies which have examined the effect of E2 on DA in this region have found differences in baseline DA levels and it is possible that the lack of significance here is due to a high rate of attrition and large variances

within groups. With the continuation of these studies and the inclusion of more subjects, it is likely that differences similar to previous studies will emerge. Nonetheless, these studies contribute further evidence which suggests that E2 can affect DA transmission in brain regions important to behavioral tasks.

9.3. A proposed framework

Taken as a whole, the studies contained in this thesis suggest that the estrogenic modulation of DA transmission in the DS and mPFC mediates behavioral performance in behavioral tasks. Based on these findings, a hypothetical framework is proposed here that may account, in part, for the influence of E2 on dopaminergic brain regions and how it may affect behavior in behavioral tasks. This framework is a speculative attempt to elucidate the direct and indirect roles of the HPC, DS, and mPFC in the modulation of DA-mediated behaviors by E2.

It is clear that the estrogenic modulation of DA in the brain alters performance in a number of DA-mediated behavioral tasks. This is most apparent in the abolition of LI by high levels of E2 and the impairment of working memory performance by low levels of E2. These learning and memory behaviors are thought to be directly regulated by DA in the DS (Lubow 1997) and the mPFC (Sawaguchi and Goldman-Rakic 1991), respectively. Accordingly, interference with dorsal striatal DA directly affects LI (Konstandi and Kafetzopoulos 1993; Ellenbroek, Knobbout et al. 1997; Jeanblanc, Hoeltzel et al. 2003) and interference with medial prefrontal cortical DA directly affects working memory performance (Sawaguchi and Goldman-Rakic 1994; Watanabe,

Kodama et al. 1997; Sawaguchi 2001; Jones 2002). E2 also affects LI (Nofrey, Ben-Shahar et al. 2008; Arad and Weiner 2009) and working memory performance (Fader, Johnson et al. 1999; Wide, Hanratty et al. 2004; Daniel, Hulst et al. 2006; Sinopoli, Floresco et al. 2006) although it is thought to do so in an indirect manner. It is possible that the estrogenic modulation of DA in the DS and mPFC alters DA transmission in these brain areas and subsequently affects the DA-mediated behaviors they support. For example, local infusions of E2 into the mPFC enhance working memory performance (Sinopoli, Floresco et al. 2006). Although direct infusions of E2 into the DS during LI testing have not been investigated, evidence showing E2 enhances dorsal striatal DA release (Becker and Beer 1986) suggests that this would result in the disruption of LI.

Local infusions of E2 into the DS have been shown to disrupt response learning in a cue-deficient maze (Zurkovsky, Brown et al. 2007). Response learning and response strategy are thought to be primarily mediated by dorsal striatal DA (Packard and McGaugh 1996; White and McDonald 2002) and high and low levels of E2 differentially bias strategy use (Korol and Kolo 2002; Korol, Malin et al. 2004; Davis, Jacobson et al. 2005). As noted above, this may be attributed to the enhancement of hippocampal function and the augmentation of dorsal striatal DA by high levels of E2. These high levels may result in a decrease in the efficacy of a response strategy while increasing the effectiveness of a place strategy. Thus, high E2 rats predominantly use a place strategy while low E2 rats primarily use a response strategy. Electrophysiological studies show that dorsal striatal neurons preferentially fire during specific movements, such as directional turns, while hippocampal neurons fire in response to general movement (Ragozzino, Leutgeb et al. 2001; Mizumori, Yeshenko et al. 2004). In addition, dorsal

striatal neurons are sensitive to alterations in the properties of rewarding stimuli while neurons in the HPC are responsive to visual and contextual changes in the environment (Knierim 2002). Hence, an enhancement of hippocampal function by high E2 may place focus on the spatial and environmental cues leading to the reward and reduce the emphasis on the performance of a directional turn. In addition, the enhancement of dorsal striatal DA transmission by high E2 may interfere with the ability of neurons in this region to effectively support an egocentric representation of the environment. In low E2 rats, DA transmission is at a baseline level and may result in the DS being more able to regulate the expression of a response strategy.

While it is clear that the DS and HPC play a direct role in the mediation of cognitive strategy, it appears that the mPFC is also important, at least in rats with low levels of E2. Local antagonism of D1Rs and D2Rs in this brain region results in a switch of strategy use, but only by low E2 rats. Because inactivation of the DS leads to a blockade of response learning (Packard and McGaugh 1996), it is unlikely that the mPFC is the primary mediator of strategy use. Therefore, there are three possible means by which D1R and D2R antagonism in the mPFC could affect use of a response strategy in low E2 rats; through reciprocal connections with the HPC, through direct projections to the DS, and/or through indirect projections to the DS via the midbrain, primarily the VTA.

A significant portion of the pathway between the HPC and the mPFC consists of hippocampal efferents (Jay and Witter 1991; Conde, Bicknell et al. 1995; Carr and Sesack 1996) but there is evidence of reciprocal communication consisting of mPFC efferents to the HPC (Goldman-Rakic, Selemon et al. 1984; Wall and Messier 2001) and

its related cortices (Sesack, Deutch et al. 1989; Takagishi and Chiba 1991). Because the HPC is central to use of a place strategy, these medial prefrontal cortical projections could influence the tendency to use a place strategy. It is also possible that direct projections from the mPFC to the DS (Alexander, DeLong et al. 1986; Sesack, Deutch et al. 1989; Takagishi and Chiba 1991; Berendse, Galis-de Graaf et al. 1992) may affect the use of response strategy. Electrical stimulation of the mPFC has been shown to enhance DA release in this brain region (Taber and Fibiger 1993) and interference with dorsal striatal DA transmission alters response learning (Daniel, Sulzer et al. 2006) and strategy use (Study 3). A third possibility is that the medial prefrontal cortical influence on the DS could occur through a more indirect route, possibly via reciprocal connections from the mPFC through the VTA (Swanson 1982; Oades and Halliday 1987; Carr and Sesack 2000) or the SN (Ferreira, Del-Fava et al. 2008). These small subsets of dopaminergic inputs mainly project to striosomes in the DS which are involved in sensorimotor processing (van Domburg and ten Donkelaar 1991).

Medial prefrontal cortical DA is also implicated in cognitive set-shifting (Ragozzino, Detrick et al. 1999; Floresco, Block et al. 2008) and behavioral flexibility (Floresco and Magyar 2006; Ragozzino 2007). For example, although the inactivation of the mPFC has no effect on the acquisition of a place or response task, deficits in this region impair cross-modal shifting when rats are required to change from place to response discrimination, and vice versa, after learning (Ragozzino, Detrick et al. 1999; Ragozzino, Wilcox et al. 1999). Similar perseverative deficits are seen after disruption of medial prefrontal cortical function when rats are required to switch rules in an 8-arm radial maze (Joel, Weiner et al. 1997) and when switching use of foraging strategies

(Seamans, Floresco et al. 1995). This deficit is not evident during performance in the dorsal striatal DA-dependent LI task (Joel, Weiner et al. 1997).

Although both strategies are generally available for use, the HPC-mediated place strategy may be more effective during early stages of training while the DS-mediated response strategy may be more effective during later stages of training (Figure 1). Chang and Gold (2003) have shown that male rats initially use a place strategy and, with extensive training (approximately 40-60 trials), switch to use of a response strategy. This suggests that, during initial learning in a maze, the HPC is required to create a spatial map of the environment and the location of the reward while, after repeated trials, the maze can be solved using a habitual response mediated by the DS. The current findings (Studies 3, 4, and 5) suggest that high levels of E2 may interfere with this ability to make the transition and that the mPFC may play a key role in this process. Likewise, the antagonism of D1Rs and D2Rs in this brain region affect the ability of low E2 rats to make this transition (Figures 2 & 3). Previous studies suggest that the mPFC is important in mediating a shift in strategy use when changing environmental conditions, such as changes in hormone levels or DA transmission, necessitate the use of a novel cognitive strategy (Ragozzino 2007). Inactivation of the DS has been shown to result in impaired behavioral flexibility but this deficit is due to an inability to learn a new response strategy rather than a perseveration of a previously learned strategy. (Ragozzino, Ragozzino et al. 2002). Because D1Rs maintain persistent levels of activity in the mPFC and D2Rs decrease inhibition of medial prefrontal cortical cells, it has been hypothesized that activation of D2Rs enables the inhibition of a previous strategy while

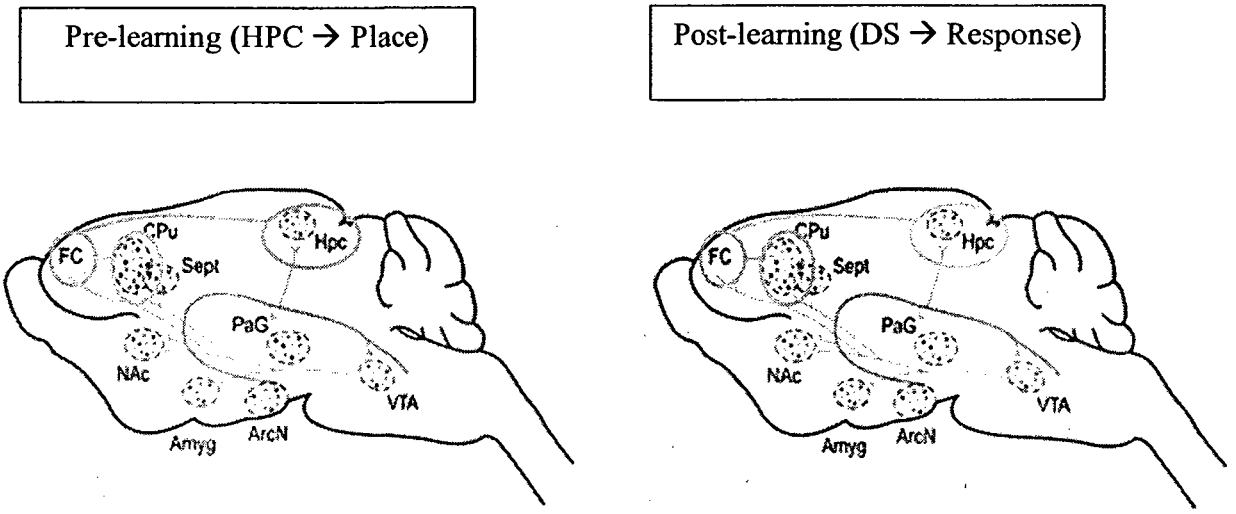


Figure 1. Speculative representation of the brain regions involved in strategy use. A place strategy (HPC) is used in the initial stages of training while a response strategy (DS) is used after repeated trials. Both are generally available for use.

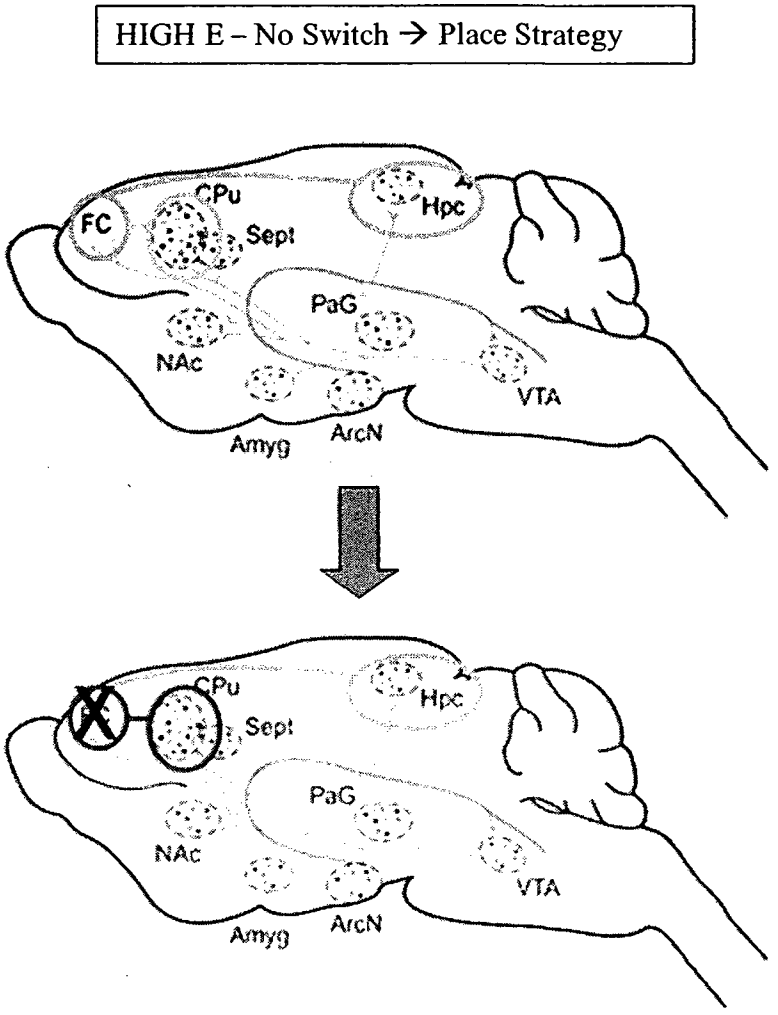


Figure 2. Speculative representation of the brain regions involved in the strategy use of high E2 females subsequent to DA receptor antagonism. High E2 enhances hippocampal function and augments dorsal striatal DA release and may result in a bias to use a HPC-mediated place strategy. DA receptor antagonism has no effect on strategy use.

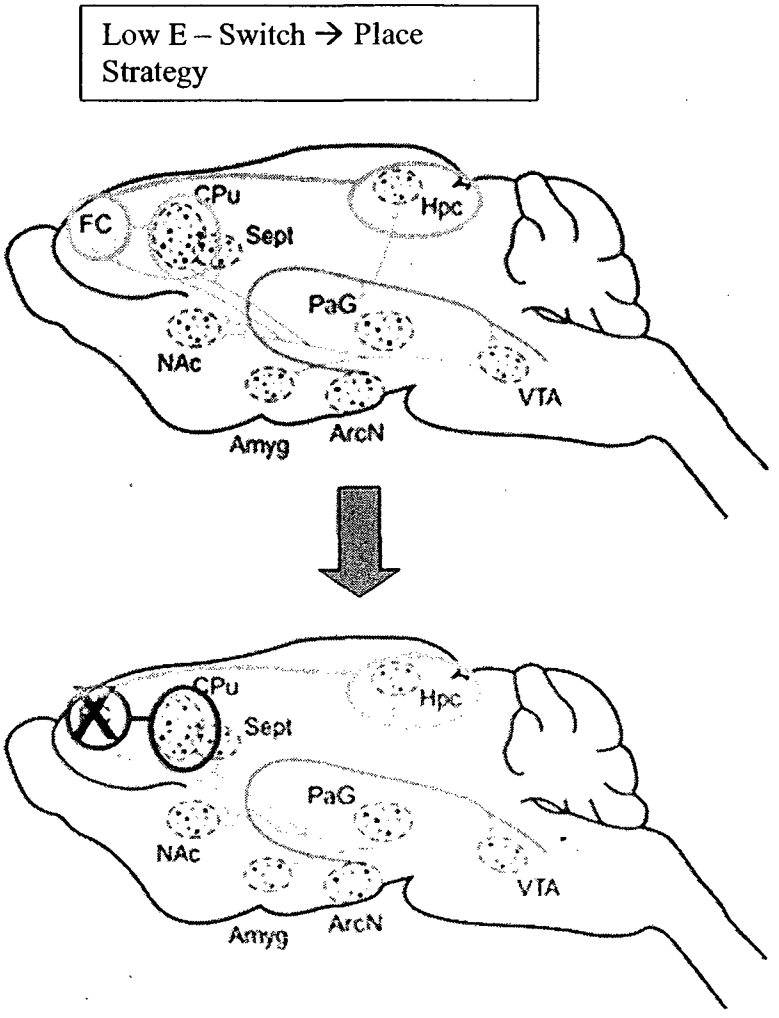


Figure 3. Speculative representation of the brain regions involved in strategy use by low E2 rats subsequent to DA receptor antagonism. Low E2 rats predominantly use a response strategy. D1R antagonism in the DS blocks use of a response strategy and causes a switch. Likewise, both D1R and D2R antagonism in the mPFC result in a switch to use of a place strategy in low E2 rats.

activation of D1Rs facilitates the stabilization of a new strategy (Floresco and Magyar 2006). Thus, it is possible that D2R antagonism in the mPFC blocks the ability of low E2 rats to inhibit the use of the previously learned place strategy and make the transition to use of a response strategy. The fact that D1R antagonism in the mPFC also alters strategy use in low E2 rats may be explained by the inability of a rat to stabilize use of the relatively new response strategy. It may also be a result of the influence of medial prefrontal cortical DA on the DS.

The studies contained in this thesis indicate that the estrogenic modulation of DA in the brain is an important factor in the performance of DA-mediated behavioral tasks such as LI, working memory, and strategy use. These studies confirmed previous findings that differential levels of E2 bias cognitive strategy use and are the first to demonstrate that D1R and D2R antagonism can alter strategy use in low E2 rats. This is also the first demonstration that the mPFC is involved in the use of cognitive strategy to solve a maze. In conjunction with previous studies, the current findings also provide evidence that these behavioral changes are based in the estrogenic modulation of DA which occurs in the DS and the mPFC in response to chronic systemic administration of E2 as well as acute intracranial infusions of E2. The studies presented here demonstrate that, when evaluating the influence of E2 on performance in behavioral tasks, it is important to consider its effects on neurotransmitter systems in the brain. The alteration of these systems, such as DA, by E2 can have a profound impact on behavior and a better understanding of such modulation may contribute to the resolution of at least some of the inconsistencies within the literature.

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