Effects of Estrogen on Cognitive Strategy and Striatal Dopamine Transmission in Female Rats: The Role of Reproductive Experience

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ABSTRACT

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Estrogen (E) influences the cognitive strategy used by female rats when solving a maze. Nulliparous rats with high levels of E tend to use an allocentric (place) strategy whereas rats with low E levels are biased towards an egocentric (response) strategy. Furthermore, striatal-dependent response learning is sensitive to dopamine (DA) transmission in that brain area, and E changes DA release in the striatum. Recent findings suggest that reproductive experience may alter how E affects DA transmission. Here the effects of E on cognitive strategy was explored in nulliparous and primiparous rats solving a t-maze task, and striatal DA release was measured after administration of an indirect DA agonist (amphetamine; 0.5 & 1 mg/kg) and an autoreceptor-activating dose of the DA agonist (apomorphine; 50 & 80 μg/kg). As expected, ovariectomized (OVX) nulliparous rats receiving low 17β estradiol (E2) predominantly used a response strategy, whereas high E2 OVX rats did not; this pattern changed in low E2 primiparous rats. High E2 rats receiving the lower AMPH dose exhibited greater DA release than low E2 rats. Interestingly, there were also hemispheric differences in striatal DA response, which suggests possible lateralization in DA function. These findings suggest that parity changes how E affects the cognitive strategy used by females. They also show that E2 affects DA transmission in the dorsal striatum in a lateralized manner.

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INTRODUCTION

The steroid hormone estrogen (E) has typically been associated with regulation of reproduction and sexual behaviour through its actions in the hypothalamus and associated hormone regulating centers in the female mammal. However, it has recently been established that E has a greater variety of effects on the female brain than previously asserted. A subset of these effects involves the role E plays in cognition, particularly learning and memory. Interest in research on this topic was invigorated after it was established that E receptors are present in extensive areas of the brain, many of which play a significant part in cognition, such as the hippocampus and cerebral cortex (Polo-Kantola et al., 1998; Shughrue & Merchenthaler, 2000; Shughrue & Merchenthaler, 2001). Animal models confirm these findings; it's been shown that high E levels are linked to improved performance on spatial memory tasks, which are dependent on hippocampal function (Korol, 2004), an area recently known to contain E receptors (Sinopoli, Floresco, & Galea, 2006).

Research carried out on E's effects in women has generated differing findings. On the one hand, it has been found that E improves working memory (Shaywitz et al., 1999), articulation (Hampson, 1990), and verbal fluency in women (Rosenberg & Park, 2002). On the other hand, E has been shown to impair male-typical tasks, such as visuospatial abilities (Hampson, 1990). Indeed, such findings hint at a subtle E-level dependent modulation of cognitive abilities in young cycling women, which further indicates the varied effects E could have on the female brain outside of sexual behaviour and reproduction.

Recent research carried out in rodents suggests that E is also involved in the type of cognitive strategy used to solve particular tasks. Specifically, E levels affect whether a hippocampus-mediated place (or allocentric) or a striatum-mediated response (or egocentric) strategy is employed when searching for a food reward in a maze (Korol, 2004; Quinlan et al., 2008). The former strategy involves the use of several spatial cues to develop a cognitive map to navigate a maze and seek a reward. For the latter, the rat relies on a particular motor response to seek the reward (e.g., always turn 90 degrees left). Korol and colleagues have shown that female rats' use of either a place or response strategy to solve a t-maze task depends on E levels.

In one study carried out with ovariectomized (OVX) rats, it was found that rats administered 17β estradiol (E2; the most common estrogen present in females) replacement were faster to acquire a place task, but slower than rats without E2 replacement in acquiring a response task (Korol & Kolo, 2002). In another study, an ambiguous t-maze task was used, in which the strategy used by the rats was unknown during training. Once rats had learned the task, which was to go to a specific maze arm to retrieve the reward, a probe test was carried out to determine which strategy was used. During this probe test, the start arm was rotated 180 degrees relative to the start arm used during training, and the goal arm was kept constant. A place strategy was recorded if the rat turned to the same spatial location that contained the reward during training; a response strategy was recorded if the rat made the same directional turn as that used during training. It was found that OVX rats with E2 replacement predominantly used a place strategy, whereas the opposite pattern was seen in the OVX rats without E2 (Korol et al., 2004).

In another study carried out by Korol and colleagues (2004), the same effect was observed in cycling females. Gonadally intact, naturally cycling females were used in order to determine whether natural fluctuations in all reproductive hormones (e.g., progesterone) would produce the same effect as that observed in OVX rats. Korol et al. (2004) found that when rats were in proestrus, the phase of the estrous cycle when endogenous circulating E is highest, they used a place strategy more often on a t-maze task. Conversely, when the rats were in estrus, or the phase when circulating E is lowest, they were more likely to use a response strategy. All of these findings indicate that E plays a key role in determining what type of cognitive strategy females use when learning a task.

Neurobiological findings support the premise that place learning and response learning are separate competing systems that rely on different brain pathways, viz. the hippocampus and dorsal striatum, respectively. When hippocampal integrity is impaired, performance on tasks that rely on spatial cues is diminished (Packard & McGaugh, 1992). Several studies have shown that hippocampal lesions and pharmacological inhibition of the hippocampus lead to impaired spatial memory and place learning (McDonald & White, 1993; Packard & McGaugh, 1992). For example, when the γ-amino butyric acid A ($GABA_A$) receptor agonist, muscimol, is injected into the dorsal hippocampus, place learning is impaired (Mao & Robinson, 1998). Furthermore, when hippocampal function is impaired using lidocaine, rats rely on response learning instead (Packard, Cahill, & McGaugh, 1994).

Conversely, damage to the dorsal striatum results in impaired response learning, but not place learning (Kesner, 1990). Lesions to the dorsal striatum result in impaired

performance on tasks dependent on response learning (McDonald & White, 1993), and lidocaine infusions into the dorsal striatum also lead to impaired response learning but not place learning (Packard & McGaugh, 1996). Inactivation of the hippocampus has been shown to result in deficient place learning in male rats, while damage to the dorsal striatum leads to impaired response learning in a cross-maze task (Packard & McGaugh, 1996); this has also been observed in the Morris water maze (White & McDonald, 2002). It could be said that as one system is compromised or unavailable, the rat must rely on the other.

Moreover, E affects these two learning systems differently. It has been shown that E has a variety of significant effects in the hippocampus: it is linked to increased spine density, as well as stimulated dendritic spine synapses in this brain region (Woolley & McEwan, 1992; Woolley, 1998); E increases excitatory synapses and synapse boutons on hippocampal neurons (Woolley et al., 1996). E also increases spine density in the CA1 area of the hippocampus, and this increase is dependent on estrous cycle phase (Woolley & McEwan, 1992). High levels of E are also linked to increased acetylcholine levels in the hippocampus, especially during place learning (Gabor et al., 2003; Marriott & Korol, 2003). Based on these findings, as well as on the sizeable body of research linking hippocampal functioning with improved place or spatial learning, it is apparent the role of E in the hippocampus is key for this type of learning strategy in females.

A large body of research indicates that place learning is dependent upon E action in the hippocampus (e.g., Woolley & McEwen, 1992; McEwen & Alves, 1999), but how does E impact the dorsal striatum and striatal-dependent response learning? Recent focus has been placed on investigating the role of dopamine (DA) in cognitive strategy and its

possible modulation by E in the dorsal striatum. Some studies have been carried out examining how E2 stimulates dopaminergic activity within this brain structure (McEwen & Alves, 1999). In general, E has been linked to increased striatal DA levels in females (Becker, 1999). It's also been shown that amphetamine-induced striatal DA release and behaviours are attenuated in OVX females, but are greater in E2-treated rats, and striatal DA receptor density increases following chronic E2 treatment (Becker, 1999; Di Paolo et al., 1981). Furthermore, Becker and Rudick (1999) showed that both acute and longer-term prior exposure to E2 results in enhanced amphetamine-induced DA release in the striatum. In addition, E2 administration has been shown to lead to potentiation of DA release and increases in behaviours associated with DA release, such as stereotypy and rotation (Becker, 1990, Castner et al., 1993, Di Paolo et al., 1981). The dorsal striatum also possesses the highest density of DA D1 and D2 receptors (D1R and D2R, respectively) in the brain (Boyson et al., 1986). All of these findings hint at a possible influence of E2 on DA transmission, which may affect striatally-mediated response learning in females.

Research investigating cognitive strategy use in response to changes in DA transmission has revealed that response learning may in fact be affected not only by different E2 levels, but also DA. Daniel, Sulzer, and Hulst (2006) tested whether E2 affected DA transmission and performance on a response task. It was found that E2 potentiated the dose-dependent D2 antagonist-induced decrease in response learning, which indicates that E2 disrupts DA-dependent response learning in the striatum and biases animals to use a hippocampally-based learning strategy instead (Daniel et al., 2006).

A recent study carried out by Quinlan et al. (2008) also investigated the possible effects of E2 on DA transmission and its effect on strategy use in a t-maze task. Low and moderate doses of D1R and D2R antagonists were systemically administered to female OVX rats receiving either low or high E2 replacement. As previously found, rats receiving low E2 showed predominant use of a response strategy, while those receiving high E2 showed the opposite pattern. Yet, following DA antagonism, low E2 rats shifted strategy use to a more hippocampal-based place strategy, indicating that disrupted DA transmission decreased the predominant use of a response strategy normally observed in low E2 rats. High E2 rats did not switch strategy selection, but seemed to maintain predominant use of a place strategy under moderate DA antagonist doses while showing equal propensity to use either strategy following the lower antagonist doses. These results further corroborate the idea that E2 changes cognitive strategy use not only by affecting hippocampal function, but also by possibly altering striatal DA transmission.

Based on the findings discussed above, it is apparent that E2 and DA influence cognitive strategy in female rats. However, these effects have been exclusively observed in nulliparous rats, or those with no reproductive experience. Females not only undergo significant changes neonatally and during puberty, but are also exposed to important changes in hormonal profile during pregnancy and the period following parturition. Research examining the effects of parity has also revealed extensive changes outside the expected hormonal alterations in the hypothalamus, such as in the hippocampus and amygdala (for review, see Kinsley & Lambert, 2008). Furthermore, since E plays an important role in cognitive function, these factors are likely to change with pregnancy, a period during which females are exposed to higher E levels. The question turns to

whether reproductive experience might therefore also play a significant role in which cognitive strategy is used by females.

Pregnancy, as well as the maternal period following it, involves many neuronal and behavioural changes. Some of the important changes that occur during pregnancy include thickening of the cortex (Diamond, Johnson, and Ingham, 1971), new synapse formation (Modney & Hatton, 1990), and an increase in E receptors (Bridges & Byrnes, 2006). Another major effect is the elevated and extensive exposure to E and progesterone (P). Specifically, during pregnancy females are exposed to E levels higher than those occurring during proestrus (Bridges et al., 1984), meaning that the female brain is subjected to a significantly elevated and prolonged exposure to E and P, which likely plays a role in adapting the female brain for maternal behaviour and leading to long-lasting neuronal changes (Bridges et al., 1984; Kinsley & Lambert, 2006). Since E influences cognitive function in females, this could lead to significant alterations in how E affects cognitive learning in reproductively experienced rats.

The effects of parity are especially prominent in the hippocampus, which is not surprising since this structure is so sensitive to E. As has been mentioned, E facilitates a variety of changes in the hippocampus, which may promote enhanced spatial and working memory. For instance, E promotes CA1 dendritic spine growth in the hippocampus in pregnant and lactating rats (Woolley et al., 1990; Kinsley et al., 2006). Kinsley et al. (2006) also demonstrated that nulliparous, OVX rats treated with pregnancy levels of the hormones E and P, showed significant increase in CA1 dendritic spine growth in the hippocampus. Based on these findings, it could be proposed that the hippocampally-mediated enhanced spatial abilities observed during parts of the estrous

cycle when E is at its highest would therefore be increased in reproductively experienced females.

For example, Kinsley et al. (1999) showed that multiparous rats (those that have given birth to and reared more than one litter of pups) performed significantly better on a radial-arm maze than their nulliparous counterparts, by learning the task more quickly. In another maze task, they also found that primiparous rats (those that have given birth to and reared one litter of pups) took less time to learn to approach food wells than nulliparous rats. Interestingly, foster females who were exposed to pups until they showed maternal behaviours learned the task almost as quickly as the primiparous rats, indicating that the act of caring for young itself can lead to similar neurobiological changes as those observed in dams. These studies suggest that maternal experience itself, regardless of pregnancy, is linked to improved spatial memory and faster learning of novel spatial environments.

Kinsley et al. (2006) posit that these parity-induced long-term changes in hippocampal function and spatial abilities play an important role in environmental adaptability. They also suggest that there are undoubtedly many evolutionary advantages to enhanced spatial cognition during pregnancy and the onset of maternity: it would aid the dam in finding food and water resources for its young, remembering the locations of these vital resources, navigate its environment, locate and recall predator areas, as well as nesting areas. Having improved spatial skills would greatly benefit the reproductively experienced female care for its offspring in an increasingly demanding environment.

Furthermore, the changes in hormonal exposure sustained during pregnancy, parturition, and pup-rearing are more dramatic than any experienced at any other point in a female's life. The prolonged and heightened exposure to E and P, as well as the associated changes in brain structures, indicates a significant reorganization of the female brain as it prepares to care for its young.

Contrary to such positive findings in rodents, it's been reported that pregnancy is accompanied by cognitive impairments in humans (Parsons et al., 2004). However, as Kinsley et al. (2006) point out, this could be an effect of undergoing significant stress as the female brain goes through major and permanent changes to accommodate the novel needs of motherhood.

In addition to parity-induced changes in hippocampal function and E sensitivity and release, striatal DA function is also altered following pregnancy. As mentioned, parity is associated with prolonged and heightened circulating levels of E (Bridges et al., 1984), and E increases striatal DA receptor density (Di Paolo et al., 1981), and modulates striatal DA release (McEwen & Alves, 1999), as well as a variety of DA-mediated behaviours. The elevated exposure to E experienced by female mammals during parity could conceivably affect DA release and activity in the dorsal striatum and forebrain. Byrnes, Byrnes and Bridges (2001) examined the effect of E2 on forebrain DA activity; they found that multiparous females showed an increased response to apomorphine, a DA agonist, compared to their nulliparous counterparts. Rats with parous experience also had higher post mortem levels of DA and DOPAC (3,4-dihydroxyphenylacetic acid, a DA metabolite) in striatal tissue (Byrnes et al., 2001; Felicio, Floro, Sider, Cruz-Casallas, and Bridges, 1996).

The effects observed during and after pregnancy demonstrate how changeable the adult female brain is in response to hormones, and many of these beneficial effects can last well into senescence. Diamond and colleagues first showed that cortical widths in isolated pregnant rats were similar to those of nulliparous rats that had been housed in an enriched environment, indicating that pregnant rats have cortices similar to those that had been enriched (Diamond, Johnson, and Ingham, 1971; Diamond, Krech, and Rosenzweig, 1964). More recently, Macbeth et al. (2008) have shown that multiparous females performed better on tests of spatial memory, non-spatial memory and object recognition than nulliparous rats in middle-age (around 12 months of age). Furthermore, these multiparous rats showed higher levels of brain-derived neurotrophic factor (BDNF), a protein important for growth and development of neurons and synapses, in the CA1 area of the hippocampus (Macbeth, Scharfman, MacLusky, Gautreaux and Luine, 2008). These findings hint at a neuroprotective effect of reproductive experience, which may protect the female brain against age-related cognitive decline.

These findings suggest that the cognitive strategy used to solve a maze for a food reward by females with reproductive experience may be different from that used by nulliparous females. Since parity is associated with altered E release and enhanced hippocampal functioning, parous rats might conceivably behave differently in response to low and high E levels. As shown previously, nulliparous rats tend to utilize a hippocampus-mediated place learning strategy when administered high levels of E2, whereas they use a striatum-mediated response strategy under low E2 levels (Quinlan et al., 2008, Korol, 2004). With increased hippocampal neuron spine density (Wooley et al., 1990; Kinsley et al., 1999; Pawluski, Walker, and Galea, 2006) and improved

performance on spatial tasks (Kinsley et al., 1999), it would be expected that parous rats will show a higher propensity to utilize a hippocampally-mediated place learning strategy, regardless of the E2 level administered.

With simultaneous increased DA sensitivity and release, parity could also affect DAergic striatal functioning. This increased E-mediated DA activity in the female brain could possibly alter striatal function, as it relates to response learning in parous rats. Thus reproductive experience could alter the way females respond to E levels in using one particular cognitive strategy over another.

Rationale and hypotheses

One goal of this thesis was to replicate the well-established effect of differing E2 levels on cognitive strategy use in young nulliparous female rats, while also exploring the role reproductive experience plays in such E2-dependent strategy use. Additionally, Wistar rats were used in this experiment in order to establish this effect that was previously observed in Sprague-Dawley rats. Based on procedures described above (Korol et al., 2004; Quinlan et al., 2008), age-matched nulliparous and primiparous rats were trained to find a reward in an ambiguous t-maze task and later tested for which cognitive strategy was used in a probe test. These two groups of OVX females were further subdivided into three hormone groups: no E2 replacement, low, chronic E2 replacement, and high daily E2 replacement. A no E2 replacement condition was added in order to observe how cognitive strategy use would change in rats not exposed to E2, as would occur in older non-cycling females, and how this group would differ from those with low E2 levels. The E2 doses for the low and high E2 groups were selected based on

previous research that established these doses mirror those observed in the low and high phases of the estrous cycle (Mannino et al., 2005; Quinlan et al., 2008). Both groups received subcutaneous implants that released a constant low E2 dose, and the high E2 group were administered additional daily E2 injections to provide a high physiological E2 replacement dose.

It was hypothesized that the nulliparous rats would use a cognitive strategy similar to those observed previously: i.e., high E2 rats would predominantly use a place strategy and low E2 rats would be more likely to use a response strategy (Korol, 2004; Quinlan et al., 2008). As for rats not receiving E2 replacement, they were hypothesized to behave similarly to low E2 rats, as OVX rats have been found to use a response strategy more often than a place strategy (Korol & Kolo, 2002; Korol et al., 2004). The primiparous rats were hypothesized to use a place strategy more often in general, due to enhanced hippocampal functioning (Woolley et al., 1990; Kinsley et al., 2006; Macbeth et al., 2008), and higher performance in spatial tasks (Kinsley et al., 1999; Macbeth et al., 2008).

Another possible mechanism by which reproductive experience may influence E's effect on cognitive strategy is by affecting DA transmission in the dorsal striatum. As stated previously, parous females show a potentiated response to DA agonists, as well as higher post-mortem DA levels in the dorsal striatum (Byrnes et al., 2001). This parity-induced change in striatal DA transmission could affect response learning in females; a higher proportion of rats could rely on a hippocampally-mediated learning strategy instead when DA function in the dorsal striatum is disrupted.

It would be important to more closely examine how reproductive experience affects DA release under different E2 levels following pharmacological manipulations of DA transmission in the dorsal striatum. However, before this can be explored in reproductively experienced animals, it is important to first understand how striatal DA release is affected by E in nulliparous females using the E2 replacement schedule used here. Amphetamine-induced DA release increases following chronic E2 treatment in females (Becker, 1999; Becker & Rudick, 1999); this suggests that DA transmission in the dorsal striatum changes with E. Furthermore, no research has been carried out to examine lateralization of dorsal striatal DA transmission in response to E2. It would be interesting to observe if there is a lateralized effect of E2 on DA release in this brain region. Therefore, prior to investigating how E alters DA transmission in the dorsal striatum in reproductively experienced females, this effect must first be further elucidated in nulliparous females using the E2 replacement paradigm used in experiment 1.

A second study was therefore carried out to explore the effects of low and high E2 on striatal DA releases. In vivo microdialysis was carried out with low and high E2 rats to measure DA release independently in both the left and right dorsal striatum in response to low and moderate doses of an autoreceptor dose of the D2 antagonist, apomorphine, and the indirect DA agonist, amphetamine. Based on previous research investigating the effects of E treatment in OVX rats on striatal DA release (Becker & Rudick, 1999), it was hypothesized that high E2 replacement would enhance amphetamine-induced DA release compared to low E2 replacement. This is further supported by findings showing that E2 replacement is associated with increased striatal DA levels and release (Becker, 1990; Korol, 2004), increased striatal DA receptor density (Di Paolo et al., 1981; Becker,

1999), and enhanced DA associated behaviours (Di Paolo et al., 1981). Furthermore, it is expected that rats receiving high E2 replacement would not only be more sensitive to amphetamine-induced increase in DA release, but to changes in DA transmission in general.

METHODS

Experiment 1

Subjects

A total of 152, three- to four-month old female Wistar rats were used in this experiment (Charles River, St-Constant, QC). Seventy-four of these rats were primiparous females, which had given birth one month prior to testing. For breeding, these rats were group-housed (5 females and 1 male per cage) in hanging cages for a maximum duration of 21 days, after which the pregnant rats were moved to single shoe-box cages. Immediately after birth, the number of pups was culled to 8 per dam. The pups were then kept with the dams for 3 weeks, after which they were all weaned and dams were then housed in pairs until surgery.

The remaining 77 rats were nulliparous females with no prior reproductive experience. Both nulliparous and primiparous groups were randomly assigned to 3 treatment conditions: no E2 (n=39), low E2 (n=58), and high E2 (n=55). All rats were housed in plastic shoe-box cages, under a 12 h reverse light-dark cycle (2100 to 0900). Rats were housed in pairs prior to OVX surgery, after which they were housed singly. All rats had *ad libitum* access to rat chow and water, except for the training and testing periods, during which they were food restricted. Starting three days prior to the beginning

of training, food was restricted and weight was maintained at 90% of free-feeding levels. The rats were handled daily prior to and throughout the experiment. All procedures involving rats were in accordance with guidelines established by the Canadian Council on Animal Care and approved by the Concordia Animal Research Ethics Committee.

Apparatus

All training and testing was carried out in a black Plexiglas t-maze situated on a table one meter above the floor. The t-maze was comprised of black walls (28cm high), grid floors, a start arm (130cm long) and two goal arms (75cm long), which were each positioned at a 90 degree angle to the start arm. The start arm contained a removable door, which created a start chamber. Each goal arm contained a white ceramic bowl placed at its end. Froot Loops (Kellogg's) were placed underneath the maze at each bowl to later avoid odor cues during testing. Two guillotine doors separated the goal arms from the choice point of the start arm and could be closed to prevent animals from leaving the goal arm once they were inside. An additional arm (identical in dimensions to the start arm) was added to the t-maze to form a plus-shaped maze. Another guillotine door closed off access to this part of the maze at all times, except during probe testing. The t-maze was situated in a room dimly lit with overhead red fluorescent lamps, a lamp facing the wall (40W light bulb), and a small 15W light bulb mounted on one of the walls. Other spatial cues included cupboards and posters on the walls.

Surgeries and estrogen administration

Ovariectomy surgery

Approximately 2 to 3 days following weaning of pups from the primiparous group, surgeries were conducted on all rats. Prior to surgery, rats were anaesthethized using Halothane gas (4% for induction, 2% for maintenance) and the ovaries were removed bilaterally through a dorsal incision using standard aseptic procedures, and the incision was then sutured using 9mm stainless steel surgical staples (EZ clips; Stoelting Co., Wood Dale, Illinois). Following surgery, rats were administered the analgesic Anafen (0.1ml/animal, s.c.) and the antibiotic Penicillin G (0.1ml/animal, i.m.). Saline was also administered to rehydrate the animals (1.5ml/animal, s.c., bilaterally). Each rat's health was monitored daily following surgery.

Silastic tube implantations

At the same time as the ovariectomy surgery, a small incision was made in the nape of the neck and a Silastic tube was inserted (1 cm long). The implant releases a low steady dose of 5% 17β-estradiol in cholesterol resulting in a 20pg/ml serum concentration (Mannino, South, Inturrisi, & Quinones-Jenab, 2005). This procedure was carried out to provide all rats with a low basal rate of E2 similar to that seen in the estrus phase of the rat estrous cycle (Quinlan et al., 2008). After an initial peak and decline of serum 17β-estradiol levels, a steady dose has been shown to last approximately 24 days post-surgery (Mannino et al., 2005).

Estrogen administration

High E2 rats were administered daily injections of 17β-estradiol (10μg/kg, SC; Sigma Chemical Co.) to provide them with a physiologically high dose of E2 (~75-90pg/ml plasma; Quinlan et al., 2008). Low E2 and no E2 rats were simultaneously given daily injections of sesame oil vehicle (0.1ml, SC). All injections took place daily between 1600-1800 hrs.

Behavioral testing

Training

Training began one week following surgery. On the first three days of training, all rats were exposed to a daily 10 min habituation session in the t-maze to familiarize them with the environment (see figure 1). All guillotine doors were open and food rewards (Kellogg's Froot Loops) were distributed around the maze. Following habituation, training was started. Rats were paired and each pair randomly assigned a direction (right or left arm), which they would subsequently be trained to enter in order to receive the reward. The reward arm remained constant relative to spatial cues around the room.

Each rat was given 10 daily choice trials (alternating with its paired partner), in which the rat was placed in the start arm and all doors removed to allow the animal to choose a directional arm. Once the rat had entered either one of the two goal arms, the guillotine doors were closed and the rat removed after having eaten the food reward at the end of the arm. If the incorrect arm was chosen, the rat was permitted enough time to explore the empty bowl before being removed from the maze. The rats were considered to have successfully learned this task once they reached a criterion of 80% correct trials

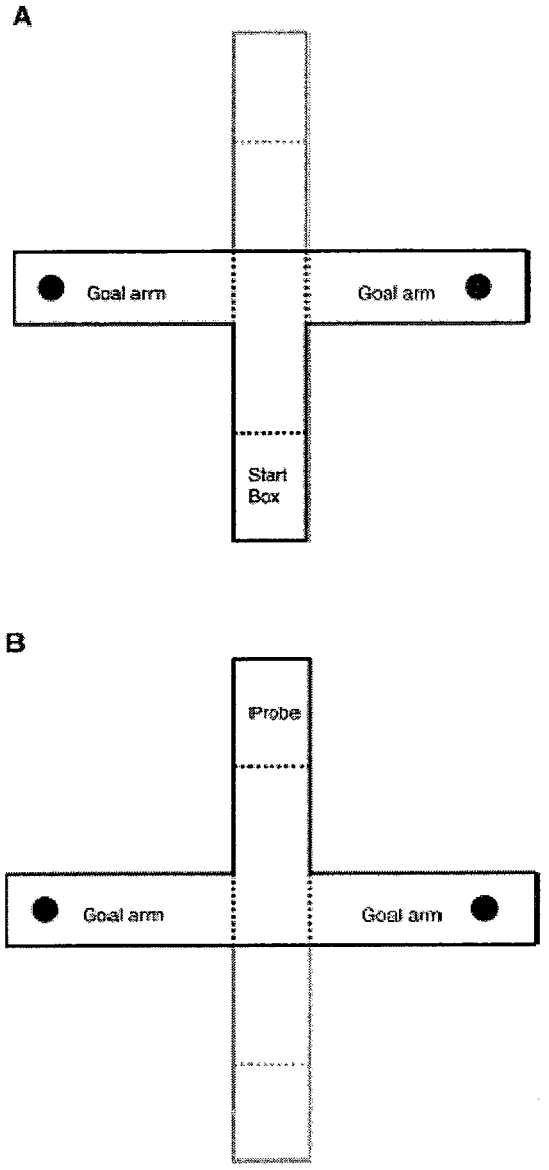

Figure 1. The modified plus maze used in this experiment as adapted from Korol et al. (2004). A) Rats were initially trained to receive a reward in either the left or right goal arm. Upon reaching criterion, rats were then placed in the opposite arm B) for the start of the probe trial.

for three days in a row. Based on previous findings (Korol, 2004), the number of days needed to reach this criterion is approximately 15-20.

Probe tests

On the third day of criterion, the rats were exposed to a probe test immediately after their tenth trial. Once the rat had completed the last trial, it was placed in the probe arm situated 180 degrees relative to the start arm (see figure 1). The start arm was closed and all other doors opened to allow the rat to enter a goal arm. If the rat entered the same arm where it received a reward during training (e.g., if the rat was trained to go to the right arm from the start arm to receive the reward, then entered the same arm during the probe test), it was deemed as using a place strategy; the rat turned to the same spatial location where the reward was found during training. If the rat entered the opposite arm to that used during training (e.g., the rat previously trained to receive the reward in the right arm during training now enters the left arm during the probe trial), it was deemed to be using a response strategy; the rat turned in the same direction as it did during training.

Statistical Analysis

This experiment was a 3 (E2 level) X 2 (parity) between subjects design. Non-parametric statistics were used for this experiment, as the dependent variable was the proportion of rats using a particular strategy. Chi square (χ^2) tests were carried out on each E2 group for both nulliparous and primiparous rats to compare strategy use within these groups. Furthermore, Kruskal-Wallis tests were used to determine whether any of the six groups differed from each other with respect to strategy use.

Experiment 2

Subjects

Forty-five, three- to four-month old nulliparous, female Sprague Dawley rats were used in this experiment (Charles River, St-Constant, Quebec). Rats were randomly assigned to either a low E2 (n=23) or a high E2 (n=22) group. All rats were housed in plastic shoe-box cages, under a 12 h reverse light-dark cycle (2100 to 0900). They were housed in pairs prior to OVX, after which they were housed singly. All rats had *ad libitum* access to rat chow and water, and were handled daily prior to and throughout the experiment. All procedures involving rats were in accordance with guidelines established by the Canadian Council on Animal Care and approved by the Concordia Animal Research Ethics Committee.

Surgeries, hormone and drug administration

Ovariectomy surgery

Ovariectomies and silastic E2 implants were carried out as described in experiment 1.

Cannula implantations

About two weeks following OVX, rats underwent bilateral cannula implantation surgery. As with previous surgeries, animals were anesthetized using Halothane gas and then placed in a stereotaxic apparatus. Twenty-gauge stainless steel tubing guide cannulae (18mm long; PlasticOne, Roanoke, VA) were bilaterally aimed at the dorsal striatum (stereotaxic coordinates from bregma: AP 0 mm, ML +/- 3.5, and DV - 3 mm);

the microdialysis probes extended 1mm beyond the length of the guide cannulae for final placement. Cannulae were anchored using skull screws and secured with dental acrylic. Following surgery, rats were provided the same post-surgical care as described earlier.

Estrogen administration

Hormone administration began about one week after cannulation surgeries and was maintained until testing ended. High and low E2 rats were given daily E2 or oil injections, respectively, as described in experiment 1.

Amphetamine and apomorphine administration

Rats in both E2 groups were randomly assigned to four drug conditions: low or moderate autoreceptor-activating doses of apomorphine (50μg/kg and 80μg/kg IP, respectively) and low or moderate doses of amphetamine (0.5mg/kg and 1mg/kg IP, respectively). Amphetamine is an indirect DA agonist that acts as a psychostimulant. The doses used in this experiment were selected to avoid stereotypic behaviours. The autoreceptor-activating doses of apomorphine used in this study were chosen based on previous studies (Doherty & Gratton, 1992; Brake, Noel, Boska & Gratton, 1997), which showed that very low doses of this drug inhibit DA transmission by acting on DA D2 autoreceptors. Each of these drug groups were comprised of both high and low E2 rats, and each rat was assigned to a single drug dose to avoid behavioural sensitization to repeated exposure to amphetamine.

Amphetamine was dissolved in 0.9% saline, and apomorphine was dissolved in 0.9% saline and perchloric acid solution in distilled water (0.02%). The drug solutions were prepared one hour prior to injection and stored at room temperature.

In vivo microdialysis

Electrochemical detection of DA release was conducted in operant chambers enclosed in soundproof outer chambers. Probes were bilaterally inserted at approximately 0930h and were removed at 1900h. The probes were comprised of a semipermeable dialysis membrane (3.5 mm exposed area; Spectrum Laboratories, Rancho Dominguez, CA) connected to internal silica tubing (Polymicro Technologies, Phoenix, AZ), allowing for the circulation of artificial cerebrospinal fluid (aCSF; 145 mM Na+, 2.7 mM K+, 1.22 mM Ca2+, 1.0 mM Mg2+, 150mM Cl−, 0.2mM ascorbate, and 2mM Na2HPO4, pH7.4±0.1). The probes were connected to polyethylene tubes (PE-20, HRS Scientific, Montreal, QC), which were linked to a dual-channel swivel and an infusion pump (Harvard Instrument, Holliston, MA) positioned above the testing chamber.

Approximately 5 hours prior to dialysate collection, rats were anesthetized with Halothane and hooked up to the apparatus by inserting the probes into the guide cannulae. The flow rate of the infusion pump was set to 1μl/min. Once dialysate collection began, samples were collected at 12 min intervals. A 60 min baseline period was followed by a vehicle injection (saline, IP). After an additional 60 min of dialysate collection, the drug was injected and sampling continued for another 144 min. Once dialysate collection was finished, animals were disconnected from the probes and returned to their home cages, and the dialysate samples were stored at -80 °C until analysis.

Analytical chemistry

High performance liquid chromatography (HPLC) was used to quantify DA levels. Each dialysate sample was loaded manually into a reverse-phase column (15 x 0.46cm SpherisorbODS2, 5 mm; Higgins Analytical, Mountain View, CA). The mobile phase (40 mg 20% acetonitrile, 0.076 M SDS, 0.1 M EDTA, 0.058 M $NaPO_4$, 0.27 M citric acid, pH = 3.35), which allowed the dialysate to circulate through the system, was pumped by Waters 515 HPLC pumps (Lachine, Quebec, Canada) at a flow rate of 1.0 ml/min. Once the dialysate was separated by chromatography, it passed through dual-channel ESA (Chelmsford, MA) coulometric detectors (Coulochem 5100, with a model 5011 analytical cell). These channels provided reduction and oxidation currents for DA and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA). The amount of DA recorded was quantified by detectors connected to a computer using an EXChrom Chromatography Data System (Scientific Software, Inc., San Ramon, CA). Dialysate samples were compared to injected standards with known quantities of DA, DOPAC, HVA, and HIAA.

Histology

Once the experiment was completed, animals were anesthetized using carbon dioxide (dry ice) and decapitated. The brains were rapidly removed and submerged in isopentane (-40°C) to rapidly freeze them, after which they were stored at -80 °C. Brains were then sectioned coronally (40 micron thickness) using a cryostat and the brain slices examined under a microscope to confirm cannula placements. Rats that did not have dialysis probes located within the dorsolateral striatum were excluded from analysis.

Statistical analysis

This experiment consisted of between subjects variables (E2 level, drug) as well as a within subjects variables (time, hemisphere). Extracellular DA levels measured during HPLC were first converted into DA concentration (pg/μl) values. The baseline DA concentration values were then averaged to generate one mean baseline value per rat. Baseline percentage values were then calculated for all sample collection time points (five baseline samples, five samples following saline injection, and 12 samples following drug injection) for all rats. These percentage values were then averaged across rats for each drug type, dose, E2 level and hemisphere.

Two-tailed t-tests were then carried out to analyze mean baselines. These tests were conducted to determine whether baselines differed across low and high E2 animals. No significant differences were observed and thus it was deemed that it was statistically valid to compare DA levels as a percentage of baseline following drug administration. Following these analyses, analysis of variance (ANOVA) statistical analyses were conducted on percentage baseline measures.

To analyze DA levels following apomorphine administration, four, 2 (hemisphere) X 22 (time) mixed-factor ANOVAs were carried out to determine whether DA levels differed between hemispheres across sample collections for both drug doses in low and high E2 groups. No significant differences were observed between hemispheres in the DA response to apomorphine, thus the data from both hemispheres were combined.

Using the combined data from both hemispheres, the effects of apomorphine on DA levels in high and low E2 groups were examined for both drug doses using four, 2

(drug) X 5 (time) repeated measures ANOVAs. For these analyses, the five saline and first five drug time points were compared. DA responses to amphetamine were also analyzed using repeated measures ANOVAs. As with the apomorphine analyses, the five saline and first five drug time points were included in order to determine whether amphetamine resulted in a significant change in DA compared to saline. Furthermore, two 2 (E2 level) X 2 (hemisphere) X 10 (time) 3-way mixed-factor ANOVAs were carried out for each drug dose to determine whether the high and low E2 groups, as well as the right and left hemisphere, differed from each other with respect to DA levels following amphetamine administration.

RESULTS

Experiment 1

A Pearson χ^2 test was carried out on all E2 groups in both nulliparous and primiparous rats to test whether the proportion of rats using a place or response strategy significantly differed from chance. Findings revealed a significantly higher proportion of response strategy use in low E2 nulliparous rats ($\chi^2 = 3.903$, $p = .048$), supporting previous findings (Korol, 2004; Quinlan et al., 2007; figure 2). None of the other groups showed any significant differences, though high E2 groups in both nulliparous and primiparous rats showed a trend towards using a place strategy.

Kruskal-Wallis analyses were also carried out to test whether any of the three E2 conditions differed from each other with respect to strategy use in the nulliparous and primiparous groups. Findings revealed only one significant difference: low E2 and high E2 nulliparous rats were significantly different from each other ($\chi^2 = 5.374$, $p = .020$),

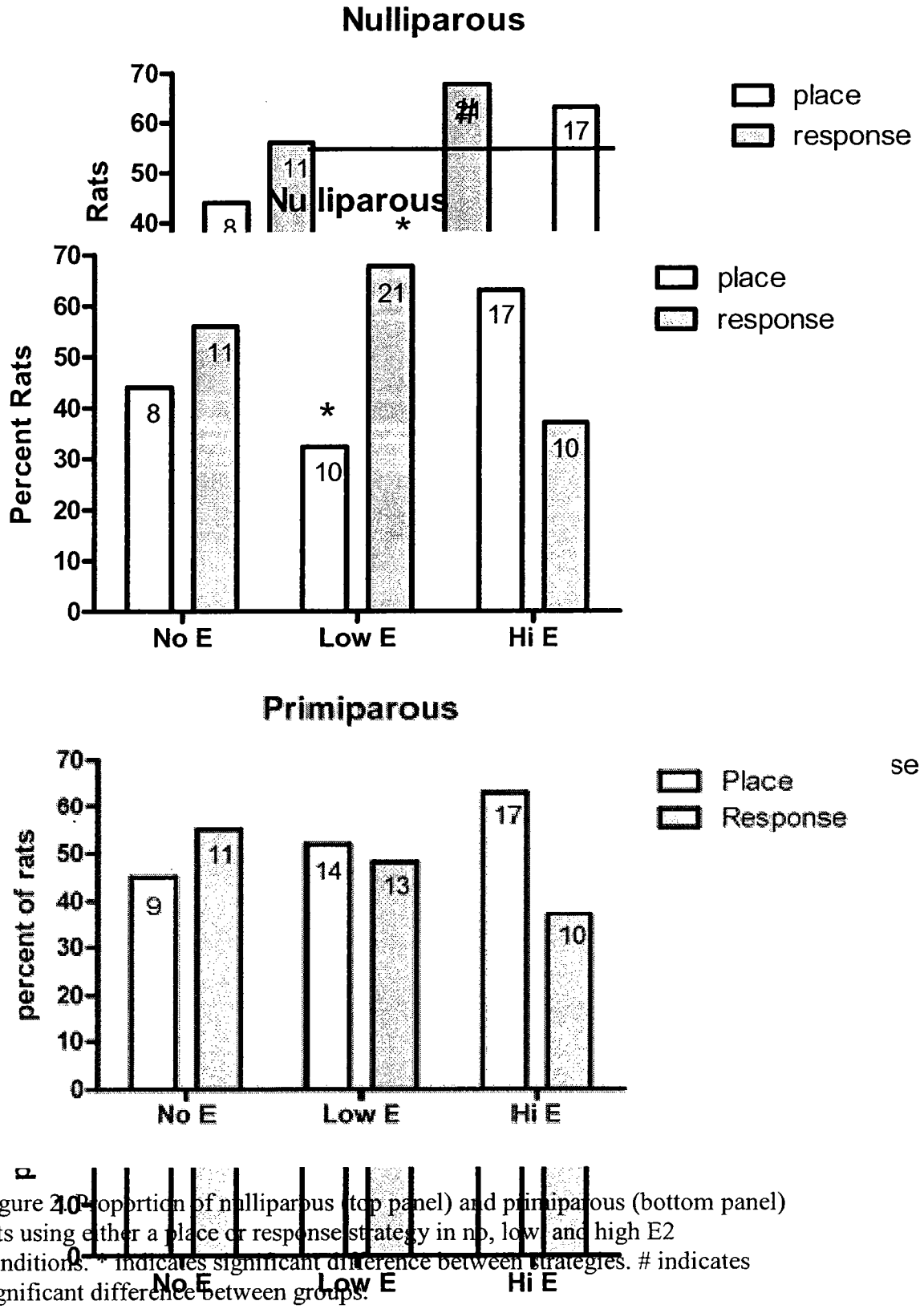

Figure 2. Proportion of nulliparous (top panel) and primiparous (bottom panel) rats using either a place or response strategy in no, low, and high E2 conditions. * indicates significant difference between strategies. # indicates significant difference between groups.

indicating that the proportion of rats using a response or a place strategy was reversed in these two conditions, with a larger proportion of low E2 rats using a response strategy, and vice versa. Again, these data support previous findings showing that low E2 rats predominantly use a response strategy, and high E2 rats a place strategy (Korol, 2004; Quinlan et al., 2007; figure 2).

Experiment 2

Cannula placements for rats used in this experiment are shown in figure 3. In all cases, cannula tracts were contained well within the dorsal striatum between Bregma coordinates 0.24mm and -0.60mm. Thus, no rats were excluded from analysis based upon histological confirmation of placements.

Preliminary two-tailed t-tests were carried out on all mean baselines to determine whether low E2 and high E2 groups differed from each other. Findings revealed no significant differences between mean baselines in low and high E2 rats, indicating that baseline DA levels were equivalent across rats and, therefore, DA percentages from baseline could be compared following drug administration.

Apomorphine data were combined across hemispheres after analysis showed no significant differences between right and left hemisphere DA levels in response to both drug doses in all rats. After data from both hemispheres were collated, four 2 (drug) X 5 (time) repeated measures ANOVAs were carried out to analyse the effects of low and high apomorphine on DA levels in low and high E2 rats. ANOVA revealed a significant main effect of drug in low E2 rats for the 50μg/kg apomorphine dose ($F_{(1,16)} = 17.514$, $p = .001$) and the 80μg/kg apomorphine dose ($F_{(1,9)} = 16.729$, $p = .003$), indicating that

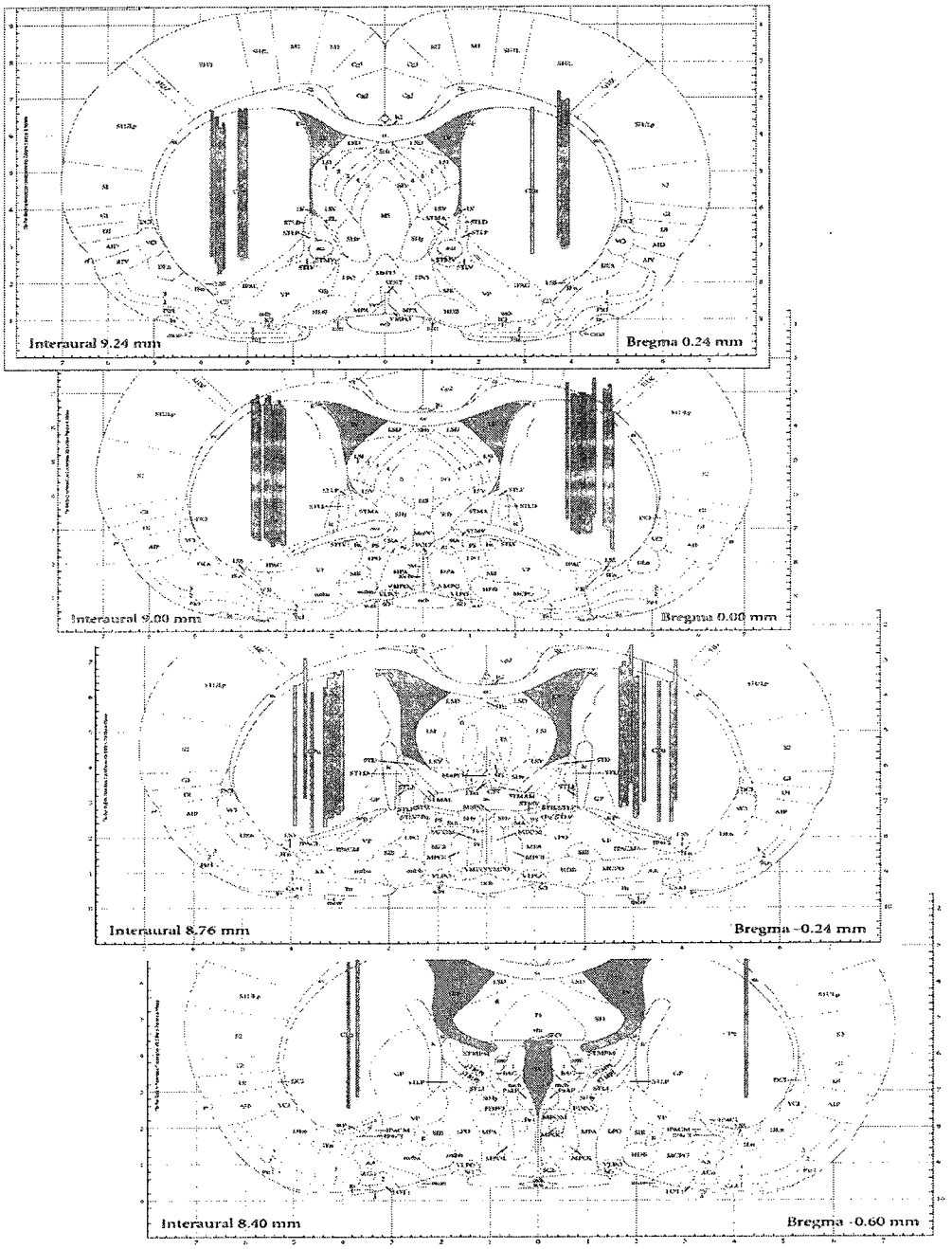

Figure 3. Coronal sections showing cannula placements in the dorsolateral striatum of rats used in experiment 2 at bregma 0.24, bregma 0.00, bregma -0.24, and bregma -0.60 (top to bottom).

autoreceptor-activating doses of apomorphine decreased DA levels in low E2 rats for both doses compared to saline (see figure 4). No significant changes in DA levels following apomorphine administration were observed in high E2 rats.

The same analyses were carried out with the amphetamine data to test whether DA levels changed in response to low and high amphetamine administration when compared to saline for both hemispheres in low and high E2 rats. For the 0.5 mg/kg amphetamine dose, ANOVA revealed significant main effects of drug in left and right hemisphere in the low E2 condition ($F_{(1,5)} = 26.528$, $p = .004$; $F_{(1,3)} = 38.828$, $p = .008$, respectively), as well as in the high E2 condition ($F_{(1,6)} = 6.493$, $p = .044$; $F_{(1,5)} = 16.779$, $p = .009$, respectively; see figure 5). For the 1.0 mg/kg amphetamine dose, ANOVA revealed significant main effects of drug in left and right hemisphere in the low E2 condition ($F_{(1,5)} = 13.243$, $p = .015$; $F_{(1,5)} = 9.16$, $p = .029$, respectively), as well as in the high E2 condition ($F_{(1,7)} = 9.959$, $p = .016$; $F_{(1,5)} = 22.476$, $p = .005$, respectively; see figure 6). These findings indicate that both amphetamine doses resulted in a DA increase following drug administration across hemisphere and E2 condition.

Lastly, two 2 (E2 level) X 2 (hemisphere) X 10 (time) 3-way mixed-factor ANOVAs were carried out (one for each drug dose) to determine whether the high and low E2 groups, as well as the right and left hemisphere, differed from each other with respect to DA levels following amphetamine administration. Mauchly's test of sphericity was significant for both sets of analyses; Greenhouse-Geisser corrections were therefore used in these analyses.

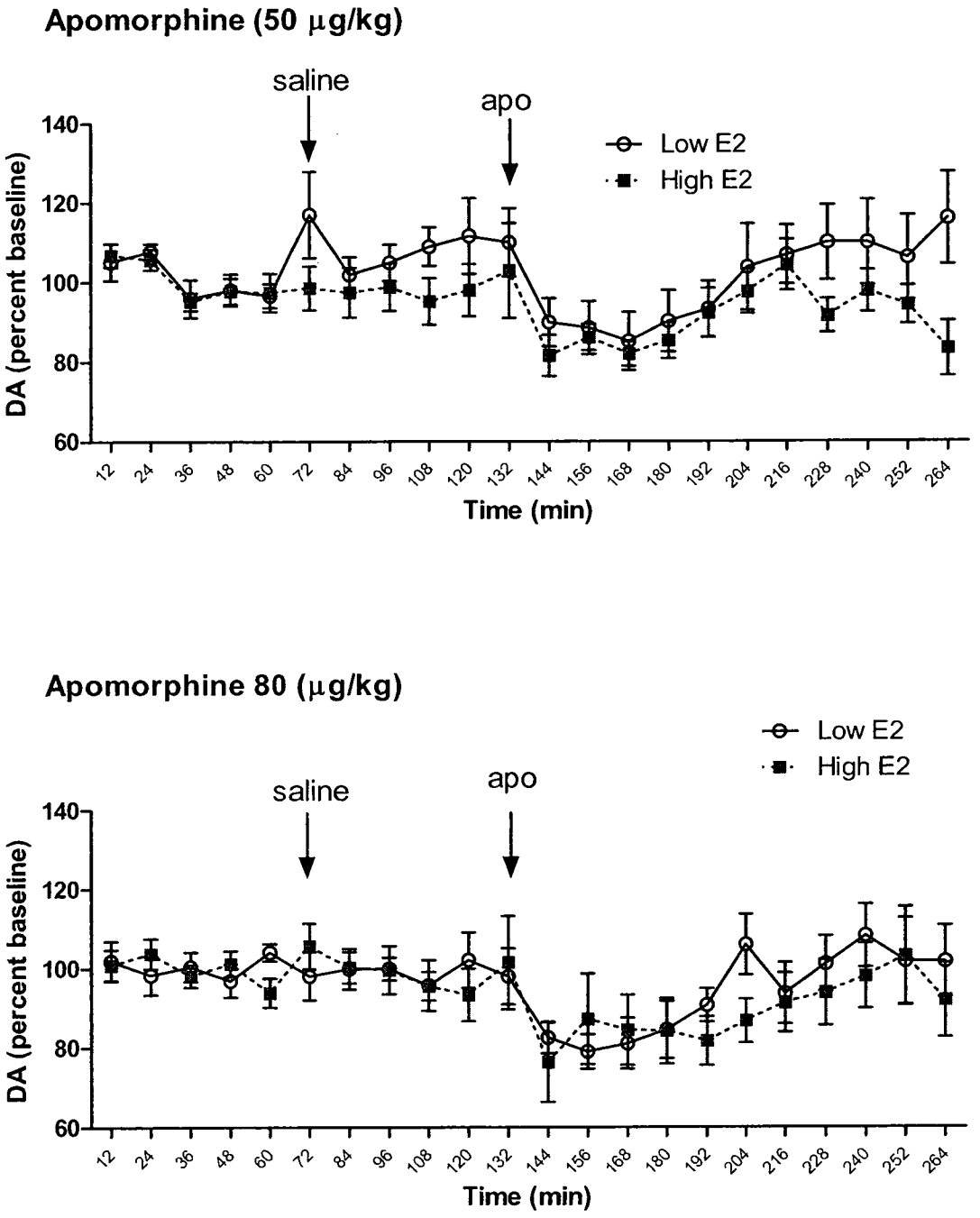

Figure 4. Percent baseline DA levels in low and high E2 rats in response to 50μg/kg (top panel) and 80μg/kg (bottom panel) apomorphine dose. DA levels decrease in low E2 rats following administration of both apomorphine doses.

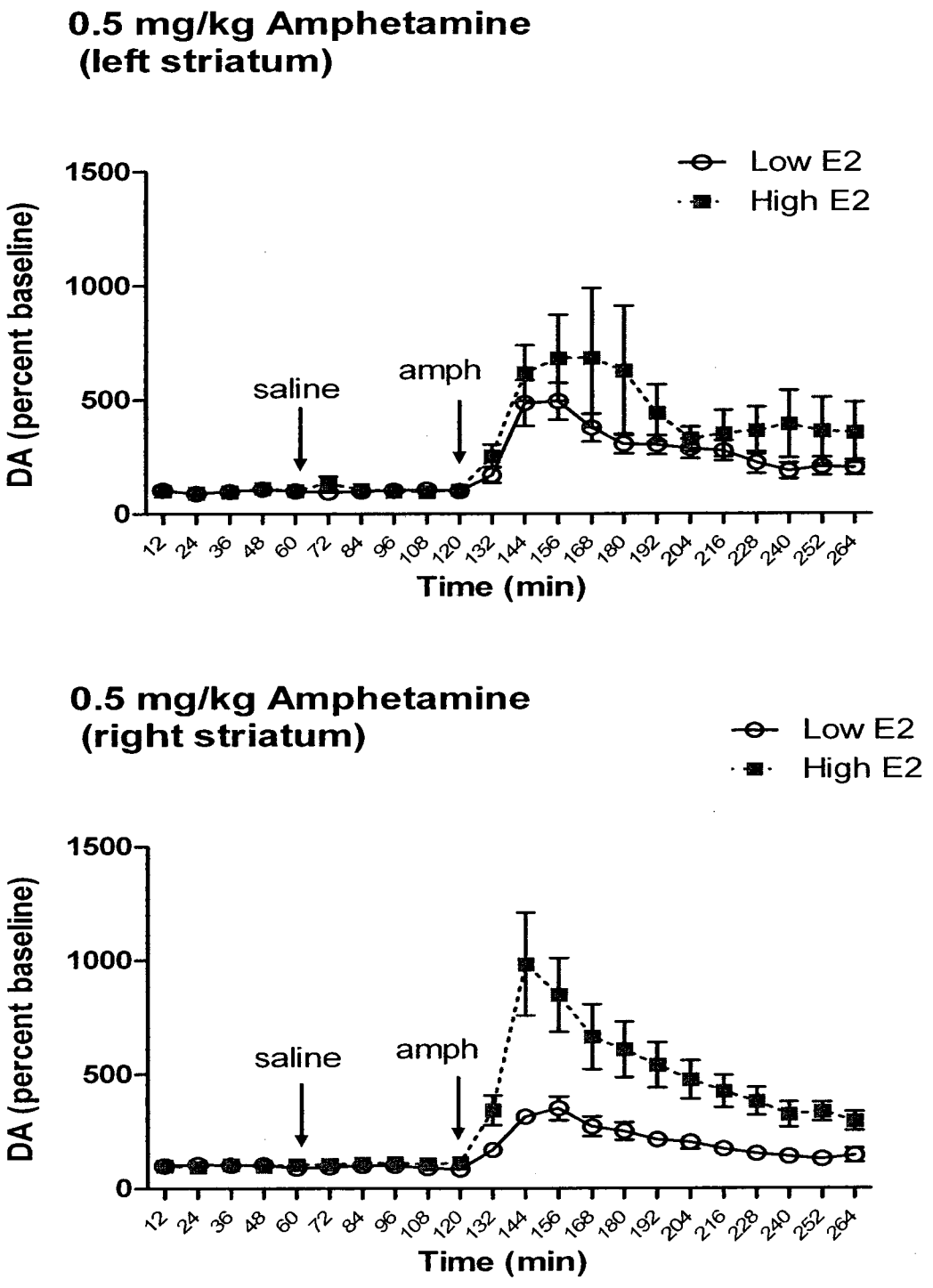

Figure 5. Percent baseline DA levels increase in low and high E2 rats in response to the 0.5mg/kg amphetamine dose in left (top panel) and right (bottom panel) hemispheres. High E2 rats generally show a higher DA response than low E2 rats in response to 0.5mg/kg amphetamine; this difference is amplified in the right hemisphere. DA levels are higher in the right hemisphere.

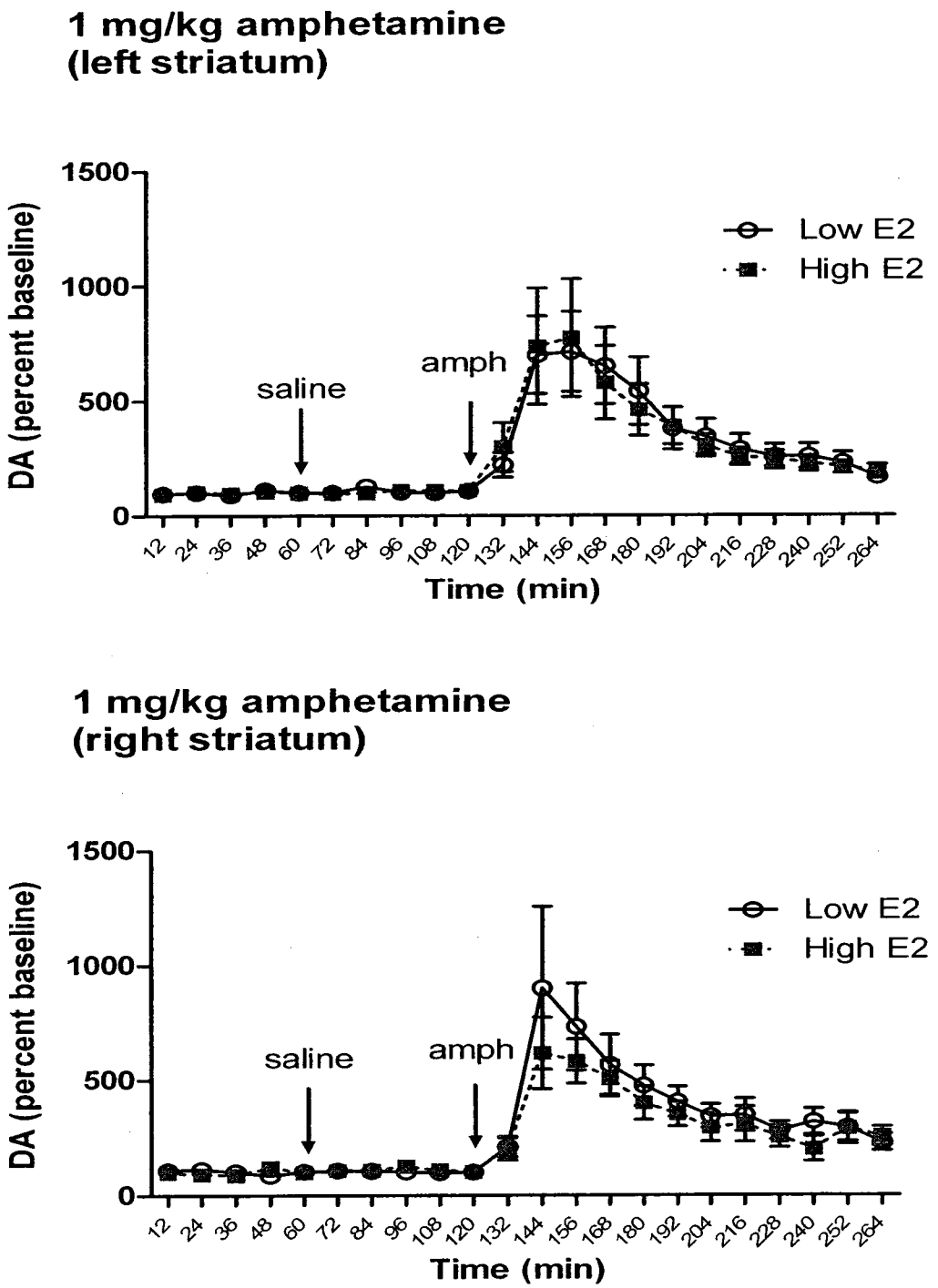

Figure 6. Percent baseline DA levels increase in low and high E2 rats in response to the 1mg/kg amphetamine dose in left (top panel) and right (bottom panel) hemispheres. DA levels are higher in the right hemisphere.

For the 0.5 mg/kg amphetamine dose, the ANOVA revealed significant hemisphere by group ($F_{(9,81)} = 5.364$, $p = .046$) and time by hemisphere ($F_{(9,81)} = 9.292$, $p = .002$) interactions. Findings also revealed significant effects for hemisphere ($F_{(1, 9)} = 15.263$, $p = .004$) and group ($F_{(1,9)} = 6.303$, $p = .033$). These data show that high E2 rats exhibited a significantly higher DA response to the 0.5mg/kg amphetamine dose than low E2 rats in general, and this group difference is amplified in the right hemisphere. Also, these data show that DA levels were generally higher in the right hemisphere under this amphetamine dose (see figure 5).

For the 1.0 mg/kg amphetamine dose, the ANOVA revealed a significant interaction for time by hemisphere ($F_{(9,90)} = 10.337$, $p = .001$), as well as significant main effects of time ($F_{(9,90)} = 5.56$, $p = .008$) and hemisphere ($F_{(1,10)} = 15.564$, .003). No other significant differences were observed, indicating that low and high E2 rats did not differ in their DA levels following administration of the 1.0 mg/kg amphetamine dose in either hemisphere (see figure 6). However, these data show that DA levels are significantly higher in the right hemisphere, as was found with the 0.5 mg/kg amphetamine dose, which suggests that DA levels are higher in the right hemisphere following amphetamine administration, regardless of dose.

DISCUSSION

The findings from experiment 1 support previous research in nulliparous rats: OVX rats receiving low E2 replacement predominantly used a striatally-mediated response strategy, and those receiving high E2 replacement showed a strong trend towards using a hippocampally-mediated place strategy, and both of these groups

significantly differ from each other (see figure 2; Quinlan et al., 2008; Korol, 2004). It was expected that OVX rats without E2 replacement would behave like the low E2 group, as previous studies have shown that oil-treated OVX rats tend to use a response strategy, as well as learn a response task faster (Korol et al., 2004; Korol & Kolo, 2002). However, the rats without E2 replacement in this study showed an equal propensity to use either strategy, and thus did not behave like the low E2 rats. One difference here from previous experiments is that Wistar rats were used whereas Sprague Dawley rats were used in previous experiments examining OVX rats without E2 replacement.

In the primiparous group, it was expected that use of a place strategy would be observed compared to response strategy, as parity is associated with potentiated hippocampal function (Wooley et al., 1990; Kinsley et al., 1999; Pawluski et al., 2006), as well as improved performance on spatial tasks (Kinsley et al., 1999). Reproductively experienced rats were therefore expected to perform better on spatial, hippocampally-mediated cognitive tasks, as their sensitivity to E2 is potentially higher than in nulliparous females. This hypothesis was partially supported: no significant differences in strategy use were observed in primiparous rats under any of the E2 doses, however, the predominant use of a response strategy observed in the low E2 condition in the nulliparous group was no longer present in the primiparous females. The high E2 primiparous group showed the same pattern as the high E2 nulliparous groups; all rats exposed to high E2 showed a strong, but non-significant, trend towards use of a place strategy (see figure 2).

Long-term exposure to high E levels during pregnancy promote hippocampal cell growth and enhanced spatial abilities, which would lead parous rats to rely on a

hippocampally-mediated place strategy more often. Additionally, low E2 primiparous rats might no longer rely on a response strategy due to disrupted DA transmission in the dorsal striatum, as reproductive experience has been shown to alter striatal DAergic function (Felicio et al., 1996); thus response learning could be compromised. This would explain why low E2 primiparous rats seem to no longer predominantly use a response strategy, and instead use both strategies equally. As with the nulliparous group, the no E2 dose did not produce any difference in strategy use in primiparous rats, thus indicating that a lack of E2 replacement in reproductively experienced rats does not alter cognitive strategy use.

As parous females are exposed to heightened levels of E for a significant period of time (Bridges et al., 1984), and show increased E receptor α (ERα) density in the pituitary gland (Byrnes et al., 2006), medial preoptic area and amygdala (Byrnes, Babb, and Bridges, 2009), females could potentially become more sensitive to E. Recent work has shown that there is an increase in ERα positive cells in the dorsal striatum of primiparous middle-aged rats, when compared to nulliparous females (Byrnes et al., 2009), which indicates that parity may also affect striatal ER expression and sensitivity to E. However, the existence of striatal ERs in young female rats remains to be investigated; current work in our lab is being carried out to explore this.

In the second study, the findings supported the hypothesis that high E2 levels would be associated with enhanced DA transmission. It was expected that high E2 rats would generally show enhanced DA release in response to amphetamine compared to low E2 rats, as it has been established that E is associated with increased striatal DA release (Becker, 1999; Becker & Rudick, 1999). This difference between E2 levels was only

observed with the 0.5mg/kg amphetamine dose; interestingly, this effect was lateralized, with high E2 rats showing greater DA levels than low E2 rats in the right, but not left, striatum (see figure 5). This lateralized effect was also observed with the 1mg/kg amphetamine dose; DA levels were generally higher in the right hemisphere across groups (see figure 6). Not much is known about lateralization of dorsal striatal DA transmission, and how E might affect this. In general, it has been established that there are hormone-induced sex differences in brain lateralization. For example, female Long-Evans rats show a higher concentration of E receptors in the right versus the left cortex (Diamond, 1991). Recent work in our lab has revealed the presence of ER α (ERα) and G-protein coupled receptor 30 (GPR30) in the dorsal striatum (preliminary findings, Brake lab, personal communication, 2010). It is possible that a similar lateralized distribution of E receptors could exist in the dorsal striatum, which could lead to differential modulation of DA in right and left hemisphere; however, this is presently unknown and thus further research is needed to investigate this.

Furthermore, a study investigating striatal DA levels in Wistar rats showed no significant differences between hemispheres (Rosen, Finklestein, Stoll, Yutzey, and Denenberg, 1984); however, higher DA levels were found in the right nucleus accumbens, which, as the authors point out, could influence striatally-mediated behaviours. It is therefore possible that DA release in the right striatum is enhanced compared to left striatum due to afferent projections from other brain areas. However, Rosen et al.'s (1984) study combined male and female rats, therefore it is unclear how E would modulate this lateralized effect.

Another theory that could explain why differences between E2 groups were only observed in the right striatum is that the high E2 group showed high variability in DA levels in the left striatum, which could be masking a statistical difference between groups (see figure 5). The reason why high E2 rats exhibit greater variability is unknown, but this phenomenon has been observed in other experiments conducted in this lab.

No group differences were found following administration of a 1.0mg/kg dose of amphetamine (see figure 6), which indicates that E2 did not differentially impact DA transmission under a higher agonist dose. This effect could be due in part to DA sensitivity being increased in general, regardless of E2 level, as all rats showed a significant increase in DA levels. It is possible that higher E2 levels potentiate striatal DA release only in response to lower amphetamine doses, and enhancement of DA levels could have reached a ceiling effect with the higher amphetamine dose, thus abolishing this E2 group difference.

Amphetamine, an indirect agonist, stimulates DA release from presynaptic terminals by acting on dopamine transporters (DATs; Feldman, Meyer, and Quenzer, 1997). It has been established that OVX depletes striatal DAT binding density, and subsequent E2 treatment increases DAT binding in female rats (Morissette & Di Paolo, 1993a; Bossé, Rivest, and Di Paolo, 1997), and striatal DAT binding is highest during proestrus in naturally-cycling females (Morissette & Di Paolo, 1993b). Thus, E2 could potentially be enhancing the amphetamine-induced increase in striatal DA levels seen in the present study by increasing the availability of DAT or altering its kinetics.

Apomorphine acts as an indirect antagonist at low doses by stimulating presynaptic D2 autoreceptors, and thus decreasing DA synthesis and release (Feldman et al., 1997). Only the low E2 rats showed a significant decrease in DA release in response to both autoreceptor doses of the D2 receptor agonist apomorphine (see figure 4). A possible explanation for the lack of a significant apomorphine-induced decrease in striatal DA levels observed in the high E2 rats could be that E2 altered D2 autoreceptor regulation, thus the effects of apomorphine could have been partially blocked in the high E2 condition by changing the availability and kinetics of presynaptic D2 autoreceptors. This suggests that E2 may also increase amphetamine-induced DA release by acting on D2 autoreceptors to alter negative feedback regulation of DA synthesis and release.

All of the previous studies exploring striatal DA transmission in response to E treatment in OVX rats have only compared E and oil-treated females. Therefore, the current findings are novel in that both low and high E2 doses were administered to OVX rats, thus striatal DA release could be observed in different E2 conditions. Therefore, if a subset of rats were given a no E2 dose, it would have been expected that DA levels would be significantly lower in response to amphetamine. This is especially interesting since similar studies looking at naturally cycling rats have found the opposite pattern: rats in estrus show potentiated amphetamine-induced striatal DA release and behaviours when compared to rats in proestrus (Becker & Cha, 1989; Becker & Ramirez, 1980; Becker, Robinson, and Lorenz, 1982). It is possible that the presence of other ovarian hormones in naturally cycling rats could be contributing to this contrasting effect. Indeed, the gonadal hormone progesterone (P) has been shown to rapidly enhance striatal DA activity, followed by inhibition of DA function 24 hours later (Dluzen & Ramirez, 1984).

Thus, the combined effects of P and E on striatal DA transmission could be causing this shift in DAergic response to amphetamine in naturally cycling rats.

Future Studies

In summary, the current studies show that reproductive experience alters the E2-modulated bias in cognitive strategy, and that E2 differentially influences striatal DA release in response to apomorphine and amphetamine. The next obvious step would be to examine striatal DA release in reproductively experienced females. It is clear that parity has a variety of significant effects on the female brain, and therefore DA transmission is likely to change following reproductive experience.

Reproductively experienced rats have been shown to be more sensitive to DA, as well as have higher striatal DA levels (Byrnes et al., 2001). Pregnancy is also linked to higher E2 levels (Bridges et al., 1984; Byrnes et al., 2006), which could lead to up-regulation of DA release and function in the striatum (Becker, 1990; Di Paolo, Poyet, and Labrie, 1981; Byrnes et al., 2001). Reproductively experienced rats could therefore show higher sensitivity to striatal DA modulation, when compared to nulliparous females. At present, it remains unclear exactly how DA function changes with parity and the period following parturition. Examining striatal DA transmission in reproductively experienced rats, and how this could change with different E2 levels, would help elucidate some of the changes that occur in the maternal brain.

It would also be interesting to investigate whether the changes observed in cognitive strategy use in parous females are permanent, or if reproductively experienced rats would eventually revert to the pattern observed in nulliparous rats. This is unlikely,

as a large body of research has shown that reproductive experience causes permanent and lifelong changes in the female (Kinsley & Lambert, 2008); for example estrous cycle-dependent changes in learning and memory appear to be permanent (Woolley, Gould, Frankfurt, and McEwen, 1990; Kinsley & Lambert, 2008).

Another interesting vein of inquiry would be to examine the role of both E and P on these behaviours and neurochemical effects. Progesterone fluctuates across the estrous cycle, and P levels increase as a result of parity (Bridges et al., 1984). As mentioned earlier, P has been shown to influence striatal DA activity (Becker, 1999; Di Paolo, Levesque, and Daigle, 1986) by immediately enhancing striatal DA release, but inhibiting DA function after 24 hours (Dluzen & Ramirez, 1984). Amphetamine-induced striatal DA release is potentiated in E-treated OVX rats administered P (Dluzen & Ramirez, 1990). Taking all of this into account, it would be interesting to examine how DAergic function in the dorsal striatum would change with E and P treatment. Becker and Rudick (1999) observed that in nulliparous OVX rats, P enhanced amphetamine-induced DA release, but only when the animals had been primed with E, which suggests that E priming is necessary for P-induced effects on DA function in the dorsal striatum. It would be of interest to explore this effect in reproductively experienced females, to observe whether E and P would interact in the same manner as in nulliparous rats.

Moreoever, one possible mechanism by which E could be influencing both learning strategy use and E-modulated striatal DA function is through GABA activity. The inhibitory neurotransmitter GABA has been shown to disrupt hippocampal activity, and $GABA_A$ receptors are widely present on hippocampal pyramidal cells (CA1-CA4 region; Mao & Robinson, 1998). Furthermore, a large proportion of spiny neurons in the

striatum are GABAergic and project to striatal DA terminals, hence affecting DA release in this brain region (Becker, 1999). It has been established that E inhibits GABA activity in the hippocampus by affecting presynaptic GABA-releasing vesicles (Ledoux & Woolley, 2005) and in the striatum by reducing CA^{2+} channel current flow in GABA neurons (Hu, Watson, Kennedy, and Becker, 2006; Mermelstein, Becker, and Surmeier, 1996; Becker, 1999). This suggests a key role for GABA transmission in how E enhances hippocampal activity and modulates striatal DA release. Indeed, E2 could be modulating learning strategy use in both nulliparous and primiparous rats through inhibition of GABA in both the hippocampus and dorsal striatum. Additionally, E2 could be facilitating striatal DA release in response to amphetamine through increased inhibition of GABA interneurons, which would increase DA release from striatal terminals. This idea is further supported by studies looking at the effects of GABA activity-altering drugs on the striatum: these studies showed that baclofen, a $GABA_B$ receptor agonist, decreased and phaclofen, a $GABA_B$ receptor antagonist, increased extracellular striatal DA levels (Smolders, De Klippel, Sarre, Ebinger, and Michotte, 1995). It is apparent that E's effects could be occurring through a variety of mechanisms, and more research is needed to further elucidate exactly how E is causing these key changes in the female brain.

This thesis has shown that reproductive experience changes how E affects cognitive strategy, as response strategy use decreases in low E2 primiparous rats, and rats receiving no E2 replacement do not differ across parity groups. Also, this thesis has explored E's effects on DAergic transmission in the dorsal striatum, and showed that females respond differently depending on circulating E2 levels. Estrogen was shown to increase striatal DA release, and this effect is lateralized, with higher DA levels observed

in the right hemisphere. Also, rats receiving high E2 replacement did not show a significant decrease in DA release following administration of an autoreceptor dose of apomorphine. These findings may be due, in part, to E2-induced changes in D2 autoreceptor function. Future studies should examine whether reproductive experience changes these E2-mediated effects on DA transmission in the dorsal striatum.

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