THE EFFECTS OF METHAMPHETAMINE AND OVARIAN HORMONES ON DOPAMINE RECEPTOR BINDING DENSITY IN THE FEMALE RAT BRAIN

A Thesis

by

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CHAPTER 1. ABSTRACT

Methamphetamine (METH) increases sexual motivation in ovariectomized female rats via direct influence on the mesolimbic dopaminergic pathway. METH’s effects on dopaminergic activity are dependent on the coadministration of ovarian hormones. In order to determine the mechanism through which METH stimulates the dopaminergic pathway, an analysis was performed on autoradiograms collected from the key mesolimbic brain regions of female rats: the medial amygdala (MeA), ventromedial nucleus (VMN), and the nucleus accumbens (NAcc). The dopaminergic receptor binding densities of both D1- and D2-type receptors were measured and compared between the groups of rats who received METH, ovarian hormones, METH and ovarian hormones, or a control injection. This analysis concluded that METH and ovarian hormone administration do not significantly change the number of bound dopaminergic receptors in any of the analyzed brain regions. The analysis also determined that there is potential lateralization of the MeA, as well as D2-autoreceptor activity in the MeA. These findings are significant in that they indicate that METH and ovarian hormone administration are activating the dopaminergic pathway, but that the change is occurring at a mechanistic location other than the number of bound dopaminergic receptors.
CHAPTER 2. INTRODUCTION

2.1 Introduction

Methamphetamine (METH) is a psychoactive stimulant drug that is highly addictive [4]. The METH epidemic of the 21st century made this drug the focus of many research studies [5]. One of the most striking findings that have come from this research is that women reported an increase in sexual motivation following METH use [14], potentially via activation of the dopaminergic reward/motivation pathways in the brain [6, 9]. Mechanistic studies indicate that this increase in sexual motivation likely occurs via an interaction between METH, dopamine, and ovarian hormones; however, the precise molecular interactions have yet to be fully elucidated. Here, the effects of METH and ovarian hormones on dopamine receptor binding within three key brain regions hypothesized to mediate sexual motivation and behavior are examined.

2.2 Dopamine and Methamphetamine

Dopamine is a monoamine neurotransmitter that plays an essential role in the reward and motivation pathways. Dopamine is synthesized within dopaminergic neurons via a series of enzyme-catalyzed reactions. Phenylalanine and tyrosine can both serve as precursors for the synthesis of dopamine [21]. In the multistep process that converts these molecules into dopamine, tyrosine hydroxylase (TH) is the rate-limiting enzyme [22].

Dopaminergic pathways have two main projections: the mesolimbocortical pathway and the nigrostriatal pathway. The nigrostriatal pathway begins in the substantia nigra and regulates motor movement while the mesolimbocortical pathway originates in the ventral tegmental area (VTA) and regulates reward and motivational behaviors [13]. The mesolimbocortical pathway is responsible
for feelings of pleasure and positive reinforcement with the incentive of both repeated and naturally occurring reward, and therefore, may mediate the enhanced sexual motivation observed following METH use. The target brain regions of the mesolimbocortical pathway contain dopaminergic receptors that are specialized into D1- and D2-type receptors. D1-type receptors include D1 and D5, which are coupled to Gs proteins that activate the production of cAMP and cAMP-activated protein kinase A (PKA), resulting in excitatory effects [2]. D2-type receptors include D2, D3, and D4, which are coupled to Gi proteins that repress the production of cAMP and PKA, resulting in inhibitory effects [2].

The dopaminergic reinforcement pathway begins in the VTA and contains axons that project to forebrain regions, including the medial amygdala (MeA), ventromedial nucleus (VMN), nucleus accumbens (NAcc), and medial preoptic area (mPOA) [3, 13], which are key neural regions that regulate sexual behavior in female rats [4]. The MeA processes olfactory inputs such as pheromones and modulates sexually motivated behaviors [13]. The MeA also projects to the VMN, which senses the hormonal status of the female rat and activates lordosis, a female copulatory position in which the female rat arches her back to allow intromission [13]. The NAcc, which plays a role in hedonic and reward behavior, also controls rejection behavior, such as avoidance [13]. The mPOA mediates other locomotor behaviors performed during sexual interactions [13]. These target brain regions contain several types of dopaminergic receptors, including both D1-types, which are excitatory, and D2-types, which are inhibitory [12].

D2 receptors can also serve an autoregulatory purpose. D2-autoreceptors are found on the dendrites, somas, and axonal projections of dopaminergic neurons in the VTA (and other midbrain regions) and modulate activity via both direct and indirect mechanisms [23]. D2-autoreceptors modulate dopaminergic neuronal activity directly by activating potassium conductance and
hyperpolarizing the neuron, preventing further activation and subsequent dopamine release [23].
D2-autoreceptors modulate dopaminergic neuronal activity indirectly by altering the expression of
TH, as well as the expression of dopamine transporter proteins in the plasma membranes of the
dopaminergic neurons [23]. Furthermore, chronic D2-autoreceptor activation inhibits dopaminergic
synapse formation [24]. These mechanisms allow D2-autoreceptors to inhibit further release of
dopamine from the neuron.

As a psychomotor stimulant, METH works by activating dopaminergic pathways in the brain
by altering the transmission and reuptake of dopamine in target regions [3, 13]. Briefly, METH
diffuses through the blood-brain barrier, simultaneously decreasing structural proteins of the
barrier, blocking vesicular monoamine transporter 2 (VMAT), and inhibiting monoamine oxidase,
which catabolizes monoamine including dopamine [8, 15]. VMAT is responsible for packaging
cytosolic dopamine into synaptic vesicles within neuronal synapses, so when it is blocked,
dopamine accumulates in excess in the presynaptic neuron and reverses the actions of reuptake
transporters [8]. METH also activates sigma receptors on target cells’ endoplasmic reticulum,
which decreases transporter functions by oxidizing cysteine residues on VMAT [3]. These actions
result in a sigma receptor-mediated release of dopamine into the synaptic cleft, disrupting the
normal pathways of action potential-mediated release of dopamine [3]. It has been observed that
METH specifically affects D1-type receptors and their corresponding neural pathways, acting
through these mechanisms to cause an intense pleasurable feeling [1, 9].

2.3 Ovarian Hormones and Dopamine

Hormones, specifically those released by the ovaries, can play a significant role in modulating
neural pathways, including those controlling dopamine release and female sexual behavior.
Estradiol (E2) is a steroid hormone secreted by the ovaries in response to gonadotropin stimulation via follicle-stimulating hormone (FSH) and/or luteinizing hormone (LH). Estradiol is the most potent form of estrogen produced by the ovaries and is the primary form present in the female body prior to menopause [18]. There are three types of estrogen receptors in the female brain: estrogen receptor α (ERα), which is stimulatory; estrogen receptor β (ERβ), which is inhibitory; and G protein-coupled estrogen receptor 1 (GPER1) [19]. E2 binds to ERα and ERβ with equal affinity; E2 can also bind to GPER1 but with a lower affinity than estrogen itself [19].

Estradiol neural modulation directly affects the dopaminergic reward and motivation pathway in female rat brains. In female rats who have been bilaterally ovariectomized (OVX), chronic E2 injections result in increased D2 binding sites in the NAcc and striatum via ERβ [16, 20]. Chronic E2 injection maintains the expression of dopamine transporter (DAT) and enhances the activation of adenylate cyclase by D1 receptors [16]. Estradiol administration also increases the protein expression and immunoreactivity of TH within the VTA and MeA [4]. By increasing the expression of TH, E2 increases the rate of synthesis of dopamine within these brain regions. Via these mechanisms, E2 has been found to enhance the dopaminergic signaling pathways in female rats’ NAcc, VTA, and MeA.

Progesterone (P) is also a steroid hormone secreted by the ovaries in response to gonadotropin stimulation. Progesterone is a type of progestogen that binds to both membrane and intracellular progesterone receptors [25]. These receptors are found in the highest densities in the hypothalamus, hippocampus, and amygdala [26]. Progesterone has a bimodal effect on dopaminergic neuronal activity, first facilitating dopamine release and subsequently inhibiting it [27]. When administered acutely, progesterone triggers spontaneous dopamine release in OVX female rats for 2-12 hours [27]. However, following a 24-hour period, dopaminergic activity levels significantly decreased.
below that of the baseline [27]. Progesterone also impacts the synthesis of dopamine; multiple populations of TH-containing cells in the VTA (and other midbrain regions) contain progesterone receptors that modulate their activity [28].

The vast majority of work examining the effects of ovarian hormones on dopamine has focused on estradiol within the NAcc, as well as its interaction with cocaine coadministration. Cocaine, another psychomotor stimulant, has been a major focus of pharmacological research due to its enhanced effectiveness in female users [31]. While cocaine also affects the dopaminergic pathways, it acts via a different mechanism than METH by binding to dopaminergic reuptake transporters and increasing the duration that dopamine is present in neural synapses [32]. Due to the enhancement of cocaine’s effectiveness in female users, the predominant focus of scientific investigation has been determining the mechanism through which E2 and its corresponding receptors facilitate an increase in the behavioral and physiological changes caused by cocaine [33, 34]. As such, there is a gap in the available literature on the effects of E2 and P on dopaminergic pathways, specifically those altered by METH coadministration.

2.4 METH and Hormones

Previous studies have demonstrated that METH increases proceptive behaviors and enhances sexual motivation in female rats, and this enhancement is mediated by the medial amygdala (MeA) [6, 7]. However, this increase in sexual motivation is only observed when rats are administered both E2 and P, suggesting that METH, and possibly dopamine, work in conjunctions with the ovarian hormones to impact sexual motivation [7]. These ovarian hormones both enhance sexual behavior and aid in the activation of key brain nuclei (e.g., the VMN and the MeA) [7].
It has been observed that only D1 pathways are affected by the presence of METH, while D2 receptors are not affected by METH administration [10]. Therefore, the effects observed when an organism is given METH can only be attributed to changes in the pathways with D1 receptors [1]. These studies isolate D1 receptors as the target of the mechanism through which METH affects neural functioning. This study evaluates the density of both D1 and D2 receptors following administration of METH in order to confirm the findings of previous literature and to prevent discounting of any conclusions from the results based on a lack of comparative data showing the activity of D2 receptors during this experiment.

The female rat requires ovarian hormones to display sexual motivation and behavior, and METH increases sexual motivation in female rats primed with ovarian hormones [4, 7]. Estradiol and progesterone administration enhances the dopaminergic response to METH administration and facilitates an increase in the release of striatal dopamine [29]. Although several regions of the brain, such as the NAcc, mPOA, VMN, and MeA, have been implicated in female sexual motivation, these regions show different patterns of activation following the administration of ovarian hormones and METH. Only the MeA and VMN show activation following administration of both ovarian hormones and METH [4]. The mPOA shows activation only when hormones are administered and the NAcc is activated only by METH [4]. The MeA was the brain region affected by both ovarian hormones and METH, resulting in an increase in proceptive behaviors [5]. Specifically, the posterodorsal medial amygdala (MePD) was subsequently shown to be the specific target of METH [6]. In addition, administering a D1 agonist results in similar effects as METH administration while D2 agonists did not have an effect [6], suggesting that METH acts by binding to D1-type dopaminergic receptors and that D2-type receptors do not interact with METH. The effects of METH on the dopaminergic pathway and subsequent behavioral changes occur due to a
mechanistic change in the functioning of neurons containing both D1 and E2 binding sites [14].
Due to the presence of receptors for both dopamine and E2 on the principal target neurons, both of
these components were taken into account and analyzed in the current study.

Subsequent studies have determined that METH increases the expression of sexually
motivated behaviors in female rats via an interaction between dopamine and progesterone receptors
in the posterodorsal medial amygdala (MePD) [4,5,6]. METH increases the extracellular levels of
catecholamines, specifically dopamine [1], making the dopamine receptors a critical focus in
determining the mechanisms by which METH increases sexual motivation and supporting the
hypothesis that METH acts via the dopamine D1-type receptors to enhance female sexual
motivation. These findings contribute to the overall understanding of how METH affects an
individual physically, cognitively, and behaviorally. However, despite the significant strides made
in the study of METH, there has yet to be an explanation for how exactly METH affects the
individual components of dopaminergic pathways to enhance sexual motivation, particularly
receptor density, and in what areas of the brain these changes are most prevalent.

2.5 Experimental Design

Previous work has determined that the E2 can increase the expression of TH, suggesting an
increased availability of dopamine in the MeA. In addition, the activation of D1 receptors is
necessary for the enhancement of proceptive behaviors by METH. However, these previous studies
did not examine any potential changes in the dopamine receptors themselves following the
administration of METH and ovarian hormones. Therefore, the study attempts to determine the
effects of METH and ovarian hormones on D1 and D2 receptor density in the MeA, VMN, NAcc,
and mPOA of the female rat brain. Autoradiography labels (dopaminergic) receptors via a
radioactive ligand binding to it, allowing the bound receptors to be visualized in postmortem brain slides. By analyzing archival autoradiographic brain slides of female rats that were administered either ovarian hormones or oil and METH or saline injections, the density of dopaminergic receptor binding can be determined. If a significant density of activated receptors is observed subsequent to METH or ovarian hormone injection, it will further explain the interactive or additive effects of METH and the ovarian hormones on D1-type and D2-type receptors in various brain regions. The variations in dopaminergic receptor binding will allow the identification of the key brain regions affected by METH usage. This will contribute information useful in creating a potential METH-specific addiction treatment that targets dopaminergic receptors and their prevalence in specific brain regions.

In addition, the female rat brain shows lateralization of specific brain regions, differing in structure and subsequent function. Specifically, the MeA has been the area of significant investigation into the difference in left versus right brain hemisphere differentiation. The MeA of female rats has been noted to be extremely lateralized, with the right MeA containing more astrocytes and the left MeA containing more complex astrocytes [17]. The left MeA also has more volume than the right MeA [17]. Therefore, this study examines the potential of lateralized effects on dopaminergic binding.

The conclusions of these studies are reported in the context of increasing sexual motivation, as part of a behavioral analysis in order to determine the effects of METH and ovarian hormones on sexual motivation. There is a gap in evidence to show that METH enhances sexual motivation via increased D1-type receptor binding density in brain regions that control sexual activity. This study will exclude any behavioral analysis and instead evaluate any potential changes to D1- and D2-type receptors by METH and/or ovarian hormones in key female brain regions involved in sexual motivation: MeA, VMN, NAcc, and mPOA. Multiple brain regions will be evaluated to test the
hypothesis that METH interacts with ovarian hormones in the MeA by the D1-type receptor, which would provide further evidence that the MeA is the target of METH and further explain the pathway through which it is affected. This research will allow scientists to further understand how the drug affects women and aid in the effort to understand and combat methamphetamine addiction.
CHAPTER 3. MATERIALS AND METHODS

3.1 Data Source (Archival Photomicrographs)

The photomicrographs were collected as part of the dissertation studies of M. K. Holder [4]. The animals used in these studies were treated as described below.

3.2 Animals

Female adult Sprague-Dawley rats (250-300 g) were bought from Charles River Laboratories (Kingston, NY). The animals were housed in the University of Maryland School of Medicine’s Laboratory Animal Facility of the Health Science Facilities. The animals had water and food available ad libitum and were housed under a reversed 12 h:12 h dark:light cycle (lights off at 1000 h). Bilateral ovariectomies (OVX) were performed on all animals under ketamine:acepromazine (80 mg:2.5 mg/kg) anesthesia. Following surgery, the animals were allowed a 10-14 day recovery period. The University of Maryland, Baltimore Institutional Animal Care and Use Committee approved all procedures, which were in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. All efforts were made to reduce the number of animals used and to minimize animal suffering.

3.3 Hormones and Methamphetamine Treatment

The animals were randomly separated into four treatment groups (Table 1). All animal injections were administered in accordance with the treatment procedures applied in previous studies [4, 5]. Forty-eight hours before the beginning of tissue collection, all animals were administered 5 μg of 17-β-estradiol benzoate (EB), followed by 10 μg of EB twenty-four hours
later. On the day of tissue collection, all animals were administered 500 μg of P. The animals received a daily injection of METH (5 mg/kg/day) or saline vehicle during the three days of hormonal priming.

<table>
<thead>
<tr>
<th>Animal Treatment Groups</th>
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<tr>
<td></td>
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<tr>
<td>Hormone Condition</td>
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</table>

Table 1. *Animal Treatment Groups*. This table depicts the number of animals (n) assigned to each treatment group, depending on Drug Condition and Hormone Condition.

### 3.4 Radioactive Ligands

[^3H] SCH23390 (85 Ci/mmol; Perkin-Helmer) was the ligand selected to label D1 dopaminergic receptors. Butaclamol hydrochloride (D1 dopaminergic receptor antagonist; obtained from Sigma-Aldrich, St. Louis, MO) was used to define the nonspecific binding.[^3H]spiperone (101 Ci/mmol; Amersham Biosciences, Piscataway, NJ) was the ligand selected to label D2 dopaminergic receptors. Haloperidol (D2 dopaminergic receptor antagonist; Sigma-Aldrich) was used to define the nonspecific binding (Table 2).

<table>
<thead>
<tr>
<th>Binding Series</th>
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<tr>
<td>Dopaminergic Receptor Type</td>
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<tr>
<td>D1</td>
</tr>
<tr>
<td>Total Binding</td>
</tr>
<tr>
<td>Non-Specific Binding</td>
</tr>
</tbody>
</table>

Table 2. *Binding Series*. This table depicts the specific binding series used depending on assigned binding evaluation and dopaminergic receptor type labeling.

### 3.5 Tissue Collection, Sectioning, and Autoradiography Processing

Four hours following P and the final METH administration, brains were collected from all subjects. The brains were rapidly dissected out of the skull and immediately frozen on powdered dry ice and stored at -80°C until all brains were used. The brains were cut into 16μm-thick sections
on a cryostat and thaw-mounted onto gelatin-coated microscope slides. Six series of slides were collected so that on each slide consecutive sections were 80µm apart. Depending on their size, 4-5 sections were mounted per slide. Four series were used for autoradiography, while another series were Nissl-stained in order to verify the location of brain regions. The slides were dried and stored at -80°C until used.

After drying at room temperature, slides were preincubated in buffer (50 nM Tris-HCl, pH 7.5 at 25°C with 1mM MgCl₂) for 30 minutes at room temperature. Slides were then incubated for 1 hour at room temperature in 2nM [³H] SCH23390 buffer with no competitor (D1 total binding, series 1) or with 2µM Butaclamol (D1 nonspecific, series 2) or 0.4nM[³H]spiperone buffer with no competitor (D2 total binding, series 3) or with 10⁻⁵M Haloperidol (D2 non-specific binding). One hour later the slides were washed twice for 5 minutes in ice-cold buffer followed by a quick dip in ice-cold distilled water. Sections were fan-dried, placed in X-ray cassettes, and exposed to BioMax MR films (Kodak, Rochester, NY) along with standards (ART-123; American Radiolabeled Chemicals, St. Louis, MO) containing concentrations of tritium ranging from 0.00 to 489.1 µCi/g. The films were developed after 4 weeks.

The density of dopamine receptor binding was analyzed in various brain regions identified by comparison with adjacent sections counterstained with the Nissl staining method. Images from the films were scanned into a Macintosh computer using a high resolution (1200dpi) scanner (Epson Perfection 3170 Photo). NIH Image (Wayne Rasband, NIH, Bethesda, MD) was used to analyze receptor density within each region. Adjacent sections were incubated in different experimental conditions or counterstained for anatomical purposes were stacked in Image J so that binding density could be measured in superimposed images. Mean density was calculated from the densities taken from the left and right sides of each nucleus at the level of its maximal extension.
The optical densities of the tritium standards that had been apposed to the film along with the sections were converted into approximate fmol/mg protein of bound \(^{3}\text{H}\) SCH23390 or \(^{3}\text{H}\)spiperone. Specific binding was determined by subtracting binding observed in the absence of Butaclamol or Haloperidol, respectively. Brain structures were identified based on plates from the atlas of the rat brain [32]. The slides were exposed on the films for four weeks. The slide images for the mPOA were unavailable for analysis, so only the scanned images for the MeA, VMN, and NAcc were included in this study.

### 3.6 Autoradiographic Image Analyses

#### 3.6.1 Selection Parameters

A total of 1105 brain slides were collected, processed, and photographed. The photographs were digitized and analyzed in ImageJ. Of the 1105 brain slide images, 1016 were included in the data analysis, with the specific number of slides per brain region depicted in Table 3. Images were removed from the analysis if artifacts obscured the corresponding brain region of focus or if the optical density measurement was reversed.

<table>
<thead>
<tr>
<th>Number of Sections Analyzed</th>
<th>MeA</th>
<th>NAcc</th>
<th>VMN</th>
</tr>
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<tbody>
<tr>
<td>D1 NS D1 NS D2 NS D2 NS</td>
<td>93</td>
<td>93</td>
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<tr>
<td>D1 NS D1 NS D2 NS D2 NS</td>
<td>93</td>
<td>93</td>
<td>92</td>
</tr>
<tr>
<td>Provided</td>
<td>93</td>
<td>93</td>
<td>92</td>
</tr>
<tr>
<td>Included in Analysis</td>
<td>86</td>
<td>86</td>
<td>89</td>
</tr>
<tr>
<td>VMN D1 NS D2 NS D2 NS</td>
<td>86</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td>Provided</td>
<td>93</td>
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<td>92</td>
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<tr>
<td>VMN D1 NS D2 NS D2 NS</td>
<td>86</td>
<td>86</td>
<td>86</td>
</tr>
</tbody>
</table>

Table 3. *Number of Brain Images Analyzed*. This table depicts the number of autoradiographic images analyzed for each brain region. Within each brain region, D1, D1 NS, D2, and D2 NS condition images were analyzed.
3.6.2 Image J

In order to measure the optical density of the appropriate brain region in each brain slide image, Image J was calibrated using a step wedge. The pixilation density was calibrated to a specific optical density, measured in microcuries. Each brain slide image was opened in Image J. Using a reference image for each brain region, a specific pixel length and width were assigned to each brain region image selection. All brain slide images were analyzed using this assigned pixel area specific to their brain region, regardless of their binding assignment or treatment condition. Once the brain region area was selected in Image J, the Analysis function was used to measure the optical density of the selected area.

![Image J Analysis](image)

Figure 1. *Pixel Selection for Analysis.* This figure depicts the pixel selection for the MeA.

3.6.3 Selection Criteria

Brain sections were collected and regionally identified by matching the structure to standard rat brain atlas plates in conjunction with the identification of landmarks (nearby neural structures) for confirmation [30]. The NAcc was identified based on Plate 13, using the corpus callosum and
the anterior commissure as landmarks [30]. The VMN was identified based on Plate 50, using the optic tracts and third ventricle as landmarks [30]. The MeA was identified based on Plate 54, using the third ventricle posterior edge of the brain and the optic tract as landmarks [30].

3.7 Statistical Analyses

The optical densities from each brain region were analyzed in GraphPad PRISM (version 9.3.1). Two-way ANOVAs were run on the data from each brain region, the left side of each brain region, and the right side of each brain region, using a p-value of 0.05. t-tests were run on the data comparing the drug experimental conditions and the hormone experimental conditions, including a left versus right comparison.
CHAPTER 4. RESULTS

4.1 MeA

The aim of this experiment was to determine any significant difference in dopaminergic receptor binding between the four treatment groups. There was a significant interaction between drug administration and hormonal treatment ($F(1,19) = 6.526, p < 0.05$) on the binding of D1 R only on the left MeA (Figure 2). There was also a significant difference between the oil groups and hormone groups in the D2 receptor binding density in the left MeA ($F(1,19) = 4.907, p < 0.05$) (Figure 3).

![Figure 2. Left Medial Amygdala D1-R Binding.](image1)

This figure depicts the density of bound D1 receptors in the left medial amygdala.

![Figure 3. Left Medial Amygdala D2-R Binding.](image2)

This figure depicts the density of bound D2 receptors in the left medial amygdala.
Further analysis comparing the left versus right side of the MeA determined a significant
difference between the D2 receptor binding density of the oil group and hormone group (F(1,42) =
6.665, p <0.05) (Figure 4).

![MeA D2 Left v. Right](image)

Figure 4. Medial Amygdala D2-R Binding. This figure depicts the density of bound D2 receptors in
the medial amygdala, comparing the left to the right side of the MeA.

### 4.2 VMN

Within the VMN, all of the different treatment conditions were statistically compared. There
was no significant difference in dopaminergic receptor binding density between any of the
hormonal or drug treatment groups. There was no significant difference in the left and right sides of
the VMN, nor when the data from both sides was combined and analyzed as a whole.

A further analysis was conducted investigating any potential difference in the hormone
conditions regardless of the drug condition did not conclude any significant differences. A similar
analysis investigating any potential difference in the drug conditions regardless of the hormone
c condition did not conclude any significant differences. Both of these analysis conditions were
repeated comparing the left side of the VMN to the right side of the VMN, as well as a compiled
analysis including the data from both sides. None of the 2-Way ANOVA tests or t-tests returned a
significant difference in binding density between the left and right brain regions or across the treatment groups.

4.3 NAcc

Within the NAcc, all of the different treatment conditions were statistically compared. There was no significant difference in dopaminergic receptor binding density between any of the hormonal or drug treatment groups. There was no significant difference in the left and right sides of the NAcc, nor when the data from both sides was combined and analyzed as a whole.

A further analysis was conducted investigating any potential difference in the hormone conditions regardless of the drug condition did not conclude any significant differences. A similar analysis investigating any potential difference in the drug conditions regardless of the hormone condition did not conclude any significant differences. Both of these analysis conditions were repeated comparing the left side of the NAcc to the right side of the NAcc, as well as a compiled analysis including the data from both sides. None of the 2-Way ANOVA tests or t-tests returned a significant difference in binding density between the left and right brain regions or across the treatment groups.
While previous studies have begun to identify the mechanism through which METH elicits physiological and behavioral changes within the body, there is still much to be determined regarding the mechanism of the drug’s action. The results of this investigation did not determine any significant difference in dopaminergic receptor binding between the METH and Saline treatment groups in either MeA, VMN, or NAcc. This finding provides evidence to support that the observed behavioral changes that occur in animals as a result of METH administration are not due to a change in the number of bound receptors within the MeA, VMN, or NAcc. The data analysis also produced incidental findings indicating potential lateralization within the MeA, as well as binding density differences indicating potential D2-autoreceptor activity in the MeA. This finding further supports the conclusion that dopaminergic activity changes are occurring in the female rat brain, specifically the MeA, but that the changes are occurring at a mechanistic location other than the number of bound dopaminergic receptors.

Previous studies have established that METH and ovarian hormone administration both increase the activity of the dopaminergic pathways. By blocking VMAT, METH prevents the packaging of presynaptic dopamine into vesicles and causes dopaminergic reuptake transporters to reverse their direction of transport, causing an efflux of dopamine into the synapse [8]. Estradiol administration causes an increase in the expression of TH, leading to an increase in the dopaminergic rate of production [4]. Therefore, following the administration of METH, EB + P, and METH and EB + P, there is a known and expected increase in the dopaminergic activity within the key brain regions. Because there was no significant difference in the D1 or D2 receptor binding densities in any of these brain regions, the findings indicate that the METH and ovarian hormone-
induced enhancement of dopaminergic activity is occurring, but not via a change in the number of bound receptors. This suggests that the change in dopaminergic activity is occurring at a different mechanistic location, such as a downstream signaling cascade or via the increase in the number of progesterone receptors [35].

In order to create physiological and behavioral changes, METH must be coadministered with E2 + P [29]. In addition, foundational studies investigating ovarian hormones determined that E2 is not sufficient to modulate sexual behavior in females, nor is P [36]. Estradiol and progesterone have to be coadministered in order to observe any significant sexual behavior in females [36]. Previous investigations have also determined that METH administration increases not only the frequency of binding of progesterone receptors but the overall number of progesterone receptors in the female rat brain [35]. Even when D1 receptors were antagonized, the effects of METH, including proceptive female sexual behaviors, were still observed when progesterone receptors were activated. These findings indicate that while METH increases sexual motivation via D1 receptor activity, the changes are modulated by P. Given this conclusion, it is not expected that the binding density of the dopaminergic receptors would change, as the effects of METH are more likely to be a result of changes to the number and binding of the progesterone receptors [35].

The secondary statistical analysis comparing the drug and hormone conditions determined several incidental findings. Firstly, there was a significant interaction between the drug condition and hormone condition during the analysis of the left MeA D1 slides. While no conclusion can be drawn regarding the effects of the treatment groups themselves, this indicates a potential lateralization of D1 receptors within the MeA. Secondly, there was a significant difference between the D2 receptor binding density of the oil group and the hormone group. This further supports the indication that there is lateralization within the MeA, not only with D1 receptors but with D2-type
receptors as well. Finally, there was a significant difference in the D2 receptor binding density in the left MeA between the oil groups and hormone groups. Given the innate autoreceptor characteristics of D2 receptors, this finding supports the conclusion that there is a change in the dopaminergic pathways’ activity within the MeA. While the change is not occurring in the number of receptors binding to dopamine, the activation of the autoreceptor pathway indicates that the D2 receptors are engaging in negative feedback in order to compensate for the E2-induced changes and overstimulation within the MeA.

This study had several limitations that could have affected the results of the experiment. The brain slides that were analyzed were archival, and not all of the methodology and notes used in the preparation of the slides were available for review. As such, any errors or malfunctions that occurred during the brain slide preparation process were unable to be noted during data analysis. As well, several of the slides included artifacts that prevented the proper analysis of the optical density in the designated brain region. This prevented their inclusion in the data analysis.

While the overall analysis included over 1,000 slides, the aforementioned limitations prevented the inclusion of 89 brain slide images. Therefore, while the standard slide number for the different treatment groups was upheld in most of the analyses, some of the treatment groups only had four slide images for analysis. Should a future study be performed, a larger sample size would allow experimenters to account for any outlying data or artifact interference without an impact on the study. As well, given that the scope of the current experiment was limited to the analysis of bound dopaminergic receptors, future studies could investigate the concentration of dopamine released, in order to further clarify the mechanism through which METH and estradiol affect the dopaminergic pathways.
While the data analysis did not return significant findings indicating a difference in dopaminergic receptor binding across the different treatment conditions, the results from the experiment provide insight into the mechanism through which METH affects the female rat brain. Given that the results of this experiment rule out a change in the number of bound receptors, the conclusions of this experiment point to a different mechanism through which METH incites behavioral effects. This finding indicates that future experiments should be performed to investigate changes in the total receptor numbers whether the behavioral changes are a result of further downstream signaling cascades or an increase in progesterone receptors secondary to METH administration. These conclusions can aid in the drive to understand the mechanism through which METH incites neural effects, specifically those that control and enhance female sexual motivation. This further contributes to the investigation and identification of the effects of METH on female users.
REFERENCES


