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Analysis of the role of D₂ receptors in methylphenidate-induced conditioned place preference

Thesis submitted in partial fulfillment of Honors

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Abstract

ADHD is one of the most commonly diagnosed disorders during adolescence. Recently, significant increases in the diagnosis of ADHD have caused the prescription of the ADHD medication methylphenidate (MPH) to increase. MPH is a psychostimulant that blocks the dopamine transporter, which is responsible for dopamine reuptake at the synapse. The blockade of the dopamine transporter results in an increase in the availability of dopamine in the synaptic cleft. This increase of dopamine accounts for the addictive properties of a MPH due to strong effects on portions of the brain's drug-reward pathway, including the striatum and nucleus accumbens. In this study, we hypothesized that dopamine D₂ receptor antagonism would block MPH-induced conditioned place preference. We also hypothesized this will be more effective in adolescent male rats as compared to adolescent female rats based on evidence that has shown a higher density of dopamine D₂ receptors in the brain's reward areas of adolescent male rats. The effects of MPH on the associative effects of MPH was analyzed using the conditioned place preference (CPP) behavioral paradigm. Results showed that MPH-induced CPP was not blocked by the dopamine D₂ receptor antagonist, likely due to its effects on the inhibitory presynaptically located dopamine D₂ autoreceptor. The importance of these findings is discussed relative to the role of the D₂ receptor in MPH addiction.

Keywords: Methylphenidate, MPH, dopamine, D₂ receptor, antagonist

Analysis of the role of D₂ receptors in methylphenidate-induced conditioned place preference

Attention deficit hyperactivity disorder (ADHD) is one of the most commonly diagnosed disorders in adolescence, and is characterized by increased impulsivity, hyperactivity, and inattentiveness (Goldman, Genel, Bezman, & Slanetz, 1998). It is suspected that this disorder affects from two to sixteen percent of school-aged children, and effects three to five percent of children globally (Rader, McCauley, & Callen, 2009; Nair, Ehimare, Beitman, Nair, & Lavin, 2006). Children who are affected by ADHD will have difficulties with decision making, motivational issues, behavioral inhibition, temporal rewards, and may engage in riskier activities when compared to their peers (Toplak, Jain, & Tannock, 2005; Nikolas & Nigg, 2013). As can be understood, ADHD can prove to be a significant impairment for adolescent children if it is not properly treated. As such, a number of different treatment options have come into existence, but the most prevalent form of therapy for ADHD is by medication through psychostimulants (Swanson, Baler, & Volkow, 2011).

One of the most commonly prescribed medications for ADHD is the psychostimulant methylphenidate (MPH) (Trade name: Ritalin; Wu et al., 2012). MPH has a therapeutic effect on ADHD symptoms as a product of working on the dopamine system by blocking the dopamine transporter, a protein that is implicated in the reuptake of the dopamine neurotransmitter from the synaptic cleft for their return to the presynaptic neuron (Schweri et al., 1995). Similarly, MPH also produces slight effects on the levels of another neurotransmitter, norepinephrine, by blocking the norepinephrine transporter (Solanto, 1998). This transporter behaves similarly to the dopamine transporter relative to the norepinephrine system. In the case of blockade of either of these two transporters that MPH effects, the result is an excess of the neurotransmitter in the

synaptic cleft, resulting in a diminished synaptic clearance of these neurotransmitters. This diminished clearance leaves abnormally high amounts of these neurotransmitters in the synaptic cleft. As with MPH, and many other drugs, this can lead to unwanted or deleterious effects for the person who is using these agents.

Dopamine plays a central role in modulating the brain's reward and pleasure systems, while norepinephrine plays a role in regulating mood. Additionally, dopamine is closely associated with the presentation of reward-seeking behaviors including approach, consumption, and addiction (Nestler, 2005). Drugs that are commonly abused by humans such as opiates, ethanol, nicotine, amphetamine, and cocaine show an increase in the dopamine levels within the mesolimbic system, with especially high concentrations found in the nucleus accumbens (Di Chiara & Imperato, 1988). Additionally, there are five identified dopamine receptors: D₁, D₂, D₃, D₄, and D₅ (Niznik & Van Tol, 1992). These receptors come in two different subtypes: the D₁-like subtype, comprised of D₁ and D₅ receptors, and the D₂-like subtype, comprised of the D₂, D₃, and D₄ receptors (Vallone, Picetti & Borrelli, 2000). Subsequent findings have revealed that the main distinction between the two different subtypes is based on the effects these receptors have on the second messenger cyclic adenosine monophosphate, as well as their differences in pharmacological profiles (Kebabian & Caine, 1979). Despite having differing pharmacological properties, both families of dopamine receptors are affected when using MPH (Swanson & Volkow, 2003). Due to the fact that MPH enhances dopamine activity, the two families of dopamine receptors should be studied to further the understanding about the effects of MPH on the dopamine system, as well as their effect on the reinforcing behaviors.

Whereas it is widely accepted that MPH is an effective treatment for ADHD and pervasive developmental disorders, investigations on the effects of MPH are critically important

(Reichow, Volkmar, & Bloch 2013). This is because MPH has been shown to have addictive liabilities, due to increases in available synaptic dopamine (Bogle & Smith, 2009). Furthermore, the use of a dopamine transporter blockade gives MPH a neuropharmacological profile that is similar to that of cocaine (Volkow et al., 1999). In addition to cocaine, MPH interacts with and activates the same drug-reward pathway that drugs such as nicotine and amphetamine do (Swanson & Volkow, 2002; Yang, Swann, & Dafny, 2006). The interaction with the drug-reward pathway that results in the increase of dopamine-related activity is prevalent in two particular brain regions, the striatum and nucleus accumbens. Additionally, MPH has also been shown to produce significant increases in locomotor activity, a behavior that is mediated by the nucleus accumbens and striatum (Salamone & Correa, 2002). Locomotor activity can simply be defined as the movement from one place to another, and, in experimental contexts, it may be measured to gauge reward-seeking behavior. The similarities in the properties and effects of commonly abused rewarding drugs, such as cocaine and amphetamine, to the properties of MPH, show the addictive potential of MPH.

It has been shown that MPH is used recreationally as an addictive agent. This is due to the fact that this drug is a stimulant. When taken recreationally, MPH is typically taken orally or snorted (Teter, McCabe, LaGrange, Cranford, & Boyd, 2006). In the case of snorting MPH, this route avoids the body's first pass metabolism by the liver, which delivers higher concentrations of the drug to the brain when compared to taking the drug orally. The results of avoiding first pass metabolism will be a more intense drug effect that typically has a faster onset. Due to the fact that people both frequently use and abuse this drug, one could consider it a major concern for public health due to a possibility that long-term effects on the brain could be present (Carlezon & Konradi, 2004). Consequently, further studies on the long and short-term effects of

MPH use are desperately needed to ensure that using medication with MPH is not having a damaging effect on the brain.

Additionally, our laboratory has also shown the use of MPH causes an increase in neurotrophic factors (Brown et al., 2012). Neurotrophic factors are cellular growth factors that are important for synaptic maintenance and growth, and they have been separated into three main families: the neurotrophins, the glial-cell derived neurotrophic factor (GDNF) family, and the neuropeptidic cytokines (Boyd & Gordon, 2003). For example, in vivo studies using neurotrophic factors have been responsible for neurogenesis of mammalian neurons in two different brain regions (Hagg, 2009). However, despite the fact that neurotrophic factors facilitate the proper functioning and efficiency of neurons, significant increases of neurotrophic factors does not always correlate with flourishing and healthy neurons. In fact, elevated neurotrophic factor levels appear to play a role in the drug craving response, especially when these elevated levels are found within the mesolimbic pathway (Pickens et al., 2011). For example, increased neurotrophic factors in the mesolimbic pathway have been shown in people who use cocaine, attesting to the idea that this increase plays a role in addiction (Grimm et al., 2003).

Of the three families of neurotrophic factors discussed above, the family most pertinent to our study is the neurotrophin family. The mammalian neurotrophin family of growth factors contains four different types: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) (Lessmann, Gottmann, & Malcangio, 2003). All four different types of neurotrophins play a wide variety of roles for different bodily functions throughout mammalian development, and changes in the secretion rates and overall levels of these growth factors are implicated in a diverse array of disease states

including Alzheimer's disease, depression, pain, and asthma (Allen & Dawbarn, 2006).

Additionally, Allen and Dawbarn (2006) note that one particular growth factor, BDNF, plays a special role in activity-dependent synaptic plasticity, something that could be indicative of either learning occurring or memory formation. Typically learning and memory formation is a sign of proper function of neurons, but in the context of drug addiction, it proves to be the fuel to help this maladaptive process set in. This growth factor, BDNF, is of primary interest for this particular study.

BDNF is a growth factor protein that is found throughout the central nervous system that helps to support the survival of neurons, as well as to promote the growing of new synapses in the brain (Conner, Lauterborn, Yan, Gall, & Varon, 1997). When describing the role and effect of BDNF, authors Robert Julien, Claire Advokat, and Joseph Comaty (2010) write, "...when injected into the brain of rats, BDNF not only prevents the spontaneous death of some neurons, but also helps to protect neurons that have been poisoned with various toxins. Conversely, in animals, chronic stress decreases the production and amount of BDNF (and other neurotrophic substances) in the brain and increases cell death." This excerpt shows that BDNF is critical to neural functioning, so, naturally, a lack of this neurotrophic factor can be severe in consequence. Interestingly, Corominas-Roso et al. (2013) have also reported that, "Low BDNF levels may contribute to the neurodevelopmental deficits of ADHD and to the persistence of the disorder into adulthood." This finding is extremely interesting because it gives more information on why MPH works so well as an ADHD medication. By using MPH, you increase BDNF levels throughout the brain, and, with respect to these findings, this increase may also be part of the therapeutic action that MPH takes when alleviating symptoms of ADHD. However, it should be noted that polarization of BDNF levels in either direction can cause deleterious effects on the

brain, and that the healthiest level of this protein can be found in the middle ground. In this study, we will be harvesting brain tissues from two dominant areas in the drug-reward pathway, the nucleus accumbens and the striatum, and we will be running a chemical analysis to determine the amount of BDNF in these tissue samples.

Another interesting and important area to consider when trying to fully understand the effects of MPH with D₂ antagonism is sex differences. Our laboratory has shown that a relatively high dose of MPH (5mg/kg) (a dose which is comparable to dose taken by addicts, rather than medicinal users) results in robust sex differences in the behavioral response to this drug (Chase, Carrey, Soo, & Wilkinson, 2007; Brown et al., 2012). This data shows that adolescent female rats demonstrate a 100% increase in locomotor activity compared to adolescent males given the same dose at peak responding, and the results from this study indicate that female adolescent rats sensitized to MPH, whereas males administered the same dose and regimen of MPH failed to produce sensitization (Brown, et al., 2012). Drug sensitization is a phenomenon produced by psychostimulants when an increased behavioral effect of a drug occurs following repeated doses. In essence, drug sensitization is the opposite of acquiring a drug tolerance, which means that the female adolescent rats may have a higher sensitivity to the addictive properties of MPH as compared to adolescent male rats. These behavioral and pharmacological results indicate a raised probability that dopamine-dense brain areas will have increased sensitivity to the addictive properties of MPH (Meredith, Callen, & Scheuer, 2002).

In our present study, we intend to investigate the neurobehavioral mechanisms of the rewarding effects of MPH by focusing on the dopamine D₂ receptor to evaluate its role in this phenomenon. We will assess the role of D₂ receptors in MPH-induced CPP by conditioning rats in a five day CPP behavioral paradigm. By using a D₂ receptor antagonist (eticlopride), we will

reduce the functionality of the D₂ receptors, and subsequently gauge whether or not MPH-induced CPP occurs. Following a posttest to see whether or not CPP has occurred, brain tissues will be taken to be analyzed for BDNF concentrations within the nucleus accumbens and striatum. Finally, group differences will be analyzed using ANOVA. Our hypothesis is that the use of eticlopride, the dopamine D₂ antagonist, will block CPP produced by MPH in adolescent rats, and that this will be more effective in adolescent male rats as compared to adolescent female rats. This hypothesis is based on work by Andersen & Teicher (2000) that have reported a higher density of dopamine receptors in the striatum of males during adolescence when compared to adolescent female rats. This study will add to the information on the underlying actions and mechanisms of the rewarding effects of MPH.

Method

Participants

The animals for this experiment arrived at East Tennessee State University at postnatal day 21 (p21) and were ordered from Harlan, Inc (Indianapolis, IN, USA). In total, there were 45 subjects (23 female, 22 male) in our study. All rats were housed in an AAALAC-accredited animal facility at East Tennessee State University with a 12 hour on/off light/dark cycle. Food and water were available ad libitum.

Research Design

Animals were divided into four separate experimental groups: a low dose eticlopride group (0.01 mg/kg), a high dose eticlopride group (0.03 mg/kg), MPH (5 mg/kg) group, and the saline control group. Both eticlopride groups were administered eticlopride followed 15 minutes later by MPH, whereas the MPH group was first given an intraperitoneal (ip) injection dose of

saline followed 15 minutes later by MPH. Approximately 10-15 minutes after MPH injection, animals were placed into their respective conditioning contexts, which were randomly assigned. Saline control groups received two injections of saline. By analyzing two doses of eticlopride, we can compare the differences between the low dose and high dose of eticlopride, which will help discern dose-dependent effects of this drug. The incorporation of MPH and saline groups will allow for the comparison of the D₂ antagonist groups with MPH relative to CPP.

Materials and Procedure

The CPP apparatuses measure 90 cm on each side, and are divided into three equivalent sized compartments. In our lab, we have two CPP apparatuses that are placed directly beside one another, and the three contexts are separated by removable wooden dividers. By doing this, we are able to simultaneously condition multiple animals in order to help facilitate the time spent during conditioning trials.

Additionally, each one of the individual compartments is separated by a removable wooden divider that is only removed during both the pretest and posttest of the experiment. A picture of this CPP box setup can be viewed in Figure 1. Both of the far ends of this box are painted with black and white paint, but the painting differs in its orientation. One of the far sides of the box has vertical stripes, while the other end has horizontal stripes. The middle compartment is painted solid gray. In addition to the varying visual areas, the CPP chamber also has different tactile surfaces along the floors of the boxes that help to make the regions more distinct. One of the compartments features wire-mesh flooring, while the other has roller pin flooring. The physical and perceptual differences between the compartments plays a crucial role in the post-conditioning test; the main measure of CPP.

After the pretest was conducted, we began the conditioning phase of the experiment. During this portion of the experiment, the wooden dividers are placed back into the CPP apparatus. This setup can be viewed in Figure 2. Throughout the next five days (the conditioning phase), subjects were first administered either a dopamine D₂ receptor antagonist (eticlopride) (0.1 mg/kg, 0.3 mg/kg) or saline (0.1 mg/kg), and, following a 15 minute period to ensure the drug has been distributed, the same animals will be administered a dose of either MPH (5 mg/kg) or saline. Approximately ten minutes later, animals were placed into pre-determined contexts of the CPP apparatus. The animals spent 10 minutes in the CPP box, enclosed in one singular compartment so that they cannot explore the other regions of the box. During the conditioning phase, there were two different sessions; one in the morning and one in the afternoon. In the morning sessions, we give all 45 animals two injections of saline. This is to pair one context with saline in the drug condition, and to provide equal exposure to all contexts across all groups. During the afternoon sessions, animals were given their context-dependent drug treatments, and were placed in the CPP box to elicit proper conditioning. On the sixth day, when the animals are 38 days of age (P38), we conducted a post-conditioning preference test where the wooden dividers are removed, and the preference of each animal was measured. Throughout the entire experiment, the animal's behavior was tracked using AnyMaze behavioral scanning software (Stoelting Co, Wood Dale, IL).

The day after the posttest, brain tissue will be taken and analyzed for BDNF concentrations. The first step in analyzing brain tissue is to kill the animal. In order to preserve the integrity of the brain tissues that we are trying to retrieve, this death is done as a live decapitation. This method, while seemingly brutal, allows for a quick and painless death while also preserving the brain tissue of the subjects. Other common forms of disposal can easily

affect the brain regions in which we are trying to analyze, effectively defeating the purpose of the whole experiment. After the rat's head has been removed, the outer layers of hair, skin, and the meninges are removed from the top of the head, and the whole brain is removed after removal of the upper region of the skull. Immediately following removal, the entire brain is submerged into Isopentain for proper storage. Eventually, we will harvest tissues from the striatum and the nucleus accumbens and analyze them using an Enzyme Linked Immunosorbent Assay (ELISA) kit. As previously described, the nucleus accumbens and striatum were chosen because of their important role in the drug reward pathway, as well as the fact that previous work in our laboratory using a D₁ receptor antagonist also involved taking tissue from these areas. In addition to tissue analysis, we will compare all of the groups in a between-groups design ANOVA to compare their means on a statistical level. At the present moment, the BDNF analysis has not been completed, but will be analyzed at a later time to reap more information about D₂ antagonism in the effects of MPH. Both of these types of post-experiment analysis will help aid in the comparison of these two studies to help further expand the pharmacological profile of MPH.

Results

The results from our experiment can be viewed in the appendix in Table 1 and Table 2 and were computed using SPSS. The most important numbers to view in Table 1, the descriptive statistics table, is the means column, which accords to the preference ratio. The preference ratio gives us a numerical statistic to represent the strength of a CPP that is formed by the animals, and is calculated as time spent in their paired context divided by the time spent in both the paired and non-paired contexts. This ratio can be interpreted as a description of preference, whereas zero shows complete indifference, and 1.00 shows a total adherence to their contextual area. In

this column we see that the preference ratios that were formed between the MPH group and the high dose eticlopride group were similar, with the averages of these groups being 0.644 and 0.664, respectively. As will be discussed in the next section, these two groups both formed a CPP. Additionally, we can see that the low dose eticlopride group was relatively close to saline controls, indicating that a CPP was not formed for either group.

In the next table, we have the results from our ANOVA of the preference ratio. This table shows us that there was a significant main effect of drug treatment ($F = 5.152$, $p = 0.004$). This table also demonstrates that the MPH and high dose eticlopride groups were not statistically significant, but the low dose eticlopride group and the saline group were statistically equivalent. This implies that the high dose eticlopride group produced a CPP that was equivalent to that of MPH. In this table we can also see that no sex differences were detected between any of the groups, even when sex was paired with the antagonist to check for statistical significance.

Lastly, we harvested tissues from the nucleus accumbens and striatum to prepare the samples for an ELISA. This gives us the ability to quantitatively analyze the BDNF levels within these areas. Unfortunately, the ELISA results are pending at the moment, and may not be evaluated until later during the present year. This is in part due to time constraints, but I intend to amend this paper at a later time in order to add these important results. However, we expect to see comparable BDNF levels between the high dose eticlopride group and MPH group, due to the fact that the preference ratios between the MPH and eticlopride groups were very similar. At this moment, it would be difficult to hypothesize about the BDNF concentrations in striatum and nucleus accumbens of the saline and low dose eticlopride groups since those groups' means were not statistically significant against any other groups.

Despite the fact that our hypothesis was that the dopamine D₂-like antagonist (eticlopride) would block MPH-induced CPP with greater effect in adolescent males compared to females, our data suggests that our hypothesis was incorrect. In fact, the animals who were administered the high dose of eticlopride formed a CPP themselves, in addition to those animals who were given MPH. However, despite our incorrect hypothesis, these data have important implications towards MPH addiction.

Discussion

As previously described, our results indicated that both MPH and a high dose (0.03 mg/kg) of eticlopride resulted in CPP in adolescent male and female rats. These results, while not being hypothesized correctly, actually are consistent with past works on D₂ receptors and the actions of MPH and other psychostimulants. To explain, some D₂ antagonist drugs, including eticlopride, will block all types of D₂ receptors that are in the synaptic cleft. In the case of D₂ receptors, there are two locations of receptors in the synaptic cleft. Dopamine D₂ receptors exist as both autoreceptors on the presynaptic side of the synaptic cleft, and postsynaptic receptors. Autoreceptors are the receptors that are found on the presynaptic side, on the presynaptic terminal where neurotransmitters are released into the synaptic cleft. The D₂ autoreceptors play a role in a feedback loop that modulates the reuptake of excess dopamine in the synaptic cleft (Dreyer & Hounsgaard, 2013). Eticlopride blocks these inhibitory autoreceptors, which causes an increase in synaptic dopamine as a result.

In contrast, dopamine D₂ are located postsynaptically, and will bind with dopamine. However, using a high dose of eticlopride will block these receptors as well as the autoreceptors. Therefore, MPH causes an increase in the amount of dopamine released into the synaptic cleft,

and eticlopride prevents an increase in the availability of dopamine due to the blocking of the mechanism responsible for inhibition of dopamine release. This is accomplished via delineation of autoreceptor functioning. In the case that only the post synaptic D₂ receptors would be blocked, we would expect to see very similar results to those in our D₁ study, which was blockage of MPH-induced CPP. However, eticlopride blocks both presynaptically as well as postsynaptically located D₂ receptors, so we see that CPP was produced, and we can conclude that D₁ antagonism works to block MPH-induced CPP, while D₂ antagonism does not.

Furthermore, as can be viewed in Table 1 in the means column, the high dose, or 0.03 mg/kg dose, produced a preference ratio of 0.63 in females, and 0.65 in males. Interestingly, these numbers are virtually equivalent to the preference ratios that formed in females and males who were administered MPH (0.65, 0.67, respectively). Nonetheless, the low dose of eticlopride resembled the results of the saline group, so at a dose of 0.01 mg/kg, the drug may have not caused full blockage of the D₂ receptors. These results help in ushering in the notion of a dose-dependent effect in eticlopride-induced CPP.

In attempt to apply these results to real life translationally, we can argue that these results confirm that MPH, while being relatively safe, is still addictive in nature due to its interactions with dopamine. Other studies have shown that, despite being the best available medication for ADHD, patients may still exhibit residual symptoms from the disorder, even after medication has been added. In order to increase the quality of treatment for ADHD patients, one should first consider the risks of medicating using methylphenidate. If it is decided that the patient can properly use MPH, then the addition of a non-pharmacological therapy may help improve their therapeutic effects. Behavioral therapies such as cognitive-behavioral therapy (CBT), or even

just the implementation of a regular exercise regimen, have shown to have efficacy in treating the symptoms of ADHD.

CBT can be described as working with a patient to change their thinking patterns so that they can relieve the negative emotions that they associate with themselves. This changing in self-perception and thinking patterns also helps the patient change maladaptive behaviors that may be exacerbating the symptoms of the disorder. This form of therapy has been identified as an effective treatment option for the treatment of ADHD, but evidence shows that multimodal interventions seem to be the most successful for adolescents (Young & Amarsinghe, 2010). For example, research has shown that CBT may be more effective for adolescents with ADHD and comorbid anxiety/depression, than in adolescents with ADHD and oppositional defiant disorder (Antshel, Faraone, & Gordon, 2012). While CBT can often times be used as the sole therapy for ADHD patients, it should be recommended that pharmacological therapy be added with it to increase the efficacy of treatment for the patient.

Additionally, another treatment that can be considered is the incorporation of physical activity. Recent studies have shown that intense aerobic exercise has a positive effect on children with ADHD symptoms, and this is thought to be due to increases in brain structure and function as a product of exercise (Berwid, Halperin, 2012). In one study by Chang, Liu, Yu, and Lee (2012), the effects of acute physical activity on executive functioning of children with ADHD were measured using the tests (the Stroop Test and the Wisconsin Card Sorting Test). Results from this study showed that acute aerobic activity increased ratings on both neuropsychological tests, and authors postulate that this is due to the brain allocating attention resources, via influence from the dorsolateral prefrontal cortex, resulting in dopamine release (Chang, Liu, Yu, & Lee, 2012). This is evidence shows slight effects in the alleviation of ADHD

symptoms due to decreases in executive function, and should also be considered a viable treatment additive for adolescents in this category.

In conclusion, the results of our study showed that the dopamine D₂ antagonist eticlopride failed to block MPH-induced CPP and that sex was not influential in the effects of this drug. When compared to previous work, it seems that D₁ antagonism works for blocking the rewarding effects of methylphenidate, but D₂ antagonism does not block these effects. This would indicate that the D₁ receptor is more important for mediating the rewarding effects of MPH. Therefore, when considering implications of ecological validity, one can argue that this information adds to the profile of MPH, and shows even more evidence for the similarities between MPH and other psychostimulants. Further research is needed to assess the role of MPH in mesolimbic system.

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Table 1

Descriptive Statistics

Descriptive Statistics
Dependent Variable: PREFRATIO

ANTAG	SEX	Mean	Std. Deviation	N
0.01ETIC	F	.5243845	.18281178	7
	M	.5456220	.22591350	5
	Total	.5332334	.19211361	12
0.03ETIC	F	.6360676	.18056022	6
	M	.6518788	.13298998	6
	Total	.6439732	.15141503	12
MPH	F	.6552301	.02406915	5
	M	.6725337	.03571545	5
	Total	.6638819	.03012605	10
SAL	F	.4730559	.06908358	5
	M	.4507395	.06622748	6
	Total	.4608833	.06509900	11
Total	F	.5708055	.15210493	23
	M	.5775677	.15421887	22
	Total	.5741115	.15142931	45

Note. The differences in the numbers of individuals in each group are due to our pretest results, and were modified to ensure that no animals showed an initial preference. Additionally, it should be noted that our previous experiments using D₁ antagonism also includes MPH and saline data, which made it less important to have their number of participants equal to that of the eticlopride groups.

Table 2

*ANOVA Table***Tests of Between-Subjects Effects**

Dependent Variable: PREFRATIO

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.304 ^a	7	.043	2.284	.049
Intercept	14.726	1	14.726	773.350	.000
ANTAG	.294	3	.098	5.152	.004
SEX	.001	1	.001	.037	.848
ANTAG * SEX	.003	3	.001	.060	.981
Error	.705	37	.019		
Total	15.841	45			
Corrected Total	1.009	44			

a. R Squared = .302 (Adjusted R Squared = .170)

Figure 1

Conditioned Place Preference Boxes- Pretest and Post Test

Note. The picture above shows the conditioned place preference boxes without the wooden dividers in. This is how the box was set up during the pretest and posttest to allow free roam by the animals. During the pretest and posttest, the behavioral counts were measured and a preference ratio was computed.

Figure 2

Conditioned Place Preference Boxes- Conditioning Trials

Note. During the 5 days of conditioning trials the wooden dividers were inserted, and the animals were put into one of the four end compartments after they were given their drug treatment. By doing this, we tether their surroundings with their rewarding effects, or lack thereof, from the drug treatment. Additionally, the differences in tactile and visual cues, a very important aspect of our CPP paradigm, can be viewed above.