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REPEATED TREATMENT WITH 5-HT1A AND 5-HT1B RECEPTOR AGONISTS: EVIDENCE OF TOLERANCE AND BEHAVIORAL SENSITIZATION

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REPEATED TREATMENT WITH 5-HT$_{1A}$ AND 5-HT$_{1B}$ RECEPTOR AGONISTS: EVIDENCE OF TOLERANCE AND BEHAVIORAL SENSITIZATION

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Jordan Amy Taylor

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ABSTRACT

Serotonin has been found to regulate several cognitive and physiological functions, and its role in depression and other neuropsychiatric disorders has been a focus of research. More specifically, a wealth of research regarding serotonin focuses on serotonergic medications in the treatment of neuropsychiatric disorders, such as depression and anxiety, and stimulates the 5-HT_{1A} and 5-HT_{1B} receptors. Within the last decade, there has been an increase in prescriptions of psychotropic medication for children, however, the efficacy and adverse effects of these drugs have not been evaluated in younger populations. While antidepressants reduce symptoms of depression in adults, they are less effective in reducing symptoms in children, and can result in an increased risk of suicidal behavior or ideation, social impairments, and substance abuse. For this reason, there is an urgent need to better understand how the 5-HT_{1A}, and 5-HT_{1B} receptors work in different age groups to find treatments that are efficacious in pediatric populations. RU 24969 is a preclinical serotonergic drug that binds to the serotonin 5-HT_{1A} and 5-HT_{1B} receptors, that possesses antidepressant qualities in animal models. Therefore, the purpose of the present study was to investigate whether RU 24969 treatment is unique to this preclinical drug or if it is primarily mediated by the stimulation of the 5-HT_{1A}, 5-$HT_{1B,}$ or both receptors. A total of 128 male and 128 female Sprague-Dawley rats were used for this study. In examining the single-trial effects of each drug, *Experiment 1A*: rats were pretreated with saline (*n* = 32) or 0.1, 0.2, 0.4, or 0.8 mg/kg 8-OH-DPAT. After a

24 hr abstinence period, rats previously treated with saline received 0.1, 0.2, 0.4, or 0.8 mg/kg 8-OH-DPAT (*n* = 8 per group). Subjects initially treated with 8-OH-DPAT were injected with the same dose of 8-OH-DPAT again (*n* = 8 per group). In *Experiment 1B:* The procedure was identical to Experiment 1A, except higher doses of 8-OH-DPAT (1, 2, 4, or 8 mg/kg) were administered on PD 20 and PD 22. In *Experiment 2:* The procedure was identical to Experiment 1A, with the exception that CP 94253 (2.5, 5, 10, or 20 mg/kg) was administered instead of 8-OH-DPAT. In *Experiment 3:* rats were injected with saline or the combined treatment of 4 mg/kg 8-OH-DPAT plus 5 mg/kg CP 94253. *Experiment 4:* In examining the repeated effects of each drug during a PD 17–20 pretreatment phase, rats were injected with saline (*n* = 24) or 4 mg/kg 8-OH-DPAT, 5 mg/kg CP 94253, or DPAT+CP (*n* = 8 per drug group) and immediately placed in activity chambers. On PD 22, rats previously administered saline were injected with 8-OH-DPAT, CP 94253, or DPAT+CP (*n* = 8 per group), whereas rats initially given one of the drug treatments were given the same drug treatment again (*n* = 8 per group). Distance traveled and motoric capacity were measured during each 45-minute session, while axillary temperatures were measured immediately after each session. Our findings revealed (across all experiments), all drug groups (8-OH-DPAT, CP 94253, and DPAT+CP) increased locomotor activity and caused hypothermia on the first pretreatment day. In our single-trial studies (*Experiments 1-3*), only 8-OH-DPAT and CP 94253 caused tolerance to the hypothermic effects. Additionally, CP 94253 causes tolerance to motoric

impairment, while DPAT+CP causes a sensitized motoric impairment. When given repeatedly (*Experiment 4*), 8-OH-DPAT caused locomotor sensitization and tolerance to axial temperatures. CP 94253 caused tolerance to locomotor activity and axial temperatures. Lastly, DPAT+CP caused locomotor sensitization and tolerance to axial temperatures. In summary, the effects observed through single and multi- trial paradigms with the direct agonists 8-OH-DPAT and CP 94253 administered alone and in combination differ from the mixed agonist RU 24969. This suggests that therapeutic effects observed with RU 24969 treatment are unique and may possibly stimulate receptors that are still undiscovered.

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CHAPTER ONE:

INTRODUCTION OF SEROTONIN

In the early 1930s, Dr. Vittorio Erspamer combined his interests in pharmacology and physiology, and began to explore the effects of endogenous and naturally occurring compounds (Whitaker-Azmitia, 1999). He was primarily interested in substances that initiated smooth muscle constriction and contraction in enterochromaffin cells found in the gut. From these experiments, a substance was found to initiate contraction, and this substance was named serotonin (Whitaker-Azmitia, 1999; Berger, 2009). Since the time of these early experiments, serotonin has been found to regulate a number of cognitive and physiological functions, such as mood, perception, memory, reward, thermoregulation and locomotor activity to name a few (Roth et al., 2004; Sari, 2004; Arian et al., 2007; Canli et al., 2007; Berger et al., 2009; Rojas et al., 2014). Additionally, serotonin is critical for prenatal and postnatal neurodevelopment, because it is responsible for the growth and survival of neurons (Sari, 2004; Rojas et al., 2014).

Serotonin and Mental Health

Although serotonin is involved in the regulation of many important functions, its role in depression and other neuropsychiatric disorders has been a particular focus of research. More specifically, a wealth of research regarding

serotonin focuses on serotonergic medications in the treatment of neuropsychiatric disorders, such as depression, anxiety, autism, and Tourette's syndrome (Guo et al., 2020). For example, in a study examining the postmortem brain tissue of individuals diagnosed with major depressive disorder (MDD), a portion of them having commit suicide, resulted in a significant decrease in serotonin levels in the depressed and suicidal individuals compared to a non-depressed control group (Shaw, 1967). This finding led researchers to focus on serotonin as a therapeutic target for antidepressant drugs, and was the rationale for the serotonin hypothesis, which proposed that depression is caused by a significant reduction in serotonin in the synaptic cleft (Tiger, 2018).

Developmental Differential Responses to Psychotropic Medication

Children and adolescents often suffer from the same psychiatric disorders as adults and are treated with the same medications. According to a 2016 survey from National Health and Nutrition, 45.8% of the population in the United States has filled a prescription for psychotropic drugs within a span of 30 days, and 18% of these individuals are under the age of twelve (Martin, 2019). According to the Center for Disease Control (CDC), 60% of children diagnosed with bipolar disorder are prescribed mood stabilizers and 57% of children with depression are prescribed antidepressant medications (Bridge et al., 2007); however, the efficacy and adverse effects of these drugs have not been evaluated in younger populations (Hollander, Phillips & Yeh, 2003; Brummelte et al., 2017; Hirsch,

2018; Salokangas et al., 2018; Langerberg et al., 2019). Unfortunately, most of these treatments are less effective in pediatric populations than in adults. For example, studies examining the efficacy of pharmacological and psychological interventions on adult depressive disorders found that all interventions are effective in reducing depressive symptoms (Barth et al., 2013; Cipriani et al., 2018; Zhou et al., 2020). Conversely, the same study examining psychological and pharmacological interventions in children with depressive disorders found that fluoxetine alone or in combination with cognitive behavioral therapy (CBT) were more effective in decreasing depressive symptoms in children than fifteen other pharmacological interventions (Zhou et al., 2020). While antidepressants reduce symptoms of depression in adults, they are less effective in reducing symptoms in children, and antidepressants can result in an increased risk of suicidal behavior or ideation, social impairments, and substance abuse (Zhou et al., 2020).

Within the last decade, there has been an increase in prescriptions of psychotropic medication for children by clinicians who believed they were enhancing the quality of life of the pediatric population, despite the limited research on this age group (Lakhan, 2007; Spetie & Arnold, 2007). These medications may alter the underlying mechanisms of neuropsychiatric disorders that heavily influence how drugs affect their therapeutic target in the fully developed adult brain compared to the developing brain of children (Lakhan,

2007; Spetie & Arnold, 2007). For this reason, there is an urgent need to find treatments that are efficacious in pediatric populations.

In the following chapters, I will discuss serotonin systems and developmental differences in response to serotonergic drugs.

CHAPTER TWO:

THE SEROTONIN SYSTEM

Serotonin (5-hydroxytryptamine) is a monoamine neurotransmitter involved in affective, cognitive, and physiological functioning. Approximately 95% of serotonin is found in the peripheral nervous system (PNS), where it mediates vasoconstriction and vasodilation (Whitaker-Azmitia, 1999; Berger, 2009). The remaining serotonin is located in the central nervous system (CNS), which carries out processes such as behavioral inhibition, which can be expressed as distress and withdrawal, and behavioral disinhibition, which can be expressed as impulsive and sensation-seeking behavior (Whitaker-Azmitia, 1999; Berger, 2009). Serotonin is primarily synthesized in the raphe nuclei in the brain stem, where it projects to the cortical, limbic, midbrain and hindbrain regions, and influences motor activity, mood, sleep, internal temperature, and cognition (Whitaker-Azmitia, 1999; Berger, 2009). The synthetic pathway of serotonin begins with the amino acid tryptophan (Shaw, 1967; Frazer, 1999; Berger, 2009). Tryptophan is an essential amino acid, meaning serotonin levels can be regulated by intake of tryptophan- containing foods. Tryptophan is then converted to 5-hydroxytryptophan in the presence of tryptophan hydroxylase, where 5-hydroxytryptophan is converted to serotonin (5-hydroxytryptamine) in the presence of aromatic amino acid decarboxylase (Whitaker-Azmitia, 1999; Berger, 2009). Intracellular serotonin levels are regulated by monoamine oxidase, which

metabolizes serotonin and converts it to 5-hydroxyindole acetic acid (5-HIAA). The serotonin transporter (SERT) removes serotonin from the synaptic cleft to inactivate serotonin activity after release.

Ontogeny of Serotonin Systems

There are age-related changes in the expression of serotonergic neuronal components that begin at birth and continue through adulthood. For example, the density of synapses in the basal forebrain that are overexpressed from birth to postnatal day (PD) 14. Then, from PD 14 to PD 21 the synapses in the basal forebrain undergo pruning (Spear, 2000), in which dendritic refinement takes place, and synaptic density decreases below adult levels (Brenhouse, 2011). PD 60, which is analogous to early adulthood in the rat, is marked by decreased 5-$HT_{1A/B}$ receptor density in the neocortex, hippocampus, and striatum when compared to 5-$HT_{1A/B}$ receptor densities of preweanling rats (Davidoff & Lolova, 1991).

Serotonin Receptors

Serotonin receptors have been extensively studied through human postmortem tissue samples and imaging, as well as through animal studies using monkey and rodent models (Shaw, 1967; Frazer, 1999; Garcia-Garcia, 2014, Hillhouse, 2015). The serotonin family of receptors consists of fourteen receptor subtypes, which have been divided into seven families. According to a

radioligand study conducted by Frazer et al. (1999), thirteen of the serotonin subtypes are metabotropic, meaning they are G-protein coupled receptors (GPCRs) and one receptor (5-HT_3) is ionotropic (Raymond et al., 1999; Yudin & Rohacs, 2018). These families consist of the 5-HT_1 family (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, & 5-HT_{1F}), 5-HT2 family (5-$HT_{2A,}$ 5-$HT_{2B,\ \&}$ 5-HT_{2C}), 5-HT_3 family (the only 5-HT receptor that is a ligand gated ion channel), 5-HT_4 family, 5-HT_5 family (5-HT_{5A}, 5-HT_{5B}), the 5-HT_6 family, and the 5-HT_7 family (Frazer et al., 1999). When stimulated, the 5-HT_1 and 5-HT_5 families inhibit adenylyl cyclase activity, the 5-HT_2 family stimulates phospholipase C, and the 5-$HT_{4,}$ 5-$HT_{6,}$ and 5-HT_7 receptors stimulate adenylyl cyclase activity (see Table 1) (Frazer et al., 1999).

Table 1.

Serotonin Receptor Subtypes

Receptors	G-Protein Subunit	Signaling Mechanism
5-HT1A	$G\alpha_{i/o}$	Inhibition of adenylyl cyclase
5-HT1B	$G\alpha_{i/o}$	Inhibition of adenylyl cyclase
5-HT1D	$G\alpha_{i/o}$	Inhibition of adenylyl cyclase
5-HT1E	$G\alpha_{i/o}$	Inhibition of adenylyl cyclase
5-HT1F	$G\alpha_{i/o}$	Inhibition of adenylyl cyclase
5-HT2A	$G\alpha q/_{11}$	Stimulation of phospholipase C
5-HT2B	$G\alpha q/_{11}$	Stimulation of phospholipase C
5-HT2C	$G\alpha q/_{11}$	Stimulation of phospholipase C
5-HT3	N/A	Ligand gated ion channel
5-HT4	$G\alpha_{s}$	Stimulation of adenylyl cyclase
5-HT5A	$G\alpha_{i/o}$	Inhibition of adenylyl cyclase
5-HT5B		Unknown
5-HT6	$G\alpha_{s}$	Stimulation of adenylyl cyclase
5-HT7	$G\alpha_{s}$	Stimulation of adenylyl cyclase

Although the behavioral role of each 5-HT receptor subtype remains largely unknown, there are some known functions. For example, the 5-HT_{2c} receptor, which is distributed throughout the cortex, limbic system, and basal ganglia, has a role in locomotion and reward processing (Berger et al., 2009). The 5-HT_4 receptors, which are densely located in the nigrostriatal and mesolimbic areas, play a role in spatial memory and cognition. Whereas, the 5-HT_7 receptor subtype, which is densely located in the thalamus, hypothalamus, and hippocampus, is associated with circadian rhythms and sleep (Barnes & Sharpe, 1999; Terry, Buccafusco, & Wilson, 2008).

The serotonin subtypes that are most intensively studied are those in the 5-HT_1 receptor family, because of their relevance to therapeutic drugs (Harrison, 1999; Nautiyal, 2017). Specifically, 5-HT_{1A} receptors, which are somatodendritic autoreceptors that modulate 5-HT neurotransmission, can be found in the raphe nucleus in the brain stem, and project to cortical, limbic, midbrain and hindbrain regions. Additionally, 5-HT_{1A} receptors are found in high densities in limbic areas, such as the hippocampus, lateral septum, cingulate and entorhinal cortex. The dorsal and median raphe nuclei are the targets for drug therapies in disorders such as anxiety and depression (Liu et al., 2005; Nautiyal et al., 2017). 5-HT_{1B} receptors are autoreceptors and heteroreceptors that are located on the axon terminals of serotonergic, as well as non-serotonergic, neurons (Maroteaux, 1992; Nautiyal et al., 2017). 5-HT_{1B} receptors are densely located in the basal ganglia modulate locomotion, aggression, and reward mechanisms (Przegalinski,

2001; Roth, 2004, Garcia- Garcia et al., 2014; Tiger, 2018). 5-$HT_{1\ A/B}$ receptors are important for the modulation of neurotransmitter systems other than serotonin, such as dopamine and acetylcholine (Sarhan et al., 1999; Fink & Göthert, 2007; Hagan et al., 2012).

CHAPTER THREE:

BEHAVIORAL SENSITIZATION AND TOLERANCE

Effects of Repeated Drug Administration

Repeated exposure to a drug can lead to a greater (behavioral sensitization) or a decreased response (tolerance) or both. Behavioral sensitization is a phenomenon that occurs when repeated drug treatment (often psychostimulant drugs, such as cocaine, amphetamine, etc.) results in an enhanced response to the drug with each subsequent administration (Miller et al., 1998; McDougall et al., 2007, 2011; Herbert et al., 2010). In rodent studies, behavioral sensitization is often characterized by increases in locomotion, and enhanced stereotyped behaviors (i.e., grooming) that are seen after a single or multiple drug pretreatment day(s). Behavioral tolerance is a phenomenon in which repeated administration of a drug (i.e., alcohol and opioids) results in a reduction of the drug's effect. Drug tolerance is characterized by a reduction in response to the drug with each subsequent administration of the same dose during the pretreatment phase, such that the initial effects of a drug on the first pretreatment day may decrease by as much as 80% by the fourth pretreatment day (Oberlander et al., 1987; McDougall et al., 2020 a, b). These reductions in response can be restored with a higher dose of the drug (Krasnegor, 1978; Oberlander et al., 1978; McDougall et al., 2020 a, b).

Behavioral Sensitization

Behavioral sensitization occurs when repeated administration of a drug leads to an increase in the behavioral response (Przegalinski et al., 2001; Pentkowski et al., 2009; Sari et al., 2013). Sensitization is typically observed using psychostimulants, such as cocaine, methamphetamine, and MDMA, and can be assessed by monitoring changes in behavior after one or multiple exposures of the drug. For example, repeated treatment with cocaine leads to a significant increase in locomotor activity each time the drug is administered (Anagnostaras & Robinson, 1996; Przegalinski, 2001; Pentkowski, 2009). Although different behavior can be used to assess behavioral sensitization, locomotor activity is commonly used in rodent studies (Anagnostaras et al., 1996; Anagnostaras & Robinson, 1997; Przegalinski et al., 2001; Pentkowski et al., 2009).

Behavioral Tolerance

Tolerance occurs when repeated administration of a stimulus (i.e., drug) has a progressively diminished effect over time (Bespalov et al., 2016). Behavioral tolerance is typically observed with alcohol and opioids, such as morphine, and can be assessed by monitoring changes in behavior after one or multiple exposures of the drug. Tolerance takes multiple forms: acute tolerance, which develops within a single exposure, rapid tolerance, which develops twenty-four hours after the initial administration, and chronic tolerance, which develops

after repeated exposure of the drug over the course of many days or weeks (Silveri & Spear, 1999).

Adult Sensitization and Tolerance

Single- trial versus Multi-trial Sensitization and Tolerance

Behavioral sensitization and tolerance can occur after a single exposure or multiple exposures to a drug. To assess behavioral sensitization in a single-trial procedure, the subject is typically pre-exposed to a high dose of the drug (Herbert et al., 2010; McDougall et al., 2011), followed by a period of abstinence (i.e., one or more days free from drug exposure and testing). Lastly, the subjects are given a challenge injection with a low dose of the same drug, and changes in behavioral response are examined. To assess single-trial behavioral tolerance, the same procedure is followed, however, the subjects are pretreated with a moderate drug dose and challenged with a higher drug dose (McDougall et al., 2020b). A sensitized response is evident when the subject has increased locomotion or other behavioral responses, while tolerance is characterized by diminished response compared to a drug naïve subject or the response after the initial drug treatment. During a multi-trial procedure, the subject undergoes multiple pre-treatment days with moderate drug dose, followed by a period of abstinence (McDougall et al., 2007; Herbert et al., 2010). Lastly, the subject's behavioral response to a low dose (to assess sensitization) or a high dose (to assess tolerance) of the challenge drug is examined.

For both sensitization and tolerance, a wealth of literature has shown that dose, number of trials, and context are factors that can greatly influence the size of the effect and how long it will persist (Anagnostaras & Robinson, 1996; Przegalinski et al., 2001; Pentowski et al., 2009; Sari et al., 2013). Contextual factors work through associative learning, which occurs when two previously non-related stimuli (i.e., novel environment [CS], and a drug treatment [US]) elicit a conditioned response when they are paired together (McDougall et al., 2007, 2011; Herbert et al., 2010). For example, it is only evident in adult rats and mice, when the drug treatment (i.e., cocaine, MDMA, amphetamine) is paired with a novel environmental context single-trial behavioral sensitization (Anagnostaras, & Robinson, 1996, 1997, 2002; McDougall et al., 2007, 2011; Herbert et al., 2010). This phenomenon will not occur if the drug pretreatment and challenge drug are not administered in the same environment. In contrast, adult rats undergoing multiple-trial sensitization, will show a context-independent sensitized response (i.e., animals are trained and tested in different environments); however, even in multi-trial sensitization the response is often stronger if the pretreatment and test environments are the same (Herbert et al., 2010). In adult rats, the sensitized response can persist for months after receiving the last drug pretreatment.

Pavlovian conditioning not only produces behavioral sensitization, but it also produces associative tolerance by pairing drugs such as morphine and alcohol to a novel environment (Siegel, 1991; Bespalov et al., 2016, McDougall et al., 2020). Siegel (1991) and Azorlosa et al. (1994) found that humans and

animals show a greater tolerance to morphine when it is repeatedly administered in the same context as compared to a novel environment.

Preweanling Sensitization and Tolerance

Adult and preweanling rats differ in how many trials are needed to produce behavioral sensitization, the importance of environmental context, as well as how long the sensitized response will last after the final drug exposure. In single trial sensitization, adult rats only show a sensitized response if they are trained and tested in the same context (Anagnostaras & Robinson, 1996, 1997, 2002; McDougall et al., 2007, 2011; Herbert et al., 2010). Conversely, in the single trial procedure context is irrelevant for preweanling rats to show sensitization (McDougall et al., 2007, 2011; Herbert et al., 2010). Thus, preweanling rats will emit a sensitized response if the context where they receive the pretreatment drug is the same or different from the context in which they are tested (McDougall et al., 2007, 2011; Herbert et al., 2010). The persistence of the sensitized response induced after a single pretreatment is also not the same in adult and preweanling rats, because the sensitized response of adult rats persists for months, while the sensitized response of pups last no longer than two weeks (McDougall et al., 2007, 2011; Herbert et al., 2010; Mohd-Yusof et al., 2016). There are also developmental differences in the onset and duration of single-trial (acute) tolerance (Silveri & Spear 1998; McDougall et al., 2020). For example, Silveri and Spear (1998) examined sensitivity to the effects of ethanol

(3.5, 4.0, 4.5, or 5.0 g/kg) in rats aged PD16, PD26, PD36, PD46, PD56, or PD96. They found that single-trial tolerance was most apparent at PD16 and gradually declined until PD36, suggesting that there is a mechanism responsible for reduced sensitivity to ethanol in younger rats.

Behavioral Sensitization and Tolerance Using 5-HT$_{1A/B}$ Agonists

The effects produced by repeated administration of the 5-HT$_{1\ A/B}$ agonist RU 24969 results in decreased core body temperature, motoric impairment, and results in a profound decline in locomotor activity with each subsequent administration indicating that it produces tolerance (Oberlander et al., 1987; Harrison et al., 1997; McDougall et al., 2020). The acute and chronic effects of RU 24969 (2.5 or 5 mg/kg) on locomotor activity, core body temperature, and motoric capacity were examined for one or four days (McDougall et al., 2020). Evidence showed that an acute administration of RU 24969 resulted in an increase in locomotor activity and motoric problems, while decreasing core body temperatures. Rats receiving repeated injections of RU 24969 (2.5 or 5 mg/kg) had decreased locomotor activity, less motor issues, and an increase in core body temperature relative to acutely treated control groups. Therefore, these results suggest that RU 24969 produces tolerance after multiple treatments (McDougall et al., 2020). The tolerance produced by RU 24969 also occurs with intracranial self-stimulation (ICSS) because RU 24969 administration leads to an

increase in ICSS threshold after the second administration (Harrison et al., 1997).

Studies administering the 5-HT_{1A} direct agonist 8-hydroxy-2- (di-n - propylamino) tetralin (8-OH-DPAT) report decreases in core body temperature and motoric impairment (i.e., balance problems, dragging, rolling over) after initial drug administration (Goodwin et al., 1987; Hillegaart et al., 1989). It is unclear whether repeated administration of 8-OH-DPAT results in sensitization or tolerance. Some studies have reported that 8-OH-DPAT increases locomotor activity (Tricklebank et al., 1984; Evenden 1992; Evenden, Ryan & Palejko, 1995; De La Garza & Cunningham, 2000), while other studies have shown that it decreases activity (Carli et al., 1989; Hillegaart et al., 1989; Johnson & Ahlenius, 1989; Evenden & Angeby-Moller, 1990). For instance, adult rats were given repeated treatments of 8-OH-DPAT (0.01 or 0.2 mg/kg) for seven consecutive days and locomotor activity was measured (De La Garza & Cunningham, 2000). They found that repeated administration of 8-OH-DPAT led to an increase in locomotor activity across all days, suggesting that it results in behavioral sensitization. Conversely, other studies have found that repeated administration of 8-OH-DPAT decreases locomotor activity. For instance, in a study investigating the effects of alcohol (0.3 or 1.0 g/kg) and selective serotonergic drugs (including 8-OH-DPAT) on response choice; they found that acute administration of 8-OH-DPAT caused hypothermia and motoric impairment with

its initial administration, and each subsequent administration of 8-OH-DPAT (0.3 mg/kg) produced a rapid tolerance to these effects (Evenden & Ryan, 1999).

Studies administering the 5-HT_{1B} direct agonist 5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1Hpyrrolo[3,2-b] pyridine (CP 94253) found conflicting evidence regarding its effect on locomotor activity (Dalton et al., 2003). In adults, CP 94253 administered alone does not appear to affect locomotor activity, although it seems to promote the sensitization of other drugs (Przegalinski et al., 2001; Pentkowski et al., 2009; Der-Ghazarian et al., 2017). For example, the effects of locomotor sensitization were examined in rats that were administered saline or cocaine (10 mg/kg) for five consecutive days, and then challenged with CP 94253 or cocaine, or co-administration of CP 94253 + cocaine (Pentkowski et al., 2009). Results from this study revealed that the behavioral response of subjects pretreated with cocaine and challenged with cocaine + CP 94253 displayed dose-dependent increases in locomotor activity compared to rats that were challenged with cocaine alone, implying that it increases behavioral sensitization of psychostimulant drugs. Results from the same study revealed that rats pretreated and challenged with CP 94253 (3.0, 5.6 or 10 mg/kg) did not affect locomotor activity at any dose. Although CP 94253 treatment does not appear to affect locomotor activity in adults, there is evidence showing that it increases activity in mouse pups, because CP 94253 (0.03- 30.0 mg/kg) produced a dose-dependent increase in grid crossings in seven-day-old mice (Fish et al., 2000).

Combined administration of the 5-HT_{1A} direct agonist 8-OH-DPAT (0.05 or 1.0 mg/kg) and the 5-HT_{1B} direct agonist CP 94253 (1.75 or 2.5 mg/kg) is effective in reducing impaired voluntary movement in animal models of Parkinson's disease (Munoz et al., 2008). Specifically, neither agonist significantly suppressed abnormal movements alone, but their combined effect resulted in an 80% decrease in L-dopa-induced dyskinesia in MPTP-treated rats. Additionally, neither 8-OH-DPAT (0.5 mg/kg) nor CP 94253 (20 mg/kg) increased locomotor activity alone, but combined treatment with the two drugs resulted in a significant increase in locomotor activity. These findings imply the combined stimulation of 5-HT_{1A} and 5-HT_{1B} receptors is necessary for the initial increase in locomotor activity observed with RU 24969, but it is unknown whether this stimulation is necessary for the later development of tolerance.

CHAPTER FOUR:

SUMMARY THESIS STATEMENT

RU 24969 is a preclinical serotonergic drug that binds to the serotonin 5-HT $_{1A}$ and 5-HT$_{1B}$ receptors. Administration of RU 24969 produces hyperactivity in rodents, but subsequent administration leads to a profound tolerance characterized by a large reduction in activity (Oberlander et al., 1987; McDougall et al., 2020). The effects of this compound are important because it has purported antidepressant qualities in animal models (Tiger et al., 2018) and may lead to a new class of medications. Unfortunately, most of what is known about RU 24969 is from research using adult animals and the effect may differ in younger age groups. Furthermore, in a meta-analysis examining current psychological and pharmacological interventions in children with depressive disorders, it was found that only fluoxetine alone or fluoxetine in combination with cognitive behavioral therapy (CBT) was effective in decreasing depressive symptoms in children as compared to fifteen other pharmacological interventions (Zhou et al., 2020).

The tolerance resulting from repeated administration of RU 24969 is interesting because stimulation of the 5-HT$_{1A}$ receptors alone initially results in a hypothermic response and motoric dysfunction (i.e., balance problems, dragging, rolling over), while subsequent injections result in tolerance to these effects (Evenden, 1992; Evenden et al., 1995). While there is evidence reporting that

8-OH-DPAT could potentially increase or decrease locomotor activity, there is conflicting evidence that has shown that repeated treatments of 8-OH-DPAT results in behavioral sensitization (De La Garza & Cunningham, 2000). Additionally, stimulation of the 5-HT_{1B} receptors alone appears to have no effect in adults, although it promotes sensitization to other drugs (Przegalinski et al., 2001; Der-Ghazarian et al., 2017). In contrast, stimulation of 5-HT_{1B} receptors increases locomotor activity in pups, which is consistent with the effects of RU 24969 (O'Neill et al., 1997; Fish et al., 2000). Thus, the aim of the present study is to investigate if the tolerance effect seen after RU 24969 treatment is unique to this preclinical drug or if stimulation of the 5-HT_{1A}, 5-$HT_{1B,}$ or both receptors would also induce behavioral tolerance.

In our first set of experiments, we will administer the 5-HT_{1A} agonist 8-OH-DPAT (0.1, 0.2, 0.4, 0.8, 1, 2, 4, or 8 mg/kg), and measure distance traveled, axillary body temperature, and motor impairment using a scale adopted by McDougall and colleagues (2017). Our second experiment will be identical to the first, except we will use 5-HT_{1B} agonist CP 94253 (2.5, 5, 10, or 20 mg/kg). Our third set of experiments will use the same procedures except that 8-OH-DPAT (4 mg/kg) and CP 94253 (5 mg/kg) will be co-administered. By assessing the effects of repeated administration of each agonist alone and in combination, we will examine whether the stimulation of a single receptor, or the simultaneous stimulation of both receptors, are important for behavioral sensitization and tolerance.

Although the underlying neuronal mechanisms of repeated stimulation of the 5-$HT_{1A/B}$ receptors and its relation to tolerance are not well understood, the following six hypotheses have been formulated:

Experiments involving the 5-HT_{1A} agonist 8-OH-DPAT

8-OH-DPAT will result in a dose-dependent increase in locomotor activity, motoric impairment, and a decrease in core body temperature upon initial administration. Past studies have shown that a single administration of 8-OH-DPAT results in motoric impairment, hypothermia, and an increase in locomotor activity (Tricklebank et al., 1984; Evenden & Angeby-Moller, 1990; Chojnacka-Wojcik, 1992; Evenden 1992; Evenden, Ryan & Palejko, 1995; De La Garza and Cunningham, 2000; Dalton et al., 2003). Based on these findings, I hypothesize that larger doses of 8-OH-DPAT will result in stronger behavioral sensitization.

Multiple injections of 8-OH-DPAT will decrease motor impairment and core body temperature, producing a tolerance effect at all doses compared to saline treated animals. Although there is conflicting evidence showing that repeated treatment of 8-OH-DPAT could result in either behavioral sensitization or tolerance (Evenden, 1992; Evenden et al., 1995), the wealth of evidence has shown that 8-OH-DPAT leads to locomotor sensitization. Based on past literature, I hypothesize that 8-OH-DPAT will decrease core body temperature and motor impairment, resulting in tolerance, whereas, locomotor activity will

show a progressively elevated response after repeated injections, resulting in sensitization.

Experiments involving the 5-HT_{1B} agonist CP 94253

A single administration of CP 94253 will result in behavioral sensitization, as shown by an enhanced behavioral response and a dose-dependent increase in locomotor activity. Acute administration of CP 94253 will have no effect on core body temperature or motor impairment. Based on past literature, drugs that stimulate the 5-HT_{1B} receptor alone increase locomotor activity in mice pups, with no evidence of motor impairment or core body temperature (Fish et al., 2000; Der-Ghazarian et al., 2017).

Multiple injections of CP 94253 will increase locomotor activity at all doses, with no effect on core body temperature or motoric impairment. Past literature demonstrates drugs stimulating the 5-HT_{1B} receptor initially increase locomotor activity, though this effect does not last past a two-week abstinence period (Der-Ghazarian et al., 2017). To that end, I hypothesize that repeated treatments of CP 94253 will result in behavioral sensitization rather than tolerance.

Experiments concerning the combined treatment of 5-HT $_{1A}$ and 5-HT$_{1B}$ agonists (8-OH-DPAT and CP 94253)

The co-administration of 8-OH-DPAT and CP 94253 will result in an increase in locomotor activity, motoric impairment, and a decrease in core body temperature upon initial administration. Based on past literature, experiments found hypothermic responses with its first injection showing that there is an underlying mechanism responsible for inducing a decrease in core body temperature (Dalton et al, 2004; Munoz et al., 2008). Based on these findings, I hypothesize that a single injection of 8-OH-DPAT and CP 94253 co-administered will result in elevated locomotor activity, hypothermia, and motoric impairment.

Multiple injections of 8-OH-DPAT and CP 94253 given in combination will decrease motor impairment, increase core body temperature, and induce tolerance at all doses. Munoz et al., (2008) found that the co-administration of the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor agonists suppressed abnormal involuntary movements in $_{L}$-DOPA-induced dyskinesia in the MPTP-treated subjects by 80% after the fourth administration. In addition, repeated administration of RU 24969 induces an enhanced behavioral effect upon initial administration, but then causes tolerance to the locomotor activity inducing effect that can be observed after the second administration (Oberlander et al., 1987; Harrison et al, 1997; McDougall et al., 2020). Because of the consistent findings, I hypothesize that the combined administration of 8-OH-DPAT and CP 94253 will cause tolerance

across all three measures: core body temperature, motoric impairment, and locomotor activity.

Because of the age of our subjects, we do not believe sex will affect the sensitization or tolerance induced by our test drugs. However, sufficient numbers of male and female rats will be used so that sex effects can be detected if they exist.

CHAPTER FIVE:

METHODS

Subjects

Subjects were 256 male (*n* =128) and female (*n* =128) preweanling (*n* = 8 subjects per group) Sprague-Dawley (Charles River; Hollister, CA) rats that were bred and raised in the vivarium of the Psychology Department at California State University, San Bernardino (CSUSB). Litters were culled to 10 pups on postnatal day (PD) 3 and weaned on PD 23. All litters received food and water ad libitum and kept on a 12h light and 12h dark schedule in a colony room that was maintained at 22-23 ℃. Behavioral testing was conducted in a separate experimental room during the light cycle, with subjects being returned to their home cage after testing. All subjects were cared for according to the “Guide for the Care and Use of Laboratory Animals” (National Research Council 2010) under a research protocol approved by the Institutional Animal Care and Use Committee (IACUC) of CSUSB.

Apparatus

All behavioral testing was performed in activity monitoring chambers (26 × 26 × 41 cm) from Coulbourn Instruments (Whitehall, PA). Each chamber includes four acrylic walls, a grey plastic floor, and open top. To measure distance traveled (cm), each chamber included an X–Y photobeam array, with 16

photocells and detectors. Axillary temperatures were measured using a BAT–12 microprobe thermometer (Physitemp Instruments, Piscataway, NJ).

Drugs

8-OH-DPAT [(R)-(+)-8-hydroxy-DPAT] and CP 94253 (Tocris, Minneapolis, MN) were dissolved in saline and injected intraperitoneally (IP) at a volume of 2.5 ml/kg.

Procedure

Experiment 1a. Effects of repeated low dose (0.1–0.8 mg/kg) treatment with the 5-HT$_{1A}$ agonist 8-OH-DPAT on the locomotor activity, motoric capacity, and axillary temperatures of male and female preweanling rats: Two-day procedure

Prior to the start of testing, all subjects were randomly assigned to treatment groups. On PD 20, rats were injected with saline (n = 32) or 0.1, 0.2, 0.4, or 0.8 mg/kg 8-OH-DPAT (n = 8 per drug group) and immediately placed in activity chambers where distance traveled, and motoric capacity were measured for 45 min. On test day (PD 22), rats previously treated with saline received 0.1, 0.2, 0.4, or 0.8 mg/kg 8-OH-DPAT (n = 8 per group). Subjects initially treated with 8-OH-DPAT were injected with the same dose of 8-OH-DPAT again (n = 8 per group). Axillary temperatures were recorded immediately after completion of behavioral testing on PD 20 and PD 22.

To assess motoric capacity, the behavior of rats in the activity chambers was coded using a motoric capacity rating scale (adopted from McDougall et al.,

2019, 2021b) in which: 0 = asleep or inactive, 1 = normal forward locomotion (no balance disturbances), 2 = forward locomotion with minor balance problems (upright walking, but slightly splayed rear legs), 3 = forward locomotion with moderate balance problems (not dragging, but awkward leg movements), 4 = forward locomotion with major balance problems I (not dragging, but occasional rolling over), 5 = forward locomotion with major balance problems II (minor dragging and rolling over, but focused forward movement), 6 = predominate dragging (prominent dragging and rolling over, with random forward movement), 7 = circular dragging (forward dragging in a circular pattern), and 8= splayed or “swimming” movements (see Table 2 below).

Table 2.

Motoric Capacity Scale

Rating	Behavior
0	Asleep or inactive
1	Normal forward locomotion (no balance disturbances)
2	Forward locomotion with minor balance problems (upright walking, slightly splayed rear legs)
3	Forward locomotion with moderate balance problems (not dragging, awkward leg movements)
4	Forward locomotion with major balance problems (not dragging, but rolling over)
5	Forward locomotion with major balance problems (forward focused movement, minor dragging and rolling over
6	Predominate dragging (major dragging and rolling over, with random forward movement)
7	Circular dragging (forward dragging in a circular pattern)
8	Spayed or “swimming” movements

Note: This scale was adopted from McDougall et al. (2019, 2021b). The behavior of each subject was monitored for 15 s every five-min interval by an observer blind to treatment conditions.

Experiment 1b. Effects of repeated high dose (1–8 mg/kg) treatment with the 5-HT$_{1A}$ agonist 8-OH-DPAT on the locomotor activity, motoric capacity, and axillary temperatures of male and female preweanling rats: Two-day procedure

The procedure was identical to Experiment 1a, except higher doses of 8-OH-DPAT (1, 2, 4, or 8 mg/kg) were administered on PD 20 and PD 22.

Experiment 2. Effects of repeated treatment with the 5-HT$_{1B}$ agonist CP 94253 on the locomotor activity, motoric capacity, and axillary temperatures of male and female preweanling rats: Two-day procedure

The procedure was identical to Experiment 1, with the exception that CP 94253 (2.5, 5, 10, or 20 mg/kg) was administered instead of 8-OH-DPAT.

Experiment 3. Effects of combined treatment with 8-OH-DPAT and CP 94253 on the locomotor activity, motoric capacity, and axillary temperatures of male and female preweanling rats: Two-day procedure

On PD 20, rats were injected with saline or a cocktail of 4 mg/kg 8-OH-DPAT plus 5 mg/kg CP 94253 before being placed in activity chambers. Distance traveled and motoric capacity were measured in 5-min time blocks for 45 min. On PD 22, both groups of rats received a co-administration of 8-OH-DPAT plus CP 94253 and behavior and axillary temperatures were recorded. For comparison purposes, a separate control group was given an injection of saline on both PD 20 and PD 22.

Experiment 4. Effects of 8-OH-DPAT and CP 94253, administered alone or together, on the locomotor activity, motoric capacity, and axillary temperatures of male and female preweanling rats: Five-day procedure

On PD 17–20, rats were injected with saline (*n* = 24) or 4 mg/kg 8-OH-DPAT, 5 mg/kg CP 94253, or DPAT+CP (n = 8 per drug group) and immediately placed in activity chambers. On PD 22, rats previously administered saline were injected with 8-OH-DPAT, CP 94253, or DPAT+CP (*n* = 8 per group), whereas rats initially given one of the drug treatments were given the same drug treatment again (*n* = 8 per group). This behavioral analysis is identical to Experiment 1.

Statistical Analysis

Distance traveled and axillary body temperatures from each experiment were analyzed by separate (pretreatment × test day treatment) between group univariate analysis of variance (ANOVA). Experiments involving multiple pretreatment days were analyzed using repeated measures ANOVA to analyze day-dependent changes on PD 17-20. The pretreatment analyses for all experiments will include Group x Dose for saline vs. all drug treatment groups (*n* = 4), whereas the test day treatment analyses will include all sal-drug vs all drug-drug Group effects (*n* = 8). In the event that Mauchly's test of sphericity is violated, we will use the Huynd-Feldt correction (denoted by the superscript ^{a}) if the epsilon (ε)>.75 (Huynd & Feldt, 1976). Additionally, we will round the degrees of freedom to the nearest whole integer. Post hoc analyses will be made with Tukey tests. Because the motoric capacity rating scale provides nonparametric

data, we will analyze these measures using either Mann-Whitney *U* tests, Kruskal-Wallis *H* tests, and Wilcoxon signed-rank tests. Post hoc analyses of Kruskal-Wallis *H* tests will be made with Dunn's tests. The level of significance will be set at 0.05 for all statistical tests.

CHAPTER SIX:

RESULTS

Experiment 1A

Experiment 1a examined the effects of repeated low dose (0.1–0.8 mg/kg) treatment with 8-OH-DPAT on locomotor activity, motoric capacity, and axillary temperatures of male and female preweanling rats. It was predicted that administration of 8-OH-DPAT in this dose range would result in a dose-dependent increase in locomotor activity, motoric impairment, and a decrease in core body temperature.

Locomotor Activity

Administration of 8-OH-DPAT on PD 20, resulted in a main effect of dose observed with locomotor activity scores [Pretreatment main effect, F (4, 59) = 29.51, p<0.001] (Figure 1, upper graph). Specifically, rats treated with 0.4 and 0.8 mg/kg 8-OH-DPAT (but not 0.1 and 0.2 mg/kg) exhibited greater locomotor activity scores compared to saline-treated rats [Tukey test, $p < 0.05$]. Moreover, the higher doses of 8-OH-DPAT (0.4 and 0.8 mg/kg) differed from both lower doses (0.1 and 0.2 mg/kg) but did not differ from each other [Tukey tests, p<0.05].

On PD 22, the test day dose altered locomotor activity and this effect was not affected by treatment on the pretreatment day (e.g., saline vs drug treatment [Group main effect, F (7, 56) = 15.524, $p < 0.001$]) (Figure 1, lower graph). Rats treated with saline and challenged with the higher doses of 8-OH-DPAT (0.4 and

0.8 mg/kg) (Sal- DPAT group) had greater distance traveled scores on PD 22 than on PD 20. Additionally, rats receiving their second treatment of 8-OH-DPAT (0.4 and 0.8 mg/kg) (DPAT- DPAT group), did not significantly differ in locomotor activity than the rats that only received one treatment (Sal- DPAT group). Animals that received lower doses of 8-OH-DPAT (0.1 and 0.2 mg/kg) (Sal- DPAT group) did not significantly differ in locomotor activity on the test day [Tukey tests, $p<0.05$]. The non- significant pretreatment effect indicates that doses of 8-OH-DPAT from 0.1 to 0.8 mg/kg did not induce tolerance or sensitization after a single pre-exposure in our study.

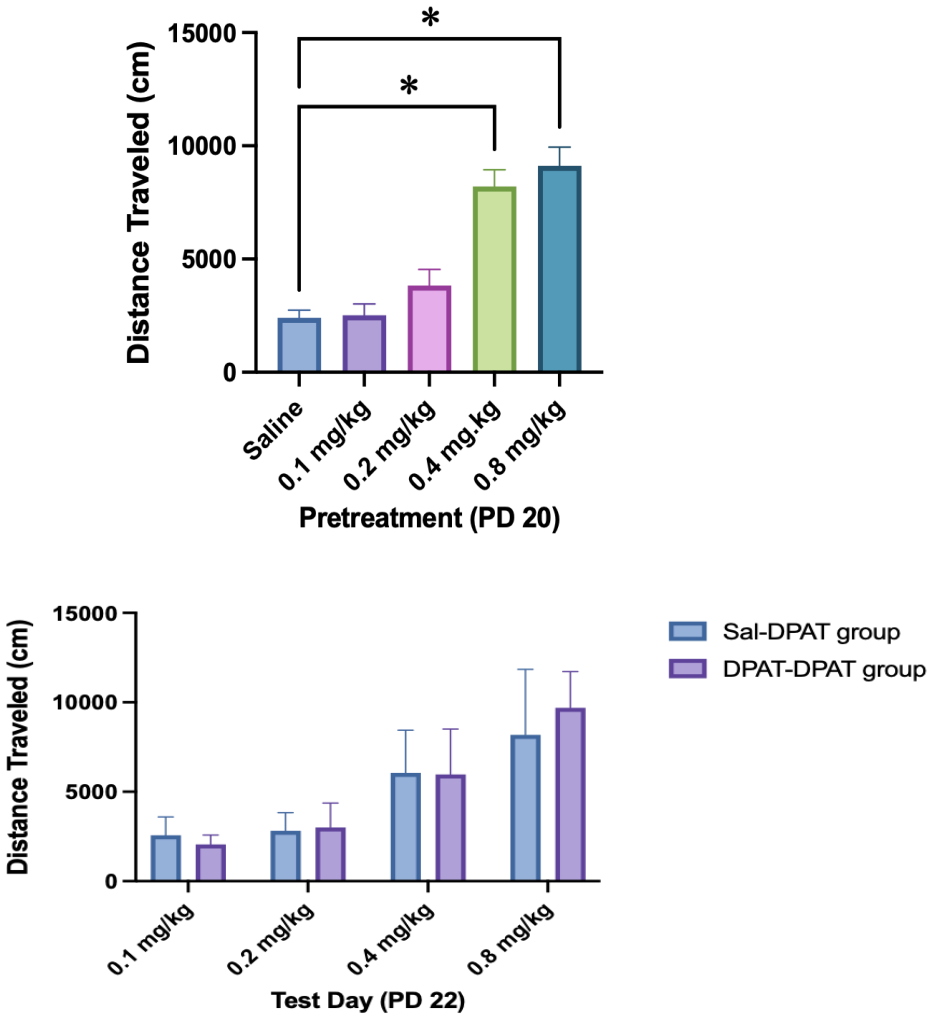

Figure 1. Mean (±) distance traveled on the pretreatment day (upper graph) and test day (lower graph) of male and female preweanling rats. Rats were injected with saline or 8-OH-DPAT (0.1, 0.2, 0.4, 0.8 mg/kg, ip) on the pretreatment day (PD 20). On the test day (PD 22), 8-OH-DPAT pretreated rats were injected with the same dose again while saline pretreated rats were injected with one of the four 8-OH-DPAT doses.
* Significant difference between Saline and DPAT groups on PD 20.

Axillary Temperature

Pretreatment with 8-OH-DPAT (PD 20) resulted in an effect of dose on axillary temperatures, [Pretreatment main effect, F (4, 59) = 21.10, p<.001] (Figure 2, upper graph). Specifically, animals pretreated with higher doses of 8-OH-DPAT (0.4 and 0.8 mg/kg) had significantly decreased axillary temperatures, while animals treated with saline or lower doses of 8-OH-DPAT (0.1 and 0.2 mg/kg) did not [Tukey test, p<.05]. Moreover, the higher dose of 8-OH-DPAT (0.8 mg/kg) differed from all lower doses (0.1, 0.2, and 0.4 mg/kg) [Tukey tests, p<0.05].

On test day (PD 22), there was an effect of dose observed on axillary temperatures [Group main effect, F (7, 56) = 5.319, p < .001] (Figure 2, lower graph). Animals previously administered saline on PD 20 and challenged with 8-OH-DPAT (0.4 and 0.8 mg/kg) on PD 22 (Sal- DPAT groups), had a significant decrease in axillary temperatures on PD 22 compared to PD 20. Conversely, animals receiving a second treatment (DPAT- DPAT groups) with the higher doses of 8-OH-DPAT (0.4 and 0.8 mg/kg) resulted in an increase in axillary temperatures compared to the pretreatment day [Tukey tests, p<0.05]. Higher doses of 8-OH-DPAT (0.4 and 0.8 mg/kg) induced tolerance after a single pre-exposure in our study, while lower doses (0.1 and 0.2 mg/kg) did not.

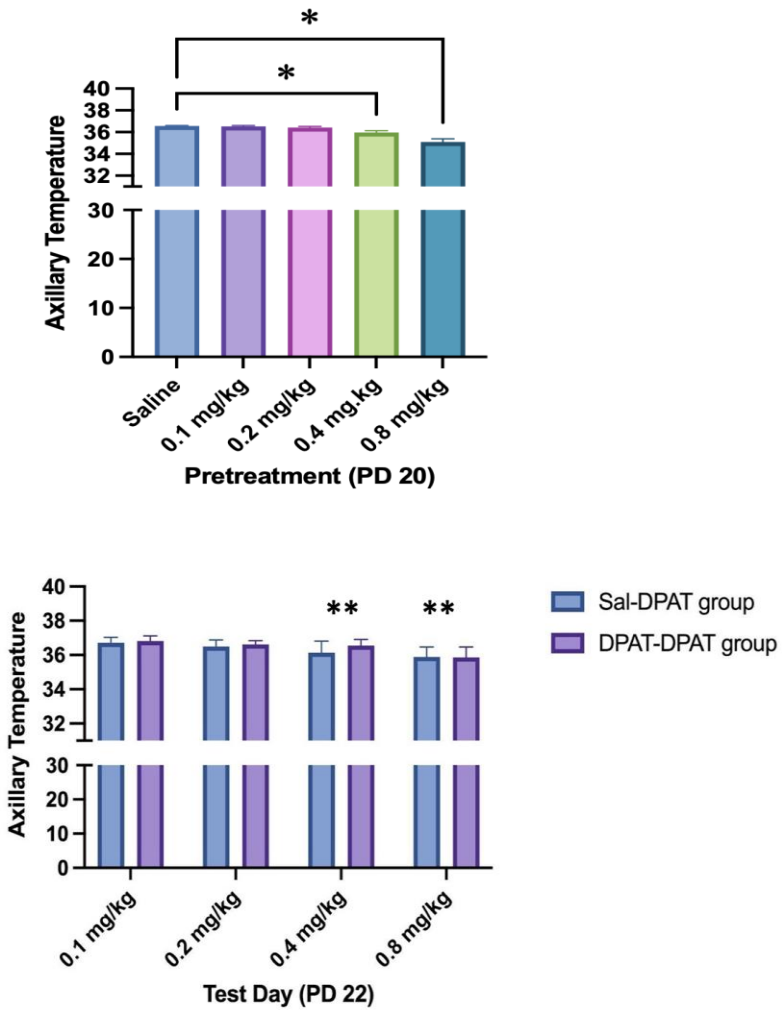

Figure 2. Mean (±) axillary temperatures on the pretreatment day (upper graph) and test day (lower graph) of male and female preweanling rats. Rats were injected with saline or 8-OH-DPAT (0.1, 0.2, 0.4, 0.8 mg/kg, ip) on the pretreatment day (PD 20). On the test day (PD 22), 8-OH-DPAT pretreated rats were injected with the same dose again while saline pretreated rats were injected with one of the four 8-OH-DPAT doses.
* Significant difference between Saline and DPAT groups on PD 20.
**Significant difference in axillary temperatures between SAL-DPAT and DPAT-DPAT groups on PD 22

Motoric Capacity

Low doses of 8-OH-DPAT (0.1 to 0.8 mg/kg) had no effect on motoric capacity on either the pretreatment day (PD 20) or the test day (PD 22) (data not shown).

Experiment 1B

Experiment 1b examined the effects of repeated high dose (1–8 mg/kg) treatment with 8-OH-DPAT on the locomotor activity, motoric capacity, and axillary temperatures of male and female preweanling rats. It was predicted that 8-OH-DPAT would result in a dose-dependent increase in locomotor activity, motoric impairment, and a decrease in core body temperature.

Locomotor Activity

Pretreatment with 8-OH-DPAT (PD 20) resulted in an effect of dose observed with locomotor activity scores [Pretreatment main effect, F (4, 59) = 18.11, p <.001] (Figure 3, upper graph). Specifically, animals treated with higher doses of 8-OH-DPAT (1-8 mg/kg) exhibited greater locomotor activity scores compared to saline treated animals [Tukey test, $p < 0.05$]. Moreover, all the higher doses of 8-OH-DPAT (1-8 mg/kg) differed from saline, but did not differ from each other [Tukey tests, p<0.05].

Distance traveled scores on PD 22 revealed that the main effect of Group was not significant [Group main effect, F (7, 56) = 1.419, p >.05, *ns*] (Figure 3, lower graph). The non- significant Group effect indicates that higher doses of 8-

OH-DPAT from 1 to 8 mg/kg did not induce tolerance or sensitization after a single pre-exposure in our study.

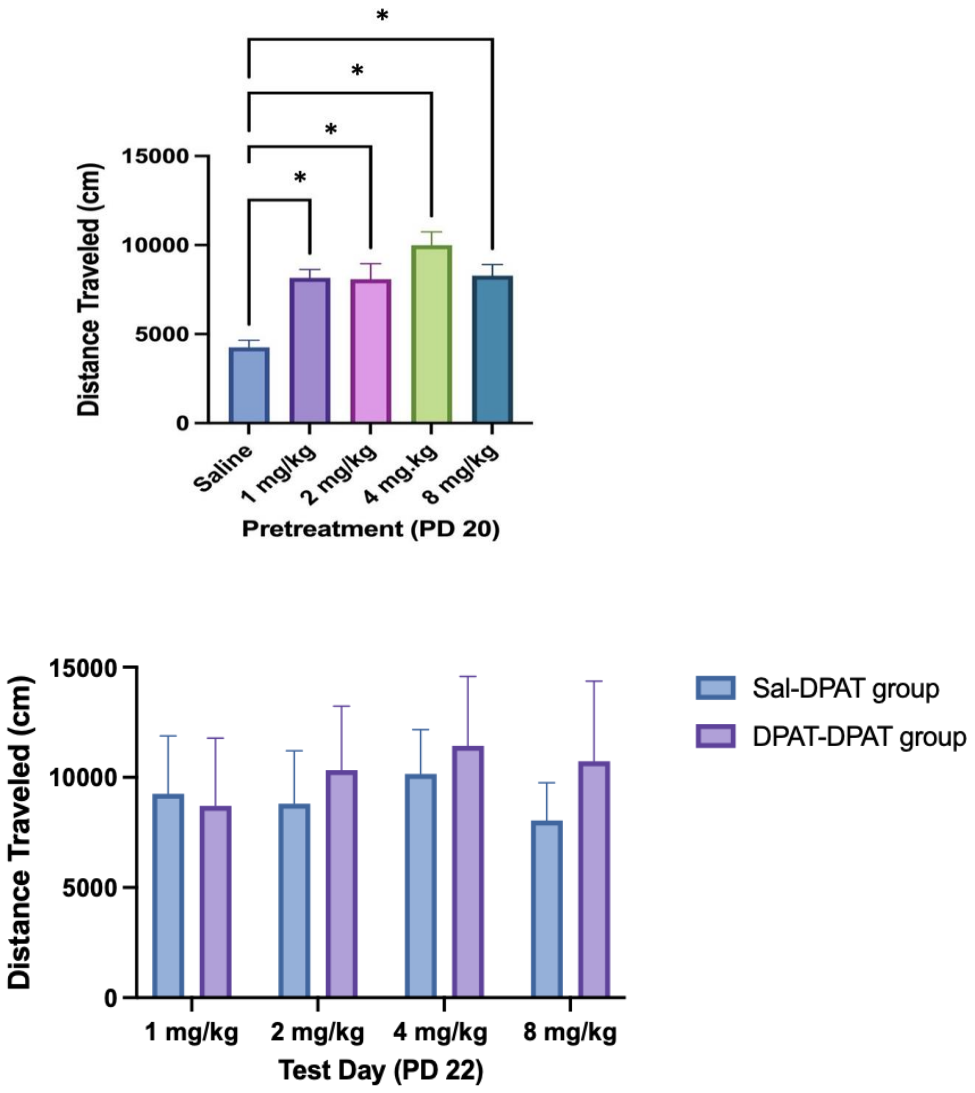

Figure 3. Mean (±) distance traveled on the pretreatment day (upper graph) and test day (lower graph) of male and female preweanling rats. Rats were injected with saline or 8-OH-DPAT (1, 2, 4, 8 mg/kg, ip) on the pretreatment day (PD 20). On the test day (PD 22), 8-OH-DPAT pretreated rats were injected with the same dose again while saline pretreated rats were injected with one of the four 8-OH-DPAT doses.
* Significant difference between Saline and DPAT groups on PD 22.

Axillary Temperature

Pretreatment with 8-OH-DPAT (PD 20) resulted in a significant main effect of dose observed on axillary temperatures, [Pretreatment main effect, F (4, 59) = 56.80, p<.001] (Figure 4, upper graph). Animals treated with higher doses of 8-OH-DPAT (1-8 mg/kg) on PD 20 exhibited a decrease in axillary temperatures at all doses compared to saline-treated animals. [Tukey test, $p < 0.05$]. Moreover, the higher doses of 8-OH-DPAT (4 and 8 mg/kg) differed from both lower doses (1 and 2 mg/kg) but did not differ from each other [Tukey tests, p<0.05].

On test day (PD 22), there was a significant main effect of Group observed on axillary temperatures [Group main effect, F (7, 56) = 4.949, p<.001] (Figure 4, lower graph). Animals previously administered saline on PD 20 and challenged with 8-OH-DPAT (1-8 mg/kg) on PD 22 (Sal-DPAT groups), had a dose-dependent decrease in axillary temperatures compared to animals that received two injections of 8-OH-DPAT (DPAT- DPAT groups) [Tukey tests, p<0.05]. Moreover, rats treated with 4 or 8 mg/kg 8-OH-DPAT (DPAT- DPAT groups) had significantly greater axillary temperatures on PD 22 than on PD 20 while rats that received 1 or 2 mg/kg 8-OH-DPAT did not. Higher doses of 8-OH-DPAT (4 and 8 mg/kg) induced tolerance after a single pre-exposure in our study, while lower doses (1 and 2 mg/kg) did not.

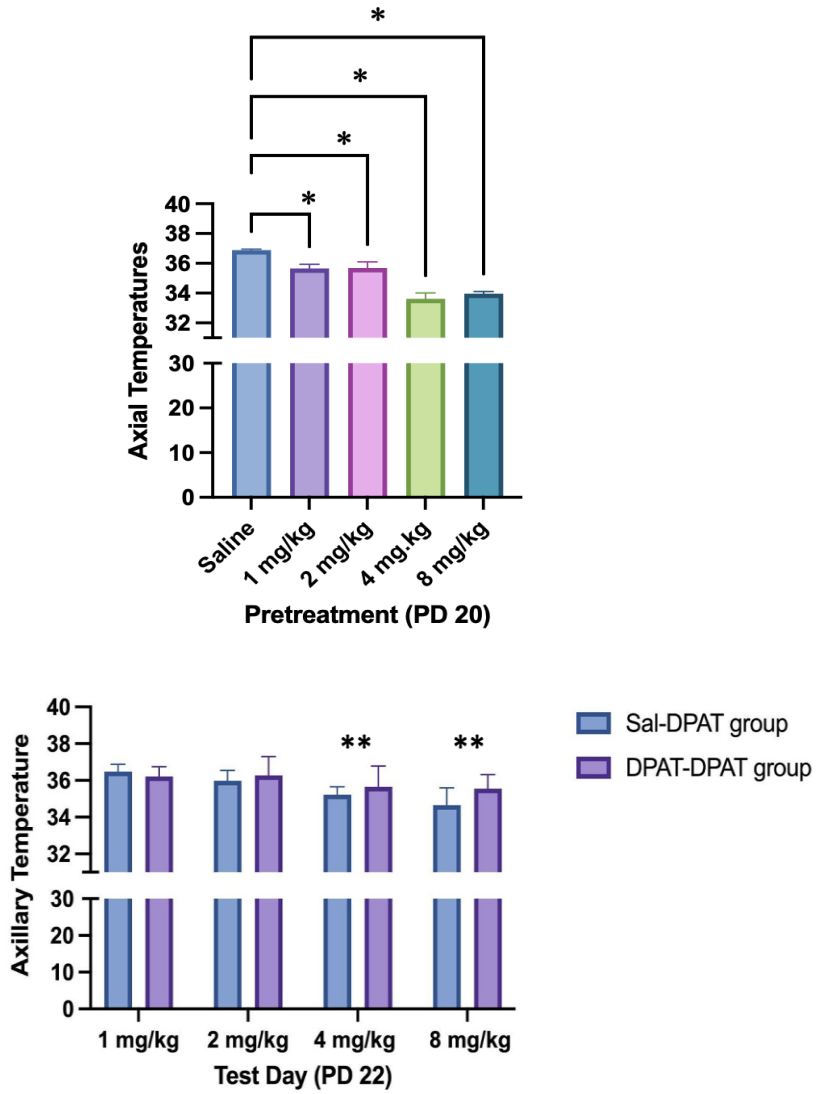

Figure 4. Mean (±) axillary temperatures on the pretreatment day (upper graph) and test day (lower graph) of male and female preweanling rats. Rats were injected with saline or 8-OH-DPAT (1, 2, 4, 8 mg/kg, ip) on the pretreatment day (PD 20). On the test day (PD 22), 8-OH-DPAT pretreated rats were injected with the same dose again while saline pretreated rats were injected with one of the four 8-OH-DPAT doses.
* Significant difference between Saline and DPAT groups on PD 20.
**Significant difference in axillary temperatures between SAL-DPAT and DPAT-DPAT groups on PD 22.

Motoric Capacity

Pretreatment with 8-OH-DPAT on PD 20 resulted in a significant main effect of dose observed on motoric capacity for 15 minutes post-treatment [Kruskal- Wallis H = 15.02 to 10.29, p< .05, Dunn's test, p<.05] (See Table 3). For the first 15 minutes, subjects pretreated with higher doses of 8-OH-DPAT (2-8 mg/kg) exhibited moderate balance problems (not dragging, but awkward leg movements; see motoric capacity scale on Table 2) while animals treated with saline or 1 mg/kg 8-OH-DPAT did not [Dunn's test, p<.05].

On PD 22, there was no significant difference in motoric capacity between subjects that received a single or multiple treatments of 8-OH-DPAT (1-8 mg/kg). The non-significant Group effect indicates that doses of 8-OH-DPAT (1 to 8 mg/kg) did not induce tolerance or sensitization after a single pre-exposure in our study.

Experiment 2

Experiment 2 examined the effects of repeated treatment with CP-94253 (2, 5, 10, & 20 mg/kg) on the locomotor activity, motoric capacity, and axillary temperatures of male and female preweanling rats. It was predicted that CP-94253 would result in a dose-dependent increase in locomotor activity, have no effect on core body temperatures, or motoric impairment.

Locomotor Activity

On PD 20, there was a significant main effect of dose observed with locomotor activity scores [Pretreatment main effect, F (4, 59) = 6.612, p <.001] (Figure 5, upper graph). Specifically, animals treated with 2.5, 10, or 20 mg/kg CP 94253 exhibited significantly greater locomotor activity scores compared to animals treated with 5 mg/kg or saline [Tukey test, $p < 0.05$].

Distance traveled scores on PD 22 revealed that the main effect of Group was not significant [Group main effect, F (7, 56) = 1.418, p =.217, *ns*] (Figure 5, lower graph). The non- significant Group effect indicates that CP 94253 did not induce tolerance or sensitization after a single pre-exposure in our study.

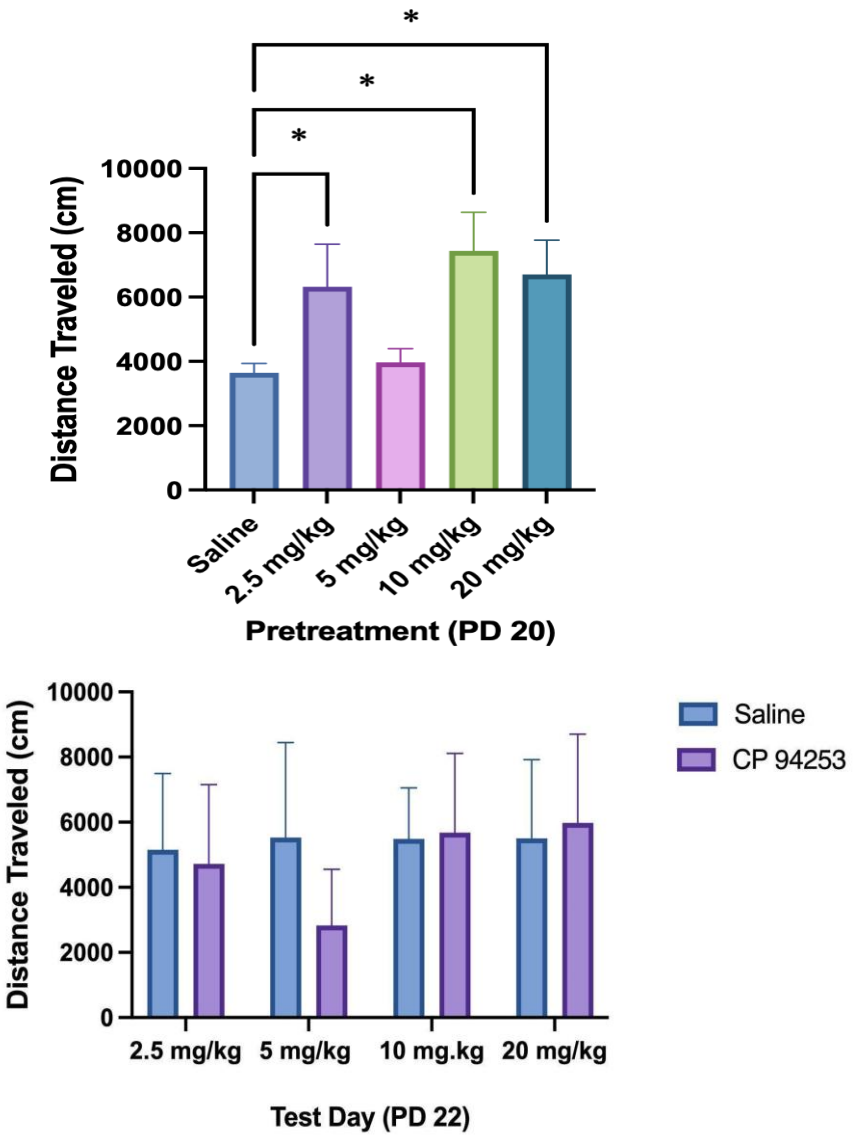

Figure 5. Mean (±) distance traveled on the pretreatment day (upper graph) and test day (lower graph) of male and female preweanling rats. Rats were injected with saline or CP 94253 (2.5, 5, 10, or 20 mg/kg, ip) on the pretreatment day (PD 20). On the test day (PD 22), CP 94253 pretreated rats were injected with the same dose again while saline pretreated rats were injected with one of the four CP 94253 doses.

* Significant difference between Saline and CP groups on PD 20.

Axillary Temperature

Pretreatment with CP 94243 (PD 20) resulted in a significant main effect of dose observed on axillary temperatures, [Pretreatment main effect, F (4, 101) = 125.566, p<.001] (Figure 6, upper graph). Animals treated with CP 94253 on PD 20 exhibited a decrease in axillary temperatures at all doses compared to saline treated animals [Tukey test, $p < 0.05$].

On test day (PD 22), there was a significant main effect of Group observed on axillary temperatures [Group main effect, F (7, 64) = 53.621, p<.001] (Figure 6, lower graph). Animals previously administered saline on PD 20 and challenged with CP 94253 (2.5, 10, and 20 mg/kg) on PD 22 (Sal-CP groups), had lower axillary temperatures compared to animals that received two injections of CP 94253 (CP-CP groups) [Tukey tests, p<0.05]. However, there is no significant difference in axillary temperatures in animals that received two injections of 5 mg/kg CP 94253 or saline [Tukey tests, p<0.05]. Moreover, 2.5, 10, and 20 mg/kg CP 94253 induced tolerance after a single pre-exposure in our study.

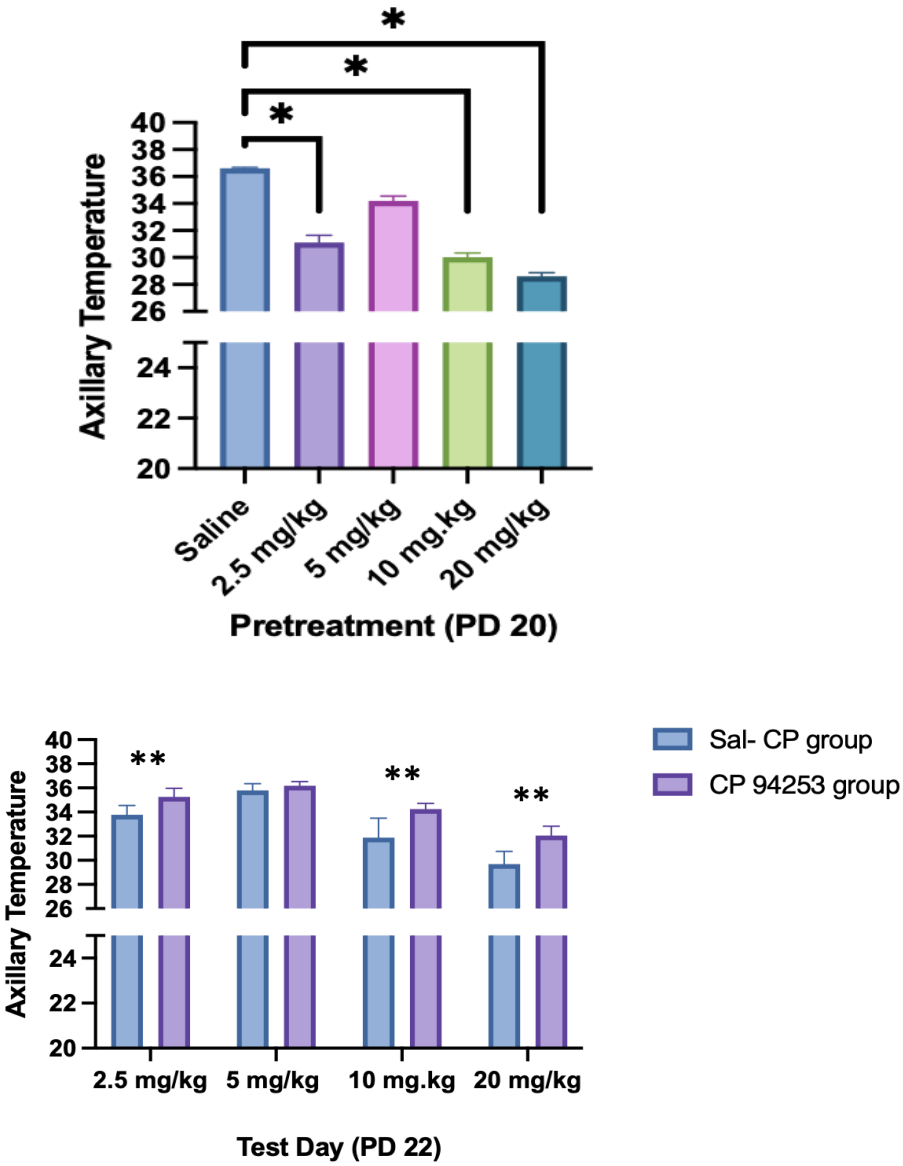

Figure 6. Mean (±) axillary temperatures on the pretreatment day (upper graph) and test day (lower graph) of male and female preweanling rats. Rats were injected with saline or CP 94253 (2.5, 5, 10, or 20 mg/kg, ip) on the pretreatment day (PD 20). On the test day (PD 22), CP 94253 pretreated rats were injected with the same dose again while saline pretreated rats were injected with one of the four CP 94253 doses.
* Significant difference between Saline and CP groups on PD 20.
**Significant difference in axillary temperatures between SAL-CP and CP- CP groups on PD 22

Motoric Capacity

Pretreatment with CP- 94253 on PD 20 resulted in a significant main effect of dose observed on motoric capacity throughout the 45 min session [Kruskal-Wallis H = 21.73 to 12.89, p< .05, Dunn's test, p<.05] (See Table 4). Specifically, animals treated with 20 mg/kg CP-94253 (CP + CP group) exhibited forward locomotion with major balance problems (forward focused movement, minor dragging and rolling over, predominate dragging (major dragging and rolling over, with random forward movement), circular dragging (forward dragging in a circular pattern), and spayed or "swimming" movements (See Table 4). There was no significant difference in motoric capacity ratings for animals treated with saline or 2.5, 5, or 10 mg/kg CP-94253 [Dunn's test, p<.05].

On PD 22, subjects that received multiple treatments of CP-94253 resulted in a significant main effect of dose observed on motoric capacity [Mann-Whitney U-tests, p<.05] (Table 4). Specifically, animals that received a second treatment of 20 mg/kg CP- 94253 (CP + CP group), had a significant decrease in motoric capacity on PD 22 during time blocks 6- 9 compared to PD 20 [Wilcoxon signed-rank tests, p<.05] (See Table 4). In addition, animals receiving their first treatment of 20 mg/kg CP- 94253 (Sal- CP group) exhibited greater motoric impairments than the CP + CP group on time blocks 4, 5, and 7 (See Table 4). Compared to PD 22, subjects that received multiple injections of 20 mg/kg CP-94253 displayed greater motoric incapacity on PD 20, indicating that they developed tolerance to the drug's initial effects after multiple treatments.

Experiment 3

Experiment 3 investigated the effects of combined treatment with 8-OH-DPAT (4 mg/kg) and CP 94253 (5 mg/kg) on the locomotor activity, axillary temperatures, and motoric capacity of male and female preweanling rats. It was hypothesized that a single injection of DPAT+CP co-administered will result in hypothermia, and elevated locomotor activity and motoric impairment.

Locomotor Activity

Pretreatment with DPAT + CP (PD 20) resulted in a significant main effect of dose observed with locomotor activity scores [Pretreatment main effect, F (1, 22) =88.93, p<.001] (Figure 7, upper graph). Specifically, animals that received the combined treatment of DPAT+CP exhibited greater locomotor activity scores compared to saline treated animals.

On test day (PD 22), there was a significant main effect of dose observed with distance traveled scores [Group main effect, F (1, 22) = 29.56, p < .001] (Figure 7, lower graph). Specifically, animals challenged with DPAT+CP (Sal-DPAT+CP and DPAT+CP group) on PD 22, had a significant increase in distance traveled scores compared to the control group (Sal-Sal group) [Tukey tests, p<0.05]. Interestingly, there was no significant difference in locomotor activity scores between subjects that were given their first injection of DPAT+CP on PD 20 (DPAT+CP- DPAT+CP group) compared to those that only received it on PD 22 (Sal- DPAT+CP group) [Tukey tests, p<0.05]. The non-significant difference between the Sal-DPAT+CP and the DPAT+CP- DPAT+CP groups

indicates that the combination of CP-94253 and 8-OH-DPAT did not induce tolerance or sensitization after a single pre-exposure in our study.

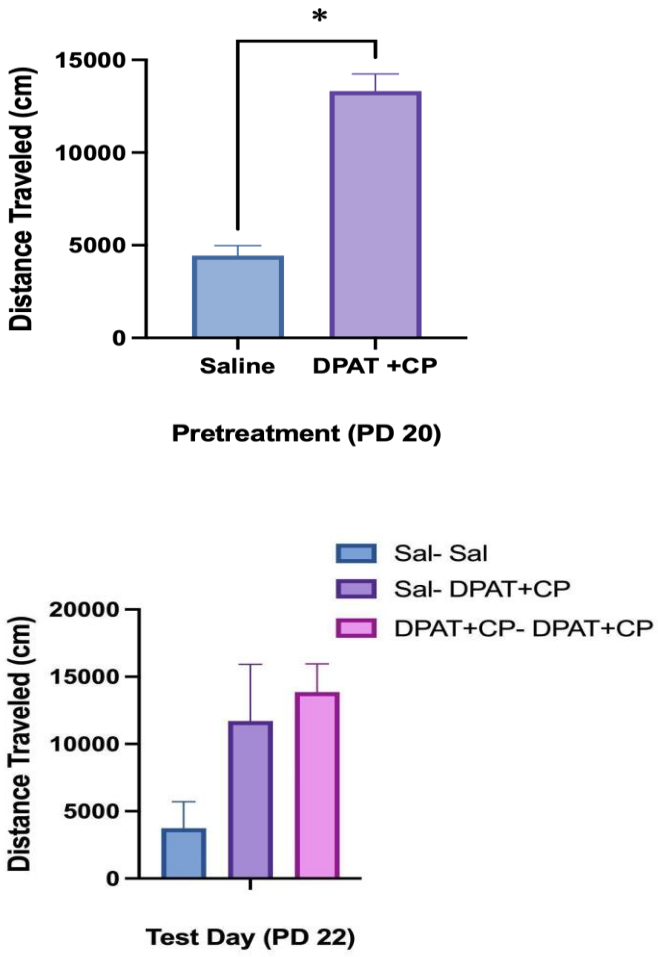

Figure 7. Mean (±) distance traveled on the pretreatment day (upper graph) and test day (lower graph) of male and female preweanling rats. Rats were injected with saline or were co-administered with 8-OH-DPAT (4 mg/kg) and CP 94253 (5 mg/kg, ip) on the pretreatment day (PD 20). On the test day (PD 22), DPAT+CP pretreated rats were injected with the same dose again while saline pretreated rats were injected with DPAT+CP.
* Significant difference between Saline and DPAT+CP groups on PD 20.

Axillary Temperature

Pretreatment with DPAT+CP on PD 20 resulted in a significant main effect of dose observed on axillary temperatures, [Pretreatment main effect, F (1, 21) = 889.246, p<.001] (Figure 8, upper graph). Specifically, animals pretreated with DPAT+CP exhibited a significant decrease in core body temperatures compared to saline treated animals [Tukey test, p< .05].

On test day (PD 22), there was a significant main effect of dose observed with axillary temperatures [Group main effect, F (2, 23) =107.756, p<.001] (Figure 8, lower graph). Animals previously administered DPAT+CP and challenged with DPAT+CP had a significant decrease in axillary temperatures compared to our control group (Sal-Sal group). Additionally, animals previously treated with saline and treated with DPAT+CP (Sal- DPAT +CP group) had a significant decrease in core body temperatures compared to our control group (Figure 6, lower graph). However, there was no significant difference in axillary temperatures between animals that received one or two injections of DPAT + CP [Tukey tests, p<0.05], indicating that it did not induce sensitization or tolerance after a single pre-exposure in our study.

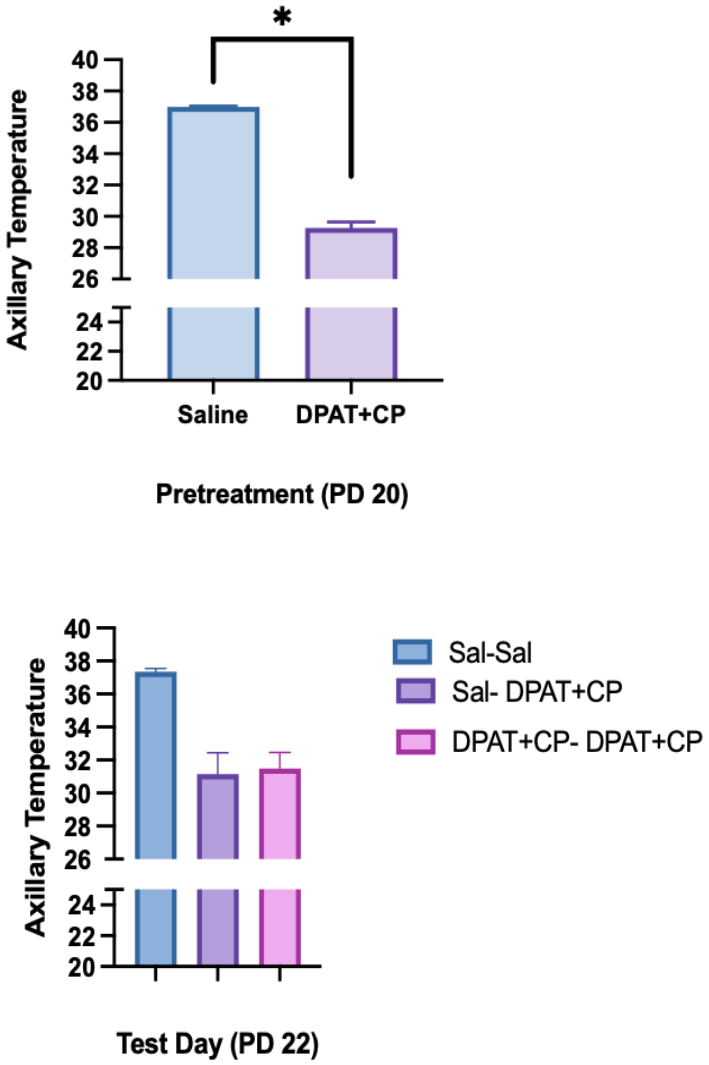

Figure 8. Mean (±) axillary temperatures on the pretreatment day (upper graph) and test day (lower graph) of male and female preweanling rats. Rats were injected with saline or were co-administered with 8-OH-DPAT (4 mg/kg) and CP 94253 (5 mg/kg, ip) on the pretreatment day (PD 20). On the test day (PD 22), DPAT+CP pretreated rats were injected with the same dose again while saline pretreated rats were injected with DPAT+CP.
* Significant difference between Saline and DPAT+CP groups on PD 20.

Motoric Capacity

Pretreatment with DPAT + CP on PD 20 resulted in a significant increase in motoric capacity throughout the 45-minute session [Mann- Whitney *U* tests, $p<.05$] (See Table 5). Animals treated with DPAT + CP exhibited focused forward movement with minor dragging and rolling over compared to saline treated animals. On PD 22, subjects that received multiple treatments of DPAT + CP also resulted in a significant increase in motoric capacity throughout the 45-minute session [Kruskal- Wallis $H = 15.02$ to 10.29, $p< .05$, Dunn's test, $p<.05$] (See Table 5). Animals receiving a second treatment of DPAT + CP exhibited focused forward movement with awkward leg movements compared to saline treated animals. Compared to PD 22, subjects displayed greater motoric incapacity on PD 20, indicating that they developed tolerance to the drug's initial effects after multiple treatments.

Experiment 4

Experiment 4 investigated the effects of 8-OH-DPAT and CP 94253, administered alone or together, on the locomotor activity, motoric capacity, and axillary temperatures of male and female preweanling rats with multiple injections. It was hypothesized that multiple injections of 8-OH-DPAT will decrease core body temperature and motor impairment, resulting in tolerance; whereas, locomotor activity will show a progressively elevated response after repeated injections, resulting in sensitization. Additionally, we predicted that

multiple injections of CP 94253 will increase locomotor activity at all doses, with no effect on core body temperature or motoric impairment, resulting in behavioral sensitization. Lastly, it was predicted that the combined administration of 8-OH-DPAT and CP 94253 will cause tolerance across all three measures: core body temperature, motoric impairment, and locomotor activity.

Locomotor Activity

Repeated administration of either saline, 4 mg/kg 8-OH-DPAT, 5 mg/kg CP- 94253, or the combined treatment of DPAT + CP during the pretreatment phase (PD 17- PD 20), there was an interaction effect of pretreatment and day observed with locomotor activity scores [Pretreatment × Day interaction, F (3, 156) = 5.383, p<.001] (Figure 9, upper graph). Specifically, over the four-day pretreatment period, rats treated with DPAT + CP, and 4 mg/kg 8-OH-DPAT exhibited significantly greater locomotor activity scores compared to rats treated with 5 mg/kg CP- 94253 and saline controls (Figure 9, upper graph) [Tukey test, $p < 0.05$], and this effect changed over days.

On PD 17, rats treated with DPAT + CP, and 4 mg/kg 8-OH-DPAT exhibited significantly greater locomotor activity scores compared to rats treated with 5 mg/kg CP- 94253 and saline controls [Pretreatment main effect, F (3, 52) = 38.936, $p < 0.001$] (Figure 9, upper graph) [Tukey test, $p < 0.05$]. Moreover, DPAT + CP produced the greatest locomotor activity, followed by 4 mg/kg 8-OH-DPAT, while 5 mg/kg CP-94253-treated animals did not differ from saline-treated animals [Tukey test, $p < 0.05$].

On PD 18, there was a main effect of dose observed with locomotor activity scores [Pretreatment main effect, F (3, 52) = 8.693, p <.001] (Figure 9, upper graph). Animals that received DPAT + CP had significantly greater locomotor activity than rats treated with 4 mg/kg 8-OH-DPAT, 5 mg/kg CP-94253, or saline [Tukey test, $p < 0.05$]. Although animals treated with DPAT + CP continue to produce the greatest distance traveled scores, there is a significant decrease in locomotor activity on PD 17 compared to PD 18.

On PD 19, there was a main effect of dose observed with locomotor activity scores [Pretreatment main effect, F (3, 52) = 21.069, $p < 0.001$] (Figure 9, upper graph). Subjects treated with DPAT + CP, and 4 mg/kg 8-OH-DPAT exhibited significantly greater locomotor activity scores compared to rats treated with 5 mg/kg CP- 94253 and saline controls [Tukey test, $p < 0.05$].

On the final day of the pretreatment phase (PD 20), there was a main effect of dose observed with locomotor activity scores [Pretreatment main effect, F (3, 52) = 39.561, $p < 0.001$] (Figure 9, upper graph). Specifically, rats treated with DPAT + CP, and 4 mg/kg 8-OH-DPAT exhibited significantly greater locomotor activity scores compared to rats treated with 5 mg/kg CP- 94253 and saline controls (Figure 9, upper graph) [Tukey test, $p < 0.05$]. Animals treated with DPAT + CP continue to produce the greatest distance traveled scores, although there is a significant decrease in locomotor activity from their initial treatment on PD 17 compared to the fourth treatment (PD 20). Rats treated with 4 mg/kg 8-OH-DPAT exhibited significantly greater locomotor activity scores

compared to rats treated with 5 mg/kg CP- 94253 and saline controls [Tukey test, $p < 0.05$].

On Test Day (PD 22), after receiving multiple injections of either saline, 4 mg/kg 8-OH-DPAT, 5 mg/kg CP- 94253, or the combined treatment of DPAT + CP, there was a main effect of dose observed with locomotor activity scores [Group main effect, F (6, 49) = 19.20, p <.001] (Figure 9, lower graph). Specifically, subjects that received consecutive treatments of 4 mg/kg 8-OH-DPAT also had greater distance traveled scores compared to the 8-OH-DPAT for the first time on PD 22. Conversely, rats that received multiple injections of 5 mg/kg CP-94253 had a significant decrease in distance traveled scores compared to animals treated with CP-94253 for the first time on PD 22 [Figure 9, lower graph; Tukey test, $p < 0.05$]. Moreover, rats that received multiple treatments of DPAT + CP exhibited greater distance traveled scores compared to rats that only received one treatment of DPAT + CP (Sal- DPAT + CP group; Figure 9, lower graph).

In sum, multiple injections of DPAT + CP during the pretreatment phase (PD 17- PD 20) resulted in a progressive decrease in distance traveled scores (Figure 9, upper graph). However, the same animals exhibited significantly greater distance traveled scores on test day (PD 22), compared to rats that received their first injection, which is indicative of locomotor sensitization. Repeated injections of 8-OH-DPAT during the pretreatment phase resulted in a gradual increase in locomotor activity (Figure 9, upper graph). On test day, the

same animals continued to have an increase in locomotor activity compared to their initial treatment on PD 17, which indicates locomotor sensitization is present. Rats treated with CP-94253 did not have a significant effect on locomotor activity scores throughout the pretreatment phase (Figure 9, upper graph). However, the same rats showed a significant decrease in locomotor activity on PD 22, indicating a tolerance effect (Figure 9, lower graph).

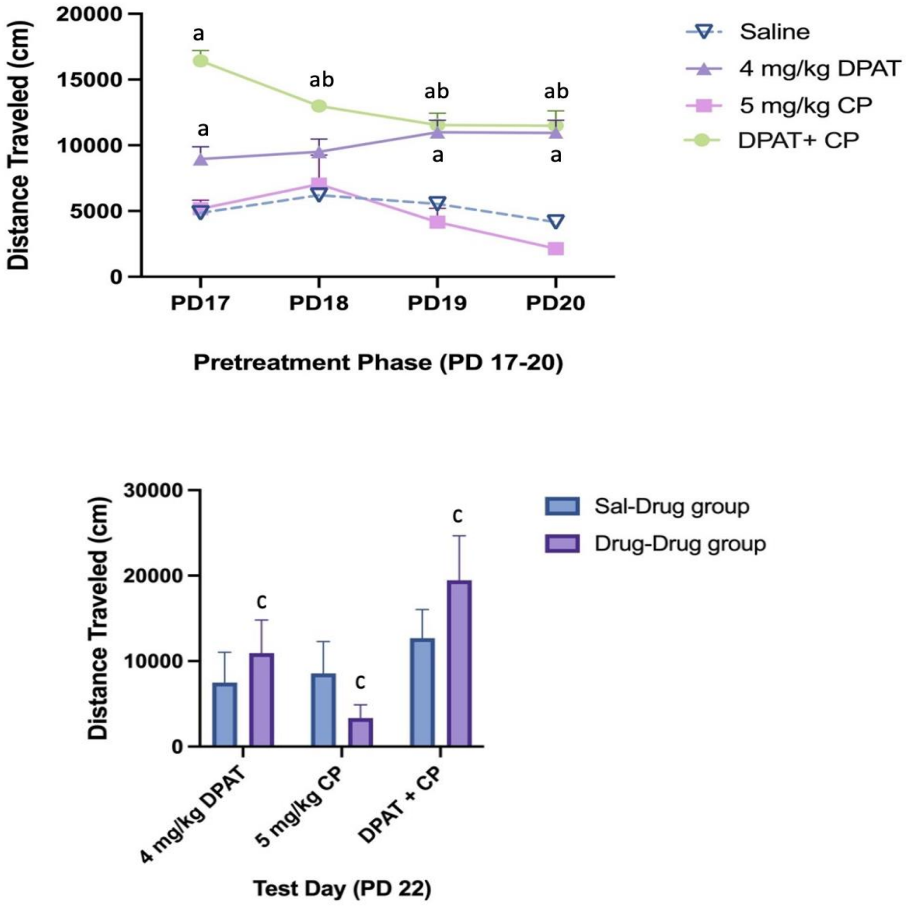

Figure 9. Mean (±) distance traveled on the pretreatment day (upper graph) and test day (lower graph) of male and female preweanling rats. Rats were injected with saline or 8-OH-DPAT (4 mg/kg) and CP 94253 (5 mg/kg, ip) alone or together on the pretreatment day (PD 20). On the test day (PD 22), 8-OH-DPAT, CP 94253, or DPAT+CP pretreated rats were injected with the same dose again

while saline pretreated rats were injected with 8-OH-DPAT, CP 94253, or DPAT+CP.
(a) Significantly different from saline-treated rats on the same postnatal day.
(b) Significantly different from the same drug group on PD 17.
(c) Significantly different from rats given the same drug for the first time on PD 22 (i.e., Sal- DPAT, Sal- CP, Sal- DPAT+CP groups).
(d) Significantly different from rats treated with 8-OH-DPAT (4 mg/kg) or CP 94253 (5 mg/kg) alone.

Axillary Temperature

Repeated administration of either saline, 4 mg/kg 8-OH-DPAT, 5 mg/kg CP- 94253, or the combined treatment of DPAT + CP during the pretreatment phase (PD 17- PD 20), there was an overall an interaction effect of pretreatment and day observed with axial temperatures [Pretreatment × Day interaction, ^{b}F (2, 156) = 257.188 p<.001] (Figure 10, upper graph). Specifically, over the four-day pretreatment period, rats treated with DPAT + CP, 4 mg/kg 8-OH-DPAT and 5 mg/kg CP- 94253 exhibited significant decrease in core body temperatures compared to saline controls (Figure 10, left graph), and this effect changed over days.

On PD 17, rats treated with DPAT + CP, 4 mg/kg 8-OH-DPAT, or 5 mg/kg CP- 94253 resulted in a significant decrease in axillary temperature compared saline controls [Pretreatment main effect, F (3, 52) = 184.81, p <.001] (Figure 10, upper graph) [Tukey test, p < 0.05].

On PD 18, there was a main effect of dose observed with core body temperatures [Pretreatment main effect, F (3, 52) = 168.97, p <.001] (Figure 10, upper graph). Animals that received multiple injections of DPAT + CP or 4 mg/kg

8-OH-DPAT, had a significant increase in axillary temperatures compared to their initial treatment on PD 17, while the temperatures of rats that received 5 mg/kg CP-94253 were not significantly different than our saline- treated rats [Tukey test, $p < 0.05$].

On PD 19, there was a main effect of dose observed with axillary temperatures [Pretreatment main effect, F (3, 52) = 137.603, p <.001] (Figure 10, upper graph). Subjects treated with DPAT + CP or 4 mg/kg 8-OH-DPAT exhibited a significant increase in core body temperatures compared to saline controls [Tukey test, $p < 0.05$]. However, 5 mg/kg CP- 9425 did not significantly differ in axillary temperatures than saline-treated animals.

On the final day of the pretreatment phase (PD 20), there was a main effect of dose observed with core body temperatures [Pretreatment main effect, F (3, 52) = 134.226, p <.001] (Figure 10, upper graph). Specifically, rats treated with DPAT + CP or 4 mg/kg 8-OH-DPAT resulted in an increase in axillary temperatures compared to saline controls (Figure 10, upper graph) [Tukey test, $p < 0.05$]. Animals treated with DPAT + CP and 4 mg/kg 8-OH-DPAT continue to produce an increase in core body temperature throughout the pretreatment phase compared to their initial treatment on PD 17. Conversely, animals treated with 5 mg/kg CP 94253 did not significantly differ from saline-treated animals.

On Test Day (PD 22), after receiving multiple injections of either saline, 4 mg/kg 8-OH-DPAT, 5 mg/kg CP- 94253, or the combined treatment of DPAT + CP, there was a main effect of Group observed with axillary temperatures [Group

main effect, F (6, 56) = 108.823, $p < 0.001$] (Figure 10, lower graph). Subjects that received their first drug treatment on PD 22 (Sal- Drug groups) exhibited an analogous pattern to the Drug- Drug groups on PD 17, such that there was a significant decrease in core body temperatures in all drug groups compared to saline controls [Tukey test, $p < 0.05$] (Figure 10, lower graph). Regarding the all Drug- Drug groups, animals that received multiple injections of 8-OH-DPAT, CP 94253, or DPAT+CP had a significant increase in core body temperatures throughout the pretreatment phase compared to the rats that received their first drug treatment on PD 22 (Sal- Drug groups) which is indicative of tolerance (Figure 10, lower graph) [Tukey test, $p < 0.05$].

In sum, multiple injections of DPAT + CP, 4 mg/kg 8-OH-DPAT, and CP-94253 during the pretreatment phase (PD 17- PD 20) resulted in tolerance to the drug's effects which were observed through the progressive increase in axillary temperatures (Figure 10, upper graph). On test day (PD 22), the same animals continued to have an increase in core body temperatures compared to their initial treatment on PD 17, although animals treated with DPAT + CP had the lowest core body temperatures (Figure 10, lower graph) [Tukey test, $p < 0.05$].

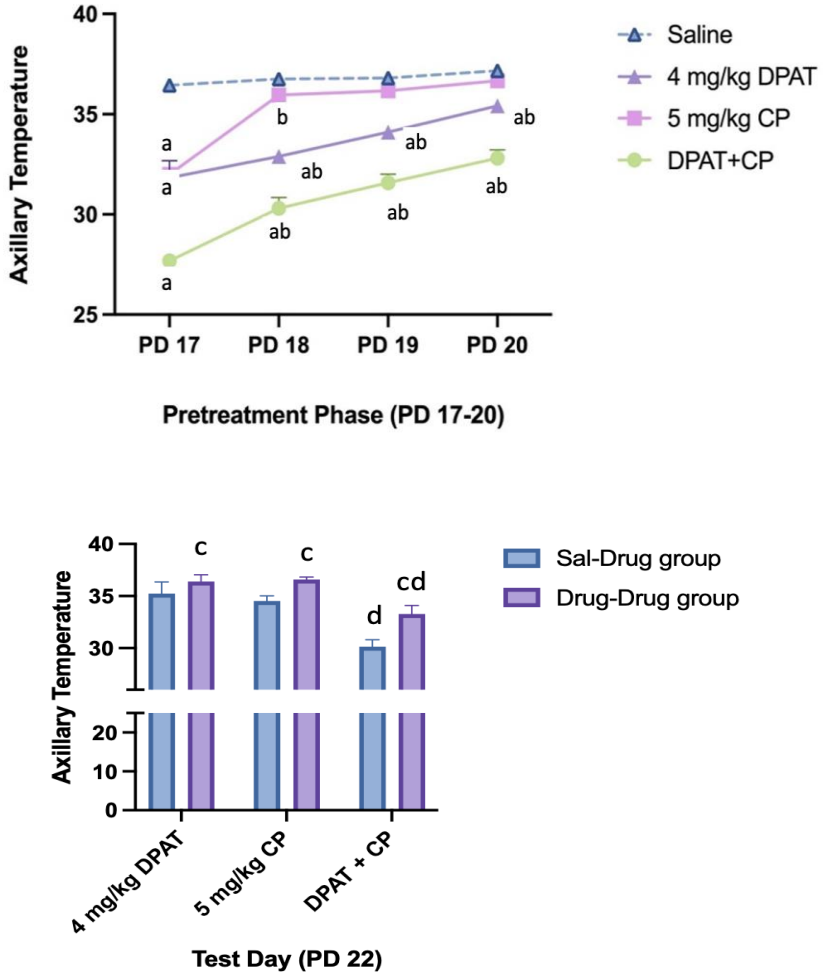

Figure 10. Mean (±) axial temperatures on the pretreatment day (upper graph) and test day (lower graph) of male and female preweanling rats. Rats were injected with saline or 8-OH-DPAT (4 mg/kg) and CP 94253 (5 mg/kg, ip) alone or together on the pretreatment day (PD 20). On the test day (PD 22), 8-OH-DPAT, CP 94253, or DPAT+CP pretreated rats were injected with the same dose again while saline pretreated rats were injected with 8-OH-DPAT, CP 94253, or DPAT+CP.

(a) Significantly different from saline-treated rats on the same postnatal day.
(b) Significantly different from the same drug group on PD 17.
(c) Significantly different from rats given the same drug for the first time on PD 22 (i.e., Sal- DPAT, Sal- CP, Sal- DPAT+CP groups).
(d)Significantly different from rats treated with 8-OH-DPAT (4 mg/kg) or CP 94253 (5 mg/kg) alone.

Motoric Capacity

Animals receiving a single or multiple injections of 4 mg/kg 8-OH DPAT, 5 mg/kg CP- 94253, or the combination of DPAT + CP had no effect on motoric capacity during the pretreatment phase (PD 17- 20) or the test day (PD 22) (data not shown).

Table 3.
Experiment 1b. Effects of saline and repeated high dose (1–8 mg/kg) treatment with the 5-HT_{1A} agonist 8-OH-DPAT on the motoric capacity scores of male and female preweanling rats: Two-day procedure.

Age-Treatment	5- Minute Time Blocks								
	1	2	3	4	5	6	7	8	9
PD20									
Saline	0.25	0	0	0	0	0	0	0	0
8-OH-DPAT (1mg/kg)	3	3	3	1	3	0	0	0	0
8-OH-DPAT (2mg/kg)	4	5[a]	2	1	3	0	0	1	0
8-OH-DPAT (4mg/kg)	3	3.5[a]	3[a]	1	3	3	3	1	0
8-OH-DPAT (8mg/kg)	5[a]	4[a]	3	0	0	3	0	0	0
PD22									
Saline-DPAT (1mg/kg)	2.5	2	2	1.5	0	0	0	0	0
DPAT-DPAT (1mg/kg)	1.5	1	1	0.5	0	0	0	0	0
Saline-DPAT (2mg/kg)	3	2.5	2	2	1	0	0	0	0
DPAT-DPAT (2mg/kg)	2	2	0.5	0	1	0	0	0.5	0
Saline-DPAT (4mg/kg)	5	4	3	3	2.5	2.5	2	2	1.5
DPAT-DPAT (4mg/kg)	3	3	3	2	2	1	1	2	2
Saline-DPAT (8mg/kg)	4	4	4	3	1.5	3	2	2.5	0
DPAT-DPAT (8mg/kg)	4	3	3	0.5	3	3	3	2	1

Note: Higher numbers mean greater impairment in motoric capacity (See Table 2).
[a] Significantly different from saline-treated rats on PD 20 (Dunn's tests, $p<.05$).

Table 4.
Experiment 2. Effects of saline and repeated 5-HT_{1B} agonist CP 94253 (2.5- 20 mg/kg) on the motoric capacity scores of male and female preweanling rats: Two-day procedure.

Age-Treatme	5- Minute Time Blocks								
	1	2	3	4	5	6	7	8	9
PD20									
Saline	0	0	0	0	0	0.1	0	0	0.2
CP 94253 (2.5mg/kg)	0	0	0	0	0	0	0	0	0
CP 94253 (5mg/kg)	0	0	0	0	1.5	0	1.5	1.5	0
CP 94253 (10mg/kg)	0	2	1	3	3.5[a]	3	0	0	1.5
CP 94253 (20mg/kg)	5[a]	6[a]	6[a]	7.5[a]	8[a]	8[a]	8[a]	8[a]	8[a]
PD22									
Saline- CP (2.5mg/kg)	0	0	0	0	0	0	0	0	0
CP-CP (2.5mg/kg)	0	0	0	0	0	0	0	0	0
Saline-CP (5mg/kg)	0	0	0.5	0	0	0	0	0	0
CP-CP (5mg/kg)	0	0	0	0	0	0	0	0	0
Saline-CP (10mg/kg)	1.5	0	0	0	0	0	0	0	0
CP-CP (10mg/kg)	0.5	0	0	1	0	0.5	0	1	1
Saline-CP (20mg/kg)	3	5	5.5	5.5	4.5	2.5	6	4	2.5
CP-CP (20mg/kg)	3	3	4	3[b]	0[b]	1.5[c]	0[b,c]	0[c]	0[c]

Note: Higher numbers mean greater impairment in motoric capacity (See Table 2).
[a] Significantly different from saline-treated rats on PD 20 (Dunn's tests, $p<.05$).
[b] Significantly different from the Saline- CP group injected with the same dose of CP-94253 (Mann- Whitney *U*-tests, $p<.05$.
[c] Significantly different from the Drug-Drug group on PD 20 (Wilcoxon signed-rank tests, $p<.05$).

Table 5.
Experiment 3. Effects of combined treatment with 8-OH-DPAT and CP 94253 (DPAT+CP) on the motoric capacity of male and female preweanling rats: Two-day procedure

Age-Treatment	5- Minute Time Blocks								
	1	2	3	4	5	6	7	8	9
PD20									
Saline	0	0	0	0	0	0	0	0	0
DPAT+CP	5[a]	5[a]	5[a]	5.5[a]	5[a]	5[a]	5.5[a]	5[a]	5[a]
PD22									
Saline- Saline	0	0	0	0	0	0	0	0	0
Saline-DPAT+CP	3[b]	3[b]	3[b]	3[b]	2.5[b]	2.5[b]	2.5[b]	2.5	2.5[b]
DPAT+CP-DPAT+CP	1.5[b]	2[b,c]	3[b]	3[b,c]	3[b,c]	3[b,c]	3[b,c]	3[b,c]	3[b,c]

Note: Higher numbers mean greater impairment in motoric capacity (See Table 2).
[a] Significantly different from Saline-treated rats on PD 20 (Dunn's tests, $p<.05$).
[b] Significantly different from Saline-Saline group on PD 22 (Dunn's tests, $p<.05$).
[c] Significantly different from DPAT + CP group tested on PD 20 (Wilcoxon signed-rank tests, $p<.05$).

Table 6.
Experiment 4. Effects of 8-OH-DPAT (4 mg/kg), CP 94253 (5 mg/kg), or DPAT+CP on the motoric capacity of male and female preweanling rats: Five-day procedure

Age-Treatment	5- Minute Time Blocks								
	1	2	3	4	5	6	7	8	9
PD 22									
Saline-DPAT (4mg/kg)	1.5	2.5	0.5	1.5	1	0.5	0.5	0	1.5
DPAT-DPAT (4mg/kg)	1	2	2	0.5	1	1	1	0.5	1
Saline-CP (5mg/kg)	0	0.5	0	0	0	0.5	0	1	0
CP+CP (5mg/kg)	0	0	0	0	0	0	0	0	0
Saline-DPAT+CP	3	2.5	2	2.5	2.5	2	2	2	2
DPAT+CP-DPAT+CP	1.5	1.5	1	1	1	1	1	1	1

Note: Higher numbers mean greater impairment in motoric capacity (See Table 2).

CHAPTER SEVEN:

DISCUSSION

The current pharmacological interventions used in pediatric populations to treat disorders such anxiety and depression are not tested for their effectiveness in these populations. Moreover, some drug treatments have been reported to increase suicidality in adolescents and young adults (Zhou et al., 2020). The large number of children and adolescents that have psychiatric disorders coupled with the lack of validated treatments has created a large need for new effective psychotropic medications for juveniles (Hollander, Phillips & Yeh, 2003; Brummelte et al., 2017; Hirsch, 2018; Salokangas et al., 2018; Langerberg et al., 2019).

Recently, the preclinical 5-$HT_{1A/1B}$ agonist drug RU 24969 has shown efficacy in an animal model of depression (Tiger et al., 2018). This compound could be a new class of antidepressants and may have a more favorable profile for pediatric users. However, most of what is known about RU 24969 is from research using adult animals, and the effect may differ in younger age groups. A better understanding of how 5-HT_{1A} and 5-HT_{1B} agonists work at different stages of development is important for determining the utility of RU 24969 as a clinical intervention. Thus, in this study, we assessed the unlearned effects of the 5-HT_{1A} agonist, 8-OH-DPAT and the 5-HT_{1B} agonist CP 94253 in young male and female rats. Specifically, we administered the 5-HT_{1A} agonist 8-OH-DPAT (0.1- 8

mg/kg), and the 5-HT_{1B} receptor agonist CP 94253 (2.5, 5, 10, or 20 mg/kg) alone, and in combination to examine the effects of single-trial (one pretreatment day) and repeated treatment (four pretreatment days) on locomotor activity, axillary temperatures, and motoric capacity.

The Effects of a Single Pretreatment Day with the 5-HT_{1A} Receptor Agonist 8-OH-DPAT

We hypothesized that 8-OH-DPAT would result in behavioral sensitization, characterized by a dose-dependent increase in locomotor activity, a larger decrease in core body temperature, and an increase in motoric impairment. In general, our predictions on locomotor activity and motoric capacity were unsupported, whereas our predictions for axillary temperature were supported.

The present study demonstrated that single-trial administration of 8-OH-DPAT (0.1-8 mg/kg) neither increased (induced behavioral sensitization) or decreased (produced tolerance) locomotor activity at any dose. Although past studies have shown that acutely, 8-OH-DPAT increases locomotor activity (Tricklebank et al., 1984; Evenden 1992; Evenden, Ryan & Palejko, 1995; De La Garza & Cunningham, 2000), other studies have shown that 8-OH-DPAT decreases activity (Mittman & Geyer, 1989; Carli et al., 1989; Hillegaart et al., 1989; Johnson & Ahlenius, 1989; Evenden & Angeby-Moller, 1990), or has no effect on activity (Woodall et al., 1996). Limited research examining the single

trial effects of 8-OH-DPAT found that it does not cause sensitization or tolerance with two drug treatments (Sipos et al., 2000; Bert et al., 2006).

In agreement to our hypothesis however, we found that single-trial administration of 8-OH-DPAT caused tolerance for axillary temperatures at higher doses (0.4, 0.8, 4, and 8 mg/kg) (Figure 2, right graph). In line with past research findings, initial administration of 8-OH-DPAT causes hypothermia and subsequent administration produces a rapid tolerance to these effects (Hillegaart et al., 1989).

Similar to locomotor activity, the effects of 8-OH-DPAT (0.1-8 mg/kg) on motoric capacity was not altered by a second exposure to the drug at any dose. Past research has found that treatment with 8-OH-DPAT induces a behavioral syndrome expressed through flat body posture, forepaw treading, and head weaving in adult mice after two injections (Bert et al., 2006). Although there is limited information on how 8-OH-DPAT affects motoric capacity in a single-trial paradigm, there is evidence that acute administration also causes motoric incapacity as soon as 30 minutes post-injection (Tricklebank et al., 1984, Evenden & Angeby-Möller, 1990).

The Effects of a Single Pretreatment Day with the 5-HT $_{1B}$ Agonist CP 94253

We hypothesized that a single administration of CP 94253 would result in behavioral sensitization, as shown by an enhanced behavioral response and a dose-dependent increase in locomotor activity and would have no effect on core body temperature or motor impairment. In general, our predictions on locomotor activity, axillary temperatures, and motoric capacity were unsupported.

The data from our present study does not support that single-trial administration with CP 94253 (2, 5, 10, or 20 mg/kg) alters locomotor activity at any dose. There is limited information on the locomotor effects of CP 94253, mostly because research on this drug focuses on reducing the rewarding properties of cocaine.

Contrary to our hypothesis, we found that two treatments of CP 94253 resulted in tolerance for axial temperature changes (Figure 6, right graph). To our knowledge, past research has not examined the single-trial effects of CP 94253 in depth because it does not affect axial temperatures in adults (Fish et al., 1999). However, there is evidence that it acutely decreases axial temperatures in adult and preweanling rats (McDougall et al., 2022). The findings from the present study are in partial agreement with previous studies, in that treatment with CP 94253 initially causes hypothermia, and this effect reverses with a drug second treatment.

In terms of motoric capacity, tolerance was observed but only present for subjects that received two injections of 20 mg/kg CP 94253 (See Table 4).

Although past research has shown that CP 94253 does not have much of an effect on adult rodent behavior (Fish et al., 1999; Dalton et al., 2004; Der-Ghazarian et al., 2017), none of these studies have examined the motoric effects for preweanling animals (to my knowledge). The results from the present study demonstrate that two treatments of 20 mg/kg CP 94253 causes behavioral tolerance to motoric impairment in preweanling rats.

The Effects of a Single Pretreatment Day with the 8-OH-DPAT and CP 94253

It was hypothesized that single-trial treatment of 8-OH-DPAT (4 mg/kg) and CP 94253 (5 mg/kg) co-administered would result in elevated locomotor activity, a decrease in core body temperatures, and greater motoric impairment after two treatments. In general, our predictions on locomotor activity and axillary temperatures were unsupported, whereas our predictions for motoric capacity were supported.

In contrast to our hypothesis, we found that single-trial administration of DPAT+CP did not differentially affect the locomotor activity (See Figures 7 & 8, right graphs). This effect is surprising, because past research has demonstrated RU 24969 produces an enhanced behavioral effect upon initial administration, but then causes tolerance to the locomotor activity inducing effect that can be observed after the second administration (Oberlander et al., 1987; Harrison et al, 1997; McDougall et al., 2020).

Two treatments of DPAT+CP did not differentially affect axillary temperatures of animals that received one treatment or two drug treatments (See Figures 7 & 8, right graphs). This effect is surprising because past studies have found that single-trial administration of 8-OH-DPAT causes hypothermia with its initial administration, and subsequent administration produces a rapid tolerance to these effects (Hillegaart et al., 1989). Additionally, CP 94253 administered acutely also decreases axial temperatures in preweanling and adult rats (McDougall et al., 2022).

The present data support that the co-administration of DPAT+CP increases motoric incapacity (See Table 5). These results are consistent with other studies showing the stimulation of 5-HT_{1A} 5-HT_{1B} receptors is needed in order to produce motoric incapacity (O' Niell & Parameswaran, 1997; Munoz et al., 2008).

The Effects of Repeated Pretreatment with the 8-OH-DPAT (4 mg/kg), CP 94253 (5 mg/kg) or the Co-administration of DPAT+CP

8-OH-DPAT (4 mg/kg)

It was hypothesized that multiple injections of 8-OH-DPAT would show a progressively elevated locomotor response, resulting in sensitization, whereas, core body temperature and motor impairment would gradually decrease, resulting in tolerance. Our predictions on locomotor activity and axillary

temperatures were supported, while our predictions for motoric capacity were not supported.

In agreement with our hypothesis, we found that repeated administration of 4 mg/kg 8-OH-DPAT resulted in locomotor behavioral sensitization and tolerance to axillary temperatures. Our results were consistent with reports stating that repeated treatment with 8-OH-DPAT increases locomotor activity (Evenden 1992; De La Garza & Cunningham, 2000) (See Figure 9, right and left graphs). Additionally, we found that repeated treatment with 4 mg/kg 8-OH-DPAT increased axillary temperatures in our subjects, resulting in tolerance (De Souza et al., 1986; Evenden & Ryan, 1992) (Figure 10, right graph). The tolerance found with repeated treatment of 4 mg/kg 8-OH-DPAT was concluded after the initial treatment greatly decreased core body temperatures, yet the subsequent drug treatments increased axial temperatures back to its original state.

In contrast to our predictions, we found that repeated treatment with 4 mg/kg 8-OH-DPAT did not differentially affect motoric capacity in our subjects, resulting in neither sensitization nor tolerance (data not shown). This effect differs from past research that has found that multiple injections of 8-OH-DPAT causes tolerance (Evenden & Ryan, 1999). For example, in a study examining motoric capacity through lever pressing in adult rats, it was reported that 8-OH-DPAT initially caused motoric incapacity, resulting in the subject's inability to press the lever (Evenden & Ryan, 1999). However, this effect was quickly reversed, and

the subjects regained the ability to press the lever, indicating that repeated treatment with 8-OH-DPAT causes tolerance in motoric capacity.

CP 94253 (5 mg/kg)

We hypothesized that multiple injections of CP 94253 would result in locomotor sensitization, with no effect on core body temperature or motoric impairment. Our predictions on locomotor activity and axillary temperatures were not supported, while our predictions for motoric capacity were supported.

In contrast to our hypothesis, we found that repeated treatment with 5 mg/kg CP 94253 results in tolerance, which can be observed through the decrease in distance traveled scores on the test day (Figure 9, right graph). The tolerance effect found in our study is interesting, because it conflicts with past research that has found that CP 94253 increases locomotor activity in preweanling rodents after one injection (Fish et al., 2000; Der-Ghazarian et al., 2017).

Repeated treatment with 5 mg/kg CP 94253 on axillary temperatures produced interesting results. Initial treatment with CP 94253 caused a significant decrease in core body temperatures causing hypothermia, but that effect was reversed with subsequent treatments (Figure 10, right graph). Specifically, our data shows that tolerance was developed after the second drug treatment. To our knowledge, the only studies available on the effects of CP 94253 have studied it acutely and have found that it does not affect axial temperatures in adult rats (Fish et al., 2000).

Treatment with CP 94253 did not differentially affect motoric capacity for animals receiving one or multiple drug treatments, resulting in neither sensitization nor tolerance (data not shown). To our knowledge, studies have not previously examined the effect CP 94253 has on motoric capacity in adults or preweanling rodents, however, the mixed 5-$HT_{1A/1B}$ agonist RU 24969 initially causes motoric impairment, but each subsequent injection causes behavioral tolerance to this effect (Oberlander et al., 1987; Harrison et al., 1997; McDougall et al., 2020).

Co-administration of DPAT (4 mg/kg) + CP (5 mg/kg)

We hypothesized that multiple injections of 8-OH-DPAT and CP 94253 given in combination would decrease locomotor activity, increase core body temperature, and decrease motor impairments inducing tolerance at all doses. In general, our predictions on locomotor activity and motoric capacity were unsupported, whereas our predictions for axillary temperatures were supported.

In contrast to our hypothesis regarding locomotor activity, we found that rats that received multiple treatments of DPAT + CP exhibited greater distance traveled scores on PD 22 compared to rats that only received one treatment of DPAT + CP (Sal- DPAT + CP group; Figure 9, right graph) causing locomotor sensitization. This effect is interesting because the 5-$HT_{1A/1B}$ agonist RU 24969 initially increases locomotor activity, but then produces a tolerance to these effects as soon as the second drug administration (Oberlander et al., 1987; McDougall et al., 2020).

Our data supports past findings stating that repeated treatment with DPAT + CP results in behavioral tolerance for axial temperatures (Figure 9, right graph). Despite the absence of studies examining the effects of DPAT + CP on axial temperatures, we predicted that these effects would be similar to that of RU24969.

Additionally, we found that there was no difference in motoric capacity in animals that received a single or multiple injections, resulting in neither sensitization nor tolerance (data not shown). This finding is surprising, because past research has demonstrated that the co-administration of the 5-HT_{1A} and 5-HT_{1B} receptor agonists suppressed abnormal involuntary movements in MPTP-treated subjects by 80% after the fourth administration.

In comparing the effects of the direct 5-HT_{1A} agonist 8-OH-DPAT and 5-HT_{1B} agonist CP 94253 alone and in combination to the mixed 5-$HT_{1A/1B}$ agonist RU 24969, there were many differences. We found that single-trial administration of 8-OH-DPAT (0.1-8 mg/kg) does not result in locomotor sensitization nor tolerance, which is consistent with past research using the single-trial paradigm (Woodall et al., 1996; Sipos et al., 2000). Possible explanations for these differences could be attributed to the differences in the doses administered, the route of administration (i.e., intraperitoneal, subcutaneous), age, and the type of species (Perry & Fuller, 1989; Evenden & Angeby-Möller, 1990; Evenden, 1992). Additionally, we found that repeated administration of 8-OH-DPAT (0.1-8 mg/kg) does not cause sensitization or tolerance to motoric capacity in younger animals.

The different effects seen in the present study differ from the reported effects of RU 24969. Studies have established that RU 24969 initially increases locomotor activity and causes motoric impairment, but each subsequent injection causes behavioral tolerance to these effects (Oberlander et al., 1987; Harrison et al., 1997; McDougall et al., 2020).

Two treatments of CP 94253 (2, 5, 10, or 20 mg/kg) did not alter locomotor activity at any dose, however, it causes tolerance to axial temperatures. Although CP 94253 treatment does not appear to affect locomotor activity in adults, there is evidence showing that it acutely increases activity in 7-day-old mouse pups (Fish et al., 2000). In terms of axillary temperatures, there is evidence showing that CP 94253 acutely decreases axial temperatures in preweanling rats (McDougall et al., 2022). This effect partially supports the findings from the present study in that it decreases core body temperatures of preweanling rats 60 minutes post-injection. It differs from our study however, because the observed effects occurred with two injections while acute studies only examined axial temperatures on a single day.

Additionally, we found that multiple injections of CP 94253 cause tolerance to locomotor activity and axillary temperature. Possible explanations for the tolerance effect observed in our study can be attributed to the duration of testing, where Fish et al. (2000) examined the acute effects of the drug in 7-day-old (PD 7) mice for four minutes and injected their animals subcutaneously. The present study used a different methodology, where we examined the repeated

effects of CP 94253 on locomotor activity, injected intraperitoneally, and used slightly older rats (PD 17-22). In terms of axillary temperatures, there is conflicting evidence that CP 94253 acutely decreases axial temperatures in preweanling and adult rats (McDougall et al., 2022), while another study has found that it has no effect on axillary temperatures (Fish et al., 2000). A possible explanation for this effect may be due to the difference in the route of administration, where one study injected subcutaneously (Fish et al., 2000), while the other injected intraperitoneally (McDougall et al., 2022), which is the method we used for our study, and is consistent with our findings.

In summary, the effects observed through single and multi-trial paradigms with the direct agonists 8-OH-DPAT and CP 94253 administered alone and in combination differ from the mixed agonist RU 24969. This suggests that therapeutic effects observed with RU 24969 treatment are unique and may possibly stimulate additional receptors. The 5-$HT_{1\ A/B}$ receptors are important for modulation of neurotransmitter systems other than serotonin, such as dopamine and acetylcholine (Sarhan et al., 1999; Fink & Göthert, 2007; Hagan et al., 2012). Although the underlying mechanisms of RU 24969 are not well understood, studies have found that it not only stimulates the 5-HT_{1A} and 5-HT_{1B} receptors, but also stimulates the 5-$HT_{1C,}$ and 5-HT_{1D} receptors (Oliver et al., 1993), with the highest affinity for the 5-HT_{1B} receptor (Waeber, Schoeffter, and Hoyer, 1990; Harrison et al., 1999). In our study, the tolerance observed with CP 94253 treatment in two of our three measures (all except motoric capacity) were similar

to that of RU 24969. Specifically, we observed that repeated treatment of either drug (CP 94253 and RU 24969) initially increases locomotor activity and hypothermia, but tolerance to these effects occurs as soon as the second drug treatment (Oberlander et al., 1987; Harrison et al., 1997; McDougall et al., 2020). Harrison et al. (1999) found that RU 24969 facilitates dopamine release in the striatum and nucleus accumbens and suggests that its effects are not a result of reduced serotonin neurotransmission. Instead, they postulated that RU 24969's behavioral effects are caused by alterations of glutamate, acetylcholine, or GABA neurotransmission induced by RU 24969's high affinity for the 5-HT_{1B} receptor.

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