

ROLE OF SEROTONIN 5HT_{2C} RECEPTORS IN MEDIATION OF A BEHAVIOR
THAT IS A POTENTIAL ANIMAL MODEL FOR OCD

by

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Abstract:

Obsessive Compulsive Disorder (OCD) was formerly considered a relatively rare psychological disorder, yet today it is known to affect 2-3% of the global population. OCD is characterized by invasive, anxiety producing thoughts (obsessions) that cannot be controlled. In an effort to reduce anxiety, the OCD sufferer will perform repetitive rituals (compulsions). The most effective pharmacotherapy for OCD is serotonin reuptake inhibitors, such as clomipramine and fluvoxamine. Treatment with these drugs, however, is only effective in 40-50% of sufferers, takes 10-12 weeks to become effective, and does not fully alleviate symptoms.

Although it remains uncertain exactly which regions of the brain mediate the symptomatic expression of OCD, there are several brain regions that have been shown to have abnormal activity in OCD patients. Positron Emission Tomography (PET) reveals that the orbital frontal cortex, caudate nucleus and the thalamus are overactive in the brains of OCD sufferers. The cortico-basal ganglia-thalamic brain circuitry provides a mechanism through which abnormal activity of certain pathways leads to an imbalance of neuronal processes. This imbalance causes an inability to switch to a new thought or behavior because the brain remains locked in the previous thought or behavior. Currently, there is no widely accepted animal model for OCD. Development of an animal model could provide critical new insights into treatment strategies.

My research assesses whether ritualistic chewing behaviors induced by the drug mCPP (1-(3-chloro-phenyl) piperazine) can serve as an animal model for OCD. There are two reasons mCPP was chosen for study. The first reason is that clinical studies have shown that mCPP exacerbates symptoms in untreated OCD sufferers. The second reason is that mCPP is known to activate serotonin 5HT_{2C} receptors and this receptor subtype is found in abundance in the cortico-basal ganglia-thalamic brain circuit.

Taken together, my results suggest that mCPP-induced effects in the rat brain can serve as a model for some aspects of OCD. I showed that repeated administration of clomipramine and fluvoxamine attenuated mCPP- induced ritualistic chewing behaviors in rats. This reduction is congruent with their efficacy in OCD patients. Furthermore, the delayed onset of behavior attenuation is analogous to the delayed anti-OCD effects of serotonin reuptake inhibitors in humans.

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I. Introduction

A. Obsessive Compulsive Disorder:

Definition

Although formerly thought to be a rare psychiatric disorder, the diagnosis rate of Obsessive Compulsive Disorder (OCD) has increased over the past 3 decades. Today, 2-3% of the world's population – equivalent to 120 million people – are believed to be affected by OCD (Foa et al., 2005). This estimate of afflicted individuals may be under-stated because OCD patients are often ashamed or embarrassed of their symptoms and thus refrain from seeking treatment. Studies suggest that OCD is inherited (Modell et al., 1989) and affects males and females equally (Antony et al., 1998). Diagnosis of OCD most commonly occurs in early adolescence and occasionally occurs in childhood (Eddy and Walbroehl, 1998).

Diagnostic criteria for Obsessive Compulsive Disorder are defined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; American Psychiatric Association, 1994). The patient diagnosed with OCD is characterized as experiencing frequent “obsessions” – intrusive, repetitive thoughts that are both uncontrollable and anxiety producing. Although sufferers recognize that their obsessions are unreasonable, they are unable to ignore or suppress them. The obsessions are often accompanied by the performance of compulsions. A compulsion is a repetitious, ritualistic behavior or mental act with which OCD sufferers engage in an attempt to reduce their anxiety. Patients that suffer from OCD spend at least one

hour per day distracted with obsessions or performing compulsions, and they report that these symptoms interfere with their normal functioning (American Psychiatric Association, 1994). OCD is painful for both the patient and the family as the patient is powerless even though the obsessions are clearly unreasonable and the compulsions exhausting (American Psychiatric Association, 1994; Grayson 2003).

Obsessive Compulsive Disorder manifests itself in a variety of different clinical symptoms. Some of the typical obsessions include the following: fear of contamination or aggression (i.e., harming another person), concern with physical health, and fixation with symmetry or hoarding. Typical compulsions include excessive hand washing or cleaning, checking, ordering, counting, repeating rituals, and hoarding (Figure 1; Eddy and Walbroehl, 1998; Korff and Harvey, 2006).

Obsessions	Compulsions
Contamination	Hand washing Cleaning
Aggressive sexual religious somatic	Checking
Symmetry	Ordering and arranging counting repeating rituals
Hoarding	Hoarding Collecting

Figure 1: Typical Obsessions and Correlating Compulsions: Adapted from Eddy and Walbroehl , 1998, pg. 57.

Diagnosis of OCD

Diagnosis of OCD is difficult because there is heterogeneity of symptoms that are recognized as indicators of the disorder and because the symptoms occur with varying intensity (Woody et al., 1995). Accurate diagnosis of OCD requires several methods of examination, including self reporting and personal interviews with the patient. One of the most commonly used tools to evaluate a patient for Obsessive Compulsive Disorder is the Yale-Brown Obsessive Compulsive Psychiatric Screen (Figure 2; Psychiatric Associates of Atlanta, 2008). The screen is a directed interview in which intensity and frequency of symptoms are quantified on a scale of 0-4, without regard as to the nature of the symptoms. The interviewer addresses various aspects of the disorder with the patient, such as the duration of OCD episodes, the degree that the OCD disorder interferes with the patient's life goals and the amount of stress caused by the patient's symptoms (Woody et al., 1995).

Note: Scores should reflect the composite effect of all the patient's obsessive compulsive symptoms. Rate the average occurrence of each item during the prior week up to and including the time of interview.

Obsession Rating Scale (circle appropriate score)

Item	Range of Severity				
1. Time Spent on Obsessions	0 hr/day	0-1 hr/day	1-3 hr/day	3-8 hr/day	> 8 hr/day
Score:	0	1	2	3	4
2. Interference From Obsessions	None	Mild	Definite but manageable	Substantial impairment	Incapacitating
Score:	0	1	2	3	4
3. Distress From Obsessions	None	Little	Moderate but manageable	Severe	Near constant, disabling
Score:	0	1	2	3	4
4. Resistance to Obsessions	Always resists	Much resistance	Some resistance	Often yields	Completely yields
Score:	0	1	2	3	4
5. Control Over Obsessions	Complete control	Much control	Some control	Little control	No control
Score:	0	1	2	3	4

Obsession subtotal (add items 1-5) _____

Compulsion Rating Scale (circle appropriate score)

Item	Range of Severity				
6. Time Spent on Compulsions	0 hr/day	0-1 hr/day	1-3 hr/day	3-8 hr/day	> 8 hr/day
Score:	0	1	2	3	4
7. Interference From Compulsions	None	Mild	Definite but manageable	Substantial impairment	Incapacitating
Score:	0	1	2	3	4
8. Distress From Compulsions	None	Mild	Moderate but manageable	Severe	Near constant, disabling
Score:	0	1	2	3	4
9. Resistance to Compulsions	Always resists	Much resistance	Some resistance	Often yields	Completely yields
Score:	0	1	2	3	4
10. Control Over Compulsions	Complete control	Much control	Some control	Little control	No control
Score:	0	1	2	3	4

Compulsion subtotal (add items 6-10) _____

Y-BOCS total (add items 1-10)

Total Y-BOCS score range of severity for patients who have both obsessions and compulsions:

0-7 Subclinical 8-15 Mild 16-23 Moderate 24-31 Severe 32-40 Extreme

Figure 2: Rating scale of Yale-Brown Obsessive Compulsive Screen: From the Psychiatric Associates of Atlanta, LLC, 2008

B. Treatment for Obsessive Compulsive Disorder:

Pharmaceutical Treatments

Currently, it is understood that the therapeutic efficacy of anti-OCD drugs is due to their ability to inhibit the reuptake of serotonin (Insel et al., 1990; Eddy and Walbroehl, 1998; Zohar et al., 2000). Drugs without such serotonergic properties,

such as the antidepressant drug desipramine or anti-anxiety drug Xanax are ineffective in treating OCD (Zohar et al., 2000). Clomipramine (Anafranil) was the first effective treatment for OCD (Eddy and Walbroehl, 1998). Clomipramine is an inhibitor of the uptake of norepinephrine and serotonin from the synapse by the presynaptic neuron. Selective serotonin reuptake inhibitors (SSRI's), drugs that only inhibit the uptake of serotonin, are now the preferred treatment for OCD. Examples of SSRI's used to treat OCD include fluvoxamine (Luvox) and fluvoxetine (Prozac).

Comparisons between clomipramine and fluvoxamine demonstrate that there is no significant difference in the efficacy of the drugs for the treatment of OCD (Koran et al., 1996; Pigott and Seay, 1999). One benefit of the SSRI's over clomipramine is that they have fewer side effects that occur less frequently (Eddy and Walbroehl, 1998; Pigott and Seay, 1999). Less side effects result from SSRI's because they affect only the serotonin neurotransmitter system rather than both serotonin and norepinephrine systems. Since serotonin plays a significant role in sleep, hunger and sexual behaviors, it is not surprising that common side effects of serotonin uptake inhibitors are associated with these functions (Stahl, 1998). Clomipramine tends to cause dry mouth, weight gain, sexual dysfunction and cardiac conduction delays, whereas fluvoxamine causes postural hypotension, insomnia, nervousness, and nausea (Koran et al., 1996).

Behavioral Therapies

Although there are mixed reports as to the effectiveness of combined as compared to single therapy, pharmaceutical treatment of Obsessive Compulsive Disorder is often complemented by behavior therapy (Eddy and Walbroehl, 1998; Foa et al., 2005). The traditional behavior therapy that psychiatrists use is exposure and response prevention. The principle goal behind exposure and response therapy is habituation to OCD-evoking stimuli (McLean et al., 2001). In therapy, an OCD sufferer will be increasingly exposed to stress inducing stimuli that they typically avoid. Furthermore, patients are prevented from performing their rituals when they are exposed to the stimuli (Eddy and Walbroehl, 1998). One criticism of exposure and response prevention, however, is its inability to help patients with more covert, internal rituals.

A second form of behavior therapy is Cognitive Behavior Therapy (CBT). Cognitive Behavior Therapists believe that the stress caused by OCD-provoking stimuli is caused by a faulty appraisal. The faulty appraisal can be an overestimation of danger or an excessive sense of responsibility among others. CBT uses various techniques to evaluate the patient's response by helping the patient to realize the irrationality of their response (McLean et al., 2001).

C. Neurobiology of OCD:

Over activity in the OCD brain

Although the exact brain region hosting the origin of dysfunction for OCD is unknown, there are certain regions that appear overactive in Positron Emission Tomography (PET) scans of the brains of OCD patients. PET scans measure cerebral glucose metabolism in the brains of conscious patients in order to monitor brain activity. Increased areas of high glucose metabolism are interpreted to indicate areas of increased neuronal activity. PET measures of glucose metabolism in OCD patients exposed to anxiety-producing stimuli demonstrated increases in the right head of the caudate nucleus (part of the striatum) and bilateral increases in the orbital gyri, thalamus, anterior cingulate cortex and dorsolateral cortex (Baxter et al., 1992). PET studies examining the brain activity following pharmacological treatment indicated that therapy induced normalization (i.e., a decrease) of activity in the right caudate nucleus for treatment responders. In contrast, patients that did not respond to treatment did not have normalization of activity in the right caudate nucleus. Likewise, metabolic rate in the right anterior cingulate gyrus and the left thalamus both decreased with drug therapy (Baxter et al., 1996).

Basal Ganglia Circuitry

Dysregulation of activity within the cortico-basal ganglia-thalamic brain circuit is believed to underlie OCD's etiology (Rapaport and Wise, 1988; Modell et

al., 1989; Baxter et al., 1996). Elements of this circuit include the cortex, the basal ganglia and the thalamic nuclei. The cortex regulates activity through neurons that project from the cortex to the striatum. Projections from the striatum lead to different pathways within the basal ganglia. The output nuclei of the basal ganglia are the internal segments of the globus pallidus. Neurons leaving the internal segment of the globus pallidus control the activity of the neurons of the thalamus. There are neurons leading from the thalamus to the cortex, and these projections are able to influence our thoughts and behaviors.

Appropriate activity in the thalamus underlies the ability to evaluate a situation and respond with proper, adaptive behavior. This faculty includes the capacity to maintain a current thought as long as necessary, as well as the ability to suppress that thought when it is time to switch to a new thought. The activity of the thalamus is regulated through two different pathways within the basal ganglia. Figure 3 illustrates the two pathways within the basal ganglia through which the striatum (which includes the caudate nucleus) affects the internal segment of the globus pallidus. Through the Direct Pathway, inhibitory neurons from the striatum project directly to the internal segment of the globus pallidus. Although more complex, the Indirect Pathway also ultimately affects the internal segment of the globus pallidus. Through the Indirect Pathway, inhibitory neurons from the striatum project to the external segment of the globus pallidus. Then, inhibitory neurons from the external segment of the globus pallidus extend to the subthalamic nucleus. Excitatory subthalamic neurons project to the internal segment of the globus pallidus. Thus,

through the Direct Pathway, inhibitory neurons affect the internal segment of the globus pallidus, whereas through the Indirect Pathway excitatory neurons affect the internal segment of the globus pallidus. These different signals mediate activity in the thalamus.

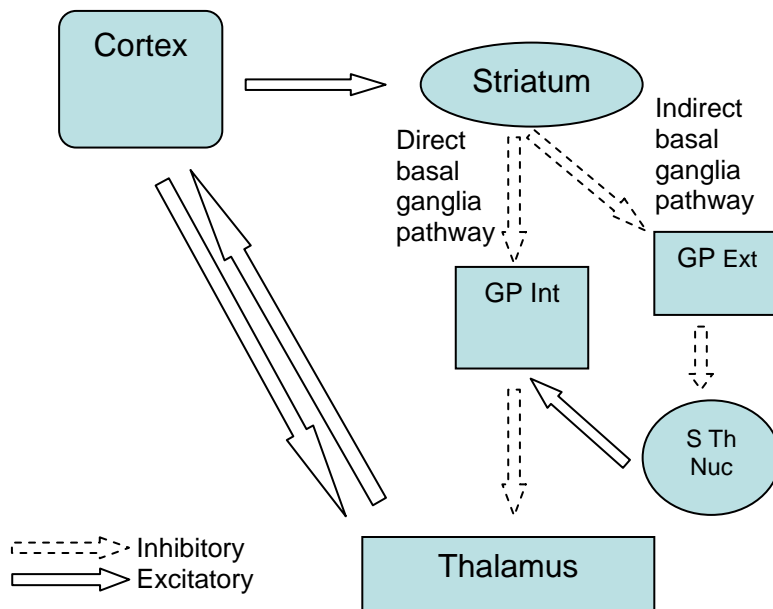


Figure 3: Direct and Indirect basal ganglia pathways: Adapted from Baxter et al., 1996, Pg. 38. Solid arrows indicate excitatory signals, whereas dashed arrows indicate inhibitory signals. GP Int = the internal globus pallidus; GP Ext = the external globus pallidus, and S Th Nuc = the subthalamic nucleus.

Proper balance between the Direct and Indirect Pathways is essential for normal cognitive functioning. Through the Direct Pathway, an individual is able to maintain their current thought because the thalamic neurons are excited and stimulate the cognitive cortex – the part of the brain that generates thoughts and worries (Figure

4A). Neurons of the internal segment of the globus pallidus typically inhibit the neurons of the thalamus. Therefore, in order for thalamic neurons to be able to become excited, activity in the internal segment of the globus pallidus must be suppressed so that the pallidal neurons no longer inhibit the thalamus.

Suppression of the globus pallidus occurs when the influence of the Direct Pathway is increased and influence of the Indirect Pathway is decreased. When the individual needs to shut off a current thought in order to switch to a new thought, the thalamic neurons must be inhibited (Figure 4B). In order to inhibit the thalamic neurons, the activity of the internal segment of the globus pallidus must be enhanced. The enhancement of the internal segment of the globus pallidus occurs when the influence of the Indirect Pathway is increased while that of the Direct Pathway is decreased.

OCD patients have difficulty switching from a current thought to a new thought. This inability to change to a new thought underlies the repetitive, persistent worries (obsessions) that lead to maladaptive behaviors that attempt to reduce the thought (compulsions). Perhaps this dysfunction can be explained by a dysregulation in the balance between the Indirect and Direct Pathways (Figure 4C). If there is an over activation of the Direct Pathway and/or an under activation of the Indirect Pathway, then the internal segment of the globus pallidus will not be activated sufficiently. Under-activity in the globus pallidus can result in an overly active thalamus and the inability to switch to a new thought.

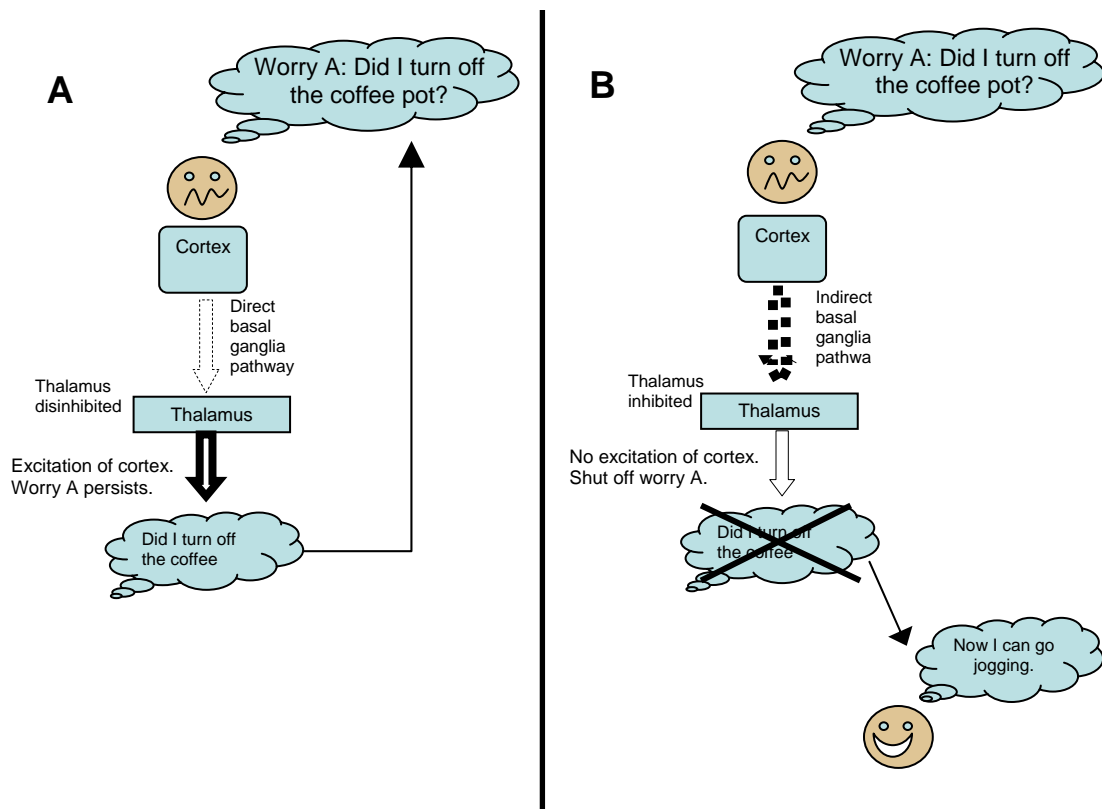


Figure 4A and B: Normal brain function and balance of pathways in adaptive behavior: Excitatory arrows are solid and inhibitory arrows are dotted. Bold lines indicate a strong signal whereas light lines indicate a weakened signal. (A) Stimulation of the cortex arises from the initial worry “did I turn off the coffee pot?” The result of the worry through the direct pathway is to not inhibit the thalamus so that it stimulates the cortex. If the thalamus is disinhibited, then the worry persists. (B) Conversely, if the thalamus is inhibited (indirect pathway), the worry is shut off and the brain switches to a new thought. A normal brain functions to balance between disinhibition and inhibition of the thalamus in order to properly evaluate problematic situations. In this situation, the coffee pot is found to be off so that the brain is able to shut off that worry in order to switch to a new thought, “Now I can go jogging.”

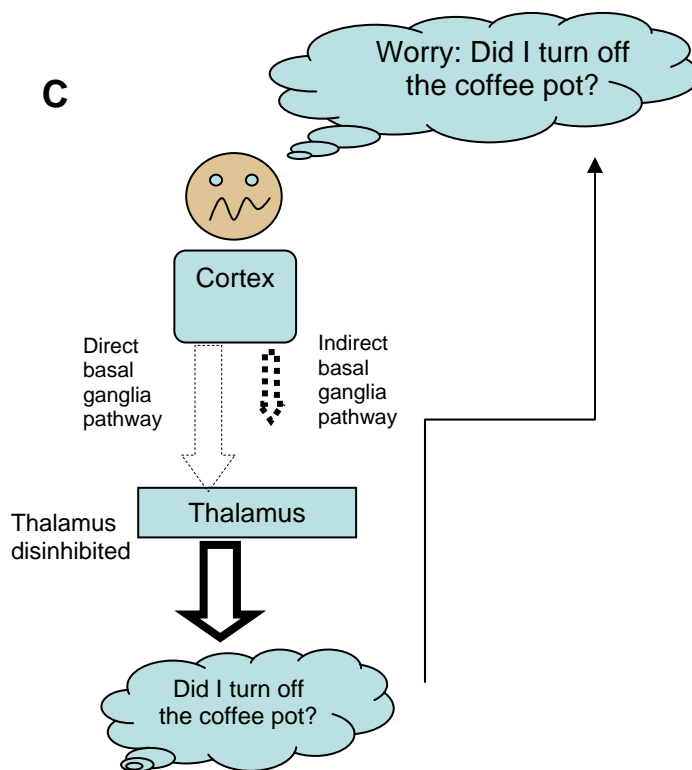


Figure 4C: Imbalance of pathways in the OCD brain: Excitatory arrows are solid and inhibitory arrows are dotted. Bold lines indicate a strong signal whereas light lines indicate a weakened signal. A worry in the OCD brain leads to an excessive disinhibition of the thalamus. This occurs either through an over activation of the Direct Pathway and/or under activation of the Indirect Pathway. The result is the persistence of the original worry and the inability to properly evaluate the gravity of a situation. The brain cannot switch to a new thought.

D. Animal Models:

Types of Validity

Because animal models cannot perfectly reflect human disorders, various types of validity are assigned to animal models so as to reflect the anticipated function of the model. There are three types of validity for animal models: predictive,

face and construct validity (Willner, 1991). Predictive validity means that the animal's physiology will demonstrate the same response to treatment as the human patient. In an animal model with face validity, the animal displays analogous symptoms as the human patient. Construct validity requires that the physiological mechanism that is responsible for the animal's physiology is identical the physiological mechanism that is responsible for the patient's condition (Willner, 1991).

Animal Models for Obsessive Compulsive Disorder

Currently, there are a number of animal models for OCD. Among the earlier animal models for OCD were behavioral models based on a repetitive behavior that demonstrated some similarity to OCD, such as rats that bury marbles and mice that excessively groom cage mates. These behaviors reflect hoarding and grooming rituals respectively, and they demonstrate face validity. Because the marble burying behavior was also found to be attenuated with serotonin reuptake inhibitors (SRIs), that model presents both face and predictive validity (Joel, 2006; Kroff and Harvey, 2006). Genetic models have been developed such that certain mutations have been found to yield behaviors analogous to OCD. The HoxB knockout mice, for example, demonstrate excessive grooming which often goes so far as to lead to hair loss; this grooming is comparable to washing rituals in humans with OCD (Joel, 2006). Genetic models such as the HoxB knockout, therefore, have face validity. The mutation, however, is not based on a known mutation in humans, so the HoxB

knockout model does not have construct validity. Naturally occurring anxiety-related behaviors are used for ethologic models, such as stress-induced as tail chasing, fur chewing, and excessive pacing. These stress responses reflect behaviors in humans such as checking or washing, and thus these models are also considered to have face validity (Kroff and Harvey, 2006). Since the neurobiology of OCD is not fully understood at this time, it is unknown whether these models demonstrate construct validity.

There is a critical need for an animal model for Obsessive Compulsive Disorder with predictive validity because the current treatment options for OCD are severely limited. The pharmaceutical treatments are only effective in 40-50% of patients (Insel et al., 1990). Furthermore, drugs often alleviate symptoms without fully eradicating them. Studies demonstrating good treatment results demonstrated a 60-70% reduction in time spent on rituals (Eddy and Walbroehl, 1998). There is also a significant delay before the drugs become effective. It can take 10-12 weeks for a patient to experience the initial response to the drug, and maximum response might not be attained until several months or more (Zohar et al., 2000). Furthermore, relapse can occur if administration of the treatment is stopped. Clinical studies report that discontinuation of the drug after one year of treatment resulted in relapse of symptoms in 90% of cases, thereby necessitating prolonged treatment (Zohar et al., 2000).

E. Summary of Current Study:

This research project was designed to ascertain whether mCPP-induced mouth movements can serve as an animal model for Obsessive Compulsive Disorder. The drug mCPP (1-(3-chloro-phenyl) piperazine) was selected for this study, because mCPP has been shown to exacerbate OCD symptoms in untreated OCD patients (Rapaport and Wise, 1988; Modell et al., 1989). The increase in Obsessive Compulsive symptoms includes both behavioral and neurophysiological augmentation. In contrast, mCPP was found to not increase symptoms in successfully treated OCD patients (Insel et al., 1990; Zohar et al., 2000). In healthy subjects, mCPP has been shown to produce anxiety, but not OCD symptoms (Insel et al., 1990).

Administration of mCPP to rats produces a number of behavioral changes, such as decreased social activity, hypolocomotion, decreased appetite and oral dyskinesia (Eberle-Wang et al., 1996; Zohar et al., 2000; Yamauchi et al., 2004). Prior work in this lab demonstrated that mCPP dose-dependency induces an increase in oral dyskinesias, defined as Ritualistic Chewing Behaviors (RCBs; Zimmer et al., 2005; Winkler, 2006). Additional studies from this laboratory have suggested that mCPP-induced RCBs (Ritualistic Chewing Behaviors) may be able to serve as an animal model for Obsessive Compulsive Disorder (Zimmer et al., 2005; Helton, 2006; Winkler, 2006; Kim, 2007; Jackson, 2007).

The experiments of this research project evaluated mCPP-induced RCBs for predictive validity through behavioral comparison following long term administration

of current treatments for OCD. Repeated administration of the SRIs clomipramine and fluvoxamine was important in order to mirror the drugs' efficacy in humans, as well as mirroring a prolonged onset of effectiveness.

In addition, the present research study determined whether mCPP-induced behavioral effects were caused through action at the serotonin 5HT_{2C} receptor subtype by pretreating the rats with a serotonin 5HT_{2C} receptor antagonist. This receptor subtype is important in that this receptor is hypersensitive in patients with anxiety disorders such as OCD (Yamauchi et al., 2004).

II. Methodology

A. Training and Care of Rats:

Before any behavioral experiments commenced, researchers acclimated the rats to their environment and the experimental setup. Because this study examines chewing behaviors as a model for the anxiety disorder OCD, it is important to eliminate any new stresses that might exacerbate the behavior. Thus, precautions were taken to control the environment as much as possible so that observed behavior can be attributed to the drug administration and not a side effect of non-drug stressors.

Rat Husbandry

Animal housing and experimental methods were in accordance with the guidelines of the National Research Council's "Guidelines for Care and Use of Lab animals" (1996, 2003), as well as UTC's Institutional Use and Care of Animals Committee (Protocol 0206 DSK-01, 2007). All rats were male Sprague-Dawley rats from Harlan (Indianapolis, IN) whose weights ranged from 120-400 g over the course of the experiment. All the rats arrived at UTC on June 5, 2008 and experienced the same care thereafter. Rats were housed in the animal colony room (Holt 317A). The temperature in the room ranged from 69.2°F to 73.8°F, and the lights were set on a 12 hour light- dark cycle. The rats were housed in pairs in clear cages with wire lids. Cages in the colony room always remained in the same location so that each cage had the same neighbors throughout the experiments. All cages had recycled paper for

bedding, which was changed once per week. Rats had constant access to 14% Protein Rodent Diet Pellets from Harlan- Tecklad (Indianapolis, IN) and a constant supply of fresh water.

Habituation

Rats are incredibly sensitive to their surroundings, which includes not only the environment but also the handlers. Thus, any researcher that was present during the behavioral observation of the rats also participated in their habituation. Behaviorists handled the rats on a regular schedule. In addition to being handled and socialized, the rats were also exposed to several novel experiences in order to prepare them for the behavior experiments. No procedure or handling took place in the colony room. Researchers transported cages via carts from the colony room (Holt 317A) to the behavior room (Holt 317D). Identification of rats required their tail to be labeled; tails were labeled regularly using permanent markers according to their number. Thus, rats were accustomed to the marker smell as well as the labeling process when behavior experiments ensued. Each rat was weighed approximately every 48 hours in order to familiarize them with weighing process and to maintain accurate records of their body weights.

Several weeks before behavioral experiments, the rats were introduced to the experimental procedure. The rats were acclimated to receiving both subcutaneous (s.c.) and intraperitoneal (i.p.) injections. Subcutaneous injections were administered by holding the rat on a table while pulling up the skin on the back with the left hand.

The injection was given underneath the subcutaneous layer of skin and above the layer of muscle lining the back. An intraperitoneal injection was administered as the researcher would hold the rat face-forwards and bend the rat slightly backwards against her own hip in order to expose the abdominal region. The needle would be stuck in the rats' abdomen above where their hind legs begin and below their ribs. Practice injections were administered with 0.9% solution of NaCl.

Several weeks before behavioral experiments, each rat also spent time in the observation bowls and heard the timer noises that would be used in the actual experiments. Observation bowls were clear, 3 gallon plastic fish globes. Beginning with 5 minutes intervals, rats spent increasingly more time in the bowls. Three days prior to behavioral observation rats spent a full 30 minutes in the bowls. Two days prior to behavioral observation, rats were placed in the bowl for 5 minutes, removed and poked as though they were getting an injection, and replaced for 25 more minutes. The day before observation they were removed at 5 minutes, injected with 0.9% saline solution and replaced for 25 minutes.

Observation

Behaviorists were trained according to guidelines established in previous departmental honors projects (Winkler, 2006). Ritualistic Chewing Behaviors (RCB's) were defined as:

...any non-directed, repetitive chewing or gaping mouth movements. Smaller movements such as oral tremors and tongues darting, were recorded as a

single RCB if three or more distinct movements were observed in a continuous bout. A 1 second pause in a repetitious bout sequence separated individual RCB's. Larger movements such as vacuous gaping, were counted individually as an RCB (Winkler, 2006, pg. 19).

Rats were excluded from a study if they exhibited fewer than 6 RCB's on first exposure to mCPP. Two students observed the behavior of the rats: Tammie Mizer and Catherine Coffman.

Treatment Preparation

All drugs used in this experiment, aside from fluvoxamine, were attained from Sigma Aldrich, (St. Louis, MO). Fluvoxamine was granted to Dr. Kreiss by Solvay Pharmaceuticals (Marietta, GA). mCPP and clomipramine were made by thoroughly mixing the dry form of the drug with 0.9% NaCl. In order to prepare SB228357 and fluvoxamine, 2-3 drops of Tween-80 was added to a mortar containing the dehydrated drug. The drug and Tween-80 were then ground into a paste using a pestle. An appropriate volume of 0.9% NaCl was added to the mortar where it was mixed with the paste. Drug solutions were all made so that the proper dosage was administered in a volume of 1 ml of drug solution per 1 kg of body weight. All drug solutions were made fresh daily.

Rat Perfusion

On the last day of an experiment, rats were perfused after behavioral observation and their brains were harvested. Approximately 100 minutes after the injection of mCPP (1 mg/kg), researchers anesthetized rats using an i.p. injection of sodium pentobarbital (50 mg/kg). Because it is so important that the rats do not experience any pain through the surgical procedure, researchers used extreme caution to ensure that the rats were fully anesthetized before and throughout any perfusion. Before the first incision, the rats were assessed for full global anesthesia. Full global anesthesia was obtained when the rat did not demonstrate any body movement when pinching their tail, exhibit an eye blink reflex nor did they withdraw from a foot pinch. If the rat did not appear fully unresponsive 10 minutes after the sodium pentobarbital injection, it received a half dose (25 mg/kg) sodium pentobarbital. Once the rat was fully anesthetized, it was taken from the behavior room to the perfusion room (Holt 318). From the beginning of the perfusion, there was only about a 5 minute time delay before the rat died as a result of exsanguinations.

The perfusion began by opening the rat's chest and separating the ribs with hemostats. A 22 gauge needle attached to a Y valve with two lines of tubing was inserted into the left ventricle of the heart, taking care to ensure that the tip of the needle remains in the left ventricle without puncturing other tissue. The needle was held in place with hemostats. A small hole was made in the right atrium of the heart to allow blood and other solutions to exit the circulatory system. 100 ml of phosphate buffered solution (PBS) was the first to pass through the animal's circulatory system.

At 100 ml, the first line was closed off, and 500 ml of 4.0% paraformaldehyde in PBS was sent through the animal through the other line. Paraformaldehyde in PBS is a suspected carcinogen, so all of the solution used was collected and “inactivated” before it was disposed. In order to inactivate the solution, 5 parts paraformaldehyde solution was mixed with one part Formalex. When the perfusion procedure was complete, the rat’s brain was carefully excised and put into a small vial containing 30% sucrose in PBS at room temperature. When the brain no longer floated (24-28 hours) it was transferred to a vial containing PBS and stored in the refrigerator (4°C). This protocol #0406DSK-02 was approved by the UTC IACUC on May 8, 2006 and re-approved on May 4, 2007.

B. Acute Administration of Serotonin 5HT_{2C} Agonist and Antagonist:

This experiment was an acute study that compared the RCB behavior of three groups of rats (figure 5): Group 1 was given a pretreatment of the serotonin 5HT_{2C} receptor antagonist (SB-22857) before the serotonin agonist mCPP; Group 2 was given a saline pretreatment before mCPP; and a Control Group was given two saline injections. The study consisted of 15 rats that were divided into three groups that received the three different treatments:

Group of Rats (n=5)	i.p. Treatment	s.c. Treatment
Group 1	SB-228357 (3 mg/kg)	mCPP (1 mg/kg)
Group 2	saline (1mg/kg)	mCPP (1 mg/kg)
Control Group	saline (1 ml/kg)	saline (1 ml /kg)

Figure 5: Groups of rats in the double treatment acute study

On observation days, rats were moved from the animal colony room to the behavior room. All rats were placed in observation bowls for 5 minutes of acclimation. After 5 minutes, the rats were removed from the bowls and given their respective i.p. treatment. They were placed back into the observation bowl. Five minutes later rats were again removed from the bowl and receive their respective s.c. treatment. Afterward, they were replaced in the bowl. Fifteen minutes after the s.c. injection, experimenters observed behavior for 10 minutes (time 25-35 minutes).

Figure 6 illustrates the timeline of this procedure.

On Day 1, all rats received 0.9% saline for both i.p. and s.c. injections, and behavior was observed. The following day, ritualistic chewing behaviors were observed in all rats with an i.p. injection of saline and an s.c. injection of 1 mg/kg mCPP. Combinations of intraperitoneal injections of saline or SB-228357 (3 mg/kg) and subcutaneous injections of saline or 1 mg/kg mCPP were given on Day 5. Between 90-120 minutes after the s.c. injections on Day 5, rats were perfused.

The experimenters who administered the drugs and observed the rats were blind with respect to the drugs that each rat received. The rat's brains were perfused the same day as the behavioral observation and preserved for future studies.

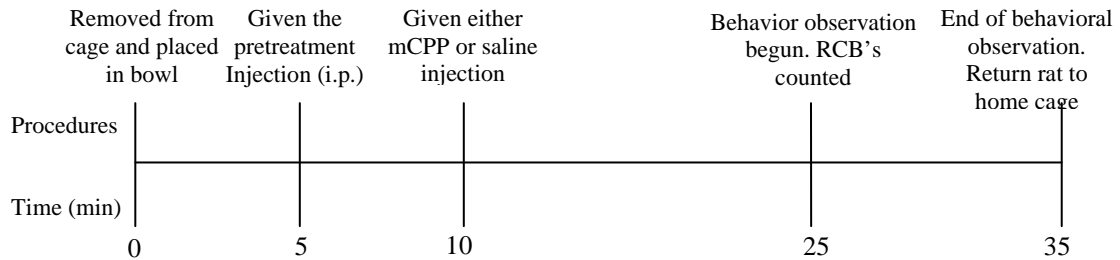


Figure 6: Double treatment injection and observation time schedule

C. Repeated administration of clomipramine and fluvoxamine:

The effects of long term, repeated administration of clomipramine and fluvoxamine on the ability of mCPP to induce RCB's in rat were examined in this study. In this experiment, three different groups of rats (16 rats per group) received chronic injections of saline, clomipramine or fluvoxamine.

Group of Rats (n=16)	Chronic Treatment (i.p.)
Group 1	clomipramine (15 mg/kg)
Group 2	fluvoxamine (15 mg/kg)
Control Group	saline (1 ml/kg)

Figure 7: Groups of rats receiving chronic injections

All rats received 1 mg/kg mCPP SC on Day 1 in order to evaluate their initial response to mCPP and establish a baseline for comparison following treatments. The rats received their respective drugs every day for 26 days (day 2- day 27) via an i.p. injection. No behavior was observed during these 26 days. On Day 28, no rat received a chronic treatment, and all rats again received 1 mg/kg mCPP s.c. Perfusion of the rat's brains occurred on this final day following behavior observation. For behavior evaluation, each rat sat in a bowl for 5 minutes in order to acclimate to the change of environment before receiving mCPP s.c. (1mg/kg). After 15 more minutes in the bowl, researchers evaluated the animal's behavior for 10 minutes. Figure 8 below clarifies time scale for treatments. Behaviorists were blind to the identity of the chronic treatment each rat received.

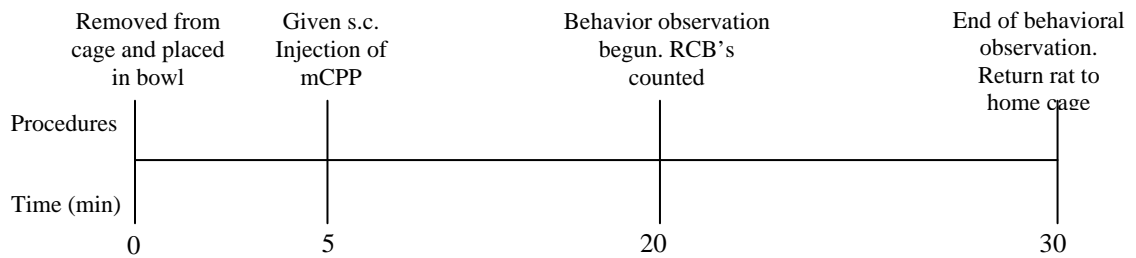


Figure 8: Single treatment injection and observation timescale

D. Time course of attenuation of the mCPP-induced behavioral effect:

This study was investigated the time frame by which clomipramine reduced the number of RCBs induced by mCPP. This study included 16 rats which were split into two groups. The experimental group of rats was treated with clomipramine (15 mg/kg) i.p., and the control group was treated with saline (1 ml/kg I.P.). On Day 0, the behavior of all 16 rats was evaluated following administration of mCPP (1 mg/kg) to establish a baseline in order to recognize attenuation of behavior. The observation of behavior followed the time schedule for single administration (Figure 4). Each rat then received their respective chronic treatment for 7 days before their behavioral response to mCPP was evaluated. Behavior was assessed on days 7, 14, 21 and 28. Rats were given their repeated treatment injections no less than 6 hours after the mCPP injection. Behaviorists were blind to drugs being administered to the rats. On day 28, following behavior, rats were perfused approximately 90-120 minutes after the s.c. administration of mCPP, and their brains were harvested.

E. Statistical Analysis:

All data reported are given as the means \pm one standard error of the mean (S.E.M). A one-way Analysis of Variance (ANOVA) was used to analyze the effects of an acute pretreatment drug on behavior by statistically comparing the mean number of RCB's for each group. Significance was determined as $p < 0.05$. Since ANOVA demonstrated that varying the pretreatment drug had a significant effect on RCBs, a Dunnett's *post hoc* test (two-sided, $p < 0.05$) was used in order to determine which pretreatments were associated with a significantly different number of RCBs. A one-way ANOVA ($p < 0.05$) was used to analyze whether repeated treatments with clomipramine and fluvoxamine significantly altered the number of RCBs induced by mCPP on day 1 versus day 28 in the same group. Since ANOVA demonstrated that varying the treatment drug had a significant effect on RCBs induced by mCPP on day 28, a Dunnett's (two-sided, $p < 0.05$) *post hoc* test was used in order to determine which treatments were associated with a significantly different number of mCPP-induced RCBs. Finally, a one-way ANOVA ($p < 0.05$) was used to analyze whether repeated treatments with clomipramine was associated with an altered number of RCBs induced by mCPP as compared to the number of RCBs induced by mCPP in the saline-treated controls.

III. Results

A. Behavioral effect of antagonist SB228357:

The mean number of RCBs induced by a mCPP injection given 5 mins after a saline pretreatment injection was 13.60 ± 3.25 (mean \pm 1 S.E.M., n=5). In comparison, the mean number of RCBs was significantly fewer in the group of rats (1.40 ± 0.51 , n=5) given a pretreatment of the 5 HT_{2C} serotonin receptor antagonist SB 228357 five minutes prior to the mCPP (Figure 9). Rats given a saline pretreatment followed by another saline treatment (rather than mCPP) also produced a significantly lower mean number of RCBs (5.20 ± 1.32 , n=5) in comparison to the first group described above.

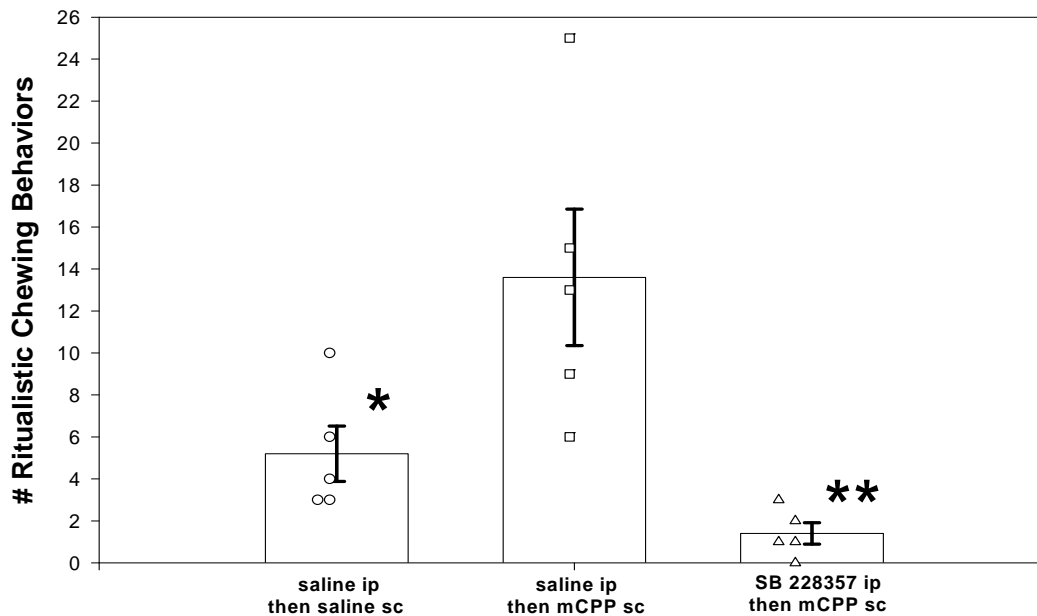


Figure 9: Bars show the mean behavioral response to 1 mg/kg mCPP or saline (1 ml/kg) 5 minutes after a pretreatment of either saline or the 5HT_{2C} antagonist SB 228357 (3 mg/kg). Error bars indicate 1 S.E.M. and individual responses are shown as circles, squares and triangles. The asterisks indicate that the saline-saline treated rats and the SB 228357- mCPP treated rats chewed significantly less than the saline-mCPP treated rats. Significance was determined by ANOVA [F(2,12)=9.31, p<.05] followed by Dunnett's post-hoc test (* p<0.05, ** p<0.01).

B. Effect of chronic administration of drugs to mCPP-induced RCB's:

Behavioral response following 26 days of administration of saline

The mean number of RCBs induced by mCPP was not altered by 26 days of chronic injections of saline. On day 1 the mean RCB count following an injection of mCPP was 18.50 ± 1.30 (n=16) and on day 28 the mean RCB count following an injection of mCPP was 17.00 ± 2.60 . Days 2-27 consisted of chronic treatments of

saline only. Figure 10 (below) demonstrates the comparison in behavior induced by mCPP before and after chronic treatment of saline.

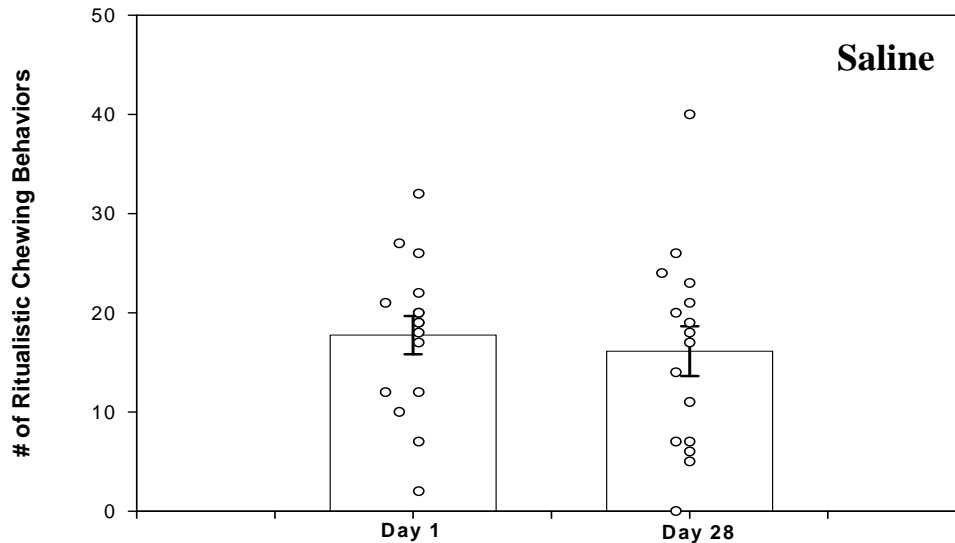


Figure 10: The bar heights indicate the mean behavioral response to 1 mg/kg mCPP on days 1 and 28 (days 2-27 consisting of chronic treatment with saline). Error bars denote ± 1 S.E.M from the mean. Individual behavioral responses are recorded as circles. ANOVA demonstrated that there was no significant difference in behavior between days 1 and 28.

Behavioral response following 26 day administration of clomipramine

Rats that were treated for 26 days with clomipramine demonstrated a significant attenuation of mCPP-induced chewing behavior following treatment. The chart below illustrates the comparison of mCPP-induced RCBs on day 1 and day 28. On day 1 rats (n=16) produced a mean RCB count of 20.69 ± 2.37 , whereas, on day 28 the rats produced a mean of 13.81 ± 0.96 RCBs, a 33% decrease.

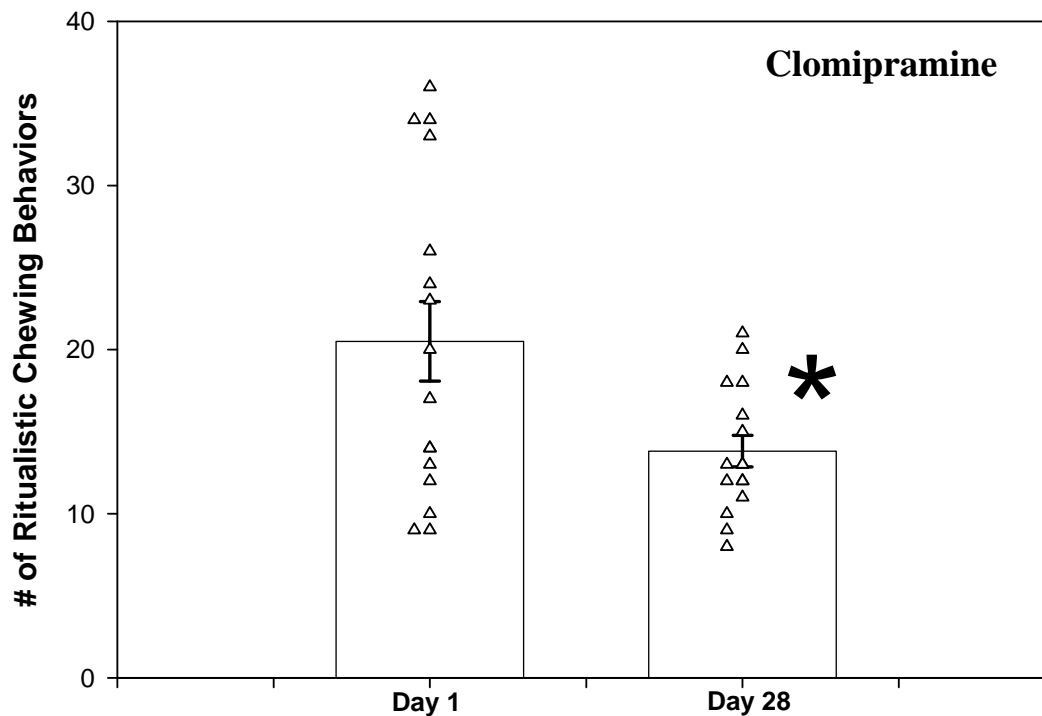


Figure 11: The bar heights indicate the mean behavioral response to 1 mg/kg mCPP on days 1 and 28. Rats were given chronic injections of clomipramine (15 mg/kg) on days 2-27. Error bars denote ± 1 S.E.M from the mean. Individual behavioral responses are recorded as triangles, and the mean behavior of the same group of rats is compared on days 1 and 28. There was a significant reduction in the mean behavioral response to mCPP between days 1 and 28, as determined by ANOVA [$F(1,30)=7.22$, $p<.05$].

Behavioral response following 26 day administration of fluvoxamine

There was a significant attenuation of behavior induced by mCPP on day 1 versus day 28 for the rats ($n=16$) that received chronic treatment of 26 days of fluvoxamine, as demonstrated by figure 12. The mean number of RCBs produced

following an injection of mCPP on day 28 (8.38 ± 0.91) was 52% less than that following an injection of mCPP on day 1 (17.31 ± 1.43).

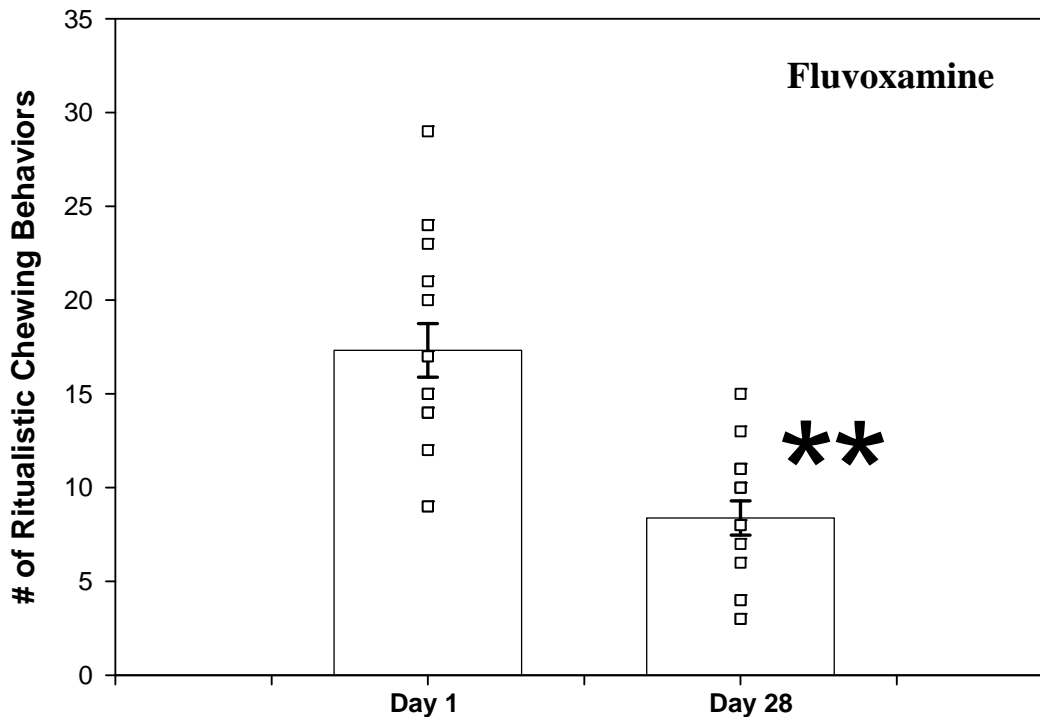


Figure 12: The bar heights indicate the mean behavioral response to 1 mg/kg mCPP on days 1 and 28. Rats were given chronic injections of fluvoxamine (15 mg/kg) on days 2-27. Error bars denote ± 1 S.E.M from the mean. Individual behavioral responses are recorded as squares and the mean behavior of the same group of rats is compared on days 1 and 28. There was a significant reduction in the mean behavioral response to mCPP between days 1 and 28, as determined by ANOVA [$F(1,30)=27.90$, $p<.01$].

Comparison of behavior for all treatment groups on day 28

Rats treated for 26 days with either clomipramine or fluvoxamine demonstrated a significant attenuation of the behavioral response to mCPP as compared to the response of the control group (administered with saline for 26 days).

As stated above, the rats given saline had an average of 17.00 ± 2.60 RCBs when given mCPP on day 28, whereas the mean RCBs induced by mCPP on day 28 of the clomipramine-treated rats was $13.81 \pm .96$ and of the fluvoxamine-treated rats was 8.38 ± 0.91 .

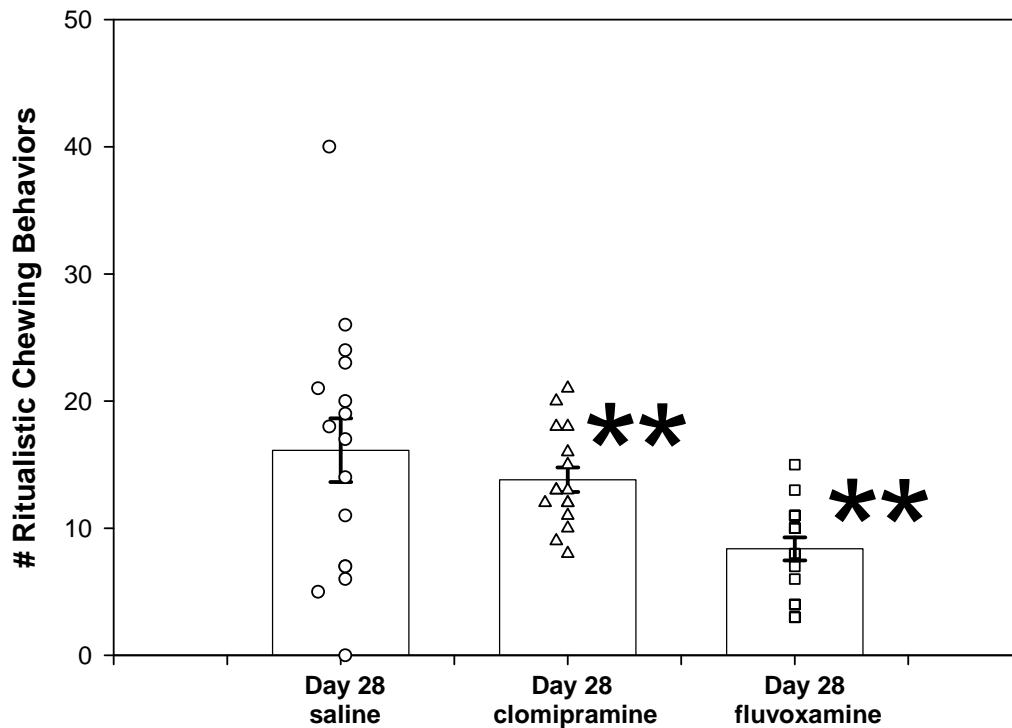


Figure 13: Bars indicate mean behavioral response to 1 mg/kg mCPP following 26 days of chronic administration of saline (1 ml/kg), clomipramine (15 mg/kg) or fluvoxamine (15 mg/kg). Error bars denote 1 S.E.M.; individual behavioral responses are recorded as circles, triangles and squares. The bars compare the mean mCPP-induced behavioral response on day 28 for the various chronic treatments. The asterisks indicate there was a significant attenuation of mean behavior for both the clomipramine and the fluvoxamine treated rats as compared to the saline-treated control group. This was determined by ANOVA [$F(2,45)=5.90$, $p<.01$] followed by Dunnett's post-hoc test (** $p<0.01$).

C. Examination of the attenuation of mCPP-induced RCBs over a 28 day period:

In this experiment, daily injections of clomipramine led to a significant reduction of behavior induced by mCPP after 21 days and after 28 days. On days 1 and 7 of treatment, analysis of behavior observation demonstrated that there was no significant difference in mCPP's ability to induce RCBs between the clomipramine treated rats and the control (saline) rats. After 14 days of daily treatments, analysis of behavior between saline treated rats and clomipramine treated rats suggested a trend towards a significant difference ($p=.06$). Twenty one daily injections of clomipramine, however, led to a significant attenuation of mCPP-induced chewing behaviors as compared to the value in rats treated with 21 daily injections of saline. The reduced mean number of RCBs in clomipramine treated rats was also evident on day 28. Data from these experimental groups on each day is presented in figure 14 and illustrated in figure 15.

Number of injections (daily treatment)	Mean number RCBs for saline (1 mg/kg) treated rats	Mean number of RCBs for clomipramine (15 mg/kg) treated rats
0	15.25 ± 1.03	15.25 ± 2.58
7	12.25 ± 2.39	12.38 ± 1.52
14	11.50 ± 1.13	7.75 ± 1.39
21	14.13 ± 2.29	5.63 ± 1.68 **
28	14.50 ± 1.59	6.50 ± 1.57 *

Figure 14: Data demonstrating attenuation of mCPP-induced behavior by clomipramine over a 28 day period as compared to the behavior following similar injections of saline. Asterisks indicate that the mean responses after 21 and 28 days of treatment with clomipramine were significantly less than that of saline (* p<0.05; ** p<0.01) as determined by ANOVA for day 21 [F(1,14)=8.98] and day 28 [F(1,14)=12.8].

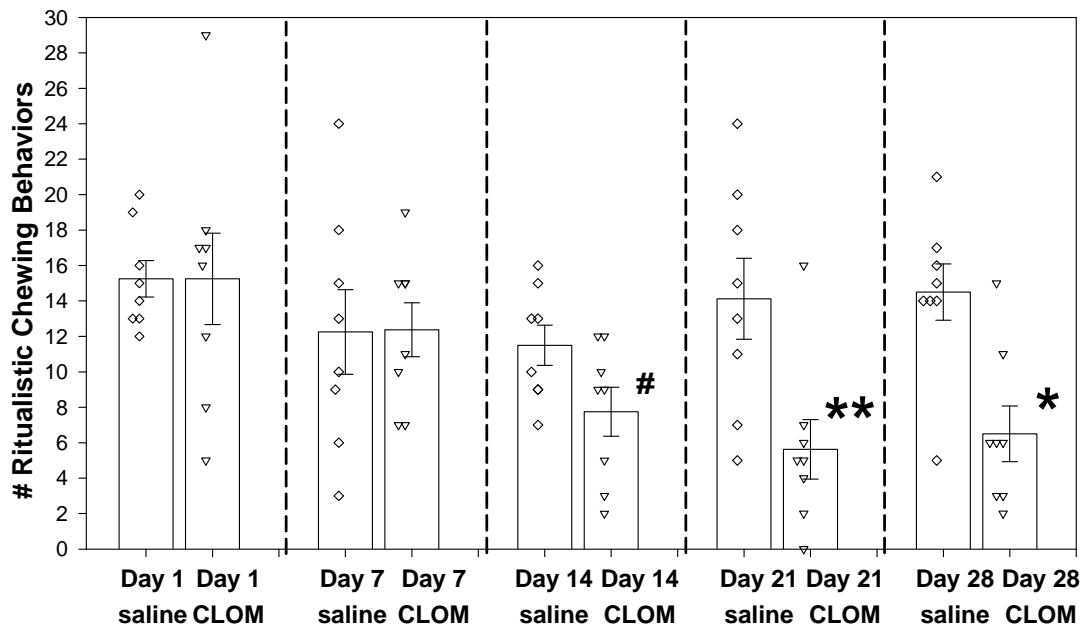


Figure 15: Bars indicate the mean behavioral response to mCPP (1 mg/kg) following chronic treatments of either saline or clomipramine. Error bars denote 1 S.E.M.; individual responses are indicated as either diamonds (saline treated) or inverted triangles (clomipramine treated). Dotted lines separate observations of the mCPP behavior conducted after 0, 7, 14, 21, and 28 days of treatment. Asterisks indicate that the mean responses after 21 and 28 days of treatment with clomipramine were significantly less than that of saline (* $p < 0.05$; ** $p < 0.01$) as determined by ANOVA for day 21 [$F(1,14)=8.98$] and day 28 [$F(1,14)=12.8$]. The pound symbol indicates a trend towards significance where $p = .06$.

IV. Discussion:

The purpose of my research was to evaluate mCPP-induced Ritualistic Chewing Behaviors (RCBs) as an animal model with predictive validity for Obsessive Compulsive Disorder (OCD). A valid animal model is an integral factor in elucidating novel approaches in treating OCD as current treatments for OCD are inadequate. mCPP was selected for this model because it exacerbates symptoms in untreated OCD patients, and it becomes ineffective in successfully treated patients. Studies have shown that anxiety producing drugs such as yohimbine and caffeine do not increase Obsessive Compulsive symptoms (Insel et al., 1990), thus suggesting that mCPP's effect is specific to Obsessive Compulsive symptoms and not merely an increase in anxiety.

A. Evaluation of serotonin 5HT_{2C} receptors:

The serotonergic hypothesis for the underlying cause of OCD proposes that OCD patients are hypersensitive to activation of their serotonergic system (Zohar et al., 2000; Yamauchi et al., 2006). This hypothesis is in line with the currently effective pharmaceutical treatments (SRIs) which are believed to affect the serotonin system by desensitizing serotonin receptors (Zohar et al., 2000; Yamauchi et al., 2006). Thus, serotonin becomes a central focus in OCD research.

Unfortunately, there are many subtypes of serotonin receptors, and different serotonin receptors have been associated with different psychiatric disorders. For instance, the serotonin 5HT_{1A} receptor subtype is associated with depression and anorexia nervosa is associated with the serotonin 5HT_{2A} receptor subtype (Blier and Abbott, 2000; Audenaert et al., 2003). Anxiety disorders, however, are believed to originate from activation at the serotonin 5HT_{2C} receptor subtype. mCPP mimics serotonin at the serotonin 5HT_{2C} receptor subtype primarily and at the serotonin 5HT_{1A} and 5HT_{1D} receptor subtypes to a lesser degree (Zohar et al., 2000). Therefore, it is important to verify that the involvement of the serotonin 5HT_{2C} receptor subtype in mCPP-induced effects on the animal behavior being evaluated as a model for OCD.

In order to verify that the serotonin 5HT_{2C} receptor underlies mCPP-induced RCBs, SB228357 was used. SB228357 is a serotonin receptor “antagonist” in that it blocks serotonin receptors from being occupied by serotonin. SB228357 was chosen for this study because of its selectivity for the serotonin 5HT_{2C} receptor subtype (Sigma-Aldrich, 2008). Results demonstrated that a pretreatment of the 5HT_{2C} antagonist significantly reduced mCPP’s ability to induce RCBs by 90%. Previous Departmental Honors projects investigated the role of serotonin 5HT₁ and 5HT_{2A} receptor subtypes in mCPP-induced RCBs. Pretreatments of antagonists for the serotonin 5HT₁ and 5HT_{2A} receptor subtypes did not block the mCPP-induced chewing behavior (Helton, 2006; Winkler, 2006; Jackson, 2007; Lee, 2007). Taken together, the prior and current experimental results support the hypothesis that

mCPP's capacity to increase RCBs is a result of action at the serotonin 5HT_{2C} receptor.

B. Change of behavior with administration of SRIs:

Evaluation of SRIs in attenuation of mCPP-induced RCBs

Since the most effective known pharmaceutical treatments for OCD are Serotonin Reuptake Inhibitors (SRIs), experiments of the present study examined the ability of mCPP to induce RCB's following treatment with two of the most commonly prescribed SRIs for OCD, clomipramine and fluvoxamine. Results indicated that the number of mCPP-induced RCBs following repeated treatment with either clomipramine or fluvoxamine was significantly attenuated. This reduction demonstrates that SRIs have an effect on this rodent behavior that is analogous to the drugs' clinical efficacy.

Evaluation of onset of attenuation of mCPP-induced RCBs

Because drugs can take 10-12 weeks to reduce symptoms in OCD patients, the effectiveness of SRIs in a valid animal model of OCD must reflect a delayed onset. In a 28 day trial, rats' behavior following administration of mCPP was observed every 7 days of repeated administration of SRI. Significant attenuation of chewing behavior induced by mCPP was evident after 21 days of clomipramine treatment. The delayed onset of effectiveness of clomipramine upon the attenuation of the given

mCPP-induced rodent behavior is analogous to the delayed onset of the drugs' clinical efficacy.

The time frame by which SRIs alter mCPP-induced RCBs adds additional support to the likelihood that desensitization of serotonin 5-HT_{2C} receptors in the cortico-basal ganglia-thalamic circuit underlies this alteration of behavioral effects induced by mCPP (El Mansari and Blier, 2006). Clinical studies have shown SRI treatment alleviates depressive symptoms after 2-3 weeks of treatment, whereas alleviation of Obsessive Compulsive symptoms requires 8-10 weeks of treatment. This time frame of efficacy is reflected in the rodent experimental studies. These studies show that repeated treatment with SRIs desensitizes serotonin 5HT_{1A} receptors in the hypothalamus and striatum weeks before the repeated treatment desensitizes serotonin 5HT_{2C} receptors in the orbital frontal cortex.

C. Conclusion of Findings:

Several significant findings resulted from these experiments:

- Serotonin 5HT_{2C} receptor subtypes mediate mCPP-induced RCBs
- mCPP-induced RCBs are attenuated by 26 day treatment with either clomipramine or fluvoxamine.
- Clomipramine reduced mCPPs ability to induce RCBs only after 21 and 28 days of repeated administration. Attenuation of mCPP-induced RCBs did not occur after 1, 7 or 14 days of treatment.

Taken together, these results support the use of mCPP-induced RCBs as an animal model of OCD that can predict the efficacy of future treatments.

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